

**ANTIOXIDANT, HYPOGLYCEMIC AND HYPOLIPIDEMIC
EFFECTS OF SELECTED AFRICAN INDIGENOUS MEDICINAL
PLANTS IN A TYPE 2 DIABETES MODEL OF RATS**



BY

BRIAN KUDAKWASHE BESENI

Student Number: 218088125

JAN 2024

**ANTIOXIDANT, HYPOGLYCEMIC AND HYPOLIPIDEMIC
EFFECTS OF SELECTED AFRICAN INDIGENOUS MEDICINAL
PLANTS IN A TYPE 2 DIABETES MODEL OF RATS**



**UNIVERSITY OF
KWAZULU-NATAL**

**INYUVESI
YAKWAZULU-NATALI**

BY

BRIAN KUDAKWASHE BESENI

Student Number: 218088125

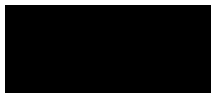
**Submitted in fulfilment of the academic requirements for the degree of Doctor of
Philosophy (PhD) in Biochemistry, School of Life Sciences, University of
KwaZulu-Natal (Westville campus), Durban 4000, South Africa**

As the candidate's supervisor, I have approved this thesis for submission

Signed:  Name: Prof MS Islam Date: January 2024

PREFACE

The information presented in this thesis represents the original work of the candidate. This work was carried out in the Department of Biochemistry, School of Life Sciences, College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Westville Campus, Durban, South Africa, from August 2018 to December 2023 under the supervision of Prof. MS Islam, and has not otherwise been submitted in any form for any degree or diploma to any other university in the world. Apart from research collaborators, all the assistance obtained from others has been duly acknowledged in the acknowledgement section.



Candidate: Brian K. Beseni



Supervisor: Prof. MS Islam

DECLARATION 1 – PLAGIARISM

I, **Brian Kudakwashe Beseni**, declare that

1. The research reported in this thesis, except where otherwise indicated, is my original work.
2. This thesis has not been submitted for any degree or examination at any other university.
3. This thesis does not contain other persons' data, pictures, graphs, or other information unless specifically acknowledged as being sourced from other persons.
4. This thesis does not contain other persons' writings unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:
 - A. their words have been re-written, but the general information attributed to them has been referenced.
 - B. where their exact words have been used, then their writing has been placed in italics and inside quotation marks and referenced.
5. This thesis does not contain text, graphics, or tables copied and pasted from the Internet unless specifically acknowledged, and the source is detailed in the thesis and in the reference section.



Signed: Brian Kudakwashe Beseni.

DECLARATION 2 – PUBLICATIONS

DETAILS OF CONTRIBUTION TO PUBLICATIONS that constitute part of the research presented in this thesis (include publications in preparation, submitted, in press and published and give details of the contributions of each author to the experimental work and writing of each publication). I, **Brian Kudakwashe Beseni**, hereby declare that the research works published in the following articles were performed and written by me, under the guidance of my supervisor, Prof. MS Islam, while the co-authors contributed partly to the work. The final editorial check on each manuscript was done by my supervisor before submission to journals for possible publication.

PUBLICATIONS FROM THIS THESIS

Published articles

1. **Beseni, B.K.**, Olofinisan, K.A., Salau, V.F., Erukainure, O.L. and Islam, M.S., 2022. *Rhus longipes* (Engl.) infusions improve glucose metabolism and mitigate oxidative biomarkers in ferrous sulfate-induced renal injury. *Asian Pacific Journal of Tropical Biomedicine*, 12(11), pp.453-465.

Prospective articles

1. **Beseni, B.K.**, Olofinisan, K.A., Salau, V.F., Erukainure, O.L. and Islam, M.S. *Trema orientalis* leaf extracts regulate glucose metabolism while concomitantly abating oxidative pancreatic tissue injury. **[In preparation]**.
2. **Beseni, B.K.**, Olofinisan, K.A., Salau, V.F., Erukainure, O.L. and Islam, M.S. *Trema orientalis* crude bark extracts inhibit carbohydrate and lipid digesting enzymes, attenuate muscular glucose uptake and ameliorate oxidative cardiac tissue injury. **[In preparation]**.
3. **Beseni, B.K.**, Olofinisan, K.A., Salau, V.F., Erukainure, O.L. and Islam, M.S. *Albizia adianthifolia* crude leaf extracts regulate glucose metabolism and ameliorate oxidative stress-linked cardiac tissue injury. **[In preparation]**.
4. **Beseni, B.K.**, Olofinisan, K.A., Salau, V.F. and Islam, M.S. *Albizia adianthifolia* crude bark extracts modulate glucose metabolism and ameliorate oxidative (FeSO₄) pancreatic tissue injury. **[In preparation]**.

5. **Beseni, B.K.**, Olofinisan, K.A., Msomi, N.Z., Mohamed, A.I., Salau, V.F., Erukainure, O.L. and Islam, M.S. *Rhus longipes* (Engl.) bark extracts inhibit carbohydrate digesting enzymes activity, promote psoas muscle and hepatic glucose uptake and mitigate FeSO₄-induced pancreatic oxidative injury. **[In preparation]**.
6. **Beseni, B.K.**, Erukainure, O.L. and Islam, M.S. *Rhus longipes* organic leaf extracts regulate glucose metabolism and abate oxidative injury in cardiac tissues. **[In preparation]**.
7. **Beseni, B.K.**, Olofinisan, K.A., Erukainure, O.L. and Islam, M.S. *Rhus longipes* (Engl.) improves atherogenic indices, glucose metabolism and ameliorates oxidative stress in streptozotocin-induced type 2 diabetic rat model. **[In preparation]**.
8. **Beseni, B.K.**, Olofinisan, K.A., Salau, V.F., Erukainure, O.L. and Islam, M.S. *Celtis africana* (N.L.Burm.) leaf extracts regulate glucose homeostasis and mitigate cardiac muscle tissue oxidative injury. **[In preparation]**.
9. **Beseni, B.K.**, Olofinisan, K.A., Salau, V.F., Erukainure, O.L. and Islam, M.S. *Celtis africana* (N.L.Burm.) bark extracts regulate glucose metabolism and mitigate pancreatic oxidative injury. **[In preparation]**.
10. **Beseni, B.K.**, Olofinisan, K.A., Salau, V.F., Erukainure, O.L. and Islam, M.S. *Celtis africana* (N.L.Burm.) improves glucose metabolism, reduces atherogenic indices, and ameliorates oxidative stress in streptozotocin induced type 2 diabetic rat model. **[In preparation]**.
11. **Beseni, B.K.**, Olofinisan, K.A., Erukainure, O.L. and Islam, M.S. *Schotia brachypetala* (Sond) crude leaf extract exhibit potent antioxidant, antihyperglycemic, and enhanced protective activities against FeSO₄ induced oxidative pancreatic cell injury. **[In preparation]**.
12. **Beseni, B.K.**, Olofinisan, K.A., Erukainure, O.L. and Islam, M.S. *Schotia brachypetala* (Sond) crude bark extract exhibit potent antioxidant, antihyperglycemic, and enhanced protective activities against FeSO₄ induced oxidative cardiac tissue injury. **[In preparation]**.
13. **Beseni, B.K.**, Olofinisan, K.A., Erukainure, O.L. and Islam, M.S. *Schotia brachypetala* (Sond) ameliorates glucose metabolic dysfunction, abates dyslipidaemia, and mitigates oxidative renal and hepatic tissue damage in streptozotocin induced type 2 diabetic rat model. **[In preparation]**.

Other published collaborative work

1. Xiao, X., Erukainure, O.L., **Beseni, B.**, Koorbanally, N.A. and Islam, M.S., 2020. Sequential extracts of red honeybush (*Cyclopia genistoides*) tea: Chemical characterization, antioxidant potentials, and anti-hyperglycemic activities. *Journal of Food Biochemistry*, 44(11), p.e13478.
2. Erukainure, O.L., Msomi, N.Z., **Beseni, B.K.**, Salau, V.F., Ijomone, O.M., Koorbanally, N.A. and Islam, M.S., 2021. Cola nitida infusion modulates cardiometabolic activities linked to cardiomyopathy in diabetic rats. *Food and Chemical Toxicology*, 154, p.112335.
3. Mohamed, A.I., **Beseni, B.K.**, Msomi, N.Z., Salau, V.F., Erukainure, O.L., Aljoundi, A. and Islam, M.S., 2021. The antioxidant and antidiabetic potentials of polyphenolic- rich extracts of *Cyperus rotundus* (Linn.). *Journal of Biomolecular Structure and Dynamics*, pp.1-13.
4. Olofinisan, K.A., Erukainure, O.L., **Beseni, B.K.** and Islam, M.S., 2022. Harpephyllum caffrum stimulates glucose uptake, abates redox imbalance and modulates purinergic and glucogenic enzyme activities in oxidative hepatic injury. *Asian Pacific Journal of Tropical Biomedicine*, 12(1), pp.9.

Training seminars

1. **UKZN Research Enrichment programme for capacity development.**2018. University of Kwazulu-Natal, Westville Campus, Durban, South Africa.
2. **Animal welfare and ethics training course.** 2018. Biomedical Resource Unit, University of Kwazulu-Natal, Westville Campus, Durban, South Africa.
3. **UKZN-Capacity Development Programme Teacher Training Program.**2019/2020. College of Agriculture, Engineering, and Sciences, University of Kwazulu-Natal, Westville Campus, Durban, South Africa.
4. **Mass Spectrometry based Metabolomics.** 2020. Virtual Workshop.
5. **Animal handling training course.**2021. Biomedical Resource Unit, University of Kwazulu-Natal, Westville Campus, Durban, South Africa.
6. **Teaching assistant and demonstrator training workshop.**2022. University of Kwazulu-Natal, Westville Campus, Durban, South Africa.

Conference presentations

1. **Beseni, B.K.**, Erukainure, O.L. and Islam, M.S. *In-vitro* antioxidant and carbohydrate digesting enzyme inhibitory activities of *Rhus longipes* (Engl)-Poster presentation. 2019. School of Life Sciences, University of Kwazulu-Natal, Westville Campus, Durban, South Africa.
2. **Beseni, B.K.**, Erukainure, O.L. and Islam, M.S. *Rhus longipes* (Engl). modulates renal oxidative injury and inhibits carbohydrate digesting enzymes.2019. **Postgraduate Research and Innovation Symposium**, University of KwaZulu-Natal, Durban, South Africa.
3. **Beseni, B.K.**, Olofinisan, K.A., Erukainure, O.L. and Islam, M.S. Aqueous leaf and bark defatted crude infusions of *Rhus longipes* (Engl) inhibit carbohydrate digesting enzymes, modulate intestinal glucose absorption and muscular glucose uptake, while ameliorating renal FeSO₄ induced oxidative stress. 2021. **Postgraduate Research and Innovation Symposium**, University of KwaZulu-Natal, Durban, South Africa.
4. **Beseni, B.K.**, Erukainure, O.L. and Islam, M.S. *Schotia brachypetala* Sond. improves atherogenic indices, glucose tolerance and ameliorates oxidative stress in streptozotocin induced type 2 diabetic rat model.2022. **Postgraduate Research and Innovation Symposium**, University of KwaZulu-Natal, Durban, South Africa.
5. **Beseni, B.K.**, Olofinisan, K.A., Erukainure, O.L. and Islam, M.S. *Rhus longipes* (Engl.) regulates hepatic glycogenic enzymes, improves glucose metabolism while ameliorating dyslipidaemia and oxidative stress in a type 2 diabetic rat model.2023. . **Postgraduate Research and Innovation Symposium**, University of KwaZulu-Natal, Durban, South Africa.

Author's contributions

Beseni, B.K.: Conceptualization; Formal analysis; Methodology; Writing-original draft. Almahi, I.M.: In silico analysis and Methodology. Erukainure, O.L.: Conceptualization and draft review, Olofinisan, K.A.: Formatting, draft review; writing review & proof editing. NZ: Writing-review and proof editing. Salau, V.F.: Writing review and proof editing. Islam, M.S.: Supervision; Project administration; Writing review & proof editing.

LIST OF ABBREVIATIONS

ALT	Alanine transaminase
AGE	Advanced glycation end products
AST	Aspartate transaminase
ATCC	American Type Culture Collection
ATP	Adenosine triphosphate
ATPase	Adenosine triphosphatase
BRU	Biomedical Research Unit
CAT	Catalase
CK-MB	Creatine kinase-myocardial band
CVD	Cardiovascular disease
DAG	Diacylglycerol
DETAPAC	Diethylenetriaminepentaacetic acid
DM	Diabetes mellitus
DMEM	Dulbecco's Modified Eagle Medium
DNS	Dinitro-salicylic acid
DPP-4	Dipeptidyl peptidase IV
DPPH	1,1-diphenyl-2-picrylhydrazyl radical
DTNB	5,5'-dithiobisnitrobenzoic acid
EDTA	Ethylenediaminetetraacetic acid
ERK	Extracellular-signal-regulated kinase
FBPase	Fructose-1,6-bisphosphatase
FBS	Foetal Bovine Serum
FRAP	Ferric reducing antioxidant power
GK	Glucokinase
GC-MS	Gas chromatography-mass spectrometry
GDP	Gross domestic product
GIT	Gastro-intestinal-tract
GLUT	Glucose transporter
GLP1	Glucagon-like peptide 1
GSH	Glutathione reductase
GWAS	Genome-wide association studies

HDL	High-density lipoprotein
HNF	Hepatocyte nuclear factor
HOMA- β	Homeostasis Model Assessment for β -cell function
HOM-IR	Homeostasis Model Assessment for insulin resistance
HTLV	Human T-lymphotropic virus
IDDM	Insulin-dependent diabetes mellitus
IDF	International diabetes federation
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
IKS	Indigenous knowledge systems
IR	Insulin receptor
IRS	Insulin receptor substrate
JNK	c-Jun N-terminal kinase
KCNJ11	Potassium inwardly rectifying channel, subfamily J, member 11
K _m	Michaelis-Menten's constant
LDH	Lactate dehydrogenase
LDL	Low-density lipoprotein
MAPK	Mitogen-activated protein kinases
MDA	Malondialdehyde
MODY	Maturity-onset diabetes of the young
NADPH	Nicotinamide adenosine dinucleotide
NO	Nitric oxide
OGTT	Oral glucose tolerance test
PI3K	Phosphatidylinositol-3-kinase
PKA	Protein kinase A
PKB	Protein kinase B
PKC	Protein kinase C
PM	Plasma membrane
PPARG2	Peroxisome proliferator-activated receptor-gamma 2
PPARs	Peroxisome proliferator-activated receptors
RAGE	Advanced glycation end products receptors

ROS	Reactive oxygen species
SGLT	Sodium glucose transporter
SGLT-2	Sodium-glucose co-transporter-2
SH2	Src homology 2
SOD	Superoxide dismutase
T1D	Type 1 diabetes
T2D	Type 2 diabetes
VLDL	Very low-density lipoprotein
WHO	World Health Organization

DEDICATION

To the Lord God almighty for his unending mercy.

To my parents **Mr. and Mrs. Beseni** for their love and unwavering support.

ACKNOWLEDGEMENTS

To my supervisor, Prof. MS Islam, for his unwavering support throughout this project. Through his guidance I have learnt several skills that have sharpened my research skills and knowledge. I believe the knowledge I have attained during this project will enable me to be a self-sufficient researcher hence forth.

Staff members in the Department of Biochemistry Dr K Pillay, Dr. OJ Poee, Ms. SR Mokhosi, Dr. N Nundkumar and Mr D. Jagganath for their cooperation during my study. Their wiliness to provide practical and academic guidance when they were approached proved pivotal to the completion of this study.

Mr. Edward Khati for his much-appreciated assistance in the field for plant collection and identification.

Staff members in the Faculty of Science, Agriculture and Engineering for the continuous support and the DVC and College Dean of Research of the College of Agriculture, Engineering and Science for providing me with a scholarship to complete my study. It is highly appreciated.

Dr. Linda Bester, Mr. David, and Rita Radebe for their support during my animal study at Biomedical Research Unit (BRU) of the University of KwaZulu-Natal, Durban, South Africa.

My colleagues, current and alumni of the Biomedical Research Lab, Department of Biochemistry including but not limited to Dr. Ochuko Erukainure, Dr Kolawole Olofinsan, Dr. Veronica Salau, Dr. Nontokozo Msomi, Mr. Almahi Idris, Dr. Rebecca Reddy, Ms. Vunene Chabalala, Mr. Mohammed Khan. They provided the much-needed emotional support throughout the study and I consider them as part of my extended family.

My friends Mr. Albert Makavanga, Mr. Charles Zungunde, Mr. Brian Tobert and Ms. Mmapula Leseilane for their friendship and support during my study.

My siblings Crystal Masaya and Lily Marshall for their love and encouragement throughout this study.

Finally, I would like to express my gratitude to the National Research Foundation through the research office at UKZN for their funding support to complete this study.

TABLE OF CONTENTS

PREFACE	i
DECLARATION 1 – PLAGIARISM	ii
DECLARATION 2 – PUBLICATIONS	iii
PUBLICATIONS FROM THIS THESIS	iii
LIST OF ABBREVIATIONS	vii
ACKNOWLEDGEMENTS	xi
TABLE OF CONTENTS	xii
LIST OF TABLES	xxiii
LIST OF FIGURES	xxvi
ABSTRACT.....	xxxv
Chapter 1: Introduction and literature review	1
1.1 Introduction.....	1
1.2 Global prevalence of diabetes	1
1.3 Global economic burden of diabetes.....	3
1.4 Global morbidity and mortality due to diabetes.....	4
1.5 Definition and diagnosis of Diabetes Mellitus (DM).....	4
1.6 Types of diabetes.....	5
1.6.1 Type I diabetes mellitus.....	5
1.6.2 Type II diabetes mellitus	5
1.6.3 Gestational diabetes mellitus (GDM).....	6
1.6.4 Other types of diabetes	6
1.7 Risk factors of diabetes mellitus	7
1.7.1 Modifiable risk factors	7
1.7.2 Non-modifiable diabetes risk factors.....	8
1.8 Normal blood glucose homeostatic.....	9
1.8.1 Insulin.....	10

1.8.1.1a Insulin-mediated glucose uptake pathway	12
1.8.1.1b Ras-mitogen-activated protein kinase (MAPK) signalling pathway	12
1.8.2 Glucagon	13
1.8.3 Pancreas.....	13
1.8.4 Liver	14
1.8.5 Muscle tissues	15
1.8.6 Adipose tissues	15
1.8.7 Glucose transporters	15
1.9 Diabetes pathogenesis and progression.....	19
1.9.1 The ominous octet of diabetes pathogenesis.....	19
1.9.2 Oxidative stress	20
1.9.3 Insulin resistance	23
1.10 Complications of diabetes	23
1.10.1 Macro-vascular complications.....	23
1.11 Diabetes management strategies	25
1.11.1 Non-pharmacological strategies	25
1.11.2 Pharmacological antidiabetic agents	26
1.11.3 Drawbacks of current diabetes management strategies	28
1.12. Traditional medicinal plants as novel antidiabetic therapeutics.....	29
1.13 Plant secondary metabolites and their antidiabetic properties	29
1.13.1 Secondary metabolites.....	29
1.14 Rationale for using medicinal plants to manage diabetes	37
1.15 Problem identification.....	37
1.16 Aim of the study.....	38
1.17 Hypotheses.....	38
1.18 Objectives	38
CHAPTER 2.....	39

Materials and methods	39
Materials	39
2.1 Chemicals.....	39
2.2 Equipment	39
Methods.....	40
2.3 Plant materials.....	40
2.3.1 Plant collection and verification	40
2.3.2 Plant extract preparation.....	40
2.4 Phytochemical characterisation and quantification	40
2.4.1 Total phenolic content	40
2.4.2 Total flavonoid content.....	41
2.4.3 Gas Chromatography Mass Spectroscopy (GC-MS) analysis.....	41
2.5 <i>In vitro</i> antioxidant analysis	42
2.5.1 Quantitative DPPH radical-scavenging activity assay	42
2.5.2 Ferric reducing antioxidant power (FRAP).....	42
2.5.3 Nitric oxide scavenging assay	42
2.6 <i>In vitro</i> enzyme inhibition analysis	43
2.6.1 α -glucosidase inhibition assay.....	43
2.6.2 α -amylase inhibition assay	43
2.6.3 Porcine pancreatic lipase inhibition assay.....	44
2.6.4 Mode of α -amylase inhibition assay	44
2.6.5 Mode of α -glucosidase inhibition assay	44
2.6.6 Mode of pancreatic lipase inhibition	45
2.6.7 Mode of inhibition analysis.....	45
2.7 Glucose uptake/transport by yeast cells	45
2.8 Cell lines	46
2.8.1 Cytotoxicity assay	46

2.9 Molecular docking	47
2.10 Animals for <i>ex vivo</i> study	47
2.10.1 Experimental animals	47
2.10.2 Intestinal glucose absorption study	47
2.10.3 Measurement of glucose uptake in isolated rat psoas muscles/liver tissues	48
2.10.4 Preparation of tissue homogenates	48
2.10.5 Fructose-1,6-bisphosphatase activity	49
2.10.6 Glycogen phosphorylase activity	49
2.10.7 ATPase enzyme assay	49
2.11 Oxidative stress biomarkers	50
2.11.1 Preparation of tissue homogenates and induction of oxidative stress <i>ex vivo</i>	50
2.11.2 Determination of lipid peroxidation level	50
2.11.3 Determination of reduced glutathione (GSH) level	50
2.11.4 Determination of superoxide dismutase (SOD) activities	50
2.11.5 Determination of catalase (CAT) activities	51
2.12 <i>In vivo</i> animal study	51
2.12.1 Animals	51
2.12.2 Animal grouping	51
2.12.3 Induction of diabetes	53
2.12.4 Intervention	53
2.12.5 Oral Glucose Tolerance Test (OGTT)	53
2.12.6 Collection of blood and organs	53
2.12.7 Fasting blood glucose and homeostatic model assessment (HOMA)	54
2.12.8 Serum lipid profiles	54
2.12.9 Serum biochemical analysis	55
2.12.10 <i>In vivo</i> antioxidant studies	55
2.12.11 Liver glycogen content	55

2.12.12 Histopathological Analysis	56
2.13 Statistical analysis	56
CHAPTER 3.....	57
<i>IN VITRO AND EX VIVO STUDIES TO INVESTIGATE THE ANTIOXIDANT AND ANTIDIABETIC ACTIVITIES OF TREMA ORIENTALIS</i> Linn. Blume.	57
3.1 <i>Trema orientalis</i>	57
3.1.1 Background and plant description	57
3.1.2 Ethnobotanical uses	58
3.1.3 Biological activities	58
3.1.4 Phytochemistry	58
3.1.5 Aim and objectives.....	59
3.2 <i>Trema orientalis</i> leaf extracts regulate glucose metabolism while concomitantly abating oxidative pancreatic tissue injury	60
3.2.1 Abstract	60
3.2.2 Introduction	61
3.2.3 Materials and methods.....	62
3.2.4 Results	63
3.2.5 Discussion	71
3.2.6 Conclusion.....	74
3.3 <i>Trema orientalis</i> crude bark extracts inhibit carbohydrate and lipid digesting enzymes, attenuate muscular glucose uptake and ameliorate	75
oxidative cardiac tissue injury	75
3.3.1 Abstract.....	75
3.3.2 Introduction	76
3.3.3 Materials and methods.....	77
3.3.4 Results	78
3.3.5 Discussion	89
3.3.6 Conclusion.....	91
CHAPTER 4.....	92

<i>IN VITRO</i> AND <i>EX VIVO</i> STUDIES TO INVESTIGATE THE ANTIOXIDANT AND ANTIDIABETIC ACTIVITIES OF <i>ALBIZIA ADIANTHIFOLIA</i> (SCHUMACH.) W.F.Wight.	92
4.1 <i>Albizia adianthifolia</i> (Schumach.) W.F.Wight.....	92
4.1.1 Background and plant description	92
4.1.2 Ethnobotanical uses	93
4.1.3 Biological activities	93
4.1.4 Phytochemistry	93
4.1.5 Aim and objectives.....	94
4.2 <i>Albizia adianthifolia</i> crude leaf extracts regulate glucose metabolism and ameliorate oxidative stress-linked cardiac tissue injury	95
4.2.1 Abstract	95
4.2.2 Introduction	96
4.2.3 Materials and methods	97
4.2.4 Results	98
4.2.5 Discussion	107
4.2.6 Conclusion.....	109
4.3 <i>Albizia adianthifolia</i> crude bark extracts modulate glucose metabolism and ameliorate oxidative (FeSO ₄) pancreatic tissue injury	110
4.3.1 Abstract	110
4.3.2 Introduction	111
4.3.3 Materials and methods	112
4.3.4 Results	113
4.3.5 Discussion	122
4.3.6 Conclusion.....	124
CHAPTER 5.....	125
<i>IN VITRO</i> , <i>EX VIVO</i> AND <i>IN VIVO</i> STUDIES FOR INVESTIGATING THE ANTIOXIDANT AND ANTIDIABETIC ACTIVITIES OF <i>RHUS LONGIPES</i> (Engl.)	125
5.1 <i>Rhus longipes</i> (Engl.).....	125
5.1.1 Background and plant description	125

5.1.2 Ethnobotanical uses.....	126
5.1.3 Biological activities.....	126
5.1.4 Phytochemistry.....	126
5.2 <i>Rhus longipes</i> (Engl.) infusions improve glucose metabolism and mitigate oxidative biomarkers in ferrous sulfate induced renal injury	127
5.2.1 Abstract	127
5.2.2 Introduction	128
5.2.3 Materials and methods	129
5.2.4 Results	130
5.2.5 Discussion	143
5.2.6 Conclusion.....	145
5.3 <i>Rhus longipes</i> (Engl.) bark extracts inhibit carbohydrate digesting enzymes activity, promote psoas muscle and hepatic glucose uptake	146
and mitigate FeSO ₄ -induced pancreatic oxidative injury.....	146
5.3.1 Abstract	146
5.3.2 Introduction	147
5.3.3 Materials and methods	148
5.3.4 Results	149
5.3.5 Discussion	163
5.3.6 Conclusion.....	165
5.4 <i>Rhus longipes</i> organic leaf extracts regulate glucose metabolism and abate oxidative injury in cardiac tissues	166
5.4.1 Abstract	166
5.4.2 Introduction	167
5.4.3 Materials and methods	169
5.4.4 Results	170
5.4.5 Discussion	180
5.4.6 Conclusion.....	184

5.5 <i>Rhus longipes</i> (Engl.) improves atherogenic indices, glucose metabolism and ameliorates oxidative stress in streptozotocin-induced type 2	185
diabetic rat model	185
5.5.1 Abstract	185
5.5.2 Introduction	186
5.5.3 Materials and methods	188
5.5.4 Results	189
5.5.5 Discussion	202
5.5.6 Conclusion.....	205
CHAPTER 6.....	206
<i>IN VITRO, EX VIVO AND IN VIVO STUDIES FOR INVESTIGATING THE ANTIOXIDANT AND ANTIDIABETIC ACTIVITIES OF CELTIS</i>	206
<i>AFRICANA</i> (Burm. F.).....	206
6.1 <i>Celtis africana</i> (Burm.f.).....	206
6.1.1 Background and plant description	206
6.1.2 Ethnobotanical uses.....	207
6.1.3 Pharmacological and biological activities	207
6.1.4 Phytochemistry	207
6.2 <i>Celtis africana</i> (N.L.Burm.) leaf extracts regulate glucose homeostasis and mitigate cardiac muscle tissue oxidative injury.....	208
6.2.1 Abstract	208
6.2.2 Introduction	209
6.2.3 Materials and methods	210
6.2.4 Results	211
6.2.5 Discussion	228
6.2.6 Conclusion.....	230
6.3 <i>Celtis africana</i> (N.L.Burm.) bark extracts regulate glucose metabolism and mitigate pancreatic oxidative injury.....	231
6.3.1 Abstract	231

6.3.2 Introduction	232
6.3.3 Materials and methods	234
6.3.4 Results	234
6.3.5 Discussion	250
6.3.6 Conclusion.....	254
6.4 <i>Celtis africana</i> (N.L.Burm.) improves glucose metabolism, reduces atherogenic indices, and ameliorates oxidative stress in streptozotocin.....	255
induced type 2 diabetic rat model.....	255
6.4.1 Abstract	255
6.4.2 Introduction	256
6.4.3 Materials and methods	258
6.4.4 Results	258
6.4.5 Discussion	271
6.4.6 Conclusion.....	275
CHAPTER 7.....	276
<i>IN VITRO, EX VIVO AND IN VIVO STUDIES FOR INVESTIGATING THE ANTIOXIDANT AND ANTIDIABETIC ACTIVITIES OF SCHOTIA BRACHYPETALA</i> (Sond.).....	276
7.1 <i>Schotia brachypetala</i> (Sond.).....	276
7.1.1 Background and plant description	276
7.1.2 Ethnobotanical uses.....	277
7.1.3 Pharmacological and biological activities	277
7.1.4 Phytochemistry	277
7.2 <i>Schotia brachypetala</i> (Sond) crude leaf extract exhibit potent antioxidant, antihyperglycemic, and enhanced protective activities against FeSO ₄ induced	278
oxidative pancreatic cell injury	278
7.2.1 Abstract	278
7.2.2 Introduction	279
7.2.3 Materials and methods	280
7.2.4 Results	281

7.2.5 Discussion	291
7.2.6 Conclusion.....	293
7.3 <i>Schotia brachypetala</i> (Sond) crude bark extract exhibit potent antioxidant, antihyperglycemic, and enhanced protective activities against FeSO ₄ induced	294
oxidative cardiac tissue injury	294
7.3.1 Abstract	294
7.3.2 Introduction	295
7.3.3 Materials and methods	297
7.3.4 Results	297
7.3.5 Discussion	314
7.3.6 Conclusion.....	317
7.4 <i>Schotia brachypetala</i> (Sond) ameliorates glucose metabolic dysfunction, abates dyslipidaemia, and mitigates oxidative renal and hepatic tissue	318
damage in streptozotocin induced type 2 diabetic rat model.....	318
7.4.1 Abstract	318
7.4.2 Introduction	319
7.4.3 Material and methods	320
7.4.4 Results	320
7.4.5 Discussion	335
7.4.6 Conclusion.....	337
CHAPTER 8.....	338
GENERAL DISCUSSION AND CONCLUSION.....	338
8.1 General discussion.....	338
8.2 General conclusion.....	346
8.3 Recommendations	347
REFERENCES	348
APPENDIX A ETHICS APPROVAL.....	394
APPENDIX B PUBLICATIONS FROM THESIS	398

APPENDIX C OTHER RESEARCH PUBLICATIONS	398

LIST OF TABLES

Table 1: The function and location of different SGLTs	16
Table 1.2: Some reference secondary metabolites, their classification and antidiabetic mode of action.....	34
Table 3.2.1: The IC ₅₀ values of the different leaf extracts of <i>T. orientalis</i> under assayed antioxidant and enzyme inhibition parameters	70
Table 3.3.1: Compounds identified in the <i>T. orientalis</i> crude bark extracts as revealed by GCMS analysis	78
Table 3.3.2: The IC ₅₀ values of different bark extracts of <i>T. orientalis</i> under different in vitro antioxidant and enzyme inhibitory parameters.....	83
Table 4.2.1: The IC ₅₀ values of <i>A. adianthifolia</i> leaf crude extracts in vitro assays	106
Table 4.3.1: The IC ₅₀ values of <i>A. adianthifolia</i> bark extracts under different antioxidant and enzyme inhibitory <i>in vitro</i> assays	116
Table 5.2.1: Phyto-compounds in <i>R. longipes</i> leaf and bark infusions	132
Table 5.2.2: IC ₅₀ values of in vitro biological activities of <i>R. longipes</i> leaf and bark aqueous infusions.....	134
Table 5.2.3: Free binding energy of <i>R. longipes</i> leaf and bark infusions secondary metabolites with carbohydrate and lipid digestive enzymes	142
Table 5.3.1: Phyto-compounds identified by Gas Chromatography-Mass Spectroscopy (GC-MS) in <i>R. longipes</i> bark extracts.....	149
Table 5.3.2: Total phytochemical contents of <i>R. longipes</i> bark extracts and IC ₅₀ values for <i>in vitro</i> bioactivity assays	154
Table 5.3.3: Free binding energy of <i>R. longipes</i> bark secondary metabolites with different protein targets.....	160
Table 5.4.1: The phyto-compounds identified by Gas Chromatography Mass Spectroscopy (GC-MS) in the different leaf <i>R. longipes</i> extracts.....	170
Table 5.4.2: Total phenolic and flavonoid content of different leaf extracts of <i>R. longipes</i> and their IC ₅₀ values under different assayed parameters.....	175

Table 5.5.1: The effects of oral treatment of hepatic glucogenic and nucleotide hydrolysing enzymes.....	195
Table 5.5.2: Effect of oral treatment on carbohydrate and lipid hydrolysing enzymes.....	196
Table 5.5.3: Effect of oral treatment on various pancreatic tissue oxidative stress linked enzymes.....	198
Table 5.5.4: Effect of oral treatment on various serum oxidative stress linked enzymes	198
Table 5.5.5: Effect of oral treatment on renal and hepatic function indices.....	200
Table 6.2.1: Phyto-compounds identified by Gas Chromatography Mass Spectroscopy (GC-MS) in <i>C. africana</i> leaf extracts.....	211
Table 6.2.2: Free binding energy of secondary metabolites from <i>C. africana</i> leaf extracts with carbohydrate and lipid digestive enzymes.....	219
Table 6.2.3: Free binding energy and interactions between of secondary metabolites from <i>C. africana</i> leaf extracts showing the best inhibitory capabilities against carbohydrate and lipid digestive enzymes.....	220
Table 6.2.4: The IC ₅₀ values of different <i>C. africana</i> extracts for different assayed parameters	222
Table 6.3.1: Phyto-compounds identified by Gas Chromatography Mass Spectrometry (GC-MS) in the <i>C. africana</i> stem bark extracts.....	234
Table 6.3.2: Free binding energy of secondary metabolites from <i>C. africana</i> stem bark extracts with carbohydrate and lipid digestive enzymes.....	243
Table 6.3.3: The IC ₅₀ values of different <i>C. africana</i> stem bark extracts for different assayed parameters	244
Table 6.4.1: The effects of oral treatment of hepatic glucogenic and nucleotide hydrolysing enzymes.....	264
Table 6.4.2: Effect of oral treatment on carbohydrate and lipid hydrolysing enzymes.....	265
Table 6.4.3: Effect of oral treatment on various serum oxidative stress linked enzymes	267
Table 6.4.4: Effect of oral treatment on various pancreatic oxidative stress linked enzymes	267

Table 6.4.5: Effect of oral treatment on renal and hepatic function indices.....	269
Table 7.2.1: The IC ₅₀ values of different leaf extracts of <i>S. brachypetala</i> under different assayed parameters	284
Table 7.3.1: Phyto-compounds identified by Gas Chromatography Mass Spectrometry (GC-MS) in <i>S. brachypetala</i> bark extracts.....	298
Table 7.3.2: IC ₅₀ values of in vitro biological activities of <i>S. brachypetala</i> bark extracts...	304
Table 7.3.3: Free binding energy of <i>S. brachypetala</i> bark extracts secondary metabolites with carbohydrate and lipid digestive enzymes.....	311
Table 7.4.1: The effects of oral treatment of hepatic glucogenic and nucleotide hydrolysing enzymes.....	327
Table 7.4.2: Effect of oral treatment on carbohydrate and lipid hydrolysing enzymes.....	328
Table 7.4.3: Effect of oral treatment on various pancreatic tissue oxidative stress linked enzymes.....	331
Table 7.4.4: Effect of oral treatment on various serum oxidative stress linked enzymes	332
Table 7.4.5: Effect of oral treatment on renal and hepatic function indices.....	333

LIST OF FIGURES

Figure 1.1: A and B: The estimated annual global prevalence of DM from 2000 to 2019...	2
Figure 1.2: Global DM incidence and future projections per region of people aged between 20 and 79.....	3
Figure 1.3: Maintenance of blood glucose levels by glucagon and insulin by different organs during the post-prandial state	10
Figure 1.4: The amino acid sequence of proinsulin showing the different cleavage points.	11
Figure 1.5: The cellular and tissue localisation of the facultative glucose transporters (GLUTs).....	17
Figure 1.6: The ominous octet involved in DM pathogenesis.....	20
Figure 2.1: Schematic representation of the in vivo study protocol.....	52
Figure 3.1: <i>Trema orientalis</i> Linn. Blume	57
Figure 3.2.1: The total phenolic content (A) and the total flavonoid content (B) of <i>T. orientalis</i> crude leaf extracts.....	63
Figure 3.2.2: Effects of <i>T. orientalis</i> leaf extracts of extracts on (A) DPPH radical scavenging activity, (B) ferric ion reducing activity, and (C) inhibition of nitric oxide production.....	64
Figure 3.2.3: Effects of <i>T. orientalis</i> bark organic extracts of extracts on (A) Pancreatic lipase inhibition activity, (B) α glucosidase inhibition activity, (C) α amylase inhibition activity	65
Figure 3.2.4: Modulatory effects of leaf crude extracts of <i>T. orientalis</i> on (A) yeast cell glucose uptake, (B) psoas muscle glucose uptake, (C) liver glucose uptake, and (D) intestinal glucose absorption.....	67
Figure 3.2.5: Effects of <i>T. orientalis</i> bark organic extracts of extracts on (A) lipid peroxidation, (B) SOD activity, (C) Catalase activity and (D) GSH activity in oxidative pancreatic injury	68

Figure 3.2.6: The modulatory effects of organic bark crude extracts of <i>T. orientalis</i> on (A) ATPase activity, (B) Glycogen phosphorylase activity and (C) Fructose 1,6 bisphosphatase activity.....	70
Figure 3.3.1: The total phenolic content (A) and the total flavonoid content (B) of <i>T. orientalis</i> crude bark extracts.....	80
Figure 3.3.2: Effects of <i>T. orientalis</i> bark organic extracts of extracts on (A) DPPH radical scavenging activity, (B) Ferric ion reducing activity, (C) Nitric oxide production inhibition.....	81
Figure 3.3.3: Effects of <i>T. orientalis</i> bark crude extracts on (A) Pancreatic lipase inhibition activity, (B) α glucosidase inhibition activity, (C) α amylase inhibition activity	82
Figure 3.3.4: The modulatory effects of bark crude extracts of <i>T. orientalis</i> on (A) yeast cell glucose uptake, (B) psoas muscle glucose uptake, (C) liver glucose uptake and (D) intestinal glucose absorption.....	85
Figure 3.3.5: Effects of <i>T. orientalis</i> bark crude extracts on (A) lipid peroxidation, (B) SOD activity, (C) Catalase activity and (D) GSH activity in oxidative pancreatic injury.....	87
Figure 3.3.6: The modulatory effects of bark crude extracts of <i>T. orientalis</i> on (A) ATPase activity, (B) Glycogen phosphorylase activity and (C) Fructose 1,6 bisphosphatase activity	88
Figure: 4.1: <i>Albizia adianthifolia</i> (Schumach.) W.F.Wight.....	92
Figure: 4.2.1: <i>A. adianthifolia</i> leaf extracts (A) total phenolic content	98
Figure: 4.2.2: The effects of <i>A. adianthifolia</i> leaf extracts on (A) DPPH radical scavenging activity, (B) Ferric ion reducing activity, (C) Nitric oxide production inhibition.....	99
Figure: 4.2.3: <i>A. adianthifolia</i> leaf extracts on (A) pancreatic lipase inhibition activity, (B) α -glucosidase inhibition activity, (C) α -amylase inhibition activity	100

Figure: 4.2.4: <i>A. adianthifolia</i> leaf crude extracts effects on (A) yeast cell glucose uptake, (B) psoas muscle glucose uptake, (C) liver glucose uptake and (D) intestinal glucose absorption.....	103
Figure: 4.2.5: Effects of <i>A. adianthifolia</i> leaf extracts on (A) lipid peroxidation, (B) SOD activity, (C) catalase activity and (D) GSH level in oxidative cardiac tissue injury.....	104
Figure: 4.2.6: The effects of <i>A. adianthifolia</i> crude extracts on (A) ATPase activity, (B) Glycogen phosphorylase activity and (C) Fructose 1,6 bisphosphatase activity	106
Figure: 4.3.1: The total phenolic content (A) of the <i>A. adianthifolia</i> crude bark extracts...	113
Figure: 4.3.2: <i>A. adianthifolia</i> bark organic extracts (A) DPPH radical scavenging activity, (B) Ferric ion reducing activity, (C) Nitric oxide production inhibition.....	114
Figure: 4.3.3: <i>A. adianthifolia</i> bark extracts (A) Pancreatic lipase inhibitory activity, (B) α - glucosidase inhibitory activity, (C) α -amylase inhibitory activity.....	117
Figure: 4.3.4: The effects of <i>A. adianthifolia</i> bark extracts on (A) yeast cell glucose uptake, (B) psoas muscle glucose uptake and (C) intestinal glucose absorption	118
Figure: 4.3.5: Effects of <i>A. adianthifolia</i> bark extracts on (A) lipid peroxidation, (B) SOD activity, (C) Catalase activity and (D) GSH activity in oxidative pancreatic injury.....	119
Figure: 4.3.6: <i>A. adianthifolia</i> bark crude extracts effect on (A) ATPase activity, (B) Glycogen phosphorylase activity and (C) Fructose 1,6 bisphosphatase activity.....	121
Figure 5.1: <i>Rhus longipes</i> (Engl.).....	125
Figure 5.2.1: <i>R. longipes</i> leaf and bark infusions total phenolic and flavonoid contents....	130
Figure 5.2.2: <i>R. longipes</i> leaf and bark infusions (A) DPPH scavenging (B) ferric reduction and (C) nitric oxide scavenging activities	132

Figure 5.2.3: <i>R. longipes</i> leaf and bark infusions (A) α -amylase (B) α -glucosidase (C) pancreatic lipase inhibitory activities.	133
Fig. 5.2.4: The double reciprocal plots of α -amylase (A-B) α -glucosidase (C-D) and pancreatic lipase (E-F) inhibition in the presence or absence of <i>R. longipes</i> leaf and bark infusions	136
Figure 5.2.5: Cytotoxic activities of <i>R. longipes</i> leaf and bark infusions against Vero cells.....	137
Figure 5.2.6: The effects of <i>R. longipes</i> leaf and bark infusion on (A) glucose uptake in yeast suspension (B) glucose uptake in excised rat psoas muscle (C) glucose absorption in the intestinal jejunum	138
Figure 5.2.7: The effects of <i>R. longipes</i> leaf and bark infusion on (A) lipid peroxidation (B) GSH level (C) SOD activity (D) CAT activities on oxidative induced kidney injury	139
Figure 5.2.8: 2D images of the molecular interactions between 24,25-Dihydroxyvitamin D and active site amino acid residues of (A) α -glucosidase (B) α -amylase (C) pancreatic-lipase	141
Figure 5.3.1: Effects of <i>R. longipes</i> bark organic extracts of extracts on (A) DPPH radical scavenging activity, (B) Ferric ion reducing activity, (C) Nitric oxide production inhibition.....	151
Figure 5.3.2: Effects of <i>R. longipes</i> bark extracts on (A) Pancreatic lipase inhibition (B) α -Glucosidase inhibition (C) α -Amylase inhibition.....	154
Figure 5.3.3: Effects of <i>R. longipes</i> bark extracts of on (A) Intestinal glucose absorption, (B) Liver glucose uptake (C) Muscle glucose uptake, and (D) Yeast cell glucose uptake	155
Figure 5.3.4: Effects of <i>R. longipes</i> bark extracts on the activities of liver (A) Glycogen phosphorylase and (B) Fructose 1,6 bisphosphatase enzymes.....	156
Figure 5.3.5: Effects of <i>R. longipes</i> bark extracts on (A) lipid peroxidation, (B) SOD activity, (C) Catalase activity and (D) GSH activity in oxidative pancreatic injury.....	157

Figure 5.3.6: Two dimensional images of diisooctyl phthalate interaction with active site amino acids of (A) α -Glucosidase (B) α -Amylase (C) Porcine pancreatic lipase (D) Fructose 1,6 biphosphatase and (E) Glycogen phosphorylase.....	159
Figure 5.4.1: The total phenolic content of the <i>R. longipes</i> crude leaf organic extracts	172
Figure 5.4.2: Effects of <i>R. longipes</i> leaf organic extracts of extracts on (A) DPPH radical scavenging activity, (B) Ferric ion reducing activity, (C) Nitric oxide production inhibition.....	173
Figure 5.4.3: Effects of <i>R. longipes</i> leaf organic extracts of extracts on (A) Pancreatic lipase inhibition activity, (B) α -Glucosidase inhibition activity, (C) α -Amylase inhibition activity	174
Figure 5.4.4: The modulatory effects of organic leaf crude extracts of <i>R. longipes</i> on (A) intestinal glucose absorption, (B) yeast cell glucose uptake (C) liver glucose uptake and (D) psoas muscle glucose uptake	176
Figure 5.4.5: The modulatory effects of organic leaf crude extracts of <i>R. longipes</i> on (A) ATPase activity, (B) Glycogen phosphorylase activity and (C) Fructose 1,6 biphosphatase activity	177
Figure 5.4.6: Effects of <i>R. longipes</i> leaf organic extracts on (A) lipid peroxidation, (B) SOD activity, (C) Catalase activity and (D) GSH activity in oxidative cardiac muscle tissue	179
Figure 5.5.1: Food and Fluid intake in different animal groups during the intervention period.....	189
Figure 5.5.2: Effect of oral treatment on mean body weight in different animal groups during the 5-week intervention period.....	190
Figure 5.5.3: Effect of oral treatment on blood glucose in different animal groups during the intervention period.....	191
Figure 5.5.4: Oral glucose tolerance test (OGTT) of different animal groups in the last week of experimental period.....	192
Figure 5.5.5: Effect of oral treatment on A) serum insulin levels B) homeostatic assessment model (HOMA-IR and HOMA- β) in different animal groups.....	193

Figure 5.5.6: Effect of oral treatment on relative liver weight and glycogen content.....	194
Figure 5.5.7: Effect of oral treatment on lipid profiles.....	197
Figure 5.5.8: Pancreatic histopathological examinations illustrating the effects of oral treatments in different animal groups during the experimental period.....	201
Figure 6.1: <i>Celtis africana</i> (Burm. f)	206
Figure 6.2.1: The total phenolic content of the <i>C. africana</i> crude leaf extracts.....	215
Figure 6.2.2: <i>C. africana</i> leaf extracts (A) DPPH scavenging (B) ferric reduction and (C) nitric oxide scavenging activities	217
Figure 6.2.3: <i>C. africana</i> leaf extracts (A) pancreatic lipase (B) α -glucosidase (C) α -amylase inhibitory activities	218
Figure 6.2.4: showing the interactions between the active site of A) α glucosidase and methoxy phenyl oxime, B) α amylase and methoxy-phenyl- oxime and C) pancreatic lipase and 3-carboxy-1H-indol-5-olate.....	221
Figure 6.2.5: The effects of <i>C. africana</i> leaf extracts on (A) glucose uptake in yeast suspension (B) glucose uptake in excised rat psoas muscle (C) glucose absorption in the intestinal jejunum	224
Figure 6.2.6: Effects of <i>C. africana</i> leaf extracts on (A) lipid peroxidation, (B) SOD activity, (C) Catalase activity and (D) GSH activity in oxidative cardiac muscle injury.....	226
Figure 6.2.7: Effects of <i>C. africana</i> leaf extracts on the activities of hepatic (A) ATPase, (B) Glycogen phosphorylase and (B) Fructose 1,6 bisphosphatase enzymes... ..	227
Figure 6.3.1: The total phenolic content of the <i>C. africana</i> crude bark extracts.....	238
Figure 6.3.2: <i>C. africana</i> bark extracts (A) DPPH scavenging (B) ferric reduction and (C) nitric oxide scavenging activities	240
Figure 6.3.3: <i>C. africana</i> bark extracts (A) pancreatic lipase (B) α -glucosidase (C) α -amylase inhibitory activities	241

Figure 6.3.4: The effects of <i>C. africana</i> bark extracts on (A) glucose uptake in yeast suspension (B) glucose uptake in excised rat psoas muscle (C) liver glucose uptake (D) glucose absorption in the intestinal jejunum.....	246
Figure 6.3.5: Effects of <i>C. africana</i> bark extracts on (A) lipid peroxidation, (B) SOD activity, (C) Catalase activity and (D) GSH activity in oxidative pancreatic injury.....	247
Figure 6.3.6: Effects of <i>C. africana</i> bark extracts on the activities of hepatic (A) ATPase, (B) Glycogen phosphorylase and (B) Fructose 1,6 bisphosphatase enzymes.....	249
Figure 6.4.1: Food and Fluid intake in different animal groups during the intervention period.....	258
Figure 6.4.2: Effect of oral treatment on mean body weight in different animal groups during the 5-week intervention period.....	259
Figure 6.4.3: Effect of oral treatment on blood glucose in different animal groups during the intervention period.....	260
Figure 6.4.4: Oral glucose tolerance test (OGTT) of different animal groups in the last week of experimental period.....	261
Figure 6.4.5: Effect of oral treatment on (A) serum insulin levels (B) homeostatic assessment model (HOMA IR and HOMA β) in different animal groups during the experimental period	262
Figure 6.4.6: Effect of oral treatment on relative liver weight and glycogen content in different animal groups during the experimental period.....	263
Figure 6.4.7: Effect of oral treatment on lipid profiles	266
Figure: 6.4.8. Pancreatic histopathological examinations illustrating the effects of oral treatments in different animal groups during the experimental period.....	270
Figure 7.1: <i>Schotia brachypetala</i> (Sond)	276
Figure 7.2.1: The total phenolic content of the <i>S. brachypetala</i> crude leaf extracts.....	281
Figure 7.2.2: <i>S. brachypetala</i> leaf extracts (A) DPPH scavenging (B) ferric reduction and (C) nitric oxide scavenging activities.....	282
Figure 7.2.3: <i>S. brachypetala</i> leaf extracts (A) pancreatic lipase (B) α -glucosidase (C) α -amylase inhibitory activities.....	283

Figure 7.2.4: The effects of <i>S. brachypetala</i> leaf extracts on (A) glucose uptake in yeast suspension (B) glucose uptake in excised rat psoas muscle (C) glucose absorption in the intestinal jejunum	286
Figure 7.2.5: Effects of <i>S. brachypetala</i> leaf extracts on (A) lipid peroxidation, (B) SOD activity, (C) Catalase activity and (D) GSH activity in oxidative pancreatic injury	288
Figure 7.2.6: Effects of <i>S. brachypetala</i> leaf extracts on the activities of liver (A) ATPase, (B) Glycogen phosphorylase and (C) Fructose 1,6 bisphosphatase enzymes	290
Figure 7.3.1: The total phenolic content of the <i>S. brachypetala</i> crude bark extracts.....	301
Figure 7.3.2: <i>S. brachypetala</i> bark extracts (A) DPPH scavenging (B) ferric reduction and (C) nitric oxide scavenging activities.....	302
Figure 7.3.3: <i>S. brachypetala</i> bark extracts (A) pancreatic lipase (B) α -glucosidase (C) α -amylase inhibitory activities.....	303
Figure 7.3.4: The effects of <i>S. brachypetala</i> bark extracts on (A) glucose uptake in yeast suspension (B) glucose uptake in excised rat psoas muscle (B) glucose uptake in excised rat liver (D) glucose absorption in the intestinal jejunum	306
Figure 7.3.5: Effects of <i>S. brachypetala</i> bark extracts on (A) lipid peroxidation, (B) SOD activity, (C) Catalase activity and (D) GSH activity in oxidative cardiac muscle injury.....	308
Figure 7.3.6: Effects of <i>S. brachypetala</i> bark extracts on the activities of hepatic (A) ATPase, (B) Glycogen phosphorylase and (B) Fructose 1,6 bisphosphatase enzymes	310
Figure 7.3.7: 2D images of the molecular interactions between 2,3-diphenyl-quinoxaline and active site amino acid residues of (A) pancreatic-lipase (B) α -glucosidase (C) α -amylase.....	313
Figure 7.4.1: Food and Fluid intake in different animal groups during the intervention period.....	321

Figure 7.4.2: Effect of oral treatment on mean body weight in different animal groups during the 5-week intervention period.....	322
Figure 7.4.3: Effect of oral treatment on blood glucose in different animal groups during the intervention period.....	323
Figure 7.4.4: Oral glucose tolerance test (OGTT) of different animal groups in the last week of experimental period.....	324
Figure 7.4.5: Effect of oral treatment on A) serum insulin levels B) homeostatic assessment model (HOMA IR and HOMA β) in different animal groups during the experimental period.....	325
Figure 7.4.6: Effect of oral treatment on relative liver weight and glycogen content in different animal groups during the experimental period	326
Figure 7.4.7: Effect of oral treatment on lipid profiles.....	330
Figure:7.4.8: Pancreatic histopathological examinations illustrating the effects of oral treatments in different animal groups during the experimental period	334
Figure 8.1: Schematic representation of the investigated plants' pharmacological activities and potential mode of action.....	346

ABSTRACT

This study investigated the antioxidant, antidiabetic and antiobesity activities of selected traditional medicinal plants including *Rhus longipes*, *Celtis africana*, *Schotia brachypetala*, *Trema orientalis* and *Albizia adentifolia* using *in vitro*, *ex vivo*, *in silico* and model rats *in vivo*. The dry stem bark and leaves of the selected plants were extracted by maceration in solvents of increasing polarity (water, methanol, ethanol, and ethyl acetate). Phyto-compounds in the extracts were quantified and their identities were characterized using gas chromatography-mass spectrometry (GC-MS) against an NIST library. The antioxidant capacity of the extracts was investigated using *in vitro* models to determine their ability to donate electrons, scavenge free radicals, and inhibit the production of proinflammatory factors. Further, *in vitro* models were used to investigate the inhibitory effects of the extracts on lipid and carbohydrate digestive enzymes. The ability of the extracts to mitigate oxidative stress was investigated using *ex vivo* models of excised heart, liver, pancreas, kidney, and brain tissues subjected to FeSO₄ oxidative injury. The modulatory effects of the extracts on oxidative stress biomarkers, including lipid peroxidation, superoxide dismutase (SOD) activity, catalase (CAT) activity, and reduced glutathione (GSH) levels, were investigated in these tissues. The effects of the extracts on glucose uptake in rat muscle and liver, and sugar absorption in the rat jejunum of the animal's small intestine were also studied. Liver homogenates were used to assess the effect of the extracts on glucogenic enzymes, including glycogen phosphorylase and glucose 6 phosphatase. The binding affinities and best-pose interactions of the identified compounds in plants with several diabetes-related proteins were investigated *in silico* using molecular docking. The biological activities of extracts showing significant antidiabetic activity (*Rhus longipes*, *Celtis africana*, and *Schotia brachypetala*) *in vitro*, *in silico*, and *ex vivo* were further investigated using model rats with type 2 diabetes *in vivo*. The plant extracts in this study contained an array of bioactive compounds, including substantial quantities of flavonoids and phenolics. These factors may have contributed to the enhanced antioxidant and digestive- enzyme inhibitory properties. The results show the hypoglycaemic potential of the extracts through their ability to modulate glucogenic enzymes and increase glucose uptake in the psoas and liver tissue while retarding intestinal glucose absorption. The extracts exhibited protective effects against oxidative imbalance in isolated rat tissues by inhibiting lipid peroxidation and augmenting the activity of intrinsic antioxidant enzymes. The *in vivo* results demonstrated the antidiabetic potential of the extracts. The extracts restored redox imbalance and euglycemic levels, mitigated dysregulated lipid metabolism, reversed tissue damage, and linked biochemical parameters to homeostatic levels in treated animals compared with those in the untreated diabetic group. The results of this study

revealed the potential of selected plant extracts to ameliorate type 2 diabetes and its associated complications through several mechanisms, including the upregulation of insulin sensitivity and secretion through the regeneration of pancreatic β -cells. Further molecular analyses and clinical trials are necessary to elucidate the precise mode of the antidiabetic action shown in the current study.

Chapter 1: Introduction and literature review

1.1 Introduction

Diabetes mellitus (DM) has been recognized as an enervating complication since ancient times, dating back to 1500 BC, according to some Egyptian manuscripts (Lakhtakia, 2013). The term "diabetes" is of Greek origin, meaning passing through, referring to a large discharge of urine (Lakhtakia, 2013). Correspondingly, "mellitus" refers to the honey-like pleasant taste of patients' excretes, which characteristically has a high glucose content (Ahmed, 2002; Lakhtakia, 2013). The discovery of diabetes was enabled by experiments on pancreatic function using dog models in the 1800s (Notelovitz, 1970; Lakhtakia, 2013). It was observed that animals that lack a pancreas develop excessive thirst (polydipsia), habitual urination (polyuria), unexplained weight loss, and frequent eating (polyphagia) (Quianzon and Cheikh, 2012). These are traditional symptoms associated with hyperglycaemia (Quianzon and Cheikh, 2012). Hyperglycaemia is a hallmark characteristic of diabetes, defined as higher-than-normal circulatory blood glucose levels, as per the diabetes diagnostic criteria. This was due to the aberrant function of insulin. Insulin is a hormone produced by the β -cells present in the islets of Langerhans of the pancreas that is central to DM pathophysiology owing to its regulatory role in glucose, protein, and lipid metabolism (Skyler, 2004). Despite ongoing research, there is no cure for DM due to its multifactorial nature, but management strategies exist (Chatterjee and Davies, 2015). Management strategies for DM include both non-pharmacological and pharmacological approaches primarily aimed at maintaining homeostatic blood glucose levels. Despite these efforts, the global prevalence of diabetes continues to rise and is projected to rise further in the impending future if the current trend persists (IDF, 2019).

1.2 Global prevalence of diabetes

DM has emerged as a worldwide health challenge, impacting individuals across diverse demographics. Unless there is a significant shift in the current management plan, its incidence is expected to continue to increase. In 2019, the International Diabetes Federation (IDF) estimated that approximately 463 million people were living with diabetes globally (Fig. 1.1A). The incidence of diabetes has continued to rise sharply over the years and has reached an unsurpassed high level (Fig. 1.1A).

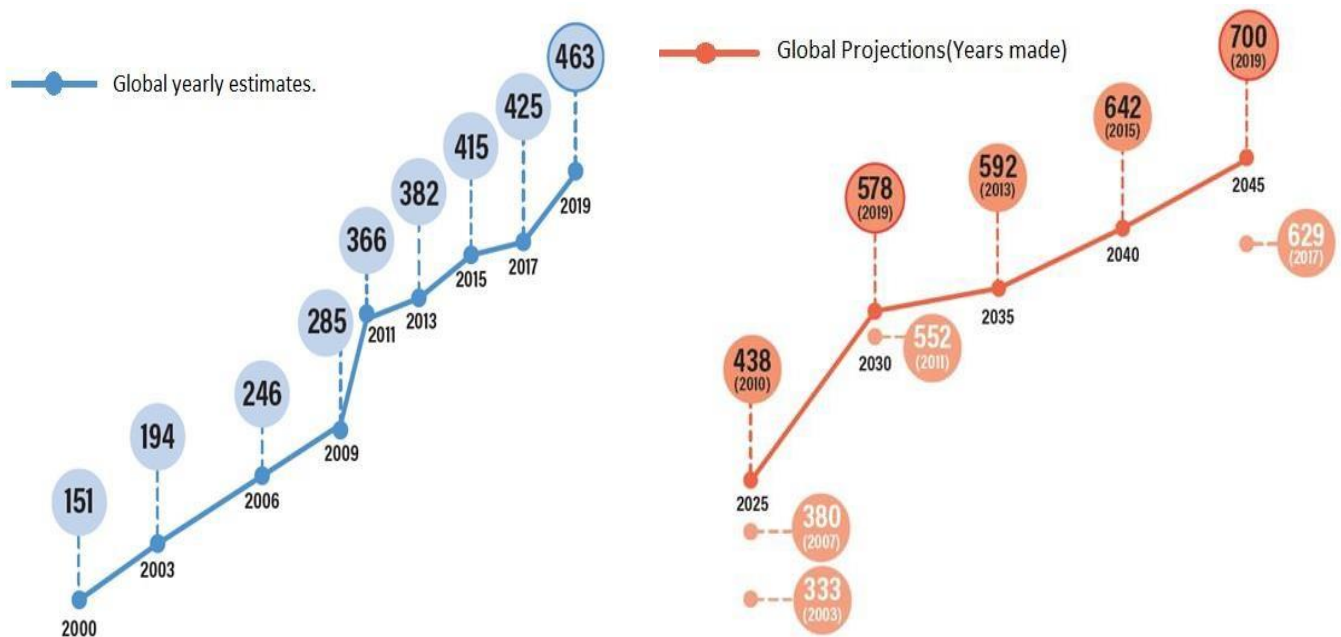


Figure 1.1: A and B: The estimated annual global prevalence of DM from 2000 to 2019. The global prevalence projections of DM from 2025 to 2045 and the years they were made. The numbers are depicted in millions. (Adapted without permission from IDF Diabetes Atlas 9TH edition (IDF,2019).

Over the years, future global prevalence rates of DM have been projected. In 2003, it was projected that by the year 2025, the prevalence of DM would be about 333 million. In 2007, the projection increased to about 380 million and further increased to 438 million in 2010 for the year 2025 as well. In 2019, the global prevalence of DM was 463 million, which was beyond the projected figure for 2025. The global prevalence of diabetes is further projected to increase to 578 million by 2030, followed by 700 million by the year 2045 (IDF, 2019). Given the current DM prevalence trajectory, current forecasts are grossly understated (Saeedi *et al.*,2019). The underestimation is due to data gaps caused by many cases not being recorded or diagnosed. The number of undiagnosed cases worldwide is estimated to be around half (50.1%) of the 463 million individuals living with DM (Saeedi *et al.*,2019). Low- and middle-income nations had the highest percentage of undiagnosed diabetes, accounting for 84.3 percent of all undiagnosed diabetics globally. The absence of regular diagnosis in these areas due to financial restrictions is the main contributor to these results. The African continent was found to have the largest number of undiagnosed DM cases (59.7%) (Saeedi *et al.*,2019). Apart from having the highest number of undiagnosed cases, Africa is also projected to have the highest increase in the prevalence of about 143%, from 19 million to 47 million between the years 2019 and 2045 (Figure 1.2). The sharp increase in the prevalence of DM in Africa is mainly attributed to the increased transition from a rural way of life to a more urbanized set-up. Historically, DM was more common in the affluent regions of the world when Africa was lagging. On the other hand, Europe is

projected to have the lowest increase from 59 million to 68 million during the same period, which is about a 15% increase (Figure 1.2) (IDF, 2019). The delayed increase in the projected prevalence in this region is attributed to effective awareness programs that have prompted a behavioural change. Globally, the prevalence of DM is projected to rise by about 51%, from 463 million in 2019 to 700 million by the year 2040 (IDF, 2019). There will be significant negative financial consequences as the regional and worldwide prevalence of DM rises. The worldwide economic burden of diabetes has immediate negative consequences for people's quality of life, particularly in low- and middle-income nations. Due to the sheer low expenditure per capita in such underdeveloped nations, more resources are required to offer basic diabetes-related healthcare (Zhang *et al.*,2010).

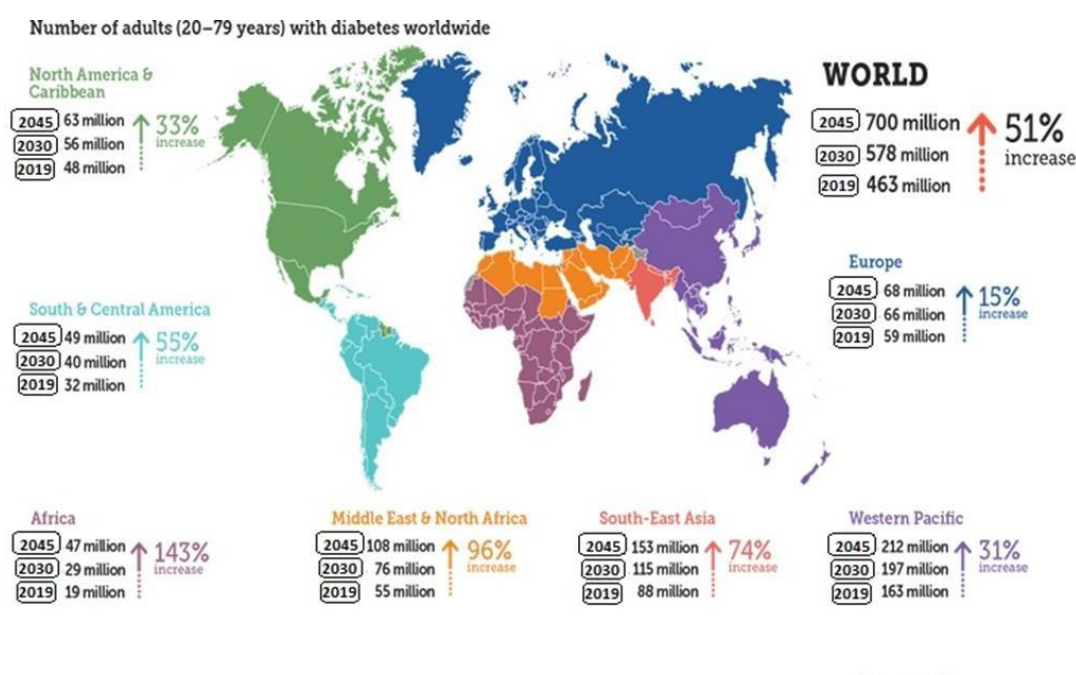


Figure 1.2: Global DM incidence and future projections per region of people aged between 20 and 79 in millions (Adapted without permission from IDF Diabetes Atlas 9TH edition (IDF,2019).

1.3 Global economic burden of diabetes

The economic burden of DM refers to the financial costs incurred because of diabetes, which can be classified as direct or indirect. Patient treatment, such as drug procurement, hospital admissions, and treatment of various diabetes-related problems, including surgery in extreme situations, are all examples of direct expenditures. Indirect expenses, on the other hand, may arise from disease and mortality, which may result in a decline in the workforce, as well as absenteeism and presenteeism (Bommer *et al.*,2017). The cost of DM is rising in tandem with the frequency of the disease. Diabetes accounted for almost 12% of total worldwide health-related expenditures in 2015 (Zhang *et al.*,2010; Ogurtsova *et al.*,2017). It should be mentioned, however, that the amount spent varies depending on

the age group, region, and income level of the country (Zhang *et al.*,2010). In 2015, after accounting for these numerous considerations, it was predicted that each DM patient received USD 1917 (Ogurtsova *et al.*,2017). According to a 2017 study by Herman, global spending on diabetes and its consequences will increase from USD673 billion in 2015 to USD802 billion by 2040. The IDF observed a similar rising trend, estimating that diabetes-related expenditures totalled USD760 billion in 2019. By 2030, this value is predicted to rise to USD825 billion, and by 2045, it will be USD845 billion. Countries with a higher GDP invest more in their healthcare systems. This is not the case with countries, particularly those in the low- and middle-income ranges. This range encompasses many African countries. Countries in the African region have enacted effective health finance reforms in recent years and are seeking novel approaches to boost health funding (WHO, 2013), yet they still trail behind. According to the World Health Organization (WHO), African countries spend roughly 6 to 7% of their entire gross domestic product (GDP) on health care (WHO, 2013). Further study of this data using Statistics South Africa population indicates that around 3,85 million South Africans may be affected by this ailment (Statistics South Africa, 2015). South Africa is known to spend about 8.9% of its GDP on healthcare, which is far higher than the World Bank's recommended 5 percent. This results in a significant financial burden for diabetic individuals as well as a decrease in their quality of life. Such economic strains only serve to slow the rate at which third-world developing countries progress toward developed-country status.

1.4 Global morbidity and mortality due to diabetes

In the year 2000, the excess global mortality due to DM was predicted to be 2.9 million (Roglic *et al.*,2005), but by 2010, this number for people aged 20 to 79 had risen to 3.96 million (Roglic and Unwin, 2010). This accounted for 5.2 percent and 6.8 percent of total global mortality cases, respectively (Roglic and Unwin, 2010; Roglic *et al.*,2005). In 2017, the number of deaths reached almost 5 million (Cho *et al.*,2018). According to data from the IDF Atlas 9th edition, DM claimed the lives of 6.7 million people aged 20 to 79, accounting for 12.2 percent of all global mortality cases. The mortality risks related to the COVID-19 pandemic, in which DM was an aggravating comorbidity, are not included in these worrisome projections (IDF, 2021). Diabetic COVID-19 patients showed a 1.7-fold higher risk of hospitalization and a 2.3-fold higher risk of death than non-diabetic COVID-19 patients (IDF, 2021). Diabetes patients had an elevated mortality risk due to cardiovascular disease (CVD), chronic lower respiratory illnesses, influenza and pneumonia, and kidney disease, according to Li *et al.*, (2019).

1.5 Definition and diagnosis of Diabetes Mellitus (DM)

DM is a multifactorial chronic metabolic disorder characterized by perpetual hyperglycaemia

(Goldenberg and Punthakee, 2013). It is attributed to relative insulin insufficiency, improper insulin function, or both (Goldenberg and Punthakee, 2013). The diagnostic parameters have evolved over the years as more information is obtained. A person may be deemed diabetic if they have a fasting blood glucose level ≥ 7 mmol/L (126 mg/dL) (Petersmann *et al.*,2018). Further confirmation of diabetes can be done by the oral glucose tolerance test (OGTT), in which an individual with blood plasma glucose levels ≥ 11.1 mmol/L (200 mg/dL) 2 hours after administration of a 75 g glucose load (Petersmann *et al.*,2018). Similarly, an individual with a random blood plasma glucose level ≥ 11.1 mmol/L (200 mg/dL) and an HbA1c level ≥ 48 mmol/mol, or 6.5%, is considered diabetic (Petersmann *et al.*,2018). However, there are transitional phases in which a person is not diabetic but is at risk of acquiring DM in the future. Impaired glucose tolerance (IGT) and impaired fasting glucose (IFG) are the terms used to describe these conditions (Petersmann *et al.*,2019). A person with a fasting blood glucose level of < 7 mmol/L (126 mg/dL) and blood glucose ≥ 7.8 mmol/L (140 mg/dL) < 11.1 mmol/L (200 mg/dL) after an OGTT is considered glucose intolerant (Petersmann *et al.*,2019). On the other hand, a person with fasting blood plasma glucose levels between 6.1 mmol/L (110 mg/dL) and 6.9 mmol/L (125 mg/dL) or 7.8 mmol/L (140 mg/dL) after an OGTT is regarded as having impaired fasting glucose (Petersmann *et al.*,2019).

1.6 Types of diabetes

1.6.1 Type I diabetes mellitus

Type I diabetes mellitus (T1DM) is an auto-immune condition caused by the local inflammatory cellular-mediated selective death of insulin-producing β -cells in pancreatic islets (Ozougwu *et al.*,2013). T1DM accounts for roughly 5–10% of all DM cases recorded (Blair, 2016). During diagnosis, many immunological markers such as auto-antibodies against glutamic acid decarboxylase, β cells, insulin, and tyrosine phosphatases IA-2 and IA-2b were found in nearly 85 percent of those with T1DM (ADA, 2014; Baynes, 2015). There is complete pancreatic β -cell destruction in almost all T1DM patients, and therefore they require intravenous insulin therapy to achieve glycaemic control (Baynes, 2015). T1DM is therefore referred to as insulin-dependent diabetes mellitus (IDDM). Additionally, T1DM is often known as juvenile-onset DM since it frequently appears early in a person's life. This is mostly determined by the rate and severity of cell destruction (Ozougwu *et al.*,2013). There have been reports of people with residual β -cell function who only need insulin in the later part of life.

1.6.2 Type II diabetes mellitus

Type 2 diabetes mellitus (T2DM) is the most common type of diabetes, accounting for more than 80% of all DM occurrences (Kerner and Brückel, 2014; Zhu and Zhang, 2016). Non- insulin-dependent diabetes (NIDD) is the common term used to refer to T2D. Although T2D commonly

appears in the later years of a person's life, it is extremely heterogeneous, affecting people across different ages and body sizes (Imam, 2013). Symptoms are typically minor, and the ailment may not be obvious until it has progressed to an advanced state, presenting clinically as ketoacidosis or nonketotic hyperosmolar coma (Zhu and Zhang, 2016). In T2D patients, the pancreatic β cells may not produce enough insulin, resulting in relative insulin deficiency (Genuth *et al.*,2018). Insulin resistance can also develop when sufficient insulin is produced but the insulin-sensitive cells are unable to adequately utilize it. Both insulin deficiency and insulin resistance appear to be the rate-limiting steps in T2D pathogenesis (Genuth *et al.*,2018). The source of the insulin secretory deficiency leading to T2D is multifactorial, but it is commonly thought to be metabolic rather than auto-immune, as seen in T1DM (Genuth *et al.*,2018).

1.6.3 Gestational diabetes mellitus (GDM)

Gestational diabetes mellitus is a type of DM that has an onset or is recognized during either the second or third trimester of pregnancy. It affects anywhere from 3% to 9% of all pregnancies, depending on the diagnosis criteria employed (Genuth *et al.*,2018). This definition has slightly evolved from the previous one, which stated that gestational DM is any form of glucose intolerance that occurs during pregnancy and did not consider that DM may have been a prior condition in women. Gestational diabetes develops during pregnancy due to insufficient insulin secretion to compensate for the significant rise in insulin resistance that occurs during pregnancy (Genuth *et al.*,2018). Consequently, gestational diabetes usually disappears or significantly improves after delivery. However, it has been shown that about 5–10% of the women continue to have DM even after delivery and require treatment to manage their blood glucose levels (Ramachandran *et al.*,2017).

1.6.4 Other types of diabetes

Other types of diabetes do occur, albeit to a lesser extent, accounting for about 1% of the total prevalence (ADA, 2015; Craig *et al.*,2009). Chronic conditions such as cancer and those resulting from viral infections, including congenital rubella, cytomegalovirus, and coxsackie virus, may result in DM (Kharroubi and Darwish, 2015). Drug-induced DM is also known to occur as a side effect of HIV/AIDS antiretroviral therapies, glucocorticoids, thyroid hormone, diazoxide, β -adrenergicagonists, thiazide diuretics, and pentamidine (ADA, 2015; Kharroubi and Darwish, 2015). Hyperglycaemia leading to DM may be induced by chronic endocrinopathies such as pheochromocytoma, hyperthyroidism, Cushing's syndrome, glucagonoma, and acromegaly (ADA, 2015). Exocrine disease-linked DM is known to be caused by extensive damage to the pancreas and pancreatic β cells caused by pancreatic carcinoma, pancreatitis, infection, pancreatectomy, and cystic fibrosis (Larger *et al.*,2012). Maturity-onset diabetes of the young (MODY) is defined by impaired

insulin secretion with minimal or no insulin action defects (ADA, 2015). It is passed down in an autosomal dominant manner. Diagnosis of MODY usually requires appropriate molecular techniques, and as such, it is frequently misdiagnosed as either T2D or T1DM (Anık *et al.*,2015). Its classification is according to the affected gene loci. The most common causes of MODY are mutations in the glucokinase (GCK) (MODY 2) and hepatocyte nuclear factor (HNF) 1A/4A (MODY 3 and MODY 1) genes (Anık *et al.*,2015). Genetic syndromes that have been linked to DM include Down syndrome, Prader-Willi syndrome, Turner syndrome, Wolfram syndrome, Laurence- Moon-Biedl syndrome, and Klinefelter syndrome (Lee and Huda, 2021).

1.7 Risk factors of diabetes mellitus

Risk factors for diabetes mellitus are divided into two categories: modifiable and non- modifiable risk factors. Modifiable risk factors are those over which an individual has control, such as their behaviour, nutrition, and physical activity habits, etc. On the other hand, age, ethnicity, genetic predisposition, and environmental conditions are non-modifiable risks over which a person has no control.

1.7.1 Modifiable risk factors

a. Unhealthy diet

Globally, urbanization has improved food accessibility and affordability. However, this has increased the consumption of processed foods, takeaways, and polished grains, all of which have high glycaemic indices (Lam and LeRoith, 2012). Obesity and weight gain are linked to such diets. Increased alcohol consumption and tobacco smoking exacerbate the role of these unhealthy diets as DM risk factors. Physical activity is lower in more urbanized areas as well.

b. Reduced physical activity

People in urban areas particularly do not engage in rigorous physical activities as opposed to those in rural areas (Chiwanga *et al.*,2016). Using inclinometers, urban dwellers have been shown to spend on average about 50% to 75% of their waking day sitting (Chaput *et al.*,2010; Craft *et al.*,2012a). Urban lifestyles are characterized by prolonged office hours and television watching. In a study conducted in the USA, it was found that men who watched television for about 40 hours a week were three times more susceptible to developing T2D than those who watched it for an hour or less (Hu, 2003). Sedentary behaviour has also been linked to a decrease in lipid and glucose metabolic rates, which results in weight gain (Hamilton *et al.*,2014). It has been shown that routine, non-intensive, and/or intensive physical activity aids in the maintenance of a healthy body mass index (BMI) (Chaput *et al.*,2010).

c. Obesity

Obesity is a condition in which a person is extremely overweight, and it was classified as a disease by the World Health Organization in 2000. The disorder is caused by an excessive quantity of fat stored in the body. The steady increase in body fat is due to a metabolic imbalance in which the body consumes more calories than it uses (Ogden *et al.*,2014; Hruby and Hu, 2015). Despite the substantial evidence linking genetic predisposition to the development of obesity, individuals can potentially postpone its onset through nutritional interventions and increased physical activity involvement. Obesity is known to be a major underlying cause of several complications such as atherosclerosis, hypertension, insulin resistance and diabetes, heart diseases and stroke (WHO, 1999; Booth *et al.*,2012). According to the World Health Organization, 42 million children under the age of five were overweight in 2014, and 1.9 billion adults over the age of 18 were overweight at the same time, with 600 million being obese. Unfortunately, these worrying figures are expected to rise. Surprisingly, a large percentage of patients with DM are or have been obese. Obesity has been demonstrated in recent studies to be more than simply a risk factor for T2D (Boucher *et al.*,2014; Burgio *et al.*,2015).

1.7.2 Non-modifiable diabetes risk factors.

a. Genetic predisposition

Diabetes has a major hereditary component to its onset and progression. It has been reported that type 2 diabetes is a polygenic disease (Sayed and Nabi, 2021). According to studies, over 40% of those with diabetic relatives develop diabetes, compared to approximately 6% of the population without a history of DM (Wu *et al.*,2014). Genome-wide association studies (GWAS) have, over the years, been used to show the polygenetic nature of diabetes and identify candidate susceptibility loci. Numerous GWAS studies undertaken in various countries and ethnic groups have found T2D susceptibility loci on the same or distinct chromosomes and have identified roughly 75 susceptibility loci (Wu *et al.*,2014). Candidate genes are KCNJ11 (potassium inwardly rectifying channel, subfamily J, member 11). KCNJ11 encodes the islet ATP-sensitive potassium channel, and PPARG2 (peroxisome proliferator-activated receptor-gamma 2) encodes a transcription factor for adipocyte differentiation. It should be noted, however, that the degree to which these gene loci influence development and advancement varies across ethnic groups.

b. Ethnicity

Race and ethnicity are useful variables for assessing the distribution of illnesses in the community and identifying at-risk populations for prevention and treatment initiatives (Katzmarzyk and Staiano, 2012). In the 2008 National Health Interview Survey, the age- adjusted prevalence of diagnosed DM for adults

was 11.0% in African Americans, 10.7% in Hispanics, 8.2% in Asians, and 7.0% in whites, indicating ethnic differences (Beckles *et al.*,2011). A 20-year follow-up study in the United States found that Asians, Hispanics, and blacks have a significantly higher risk of DM than whites, both before and after accounting for variations in BMI. Notably, compared to those on the African continent, black African communities living overseas may be at a later stage of epidemiological change (Peer *et al.*,2014). Cooper and colleagues in 2014, reported higher age-adjusted DM prevalence in black African populations living in the Caribbean (9%), USA (11%), and UK (11%), compared to Nigeria (2%). Studies conducted over two decades ago in Tanzania and South Africa reported lower DM prevalence in black Africans than in Indian communities (South Africa: 5.3% vs. 13.0%; Tanzania: 1.1% vs. 9.1/7.1%, respectively) (Mbanya *et al.*,2010; Tuei *et al.*,2010).

d. Environmental ills and diabetes

Features of the built environment that stimulate physical activity, such as walkability and green areas, as well as the food environment, have been linked to the prevalence of DM in given regions (Amuda and Berkowitz, 2019). In 2018, Dendup and colleagues discovered that regions with more walkability and green space are linked to a lower risk of diabetes. Long-term exposure to fine particles (particles having an aerodynamic diameter of 2.5 μ m (PM_{2.5})) or traffic-related air pollutants has been linked to an elevated risk of DM incidence (Lontchi-Yimagou *et al.*, 2013; Dendup *et al.*, 2018). The processes through which the environment increases the risk of DM are still unknown.

1.8 Normal blood glucose homeostatic

The homeostatic maintenance of euglycemic levels in a healthy person is a carefully regulated process aided by different hormones, cells, and tissues all working harmoniously (Fig. 1.3) (Han *et al.*,2016). The majority of total body glucose disposal occurs in insulin-independent tissues when fasted. At basal resting state, approximately one-third of all glucose utilization occurs in the brain, 25% in the splanchnic area, and the remaining 25% in the primarily muscle area (Solis-Herrera *et al.*,2021). During prolonged fasting, normal blood glucose levels are maintained by the kidney as well as hepatic glucose production via glycogenolysis and gluconeogenesis (Solis-Herrera *et al.*,2021). The rate of endogenous glucose production precisely matches the rate of basal glucose utilization, which averages 2.0 mg/kg/min (Solis-Herrera *et al.*,2021). The development of pharmacological antidiabetic therapeutic agents is therefore hinged on the understanding of blood glucose level regulation (Tiwari *et al.*,2014). Deviations from normal blood glucose levels may have catastrophic effects on the body (Yeo and Sawdon, 2013).

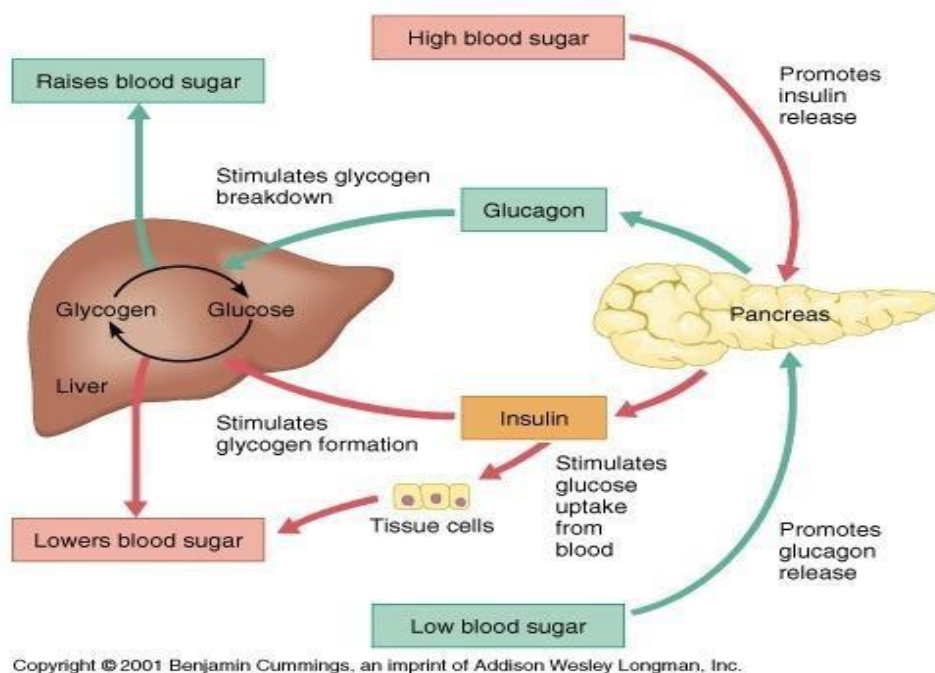


Figure 1.3: Maintenance of blood glucose levels by glucagon and insulin by different organs during the post-prandial state (Adapted without permission from Benjamin Cummings 2001).

1.8.1 Insulin

Insulin was discovered in 1921 and was one of the first polypeptides whose sequence was determined in 1952. It has critical roles in carbohydrate, lipid, and protein metabolism (Hua, 2010; Quianzon and Cheikh, 2012). Insulin is first synthesized as a single molecule (pre-proinsulin) of 110 amino acids by cells of the pancreatic islets of Langerhans (Tiwari *et al.*, 2014; Tokarz *et al.*, 2018). The cleavage of 24 amino acids converts pre-proinsulin to proinsulin (Tokarz *et al.*, 2018). Pro-insulin is composed of three chains, i.e., A-chain, B-chain, and C-chain. The C-chain of pro-insulin is composed of 33 amino acids and connects the N- terminus of the A-chain to the C-terminus of the B-chain. Cleavage of the C-chain of pro- insulin forms the active form of insulin (Liu *et al.*, 2018; Tokarz *et al.*, 2018). In its active form, insulin is a dipeptide hormone of about 6000 Daltons containing the A-chain (21 amino acids) and the B-chain (30 amino acids) that are linked together by disulfide bridges between cysteine residues A7-B7 and A20-B19 (Quianzon and Cheikh, 2012). Two structural switches between R and T states are known to modulate insulin binding to receptors, and work has been done to identify the binding residues (Hua, 2010).

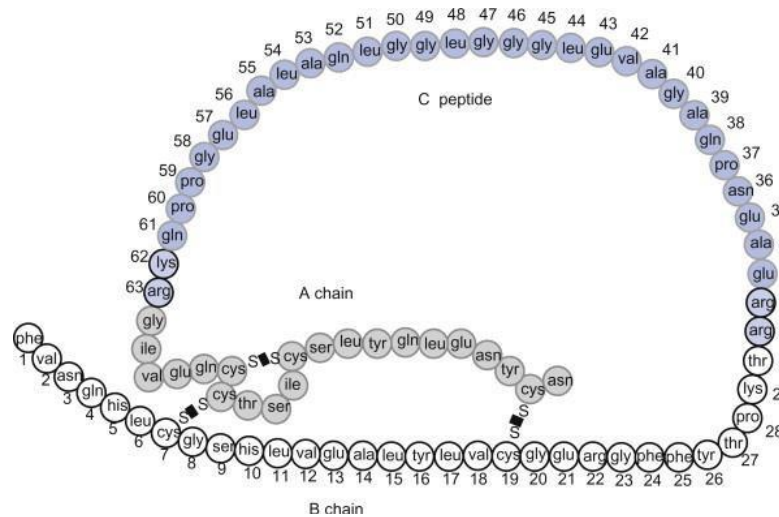


Figure 1.4: The amino acid sequence of proinsulin shows the different cleavage points that yield the A amino acids 64–84 (grey circles), B amino acids 1–30 (white circles), and C amino acids 31–63 (blue circles) chains. Adapted without permission from (Dağışan, and Erbaş, (2020).

Surges in the blood glucose levels, usually following a carbohydrate-rich meal, prompt the secretion of insulin (Yeo and Sawdon, 2013). Insulin activates various pathways that culminate in the reduction of blood glucose levels to normal levels (Saltiel, 2015) (Fig. 1.3). Insulin is secreted by the pancreatic islet β -cells, and it facilitates a cascade of reactions in its signalling pathway (Saltiel, 2015). Secreted circulatory insulin binds to its receptor in insulin-responsive tissues, including cardiac muscle, skeletal muscle, and adipose tissue (Yaribeygi *et al.*,2019). The binding of insulin mediates insulin-dependent uptake of glucose into these tissues and lowers blood glucose levels (Fig. 1.3). Moreover, insulin enhances glycogenesis, lipogenesis, and the incorporation of amino acids into proteins; thus, it performs its anabolic action as opposed to glucagon, which predominantly mediates catabolic reactions (Saltiel, 2015; Yaribeygi *et al.*,2019). Insulin acts on the pancreas in a negative feedback loop to inhibit the secretion of glucagon, which performs antagonistic actions (Yeo and Sawdon, 2013). In diabetes, insulin is given precedence over glucagon because decreased insulin production, function, and/or the body's sensitivity to insulin are at the base of the disease's pathogenesis (Al-Lawati, 2017). Furthermore, the physiological repercussions of decreased insulin secretion or function are far harsher than the physiological effects of glucagon deficiency (Al-Lawati, 2017; Yaribeygi *et al.*,2019).

1.8.1.1a Insulin-mediated glucose uptake pathway

Insulin-mediated glucose uptake is initiated by the binding of insulin to the insulin receptor (IR), which is a hetero-tetrameric bifunctional complex (Petersen and Shulman, 2018). The IR is made up of two extracellular α subunits that bind insulin and two transmembrane β subunits that have tyrosine kinase activity. Insulin binding to the α subunit triggers transphosphorylation of one β subunit by another on certain tyrosine residues located inside the cell in an activation loop, increasing the kinase's catalytic activity (Petersen and Shulman, 2018). The active IR then phosphorylates tyrosine residues on intracellular substrates, including members of the insulin receptor substrate family (IRS1 through 4), Shc isoforms, and signal regulatory protein (SIRP) family members. When phosphorylated, these substrates interact with a series of effector or adaptor molecules that include Src homology 2 (SH2) domains that identify certain phosphotyrosine motifs (Petersen and Shulman, 2018). The IRS family of proteins is the most well-studied of these substrates (Yaribeygi *et al.*,2019). The tyrosine-phosphorylated IRS protein recruits and binds to a signalling molecule with an SH2 domain, which activates either the Phosphatidylinositol-3-kinase (PI3K)-protein kinase B (Akt or PKB) pathway or the Ras- mitogen-activated protein kinase (MAPK) signalling pathway.

1.8.1.1 a Phosphatidylinositol-3-kinase (PI3K)-protein kinase B (Akt or PKB) pathway The phosphorylated IRS protein binds to the P85 subunit of PI3K and recruits its catalytic subunit P110 to activate PI3K; PI3K activates phosphatidylinositol phosphorylation to produce 3,4,5-phosphatidylinositol-3,4,5-triphosphate (PIP3), which in turn activates the serine/threonine protein kinase Akt that activates a variety of substrates and mediates the insulin effect in multiple organisms. AS160 is phosphorylated by AKT, and AS160 targets several Rabs found on GLUT4-containing vesicles, though the precise pathways beyond this are unknown. The SNARE proteins induce vesicle fusion, which results in GLUT4 integration into the plasma membrane (PM) to facilitate glucose uptake (Yaribeygi *et al.*,2019). Increased glucose absorption is directly related to GLUT-4 translocation to the cell surface membrane (Petersen and Shulman, 2018).

1.8.1.1b Ras-mitogen-activated protein kinase (MAPK) signalling pathway

The mammalian MAPK family includes the growth signalling factor p38, extracellular-signal-regulated kinase (ERK), and c-Jun N-terminal kinase (JNK). IRS1/2 activates MAPK by binding to growth factor receptor-bound protein 2 (Grb2), regulates gene transcription, and influences cell proliferation and differentiation by interacting with the PI3K-AKT pathway (Yaribeygi *et al.*,2019).

1.8.2 Glucagon

Blood glucose levels may fall below normal ranges during prolonged fasting, sleep, or between meals. As a result, the body responds by secreting glucagon. Glucagon is a catabolic enzyme that mediates the pathway transduction that results in glucose mobilization from various sources into the bloodstream (Tiwari *et al.*,2014; Gylfe and Gilon, 2014). Pancreatic α islets of Langerhans secrete glucagon. This 29-amino acid hormone is derived from the proteolytic modification of pro-glucagon. Proglucagon is found primarily in pancreatic islet α cells, intestinal enteroendocrine L cells, and, to a lesser extent, neurons in the brain stem and hypothalamus (Rix *et al.*,2015). It is secreted following protein-rich meals and when circulatory plasma blood glucose levels fall below normal due to fasting or exercise (Rix *et al.*,2015). Glucagon secretion is also insulin-dependent; low plasma insulin levels increase glucagon secretion, while high plasma insulin levels inhibit it. It promotes gluconeogenesis in the kidneys and liver and stimulates hepatic glycogenolysis by assisting in the breakdown of glycogen stored in the liver and making it available in the blood (Gylfe and Gilon, 2014). These processes result in a return to normal glycaemic levels.

1.8.3 Pancreas

The pancreas is an important organ located behind the stomach that regulates macronutrient digestion and, thus, metabolism and energy homeostasis through the release of various digestive enzymes and pancreatic hormones (Röder *et al.*,2016). Most of this secretory organ is made up of acinar (or exocrine) cells that secrete pancreatic juice containing digestive enzymes like amylase, pancreatic lipase, and trypsinogen into the ducts, which include the main pancreatic and accessory pancreatic ducts (Begg and Woods, 2013). Pancreatic hormones, on the other hand, are secreted directly into the bloodstream in an endocrine manner by the endocrine cell group called the islets of Langerhans (Begg and Woods, 2013). These cells group to form small island-like structures within the exocrine pancreatic tissue and account for only 1-2% of the total organ mass (Röder *et al.*,2016). Five different cell types release various endocrine system hormones such as insulin, glucagon, somatostatin, and amylin to keep plasma blood glucose levels within a narrow homeostatic range. Insulin and glucagon, which are formed in the β and α cells, respectively, act antagonistically, i.e., insulin is released to reduce the blood glucose levels while glucagon is released to increase them (Begg and Woods, 2013). Somatostatin is formed in the delta cells of the pancreas to regulate the action of insulin and glucagon. Amylin helps increase satiety and retards stomach emptying to avoid excessive dietary calorie intake and postprandial blood glucose spikes (Röder *et al.*,2016).

1.8.4 Liver

The liver plays an important role in glucose homeostasis regulation by controlling various glucose metabolism pathways such as glycogenesis, glycogenolysis, glycolysis, and gluconeogenesis (Han *et al.*, 2016). Under feeding conditions, the liver stores excess dietary carbohydrates as glycogen via the glycogenesis process (Han *et al.*, 2016). Glycogenesis is mediated by glycogen synthase, which facilitates the elongation of glycogen chains. Glycogen synthase acts by catalysing the formation of a new $\alpha 1 \rightarrow 4$ glycosidic linkage by adding the UDP-glucose glucose residue to the non-reducing end of an existing glycogen branch. It is inactivated by serine/threonine kinases such as casein kinase-1, protein kinase A (PKA), and glycogen synthase kinase-3 phosphorylation on several serine residues (GSK-3) (Thorens, 2015). GSK-3 phosphorylation of glycogen synthase at serine residues 640, 644, and 648 is a major inhibitory post-translational modification for glycogen synthase catalytic activity. Furthermore, increased insulin levels and signalling activate Akt in the cell, which phosphorylates and inactivates GSK-3, resulting in glycogen synthase activation (Han *et al.*, 2016). Increased concentrations of glucose-6-phosphate, which allosterically activate glycogen synthase under carbohydrate-rich conditions, are another level of glycogen synthase regulation (Thorens, 2015). Hepatic glucose production through gluconeogenesis is also under the influence of insulin, which, under conditions of increased glucose levels, inhibits phosphoenol-pyruvate and glucose-6-phosphate. These enzymes respectively catalyse the rate-limiting step and the final step of gluconeogenesis (Han *et al.*, 2016). During fasting, stored glycogen is mobilized in a process known as glycogenolysis to raise plasma glucose levels and meet the energy requirements of other tissues such as the brain, red blood cells, and muscles (Rui, 2014). The increase in the plasma concentration of the hormone glucagon mediates the cascade of kinase action that facilitates glycogenolysis. Glycogen stored in the liver is usually sufficient to sustain overnight mammalian fasting, but it depletes if the fasting period is prolonged (Rui, 2014). De novo glucose synthesis, or gluconeogenesis, produces the required glucose during prolonged fasting. The main non-carbohydrate glucose precursors used by the gluconeogenesis pathway are lactate and glycerol. Lactate is mobilized from peripheral tissues, including skeletal muscles, while glycerol is released from the adipose tissues through lipolysis. Glucose uptake in hepatocytes is mediated by GLUT2 (non-insulin-responsive), in contrast to muscle and adipose tissues, in which it is predominantly mediated by GLUT4 (insulin-responsive) (Thorens, 2015).

1.8.5 Muscle tissues

Insulin mediates the upregulation of GLUT4 translocation to the cell surface in skeletal muscle, resulting in up to a 4-fold increase in glucose influx into the tissue (Kruszynska *et al.*,2010). Skeletal muscle cells store about 1% of their weight in glycogen, which is roughly four times the amount stored in the liver considering the significantly higher muscle volume than the liver (Richter and Hargreaves, 2013). Seeing as skeletal muscle is abundant, its glucose disposal capacity is an important mechanism for controlling postprandial glucose spikes and hypoglycaemia (Kruszynska *et al.*,2010). However, unlike the liver, glycogen in muscle tissue is not directly available as blood glucose because muscles lack glucose-6-phosphatase (Richter and Hargreaves, 2013). To get around this, glycogen is first converted to lactate, which is then transported to the liver and converted to glucose (Richter and Hargreaves, 2013). Insulin stimulates glycolysis and glycogenesis while inhibiting glycogenolysis and proteolysis in skeletal muscle tissues (Kruszynska *et al.*,2010).

1.8.6 Adipose tissues

Excess dietary carbohydrates that are not stored as glycogen in the liver or skeletal muscle tissues are converted into fatty acids (Stanford *et al.*,2012). This is known as lipogenesis, and it is mediated by acetyl-CoA, which is incorporated into very-low-density lipoproteins and transported to white adipose tissue, where it is then stored. The insulin responsive GLUT4 transporter is also abundant in adipose tissue (Stanford *et al.*,2012). Insulin not only increases lipogenesis in adipose tissue but also decreases lipolysis, assisting in the maintenance of healthy serum lipid levels (Becher *et al.*,2021).

1.8.7 Glucose transporters

Glucose transporters are transmembrane proteins that facilitate the transportation of glucose into cells, where it is converted into ATP, which drives biochemical reactions. These tissue- specific glucose transporters are broadly classified as glucose transport facilitators (GLUTs) and sodium-coupled glucose transporters (SGLT) (Scheepers *et al.*,2004; Navale and Paranjape, 2016). The GLUT family consists of 14 members, 11 of whom catalyze sugar transport, while the other 3 are glucose sensors. The SGLT family consists of three sub- categories, two of which function as sodium-glucose transporters (SGLT1 and SGLT2), and the last group is involved in glucose sensing (SGLT3). Overall, the specific isotypes of glucose transporters under both families exhibit different substrate specificity, kinetic characteristics, and expression profiles. The specificity of these subtypes allows tissue-specific glucose uptake regulation for the different tissues through gene expression modulation (Scheepers *et al.*,2004).

a. Sodium-glucose transporters (SGLTs)

The SGLT family are 60- to 80-kDa transmembrane proteins with 14 transmembrane helices containing 580–718 amino acids. The amino and carboxyl terminals are in the extracellular space. These symport transporter proteins do not directly use ATP to transport glucose against its concentration gradient. SGLTs transport glucose in conjunction with sodium ions and use the gradient potential created by the sodium-potassium ATPase. SGLTs are present on the luminal surfaces of cells lining the small intestine and in renal tubules where they facilitate the dietary absorption and reabsorption of glucose from the glomerular filtrate, respectively.

Table 1.1: The function and location of different SGLTs (Adapted without permission from Navale and Paranjape, 2016)

SGLT type	Location	Function
SGLT1	Apical membranes of small intestinal cells	Absorption of glucose from intestinal content
	Straight cells (S3 cells) of the proximal tubule of the nephron	Reabsorption of remaining glucose from urine filtrate
SGLT2	The proximal convoluted tubule of nephron (S1 and S2 cells)	Reabsorption of bulk plasma glucose from the glomerular filtrate
SGLT3	Intestine, testes, uterus, lung, Brain, thyroid	Function as a glucose sensor for controlling glucose levels in the gut and brain
SGLT4	Intestine, kidney, liver, brain, lung, uterus, pancreas	Absorption and/or reabsorption of mannose, 1,5- anhydro D-glucitol, fructose and glucose
SGLT5	Kidney cortex	Transport of glucose and galactose
SGLT6	Brain, kidney, intestine	The preferred substrate is D-chiro-inositol

b. Glucose transporters (GLUTs)

GLUTs are transmembrane proteins with 12 membrane-spanning regions encoded by the *SLC2* genes (Deng and Yan, 2016). The amino and carboxyl terminals are located intracellularly. Bioinformatic multiple sequence alignment shows 28–65 % similarity of all GLUTs with GLUT 1 and these studies classify these proteins into three subclasses (Class I, II and III) of facilitative transporters (Thorens and Mueckler, 2010).

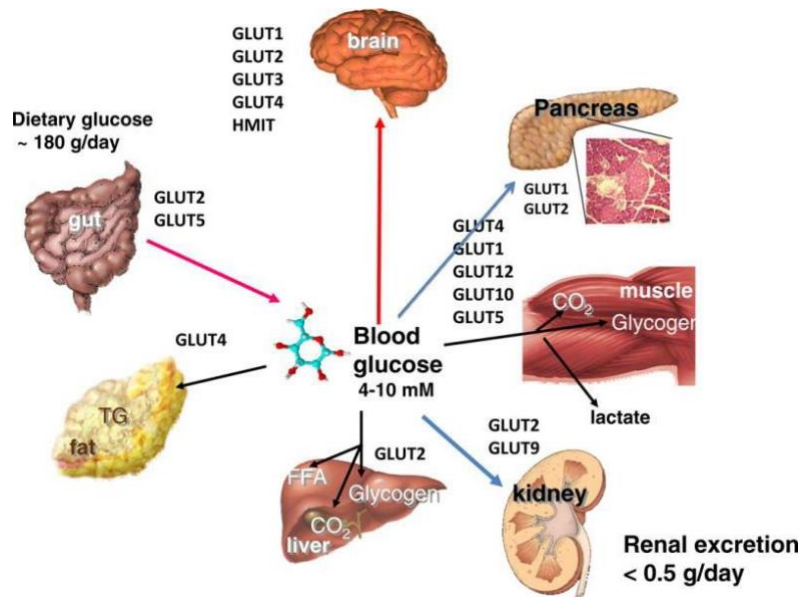


Figure 1.5: The cellular and tissue localisation of the facultative glucose transporters (GLUTs)

(Adapted without permission from Mueckler, and Thorens, 2013).

c. Class I

Class 1 comprises the classical transporters GLUT1–4 and GLUT14. GLUT1 was the first isoform of the GLUTs to be isolated and purified and is the most extensively studied member of the GLUTs (Thorens and Mueckler, 2010). GLUT1 is a ubiquitous facilitative glucose transporter present in all tissues but predominantly functions in erythrocytes and the endothelial cells' blood-brain barrier, where it is highly expressed (Deng and Yan, 2016). The protein is expressed in relatively smaller amounts in the liver, adipose tissue, and muscles, where it is reported to be the receptor for the human T-lymphotropic virus (HTLV) (Thorens and Mueckler, 2010; Mueckler and Thorens, 2013). GLUT2 is the major glucose transporter in hepatocytes, intestines, and kidneys, where it mediates hormone-mediated glucose transport. It helps in the uptake of glucose by the hepatocytes for glycolysis and glycogenesis while concurrently regulating the release of glucose from the liver cells through

gluconeogenesis. GLUT2 has a high K_m value for glucose yet a high affinity for glucosamine, and this allows it to facilitate bidirectional glucose flow depending on the fed or fasting state (Navale and Paranjape, 2016). On the brush border cells of the small intestine and renal tubule cells, GLUT2 functions to absorb the glucose from the lumen and reabsorb the glucose from the glomerular filtrate, respectively. GLUT2 in the pancreatic β cells functions in glucose sensing and mediates insulin secretion. GLUT2 isoforms appear to be developmentally regulated and are not expressed in foetal pancreatic β cells until 7 months. GLUT3 is mainly expressed in neurons, which require glucose as their sole energy source. GLUT3 has the lowest K_m value among all the GLUTs in the same class, and this enables it to facilitate glucose uptake even at low blood glucose levels. Structural features that confer high glucose affinity to GLUT3 include the first extracellular loop and the N-terminal (membrane proximal) segment of the intracellular loop

6. GLUT3 was also shown to transport mannose, galactose, and xylose but is unable to transport fructose. GLUT4 is found sequestered in intracellular vesicles of adipose tissue, skeletal muscle, and cardiac muscle. This insulin-responsive GLUT isomer translocates from intracellular vesicles to the plasma membrane in response to insulin signalling when there is an increase in blood glucose levels. Translocated vesicles fuse with the membrane and increase glucose uptake up to 10–20-fold in the tissues. Recent reports suggest that GLUT4 can be inhibited by HIV protease inhibitors, which may result in the pathogenesis of DM or its aggravation in already-diagnosed patients taking these medications.

d. Class II

Isoforms GLUT5, 7, 9, and 11 are constituents of the class II GLUTs. GLUT5 is primarily a fructose transporter expressed in the cells of the small intestine, testes, and kidneys (Navale and Paranjape, 2016). The GLUT7 isomer is located at the apical membrane of the enterocytes in the small, colon, testis and prostate and has a high affinity for both glucose and fructose. GLUT9 is expressed in the proximal tubule of the kidney and the liver and placenta with multiple isoforms known to exist. GLUT11 has about 42% sequence homology with GLUT5. Various isoforms of this GLUT are known to exist and are widely distributed and expressed in heart, skeletal muscle kidney cells, placenta, adipose tissue, and pancreas cells where it is responsible for fructose transportation.

e. Class III

Glucose facilitative transporters in Class III include the isoforms GLUT6, 8, 10, 12 and the proton-driven myoinositol transporter HMIT (GLUT13). As opposed to Class I and Class II in which the glycosylation site on loop 1 is found on loop 9 in this class. GLUT6 is expressed in the spleen, peripheral leucocytes, and the brain (Cheeseman, and Long, 2015).

GLUT8 is present in the testis germinal cells, brain, and adipose tissue. GLUT8 is a high-affinity glucose transporter located intracellularly but unlike GLUT4 its translocation is not insulin-mediated. GLUT8 facilitates glucose transportation across the mitochondrial membrane, endoplasmic reticular membrane, and lysosomal membrane but fructose and galactose inhibit this transport. GLUT10 on the other hand is reportedly expressed in the insulin-sensitive tissues of skeletal muscle, heart, lung, brain, placenta, kidney, liver, and pancreas (Scheepers, 2004). GLUT12 in the placenta, heart, small intestine, prostate, adipose tissue, and insulin-sensitive tissues (Mueckler and Thorens, 2013). GLUT12 has a high sequence homology with GLUT10. Just like GLUT4, GLUT12 translocation is mediated by insulin.

HMIT (H⁺-driven myoinositol transporter) or GLUT13 is predominantly expressed in adipose tissue, kidney cells and various parts of the brain especially in the hippocampus, hypothalamus, cerebellum, and brain stem (Navale and Paranjape, 2016). Its translocation occurs by depolarization or protein kinase C activation in neuronal cells (Uldry and Thorens 2004). GLUT13 transports inositol-3-phosphate in the brain a precursor of phosphatidylinositol whose pathway dysregulation deregulation leads to multiple psychiatric disorders.

1.9 Diabetes pathogenesis and progression

Failure in blood glucose regulatory machinery results in chronic hyperglycaemia which is at the heart of diabetes pathophysiology. Several biochemical pathways are implicated as leads in the pathogenesis and progression of diabetes.

1.9.1 The ominous octet of diabetes pathogenesis

The pathogenesis of T2D is influenced by eight key abnormalities described collectively as "the ominous octet". The development of pharmacological antidiabetic therapeutic agents follows the octet pharmacological drug development tool. Treatment for T2D should target multiple defects and include a patient-centered approach that considers aspects other than glycaemic control. This model targets the pathophysiologic abnormalities that lead to the pathogenesis and progression of diabetes. Target pathophysiologic abnormalities include β -cell

failure and insulin resistance in muscle, liver, and adipocytes, decreased incretin effect, increased glucagon secretion by α -cells and enhanced hepatic glucagon, enhanced renal glucose reabsorption, and central nervous system resistance to the anorectic effect of insulin and altered neuro-synaptic hormone secretion.

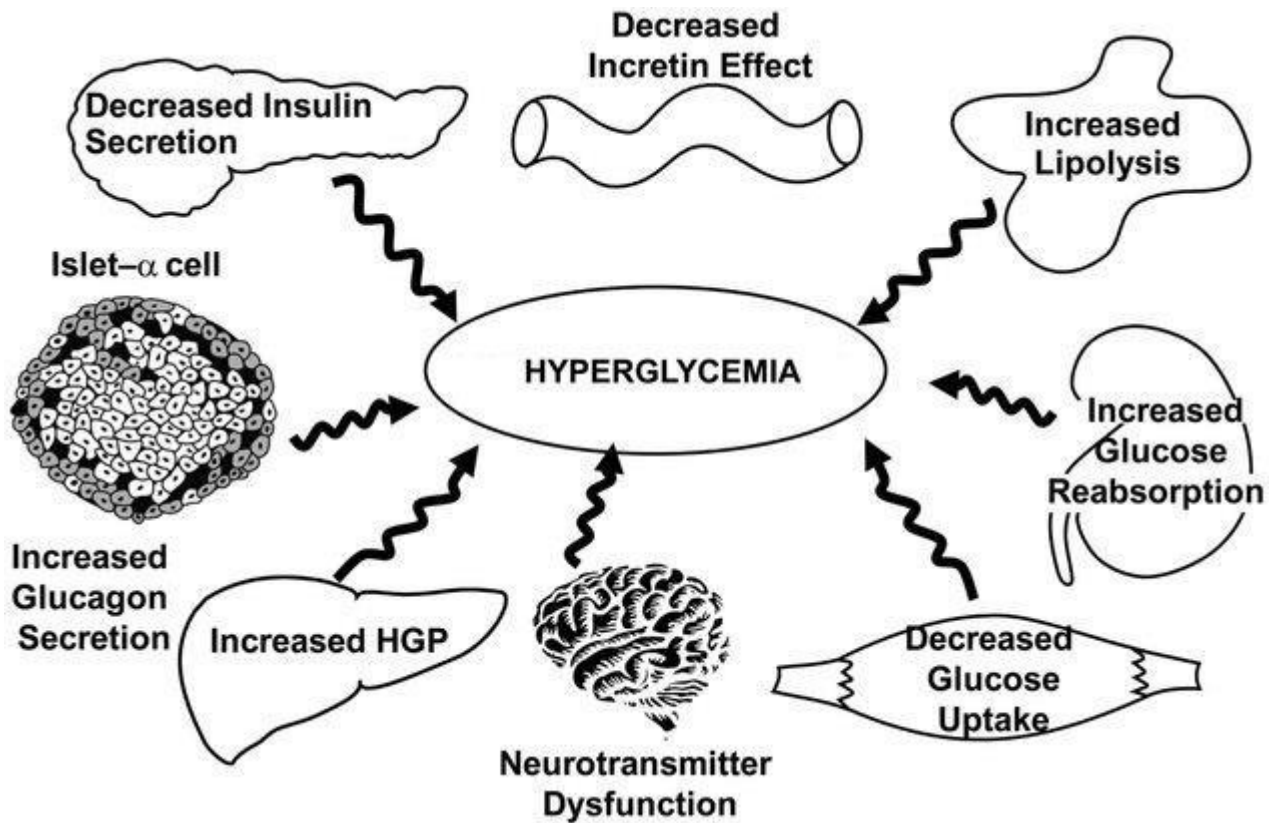


Figure 1.6: The ominous octet involved in DM pathogenesis. Available via license: CC BY-NC 3.0 adapted without permission from Triplitt *et al.*, (2010).

1.9.2 Oxidative stress

Oxidative stress, which is defined as an imbalance between the production of antioxidant molecules or enzymes and the amount of reactive species, is therefore implicated as an underlying cause in the development and progression of DM (Giacco and Brownlee, 2010). An assortment of reactive species is produced during the normal functioning of the body and play various beneficial functions such as signal mediators and in the degradation of pathogens. Various antioxidant systems exist to mop up these reactive species once they have fulfilled their purpose before they can damage other cellular systems. However, in pathological conditions, there is an excess production of these molecules, and they result in chronic irreparable damage which further results in the development of various degenerative conditions.

a. Advanced glycation end products (AGEs)

Advanced glycation end-products are formed by the spontaneous non-enzymatic reaction of proteins and glucose through the Fenton reaction. Advanced glycation end-product formation is accelerated under conditions of chronic hyperglycaemia in diabetic patients (Giacco and Brownlee, 2010). Proteins such as cell surface receptors that participate in the formation of AGEs are rendered non-functional due to conformational changes. Furthermore, AGEs interact with their receptors to exacerbate inflammation and the production of reactive oxygen species (ROS). AGEs are implicated in several diabetic-linked complications including, atherosclerosis.

b. Polyol pathway

Under conditions of hyperglycaemia, a heavy influx of glucose overwhelms the glycolytic pathway and results in the excess glucose being converted to sorbitol through the polyol pathway (Giacco and Brownlee, 2010). Under homeostatic conditions, glucose is phosphorylated to glucose-6-phosphate and undergoes glycolysis. However, in diabetic individuals, excess un-phosphorylated glucose is converted to sorbitol by aldose reductase. Sorbitol is subsequently converted to fructose by sorbitol dehydrogenase which requires reduced nicotinamide adenosine dinucleotide (NADPH) as a cofactor. The increased demand for reduced nicotinamide adenosine dinucleotide (NADPH) results in oxidative imbalance as this moiety is crucial for the generation of GSH an endogenous antioxidant. Accumulation of sorbitol produced by the polyol pathway is implicated in the pathogenesis and progression of several diabetic linked complications including diabetic retinopathy.

c. Activation of protein Kinase C isoforms

Hyperglycaemia leads to increased NADH/NAD ratio which inhibits pyruvate dehydrogenase thereby facilitating the accumulation of several glycolytic pathway intermediates such as dihydroxyacetone phosphate (Giacco and Brownlee, 2010). Dihydroxyacetone phosphate is further reduced to glycerol-3-phosphate ultimately leading to the accumulation of diacylglycerol (DAG). Increased circulation of free fatty acids caused by diabetic dyslipidaemia has also been shown to upregulate the de-novo synthesis of DAG. Diacylglycerol modulates several biochemical pathways including the stimulation of protein kinase C. PKC has been demonstrated to increase free radical production via activation of NAD(P)H oxidase in endothelial and smooth muscle tissue cells. PKC upregulation has been implicated in promoting apoptosis via the Src homology-2 domain-containing phosphatase-1 (SHP-1) pathway. The combined effect of increased PKC activation further exacerbates diabetic-related microvascular and macrovascular complications.

d. Hexosamine pathway

Chronic hyperglycaemia results increased influx of glucose into the glycolytic pathway where it is

converted to fructose 6-phosphate and ultimately glutamine fructose 6-phosphate through the hexosamine pathway (Giacco and Brownlee, 2010). Glucosamine-6 phosphate, an inhibitor of glucose-6-phosphate dehydrogenase, alters the NADPH/NADP ratio through the inhibition of the pentose phosphate shunt pathway. The decrease in the NADPH/NADP ratio results in decreased GSH regeneration. Accumulation of reactive species that would otherwise have been mopped up by GSH results in an increase in oxidative stress.

e. Obesity and inflammation

Obesity aggravates oxidative stress through the excessive generation of reactive species (Ahmadovna and Sharipovna, 2021). During the acquisition of energy from the excess fats stored as lipids in adipose tissue by β -oxidation, large amounts of reactive oxygen species are expelled as by-products. These electron-deficient or rich molecules donate or extract electrons from other molecules, rendering them non-functional (Halim and Halim, 2019). Among these molecules are cell signalling receptors, structural molecules, and functional molecules. The chronic overproduction of these molecules leads to progressive degeneration of the various cellular components within the body. Diabetes results from the damage to insulin cell surface receptors, which progressively leads to insulin insensitivity (Verma and Hussain, 2017). It may also result in damage to the insulin production machinery within the pancreas (Eguchi *et al.*, 2021). The result of both actions is insufficient insulin action and/or production. This, therefore, means obese individuals are at higher risk of developing various degenerative conditions, including diabetes. The high level of co-relationship between T2D and obesity prompted Zimmet *et al.*, (2001) to coin the term "diabesity." Management of these two conditions and their resultant complications thus should not be approached in isolation. Other macrovascular and microvascular conditions also result from the biochemical environment associated with obesity.

1.9.3 Insulin resistance

Apart from aberrant insulin production by the pancreatic β -cells, insulin resistance (IR) is the main underlying factor leading to the development and progression of DM (Baynes, 2015). Insulin resistance is a pathologic condition that leads to reduced insulin-mediated glucose uptake in the peripheral system (by muscle and fat), insufficient regulation of hepatic glucose output, and impaired triglyceride uptake by fat despite normal insulin levels in the blood (Boucher *et al.*,2014; Yaribeygi *et al.*,2019). Under conditions of insulin resistance primarily in the skeletal muscle tissue, the pancreatic β -cells compensate by increasing insulin secretion to meet the increased anomalous demand. Although increased insulin secretion can elicit a biologically homeostatic response, especially in the early stages, its relative increase results in plasmic hyperinsulinemia. Insulin resistance and hyperinsulinemia ultimately result in reduced glucose tolerance.

In patients with T2D or impaired fasting glucose, endogenous glucose synthesis is increased. Increased hepatic insulin resistance under conditions of hyperinsulinemia plays a pivotal role in T2D-induced hyperglycaemia, primarily in the early to intermediate stages of the condition. Hyperglycaemia has also been implicated in the reduction of the nitric oxide content of the vascular protective agent, which leads to endothelial dysfunction and the exacerbation of atherosclerosis.

1.10 Complications of diabetes

1.10.1 Macro-vascular complications

Macrovascular diabetic complications manifest after a considerably long period of uncontrolled chronic hyperglycaemia. Macrovascular complications result from the gradual narrowing and subsequent obstruction of arteries throughout the body (Papatheodorou *et al.*,2018). Central to this process is atherosclerosis and cardiovascular diseases.

a. Atherosclerosis

Pathogenesis of atherosclerosis results from chronic low-grade inflammation that mediates endothelial artery wall damage. Injury to the arteries prompts the accumulation of oxidised low-density lipoproteins (LDLs) on the affected site where angiotensin II promotes their further oxidation (Beckman and Creager, 2016). This signals the accumulation of monocytes which differentiate into macrophages and form foam cells by accumulating the oxidised molecules at the injury site. Foam cells further stimulate macrophages to proliferate which in turn results in the accumulation of T-lymphocytes. T-lymphocytes signal the proliferation of muscle tissue and accumulation of collagen. The process culminates in the formation of a fibrous cap and an atherosclerotic lesion which when ruptured causes an acute vascular infarction (Fowler, 2011). Diabetic patients have an increased likelihood of developing atherosclerosis as opposed to non-diabetic individuals (Chawla *et al.*,2016).

b. Cardiovascular disease (CVD)

An estimated 32.2% of the global T2D patients are affected by cardiovascular disease (Einarson *et al.*,2018). Stroke and coronary artery diseases were the major contributors to the estimated 50% mortality of diabetic patients attributed to cardiovascular diseases (Einarson *et al.*,2018). Undoubtedly, due to its high prevalence among diabetic patients, cardiovascular disease accounts for the greatest diabetic healthcare-related expenditure (Thiruvoipati *et al.*,2015).

1.8.2 Microvascular complications

Microvascular diabetic complications result from a disruption in the function of small blood vessels in various organs in the body including the kidneys, nerves, and eyes body (Papatheodorou *et al.*,2018).

a. Diabetic retinopathy

Diabetic retinopathy is the most common cause of blindness in diabetic patients (Wang, and Lo, 2018). Diabetic retinopathy is classified as either non-proliferative (background) or proliferative. Microaneurysms and haemorrhages form in the middle layers of the retina in non- proliferative retinopathy. As the condition progresses, hard and soft exudates appear as greyish areas in the retina (Wang, and Lo, 2018). Clinically observed red dots are a distinguishing feature of this type of retinopathy, which is characterized by oedema and leakage from the micro-vessels and, in severe cases, may necessitate surgical intervention.

Proliferative retinopathy differs from non-proliferative retinopathy in that new blood vessels are formed on the surface of the retina (Wang and Lo, 2018). It is clinically recognised as white spots on the retina surface. Unchecked proliferative retinopathy will progressively lead to visual occlusion and subsequent blindness through vitreous haemorrhage. Diabetic retinopathy accounts for over 5% of global blindness (Wang and Lo, 2018). Frequent monitoring and timely intervention may avert blindness in diabetic individuals (Stitt *et al.*,2016).

b. Diabetic nephropathy

Diabetic nephropathy is implicated as the major cause of kidney disease (Umanath and Lewis, 2018). Unchecked kidney disease leads to end-stage renal failure and premature mortality in diabetic patients' body (Papatheodorou *et al.*,2018). The major anatomical changes associated with diabetic nephropathy include thickening of the glomerular basement membrane, dysfunction and loss of podocytes and mesangial nodule formation (Umanath and Lewis, 2018). These changes lead to decreased glomerular filtration rates which may be clinically detected by 24-hour urine sample analysis. Individuals with impaired kidney function present with microalbumin in the urine as it leaks from glomerular capillaries and irregular microalbumin-to-creatinine ratios (Umanath and Lewis, 2018).

c. Diabetic neuropathy

Diabetic neuropathy results from the dysfunction of peripheral nervous system cells (Feldman *et al.*,2019). An estimated 50% of diabetic patients will develop neuropathy, particularly if they have poor glycaemic control (Papatheodorou *et al.*,2018). The precise mechanism of hyperglycaemic-induced neural damage is poorly understood but is thought to be related to advanced glycation end products and polyol accumulation (Feldman *et al.*,2019). The net effect of the associated processes is the damage to neurons, leading to aberrant neurotrophic signalling. Diabetic neuropathy manifests clinically in different forms, such as sensory and autonomic impairment, which cause numbness, burning sensations, and tingling pain in patients' bodies (Papatheodorou *et al.*,2018; Feldman *et al.*,2019). Diabetic patients are 15 times more likely to undergo extremity amputations, which occur after injury and ulcerations due to neuropathy and compromised wound healing (Pop-Busui *et al.*,2017). Furthermore, neurological dysfunction in other organs throughout the body is common among diabetic patients and may cause erectile dysfunction, myocardiatic ischemia, diarrhoea, and bladder dysfunction (Papatheodorou *et al.*,2018; Feldman *et al.*,2019).

1.11 Diabetes management strategies

1.11.1 Non-pharmacological strategies

Diabetes onset and progression are associated with adverse unhealthy lifestyles of affected individuals. Therefore, lifestyle intervention provides key first-line management and preventative strategy for DM (Raveendran *et al.*,2018). This non-pharmacological management strategy is hinged on behavioural changes partnering with diet and physical activity. Individuals maintaining healthy low glycaemic load diets void of refined sugars, excessive alcohol and processed meat were shown to have better glycaemic control. Healthy diets comprising of whole grains, vegetables and dairy products with low glycaemic index reduce postprandial hyperglycaemic spikes (Schwarz *et al.*,2012). Such food act mechanistically by improving insulin secretion or sensitivity as well as reducing low-grade chronic inflammation associated with lipidemic metabolism. Increased physical activity aids healthy diets as a non-pharmacological diabetic management strategy by improving the balance between calorie intake and expenditure (Jenkins and Jenks, 2017). Routine aerobic and resistance exercise increases glucose disposal by peripheral tissues by upregulating insulin action on insulin-responsive receptors in these tissues (Kirwan *et al.*,2017). A previous study by Kirwan and colleagues found a 45% increase in insulin-stimulated glucose disposal after 7 days of intensive exercise in diabetic patients (Kirwan *et al.*,2009).

1.11.2 Pharmacological antidiabetic agents

The choice of antidiabetic therapeutic agent prescribed to a patient is dependent on several factors. Practitioners, therefore, factor in the safety profiles, notable effects on weight and hypoglycaemic shock risk, tolerability, patient comorbidities, mode of administration, patient preference, and cost when deciding on a prescription. Current antidiabetic pharmacological agents fall into several classes based on their modes of action including biguanides, sulfonylureas, thiazolidinediones, dipeptidyl-peptidase 4 inhibitors (DPP-4), α -glucosidase inhibitors, Glucagon-like peptide 1 (GPL1) analogues or incretin-based therapies.

a. Biguanides

Biguanides are a class of first-generation anti-diabetic compounds that are currently used as first-line hypoglycaemic oral agents (Mane *et al.*,2012). Metformin is one of the most prescribed biguanides for newly diagnosed T2D patients and those with elevated insulin resistance (Wang *et al.*,2017). Biguanides have a general empirical base formula of $\text{HN}(\text{C}(\text{NH})\text{NH}_2)_2$, to which various alternative groups can be added to form various derivatives as needed (Kathuria *et al.*,2018). This diverse group of colourless organic compounds has a variety of hypoglycaemic effects in various tissues throughout the body (Mane *et al.*,2012). They work in the liver by inhibiting hepatic glucose output and gluconeogenesis (Meneses *et al.*,2015; Wang *et al.*,2017). Biguanides inhibit glucose absorption in the small intestine, lowering postprandial glucose spikes in diabetic patients (Wang *et al.*,2017). They also increase insulin sensitivity by modulating the action of various cell surface receptors in insulin-responsive tissues such as the muscles (Kathuria *et al.*,2018).

b. Sulfonylureas

Sulfonylureas are popular first-line prescription drugs for patients whose dietary management alone has failed to achieve appropriate glycaemic control. Sulfonylureas are oral pharmacological agents that act by stimulating insulin secretion of pancreatic β -cells (Ojha *et al.*,2019). The binding of sulfonylureas to K_{ATP} channels on β -cells decreases potassium (K^+) efflux. This action depolarizes the β -cells and induces Ca^{2+} -induced insulin release. Sulfonylureas reduce plasma insulin clearance thereby ensuring higher circulatory concentrations of the hormone. The effectiveness of sulfonylureas depends on the presence of residual circulatory insulin. Common sulfonylureas available on the market include glibenclamide, glyburide, glipizide, and glimepiride.

c. Thiazolidinediones

Thiazolidinediones, also known as glitazones, are a type of oral DM medication that was initially developed as an antioxidant but was later discovered to help with glycaemic control (Meneses *et*

al.,2015). Thiazolidinediones enhance metabolic control in diabetic patients by increasing insulin sensitivity. Thiazolidines are more commonly used in combination with other oral DM medications in patients who do not meet glycaemic targets (Mane *et al.*,2012). They act primarily on insulin-responsive tissues such as muscle, adipose and the liver. Thiazolidinediones down-regulate the synthesis of proteins commonly implicated in insulin resistance and the release of free fatty acids (Bourron *et al.*,2010). They inhibit the destruction of β -cells thereby increasing their function as opposed to sulfonylureas (Chung *et al.*,2011). They bind to peroxisome proliferator-activated receptors (PPARs) in these cells prompting multiple endocrine signalling pathways. The drug-PPAR complex then acts on response elements in promoter regions to affect the transcription of as many as 100 genes (Fowler, 2007; Chung *et al.*,2011). Thiazolidinediones up-regulate the transcription of proteins that increase insulin sensitivity, such as adiponectin in the adipose tissue and skeletal muscle. Common thiazolidinediones available on the market include rosiglitazone and pioglitazone.

d. α -glucosidase inhibitors

This class of anti-diabetic agents works by slowing carbohydrate absorption in the small intestine and can be used in conjunction with other hypoglycemic agents (Meneses *et al.*,2015). They are typically given to newly diagnosed patients after non-pharmacological interventions have failed (Kumar *et al.*,2011). They work by inhibiting the action of α -glucosidase, which aids in the breakdown of dietary polysaccharides into monosaccharides such as glucose. This enzyme is found at the small intestine's brush border, where it aids in the breakdown of disaccharides to simple sugars (glucose) before absorption into the bloodstream. α -glucosidase inhibitors are usually taken just before main meals and their capacity to reduce post-prandial glucose spikes is dependent on the amount of carbohydrates polysaccharides in the meal (Meneses *et al.*,2015). Polysaccharides are more difficult to absorb into the blood than monosaccharides. These medications have the overall effect of lowering post-prandial blood glucose levels (Kumar *et al.*,2011). In glucose-intolerant diabetics, lower carbohydrate absorption rates reduce postprandial hyperglycaemic spikes (Kumar *et al.*,2011). The most commonly available α -glucosidase inhibitors on the market are acarbose and miglitol (Meneses *et al.*,2015).

e. Dipeptidyl-peptidase 4 inhibitors (DPP-4) and incretin-based therapies

DPP-4 inhibitor is a class of new drugs for T2D that improves normal postprandial glucose homeostasis without resulting in hypoglycaemia. GLP-1 is rapidly metabolised by dipeptidyl-peptidase 4 (DPP-4) in many tissues thus resulting in almost complete loss of incretin effect (Drucker *et al.*,2010). Prevention of GLP-1 degradation by DPP-4 inhibitors increases the activity of incretin

hormones thus, provide a therapeutic option in the treatment of DM (Drucker *et al.*,2010). Vildagliptin and sitagliptin are common DPP-4 inhibitors which are presently available on the market.

f. Glucagon like peptide 1 (GLP-1) or incretin like therapies

The 'incretin' effect is defined as an increase in insulin release that is approximately three times greater for a given rise in plasma glucose when glucose is administered orally versus intravenously (Drucker *et al.*,2010). GLP-1 (glucagon-like peptide-1) is an important incretin hormone that functions as a potent insulintropic hormone (Drucker and Nauck, 2006). GLP-1 reduces postprandial glucose spikes by suppressing glucagon secretion in a glucose- dependent manner and delaying gastric emptying and excretion. Exenatide and liraglutide are two incretin-based therapies. They exert their incretin-based glycemic control by mimicking the action of GLP-1 (Drucker *et al.*,2010).

1.11.3 Drawbacks of current diabetes management strategies

Diabetes management strategies today include both pharmacological and non-pharmacological approaches. While non-pharmacological methods have the advantage of being effective and relatively inexpensive, they are often met with strong resistance, and people frequently revert to their old habits (Ahola and Groop, 2013). Pharmacological strategies, on the other hand, are expensive and fraught with unfavourable side effects and contraindications (Hung *et al.*,2012). They are generally out of reach for many DM patients, particularly those living in developing countries where access to advanced western primary healthcare systems is limited (Osadebe *et al.*,2014). Current antidiabetic pharmacological strategies include biguanides, thiazolidinediones, sulfonylureas, and meglitinides (Meneses *et al.*,2015). Biguanides such as metformin have been reported to have side effects that include diarrhoea, indigestion, nausea or vomiting, flatulence, and abdominal discomfort (Barbieri, 2003). Other metformin contraindications include renal disease, heart failure requiring pharmacologic therapy, acute or chronic metabolic acidosis, and active liver disease (Mane *et al.*,2012). Lactic acidosis has been linked to the long-term use of the biguanide drugs phenformin and buformin (Foretz *et al.*,2014). Hypoglycaemia is the most common side effect of sulfonylureas (Shorr *et al.*,1997), but weight gain, nausea, and skin reactions such as exfoliative dermatitis and hyponatraemia have also been reported (Sola *et al.*,2015). α -glucosidase inhibitors have been linked to mild to moderate gastrointestinal side effects like diarrhoea, flatulence, and abdominal bloating (Weng *et al.*,2015).

Thiazolidinediones were shown to have undesirable side effects that include hypoglycaemia, weight gain, and oedema (Barnett, 2009). Thiazolidinediones have been linked to weight gain in some patients due to increased feeding associated with PPAR-gamma receptor upregulation (Yamanouchi, 2010). They have also been linked to fluid retention as well as their ability to mediate adipose tissue maturation and increase tissue mass (Yamanouchi, 2010). Apart from being relatively expensive

compared with other commonly used oral hypoglycaemic drugs, DPP-4 inhibitors were reported to induce hypersensitivity, headaches, and nasopharyngitis (Gallwitz, 2013). GLP-1 presents no hypoglycaemic risk but has a potential neurogenic inflammation effect that increases blood pressure and enhances inflammation and allergic reactions (Drucker *et al.*,2010).

1.12. Traditional medicinal plants as novel antidiabetic therapeutics

Humanity has relied on plants since time immemorial for a plethora of uses ranging from diet, fragrances, shelter, clothing, and medicinal purposes (Abdullahi, 2011; Mahomoodally, 2013). A medicinal plant is defined as any plant that has therapeutically beneficial bioactive compounds that may be used directly or as precursors of chemo-pharmaceutical synthesis (Jain *et al.*,2019). Traditional medicinal use is widespread globally, particularly in parts of Sub-Saharan Africa, China, and India, as it offers a readily available and cheaper alternative healthcare system (Dar *et al.*,2017). People, particularly in impoverished developing countries, therefore, resort to more affordable and readily available management strategies, such as the use of traditional medicinal plants. This has resulted in a renewed interest in the development of novel therapeutic agents from natural sources as opposed to their synthetic counterparts. Pharmacognosy, which is the study of natural products' toxicology and medicinal properties, has made prominent advances in drug development. Medicinal plants have been used globally to combat viral, fungal, and bacterial infections as well as to mitigate several degenerative conditions, including Alzheimer's, cancer, cardiovascular complications (CVD), and diabetes. The use of traditional medicinal plants is gaining popularity as they provide an avenue of condition management that has fewer undesirable side effects. Regrettably, worrisome concerns are raised as the modes of action and toxicology profiles of most medicinal plants remain largely unknown. This is also true for the folkloric traditional medicinal plants that have been selected for this study. Secondary compounds have been isolated from different plants, and their modes of action have been elucidated.

1.13 Plant secondary metabolites and their antidiabetic properties

1.13.1 Secondary metabolites

Secondary metabolites are organic compounds whose production is not directly linked with the primary growth and development needs of the organism producing them (Tiwari and Rana, 2015).

Secondary metabolites are often synthesised through modified synthetic pathways of primary metabolites developed through evolving to adapt to a particular environment (Kabera *et al.*,2014). Consequently, the absence of secondary metabolites does not result in acute death, but rather in a long-term impairment of the organism's ability to ward off pests and disease, fecundity, or aesthetics (Kabera *et al.*,2014; Tiwari and Rana, 2015). They are characteristically produced by plants, bacteria,

and fungi differentially distributed among taxonomic groups (Pant *et al.*,2021). The functions of many secondary metabolites remain poorly elucidated but current evidence points to their allelopathic activities and defensive roles against parasites and predators (Tiwari and Rana, 2015). Plant secondary metabolites may either exist in their biologically active forms (constitutive) or as inactive precursors which are only activated in response to stress factors such as tissue damage and pathogen attack. The recent advancements in separation and spectroscopic techniques have shed much light on the function and structure of these bioactive natural compounds. Furthermore, with a better understanding of these compounds, their structures have been identified as the key active constituents of several therapeutic agents (Wink, 2015; Dar *et al.*,2017). This has sparked renewed research into natural secondary metabolites as a reservoir for pharmacological agent development. Phenolics, terpenes, alkaloids and saponins are among the most prominent secondary metabolites from which some current pharmacological agents have been derived.

a. Phenolic compounds

Phenolic compounds constitute the largest and most diverse group of plant secondary metabolites produced via the shikimate and acetate pathways (Lattanzio, 2013). They characteristically contain one or several hydroxylated aromatic ring structures. Common classification of phenolic compounds includes sub-groups such as simple phenols (C6 skeleton), phenolic acids (C6-C1 or C6-C3), flavonoids (C6-C3-C6) and tannins ((C6-C3- C6)_n) based on their carbon skeletons(Gani *et al.*,2021). Their functions are just as diverse and include protecting the leaves from ultraviolet radiation and browsers due to their bitter unpalatable taste (Gani *et al.*,2021). Apart from contributing to the colour of different plant tissues the ring structure in phenolic compounds has a bountiful number of electrons that can readily be donated thereby acting as antioxidants. Their bulky hydrophobic groups enable phenolic compounds to interact with various protein moieties including their catalytic active sites resulting in multiple beneficial therapeutic effects. A previous report by Ali Asgar in 2013 reported the inhibitory effects of (-)-3-O-galloylepicatechin and (-)-3-O-galloylcatechin against starch-digesting enzymes. Chlorogenic acid a phenolic acid highly abundant in coffee was previously reported to stimulate glucose uptake in the skeletal muscle cells through the activation of the 5' adenosine monophosphate-activated protein kinase pathway (Deka *et al.*,2022). They have been sought after as they retard the pathogenesis and progression of a multitude of diseases. Their modes of action include radical scavenging capabilities, quenching singlet and triplet oxygen metal chelation and providing environments for enhanced function of other biomolecules (synergism).

Simple phenols and phenolic acids are the simplest form of phenolic compounds containing one or more hydroxylated aromatic rings. They are generally low molecular weight and readily water-

soluble which include phenol (C6) with attached one (C6-C1), two (C6-C2) and three (C6-C3) carbon atoms (Kabera *et al.*,2014). While on the other hand, phenolic acids contain a phenolic ring and an organic carboxylic acid function. Several phenolic acids such as caffeic acid, p-coumaric acid and vanillic acid are known to have allelopathic activities. Caffeic acid at a concentration of 12.5 μ M was previously reported to improve glucose metabolism through the inhibition of glucogenic enzymes in insulin-resistant mouse hepatocyte model (Huang, and Shen, 2012). Furthermore, caffeic acid abated hyperglycaemia in an alloxan-induced type 1 diabetic mice model (Oršolić, *et al.*,2021). ferulic acid was previously shown to upregulate muscular muscle glucose uptake while concomitantly inhibiting carbohydrate and lipid hydrolysing enzymes (Salau *et al.*,2022).

Flavonoids are a large group of water-soluble polyphenolic compounds based on C6-C3-C6 skeleton found in the vacuoles of plant cells. In plants, they have an assortment of biological activities including providing pigmentation, fragrance and taste to fruits, flowers, and seeds. Flavonoids have strong antioxidative and hypoglycaemic properties (de Paulo Farias *et al.*,2021). Flavonoids can be widely classified into flavanols, flavones, catechins, flavanones, etc. Quercetin, which is primarily abundant in fennel, tea leaves, almonds, and lovage is among the most common flavonoids, documented to increase hepatic glucokinase activity and stimulating glucose GLUT 4 translocation and expression (Deka *et al.*,2022). Furthermore, kaempferol was reported to reduce blood glucose levels in STZ-induced diabetic mice model (Alkhalidy *et al.*,2018). Genistein an isoflavone used for the treatment of breast and prostate cancer (Yang *et al.*,2012), lowered fasting blood glucose, stimulated higher serum insulin presumably through increased islet mass in an alloxan diabetic rat model (Gilbert and Liu, 2013).

b. Steroids and Terpenes

Terpenes or terpenoids are the most diverse group of often volatile plant secondary metabolites. The concentration of these characteristically long hydrocarbon chains of alternating double and single bonded carbon atoms, phyto-compounds varies among species by usually ranging between 1% to 2% of dry matter content. They are based on an isoprene unit with a five-carbon building block. Monoterpenes are composed of two isoprene units (C10). Sesquiterpenes possess three isoprene units (C15), while diterpenes have four (C20). They are the major constituents of plant based essential oils that act as browser deterrents in plants and are used as fragrances, food spices, food colourants, food additives and medicine. A previous report documented the insulin mimetic activities of the diterpenes, stevioside and steviol, isolated from the leaves of the plant *Stevia rebaudiana* in β pancreatic cell line INS1 (Panigrahy *et al.*,2021). Steroid lipids are hydrocarbons derived from terpenes, characterised by a distinctive tetracyclic carbon structure composed of three fused six-membered rings and one

five-membered ring. Their classification, nomenclature, and functions are determined by the various conjugated groups attached to this parent carbon skeleton. A previous study documented the ability of 28Nor-22(R)Witha 2,6,23-trienolide, a novel withanolide, to restore euglycemic level tentatively through its ability to increase insulin levels in streptozotocin-induced diabetic rat model (Daisy *et al.*,2009).

c. Alkaloids

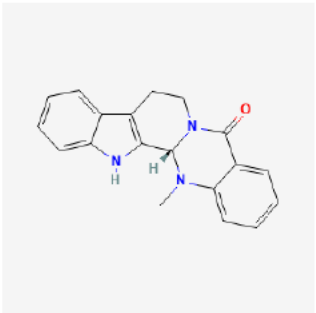
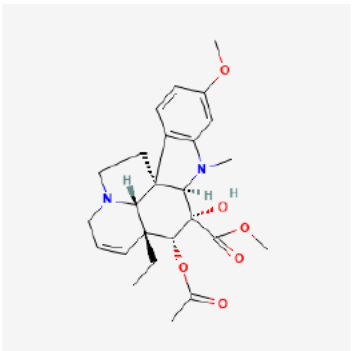
Alkaloids are nitrogenous organic compounds with complex chemical structures based on amino acids and usually contain one or more nitrogen-based cyclic rings. They are classified as pyrrolidine, pyridine, quinoline, isoquinoline, indole, quinazoline, steroidal, and diterpenoids depending on their structure (Christodoulou *et al.*,2019; Ajebli *et al.*,2021). These naturally occurring amines form part of plant defence systems and have an array of important therapeutic benefits in humans. Alkaloids are crystalline substances that exist in a free state as N-oxides or unite with acids to form salts, both readily soluble in water (Christodoulou *et al.*,2019; Ajebli *et al.*,2021). In contrast, alkaloid freebases are frequently sparingly soluble in water but soluble in organic solvents. Alkaloids usually have analgesic or toxic properties in humans (Christodoulou *et al.*,2019). The toxicity of certain alkaloids, as well as their instability at physiological pH, resulting in low bioavailability and a short half-life, may limit their clinical application. However, new pharmaceutical delivery strategies may be able to overcome such problems (Dabur *et al.*,2018). The alkaloid mahanimbine is a potent antihyperglycemic and antihyperlipidemic constituent of several medicinal plants including *Murraya koenigii* found to be active as an antidiabetic against diabetes associated with abnormalities in lipid profiles (Jain *et al.*,2012; Muhammad *et al.*,2021). Conophylline improved the insulin levels in streptozotocin-induced diabetic Sprague-Dawley rats tentatively through its ability to change originator cells to β pancreatic cells which produce insulin (Zhang *et al.*,2013).

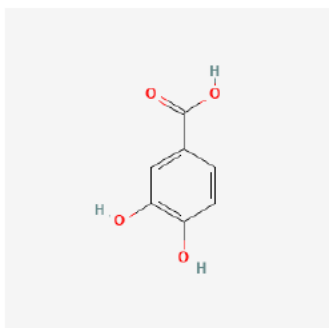
d. Saponins

Saponins distinguishably form colloidal solutions in water and produce a stable foam when they are vigorously shaken (Elekofehinti, 2015). This gives saponins a detergent characteristic that lowers water surface tension in membranes. They are glycoside derivatives of steroids which have a combination of lipophilic sugars at their ends and are capable of precipitating cholesterol (Elekofehinti, 2015). Furthermore, previous studies have demonstrated the anti- diabetic capabilities of dietary through their ability to modulate several pathways linked to diabetic pathology in various tissues including muscle, liver, pancreas, and gut (Zhou and Xu, 2022). Previous reports have shown that platyconic acid upregulates insulin sensitivity and restores glycogen reserves in peripheral tissues presumably through augmented GLUT4 translocation (Kwon *et al.*,2012). On the other hand, the

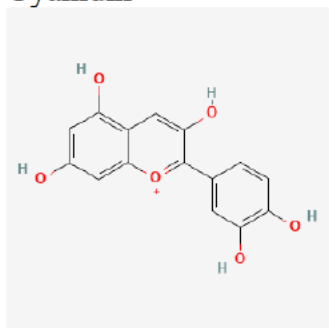
saponin arjunolic acid was found to be a potent α amylase and α glucosidase inhibitor (Hemalatha *et al.*,2010). Finally, several reports have documented the antidiabetic capabilities of Astragaloside IV through its abilities to reduce hepatic glucose output through the downregulation of the expression of hepatic glycogen phosphorylase and glucose 6 phosphatase synthesizing genes while concomitantly insulin resistance in peripheral tissues (Yin *et al.*,2004; Xiaoxing *et al.*,2006).

Table 1.2: Some reference secondary metabolites, their classification and antidiabetic mode of action.

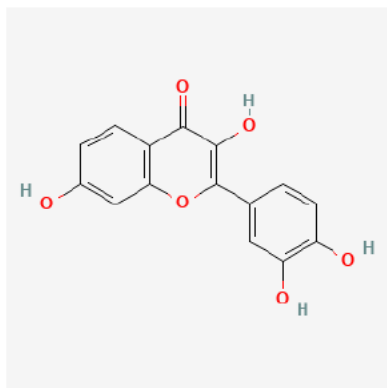
Secondary metabolite name/structure	Class	Mode of action	Reference
Evodiamine 	Alkaloid (Quinolone)	Abates peripheral tissue insulin resistance by modulating the AMPK and PPAR- γ pathways.	(Christodoulou <i>et al.</i> ,2019; Ajebli <i>et al.</i> ,2021)
Vindoline 	Indole	Insulin secretagogue Protein tyrosine phosphatase 1B (PTP1B) inhibitory activities	(Christodoulou <i>et al.</i> ,2019) (Dabur <i>et al.</i> ,2018)
Protocatechuic acid	Phenol	Inhibits α -amylase and α -glucosidase activity Enhances GLUT4 translocation Enhances adiponectin secretion	(Vinayagam <i>et al.</i> ,2016). (Alegbe <i>et al.</i> ,2019)



Cyanidin



Fisetin

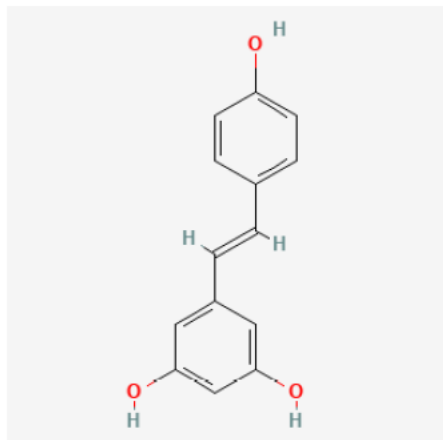


Increases the activity of hexokinase,
glucose-6-phosphate dehydrogenase
Reduces the activity of glucose 6-
phosphatase, fructose 1,6
bisphosphatase and sorbitol
dehydrogenase.

Anthocyanin Activates AMPK and GLUT4 translocation (Rupasinghe *et al.*, 2018; Al-Ishaq *et al.*, 2019)
Decreases gluconeogenesis by reduction of gene
expression of PEPCK, G6Pase
Inhibits α -amylase and α -glucosidase
Improves oxidative status
Prevents pancreatic apoptosis

Flavonoid Inhibits glycogen breakdown Decreases (Aba and Asuzu, 2018; Antika and
protein expression levels of Dewi, 2021)
gluconeogenic
genes such as phosphoenol pyruvate
carboxykinase
Inhibits gluconeogenesis Increases
plasma insulin levels

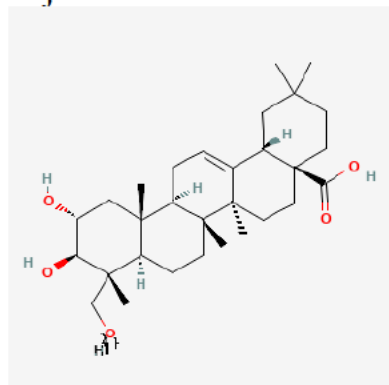
Resveratrol



Stilbenoid

Abates oxidative stress (Sun *et al.*, 2020; Shao *et al.*, 2020; Salehi *et al.*, 2020)
 Antiinflammatory capabilities
 Increases the activity of hexokinase and pyruvate kinase
 Increases hepatic glycogen content by activating glycogen synthase and inhibiting glycogen phosphorylase
 Increases GLUT-4 translocation in muscles via PI3K-Akt
 Reduces the activity of phosphoenolpyruvate carboxykinase, lactate dehydrogenase, and glucose-6-phosphatase
 Ameliorates insulin insensitivity

Arjunolic acid



Saponin.

Antioxidant activity
 Inhibits α -amylase and α -glucosidase activity (Elekofehinti, 2015; Hemalatha *et al.*, 2010)

1.14 Rationale for using medicinal plants to manage diabetes

The major drawback that is observed for the use of medicinal plants as antidiabetic agents is the poor documentation of treatment protocols. This leads to variations in dosage and preparation from one practitioner to the next. This lack of consistency leads to a lot of questions regarding the efficacy of traditional medicinal plants with the prognosis varying from patient to patient. Furthermore, the toxicological profiles of the medicinal plants that are employed remain poorly documented and this has a further risk of curing an illness while resulting in the development or exacerbation of other illnesses. This is compounded by the fact that medicinal plants are usually used in crude forms which may contain many other compounds apart from the functional compound. The modes of action of medicinal plants remain poorly understood in the scientific arena. The indigenous knowledge of the know-how of using medicinal plants is usually privy to selected individuals within the community including village elders, traditional healers, and herbalists. It is usually passed down from generation to generation through practical experience or word of mouth. While this helps cement the traditional structure of a people if the knowledgeable people pass on this knowledge is lost with them as well.

1.15 Problem identification

The incidence of DM is globally on the rise and has reached pandemic levels (Mokdad *et al.*,2003). Currently, sulfonylureas, meglitinides, insulin, thiazolidinediones and glycosidase inhibitors are pharmacologically employed in the management of DM. Current management strategies have proven to be ineffective, costly and have various adverse undesirable side effects (Dey and Attele, 2011). Additionally, they are generally inaccessible to most African people who are in ill-resourced areas and are impoverished (Whiting *et al.*,2011). Due to the unaffordability and inaccessibility of western medicine, many African patients resort to the use of traditional medicinal plants. Although the use of these medicinal plants is generally acceptable among African nationals, their modes of action and toxicology profiles are largely unknown and undocumented (Joshi, 2000). The proposed study is therefore going to employ the traditional knowledge acquired from the indigenous knowledge system (IKS) and develop a scientific understanding from it. The scientific knowledge that would result from the study will be documented and used to ascertain or refute claims that are associated with the use of plants in the management of DM.

1.16 Aim of the study

The aim of the study is therefore to document and scientifically validate the anti-diabetic effects of selected traditional medicinal plants. The study is aimed at assessing the *in vitro*, *ex vivo*, *in* and *vivo* antidiabetic potential of the selected medicinal plant-derived extracts.

1.17 Hypotheses

Medicinal plants that are reportedly used in Indigenous Knowledge Systems (IKS) for the management of various ailments including DM possess various bioactive phyto-compounds. The presence of these active therapeutic agents can thus be determined and quantified. Their subsequent activity as antioxidant and antidiabetic therapeutics can thus be ascertained using various *in vitro*, *in silico*, *ex vivo*, and *in vivo* experimental models.

1.18 Objectives

- To determine the antioxidant potential of the selected crude plant extracts.
- To determine the α -amylase and α -glucosidase inhibitory activity of the plant extracts.
- To determine the effects of the plant extracts on glucose uptake in psoas muscle tissue.
- To determine the effects of the plant extracts on the inhibition of glucose absorption in small intestinal tissue.
- To evaluate the toxicology profile of the plant extracts on experimentally induced animal model of type 2 diabetes.
- To evaluate the effects of the extracts on the antioxidant potential in serum and organ tissue extracts of treated animals.
- To evaluate the effects of plant extracts on serum insulin and glucose tolerance of the rats after treatment.
- To analyse the lipid profile and different organ function related parameters.
- To determine the histopathological morphology of the liver, kidney, and pancreas after treatment with the various plant extracts.

CHAPTER 2

Materials and methods

Materials

2.1 Chemicals

N-hexane, absolute ethanol, methanol, and ethyl acetate were obtained from Merck Chemical Company, South Africa. Folin ciocalteau, sodium hydrogen carbonate, aluminium chloride, potassium acetate, 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), potassium ferricyanide, ferric chloride, sodium chloride, sodium nitroprusside, calcium chloride di-hydrate, magnesium chloride, ferrous sulphate heptahydrate, magnesium sulphate, sodium phosphate, ascorbic acid, dinitro-salicylic acid (DNS), sulphuric acid, trichloroacetic acid, thiobarbituric acid, glucose-6-phosphate dehydrogenase, D-glucose 6-phosphate, mono-basic potassium phosphate, dibasic potassium phosphate, ethylenediaminetetraacetic acid (EDTA), sodium citrate, sodium hydroxide, Griess reagent, hydrogen peroxide, isopropanol, phenol red, soluble starch, reduced glutathione. 5,5'-dithiobisnitrobenzoic acid (DTNB), glucopyranoside (pNPG), 6-hydroxydopamine, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox), α - amylase, α -glucosidase, porcine pancreatic amylase, ammonium molybdate tetrahydrate, diethylenetriaminepentaacetic acid (DETAPAC), acarbose, citric acid, 2 deoxy-D-ribose, dibasic sodium phosphate, gallic acid, p-nitrophenyl- α -D-3-(N-morpholino) propane sulfonic acid (MOPS), p-nitrophenol, sodium acetate and streptozotocin were procured from Sigma- Aldrich, South Africa. Maltose, fructose, and glucose were bought from Associated Chemical Enterprise, South Africa whereas Metformin and Novo rapid insulin were bought from a local pharmacy (Pharmed) in Durban, South Africa. An ultrasensitive rat insulin enzyme-linked immunoassay ELISA Kit was procured from Elabscience Biotechnology Inc, USA.

2.2 Equipment

Synergy HTX Multi-mode Reader (BioTek Instruments Inc, Santa Clara USA), Shimadzu UV mini-1240 spectrophotometer (Shimadzu Corporation, Kyoto, Japan), Labmax Plenno (Labtest Inc., Lagoa Santa, Brazil), Buchi Rotavapor II (Buchi, Switzerland), Nuaire CO₂ incubator (Nuaire, Plymouth USA), Glucometer (Glucoplus Inc., Saint-Laurent, Quebec, Canada), Ultra Turrax Tube Drive Work Station Homogenizer (IKA-Works, Staufenim Breisgau, Germany), and Hettich Mikro 200 Microcentrifuge (Hettich Lab Technology, Tuttlingen, Germany) were used in this study for sample preparation and analysis.

Methods

2.3 Plant materials

2.3.1 Plant collection and verification

Leaves and bark of selected traditional medicinal plants (*Rhus longipes* Engl.; *Celtis africana* Burm.f.; *Trema orientalis* (L.) Blume; *Albizia adianthifolia* (Schumach.) W.F.Wight; *Schotia brachypetala* Sond.) were collected from Westville area in KwaZulu-Natal province, South Africa, after extensive ethnobotanical literature surveys that identified plants were used folklorically in the management of diabetes. The plant sample collection was done in summer from the same soil strata within a 2 km radius. The plant identity and authentication were done by Dr. Ramdhani and Mr. Khathi (curators) at the Ward Herbarium, School of Life Sciences, University of KwaZulu-Natal (Specimen voucher numbers: BB-003-WV10/19, BB-004- WV10/19, 13753, 108531, 12011, respectively).

2.3.2 Plant extract preparation

Air-dried leaf and bark materials protected from sunlight were ground into a fine powder using a domestic warring blender. Powdered plant material (10 g) was defatted with hexane and thereafter sequentially macerated in solvents of increasing polarity (ethyl-acetate, ethanol, and methanol) (100 ml) and allowed to stand overnight. The supernatant was filtered using a Whatman No.1 filter paper, concentrated, and poured into pre-weighed amber glass vials. The crude plant extracts were stored in air-tight amber glass vials at 4°C in the dark until further analysis. The hot aqueous infusions were prepared by infusing dry plant materials (10 g) in boiling distilled water (100 ml) for 30 min and allowed to stand overnight. The supernatants were filtered and concentrated in a water bath at 50°C, then stored in air-tight amber glass vials at 4°C until further analysis. The crude plant extract was reconstituted in distilled water to a stock solution of 1 mg/ml prior to analytical assays.

2.4 Phytochemical characterisation and quantification

2.4.1 Total phenolic content

The total phenolic content of the leaf and bark extracts was determined spectrophotometrically using the Folin-Ciocalteu phenol reagent method (Liu and Yao, 2007). Concentrations of 240 µg/ml of the different extracts (20 µl) were added to 10% Folin-Ciocalteu reagent (100 µl) and left to stand for 5 min in the dark at 25°C. Thereafter, 0.7 M sodium carbonate (80 µl) solution was added. The mixture was allowed to stand for 30 min in the dark at 25°C. Absorbance of the independent triplicates was measured at 765 nm using a microtiter-plate multimode detector (Synergy HTX). The total phenolic content was determined by linear regression from a gallic acid calibration standard curve. Results were expressed as gallic acid equivalents (GAE) in milligram per gram of dry weight.

2.4.2 Total flavonoid content

Aluminium chloride colorimetric method was used for the determination of total flavonoids (Woisky and Salatino, 1998). Concentrations of 240 µg/ml of the different extracts were prepared. Each of the extracts (10 µl) was mixed with 10% aluminium chloride (10 µl), 1 M potassium acetate (10 µl) and distilled water (200 µl). The mixture was left to stand at room temperature for 30 min. The absorbance of the reaction mixture was measured at 415 nm in triplicates. The total flavonoid content was determined by linear regression from a quercetin calibration standard curve. Results were expressed as quercetin equivalents (QE) in milligram per gram of dry weight.

2.4.3 Gas Chromatography Mass Spectroscopy (GC-MS) analysis

Selected leaf and bark extracts were subjected to gas chromatography-mass spectrometric (GC- MS) analysis to identify compounds belonging to chemical class/families including alkaloids, terpenoids (or terpenes), phenolic compounds, glycosides, saponins, fatty acids, lignans and coumarins from which therapeutic agent templates are derived. This was done using an Agilent technologies 6890 series GC coupled with (an Agilent) 5973 Mass Selective Detector and driven by Agilent Chemstation software. Briefly, a HP-5MS capillary column (30 m × 0.25 mm ID, 0.25-µm film thickness, 5% phenylmethylsiloxane) was used. Ultra-pure helium was utilized as the carrier gas and at a flow rate of 1.0 ml min⁻¹ and a linear velocity of 37 cm s⁻¹. The injector temperature was set at 250°C. The oven temperature was programmed to 280°C from 60°C at the rate of 10°C min⁻¹ with a hold time of 3 min. One microliter (1 µl) injections of the samples were made in split mode with a split ratio of 20:1. The mass spectrometer was operated in the electron ionization mode at 70 eV, while the electron multiplier voltage at 1,859 V. The ion source temperature was 230°C and the quadrupole temperature was 150°C. The solvent delay was set at 4 min, with a scan range of 50– 70 amu. Direct comparison of the retention times and mass spectral data in the NIST library was used in identifying the compounds.

2.5 *In vitro* antioxidant analysis

2.5.1 Quantitative DPPH radical-scavenging activity assay

The antioxidant activity of extracts was quantitatively determined spectrophotometrically using the DPPH free radical scavenging assay (Changlian *et al.*,2000). Equal volumes (100 µl) of 0.3 mM DPPH in methanol and different concentrations ranging from 15 µg/ml to 240 µg/ml of the extracts were incubated and protected from light at room temperature for 30 min. The DPPH in methanol solution was used as the experimental control while L-ascorbic acid (vitamin C) as a positive control. The decrease in absorbance was measured at 517 nm using a microtiter- plate multimode detector (Synergy HTX). The absorbance values obtained were converted to percentage scavenging activity using the following formula:

$$\% \text{ Inhibition} = \frac{((A_{517\text{nm}} \text{ of blank} - A_{517\text{nm}} \text{ of sample}) \times 100)}{(A_{517\text{nm}} \text{ of blank})}$$

2.5.2 Ferric reducing antioxidant power (FRAP)

The ferric ion reducing power of the different extracts was determined using Benzie and Strain (1996) method. Various concentrations (15 to 240 µg/ml) of the extracts of the different plants in deionised water (100 µl) were prepared. A blank was prepared without the extracts, while L-ascorbic acid (vitamin C) was used as the reference standard. These were then mixed with phosphate buffer (250 µl) (pH 7.4 and concentration 0.2 M) together with potassium ferri-cyanide (250 µl) and incubated at 50°C for 20 min. After incubation, aliquots of trichloroacetic acid (250 µl) were added to the mixture and centrifuged at 3000 rpm for 10 min. The supernatant (250 µl) was mixed with distilled water (250 µl) and freshly prepared ferric chloride solution (50 µl). The absorbance of the samples was measured at 700 nm using a microtiter-plate multimode detector (Synergy HTX). Percentage reducing power was calculated according to the following formula:

$$\text{Percentage reducing power} = \frac{((A_{700\text{nm}} \text{ of sample} - 1) \times 100)}{(A_{700\text{nm}} \text{ of blank})}$$

2.5.3 Nitric oxide scavenging assay

The nitric oxide scavenging assay was carried out as previously described by Rana *et al.*,2010. Plant leaf and bark extract samples (50 µl) of increasing concentrations (15 to 240 µg/ml) were incubated with equal volumes of sodium nitroprusside (10 mM) in phosphate buffer (pH 7.4) at 37°C for 2 h. Thereafter Griess reagent (50 µL) was added to the reaction mixture and mixed gently by tapping the side of the plate. The resultant chromophores' absorption was read at 546 nm and the percentage inhibition of nitric oxide generated was calculated.

$$\% \text{ Inhibition} = \frac{((A_{546\text{nm}} \text{ of blank} - A_{546\text{nm}} \text{ of sample}) \times 100)}{(A_{546\text{nm}} \text{ of blank})}$$

2.6 *In vitro* enzyme inhibition analysis

2.6.1 α -glucosidase inhibition assay

The ability of the extracts to inhibit α -glucosidase *in vitro* was carried out according to the method described by Oboh and Ademosun, 2011 with slight modifications. Plant extract samples (50 μ l) of increasing concentrations (15 to 240 μ g/ml) were incubated with equal volume of α -glucosidase (1.0 U/ml) in phosphate buffer (100 mM, pH 6.8) at 37°C for 15 min. Thereafter, 5 mM *p*-nitrophenyl- α -D-glucopyranoside (pNPG) (100 μ l) solution in phosphate buffer (100 mM, pH 6.8) was added to the reaction mixture, which was further incubated for 20 min at 37°C. Acarbose was used as a positive control (standard drug). The absorbance of the liberated *p*-nitrophenol was measured at 405 nm and the inhibitory activity expressed as a percentage of the experimental control lacking inhibitors/infusion as shown in the formula:

$$\% \text{ inhibition} = \frac{((A_{405\text{nm}} \text{ of control} - A_{405\text{nm}} \text{ of sample}) \times 100)}{(A_{405\text{nm}} \text{ of control})}$$

2.6.2 α -amylase inhibition assay

The ability of the extracts to inhibit α -amylase was done according to the method described by Shai *et al.*, 2010, with slight modifications. Plant extract samples (50 μ l) of increasing concentrations (15 to 240 μ g/ml) were incubated with equal volumes of porcine pancreatic amylase (2 U/ml) in phosphate buffer (100 mM, pH 6.8) for 10 min at 37°C. An aliquot of 1% starch solution (50 μ l) in phosphate buffer (100 mM, pH 6.8) was added to the reaction mixture and incubated at 37°C for 10 min. Dinitrosalicylate (DNS) colour reagent (100 μ l) was then added to the mixture and boiled for 10 min. Acarbose was used as a positive control (standard drug). Thereafter, absorbance was read at 540 nm and the inhibitory activity was expressed as a percentage of the control according to the formula below:

$$\% \text{ inhibition} = \frac{((A_{540\text{nm}} \text{ of control} - A_{540\text{nm}} \text{ of sample}) \times 100)}{(A_{540\text{nm}} \text{ of control})}$$

2.6.3 Porcine pancreatic lipase inhibition assay

The ability of the extracts to inhibit the lipid digestive action of porcine pancreatic lipase *in vitro* was carried out according to the method described by Kim *et al.*,2010. Plant extract samples (100 µl) of increasing concentrations (15 to 240 µg/ml) were incubated with buffered porcine pancreatic lipase (20 µl) (2.5 mg/ml). The enzyme buffer constituted 10 mM MOPS and 1 mM EDTA at pH 6.8. After the 15 min incubation at 37°C, Tris buffer (100 mM Tris– HCl and 5 mM CaCl₂, pH 7.0) (169 µl) was added. Orlistat was used as the reference standard, while enzyme only was used as the experimental control. The substrate solution of 5 µl of 10 mM *p*-nitrophenyl butyrate (*p*-NPB) in dimethyl formamide was then added to initiate the reaction. After gentle mixing, the solution was further incubated for 30 min at 37°C. The lipase activity was determined by measuring the hydrolysis of *p*-NPB to *p*-nitrophenol at 405 nm. The inhibitory activity was expressed as a percentage of the experimental control lacking inhibitors/infusion:

$$\% \text{inhibition} = \frac{((A_{405\text{nm}} \text{ of control} - A_{405\text{nm}} \text{ of sample}) \times 100)}{(A_{405\text{nm}} \text{ of control})}$$

2.6.4 Mode of α -amylase inhibition assay

The mode of inhibition of α -amylase by the leaf and bark extracts was determined using their IC₅₀ value determined previously by the inhibition assay through linear regression as described by Ali *et al.*,2006. The leaf or bark infusion (250 µl) were pre-incubated with α -amylase (250 µl) (2 U/ml) solution at 37°C for 10 min in one set of tubes. In another set of tubes, α -amylase was pre-incubated with phosphate buffer (250 µl) (100 mM, pH 6.8). Thereafter, starch (250 µl) solution at increasing concentrations (0.0–8.0 mM) was added to both sets of reaction mixtures to start the reaction. The mixture was then incubated at 37°C for 10 min. DNS (100 µl) was then added to the mixture to stop the reaction and boiled for 10 min. The amount of reducing sugars released was determined spectrophotometrically at 540 nm using a maltose standard curve and converted to reaction velocities. These were used to plot a Michaelis- Menten kinetics graph.

2.6.5 Mode of α -glucosidase inhibition assay

The mode of inhibition of α -glucosidase by the leaf and bark extracts was determined using their IC₅₀ value determined previously by the inhibition assay through linear regression as described by Ali *et al.*,2006. The leaf or bark infusion (50 µl) were pre-incubated with aliquots of 1.0 U/ml α -glucosidase (100 µl) solution at 37°C for 15 min in one set of tubes. In another set of tubes α -glucosidase was pre-incubated with phosphate buffer (50 µl) (100 mM, pH 6.8).

PNPG (50 μ l) at increasing concentrations (0.0– 10 mM) was added to both sets of reaction mixtures to start the reaction. The mixture was then incubated at 37°C for 15 min, before 500 μ l of Na₂CO₃ was added to stop the reaction. The amount of reducing sugars released was determined spectrophotometrically using a para-nitrophenol standard curve and converted to reaction velocities.

2.6.6 Mode of pancreatic lipase inhibition

The mode of inhibition of porcine pancreatic lipase by the leaf and bark extracts was determined using their IC₅₀ value determined previously by the inhibition assay through linear regression as described by Hu *et al.*,2015. The leaf or bark infusion (100 μ l) were pre-incubated with aliquots of 2.5 mg/ml porcine pancreatic lipase (20 μ l) solution at 37°C for 15 min. Porcine pancreatic lipase pre-incubated with the buffer was used as the control. The substrate solution, 5 μ l of 0.0–10.0 mM *p*-NPB was then added to initiate the reaction. After gentle mixing the solution was further incubated for 30 min at 37°C. The lipase activity was determined by measuring the hydrolysis of *p*-NPB to *p*-nitrophenol at 405 nm determined spectrophotometrically using a *p*-nitrophenol standard curve before conversion to reaction velocities.

2.6.7 Mode of inhibition analysis

The mode of inhibition for α -amylase, α -glucosidase and pancreatic lipase were analysed using Michaelis-Menten kinetics. Michaelis-Menten graph of reaction velocity (V) versus the substrate concentration (S) were converted to Lineweaver–Burk plots (double reciprocal plots (1/V versus 1/(S)). Various parameters were analysed according to the formula: $1/V = K_m/V_{max}[S] + 1/V_{max}$ where K_m is the Michaelis constant and V_{max} is the maximum rate of the enzymatic reaction.

2.7 Glucose uptake/transport by yeast cells

The effect of the extracts on glucose uptake/transport by yeast cells was carried out according to Nirupama *et al.*,2014 previously established protocol. Different amounts of the extracts were reconstituted in 1 ml of distilled water containing 25 mM glucose to desired concentrations (15 to 240 μ g/ml). The resulting solutions were incubated for 10 min at 37°C for acclimatisation. Thereafter, 100 μ l of 1% yeast (Gold star instant yeast, Rymco Ltd., Johannesburg, South Africa) suspension was added, vortexed, and incubated for a further 60 min at 37°C. Concurrently, samples incubated without yeast suspension were used as sample blank, yeast suspensions with glucose only as the control while metformin was used as the reference standard drug.

Glucose concentration of the solution was determined with dinitrosalicylic acid method, and glucose uptake (%) was calculated by the following formula:

$$\% \text{ Glucose uptake} = \frac{((A_{540\text{nm}} \text{ of sample} - A_{540\text{nm}} \text{ of control}) \times 100)}{(A_{540\text{nm}} \text{ of control})}$$

2.8 Cell lines

Vero monkey kidney epithelial cells (American Type Culture Collection [ATCC], Manassas, VA, USA) were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Sigma, South Africa), supplemented with 10% Foetal Bovine Serum (FBS), and 1% Penicillin-Streptomycin incubated at a temperature of 37°C in a humidified atmosphere with 5% CO₂. Every two to three days, the culture medium was replaced with fresh medium until cells reached 70-80% confluency. The cultured cells were constantly observed under a light microscope (Nikon TS100, Germany) for the attachment of cells, the presence of mycoplasma, their viability, and any morphological changes.

2.8.1 Cytotoxicity assay

The extracts' cytotoxicity was screened *in vitro* using a MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) cytotoxicity assay method as described by Deuschländer *et al.*, 2009. The cells were re-suspended in fresh media to a seeding concentration of 2500 cells/ml determined using a TC20 cell counter (Bio-Red). The cells were seeded into 96 well microplates for 24 hours at 37°C in a 5% CO₂ incubator and allowed to re-attach. Attached cells were exposed to increasing concentrations of the bark and leaf infusions (15 to 240 µg/ml) for a further 24 hours at 37°C in a 5% CO₂ incubator. After the exposure time, 20 µl of MTT reagent (5 mg/ml in PBS) was added to each well and the plates were incubated for 4 hours. The spent media was carefully aspirated, and the intra-cellular purple formazan crystals formed were resolubilized by the addition of acidified isopropanol (100 µl). The absorbance of the purple formazan solution was measured at a wavelength of 570 nm using a microplate spectrophotometer. The percentage of viable cells was calculated:

$$\% \text{ Cell viability} = \frac{((A_{570\text{nm}} \text{ of control} - A_{570\text{nm}} \text{ of sample}) \times 100)}{(A_{570\text{nm}} \text{ of control})}$$

2.9 Molecular docking

Virtual screening software PyRx V 0.8 was used for molecular docking. PyRx uses Autodock Vina as the docking engine (Dallakyan & Olson, 2015). The grid centre for docking was set with maximized blind co-ordinates and grid box sizes for the selected proteins. Compounds found via GC/MS in the extracts were prepared for docking. X-ray crystal structures of α -amylase (PDB ID:1B2Y, Resolution :3.20 Å), α -glucosidase (PDB ID:3TOP, Resolution: 2.88 Å) and pancreatic lipase (PDB ID:1LPB, Resolution:2.46 Å) enzymes responsible for carbohydrate and lipid hydrolysis, superoxide dismutase (PDB ID: 2C9V, Resolution :1.07 Å), catalase (PDB ID: 1QQW, Resolution :2.75 Å) antioxidant enzymes were downloaded from the Protein Data Bank website (<https://www.rcsb.org/>). The structures were prepared by the removal of all heteroatom coordinates and water molecules, added with hydrogens, Kollman charges and missing C-terminal oxygen using Biovia Discovery Studio 2017R2 Client software. The active ligands were prepared by minimizing their structural energy coefficients using Open Babel. The Binding energy for best poses was tabulated while the 3D and 2D ligand-receptor interactions were visualized using Biovia Discovery Studio 2017R2 Client software.

2.10 Animals for *ex vivo* study

2.10.1 Experimental animals

Sprague-Dawley male albino rats (36) weighing between 180–200 g were obtained from the Biomedical Research Unit (BRU), University of KwaZulu-Natal, Durban, South Africa. They were acclimatized for a week and supplied with standard pellet rat chow and water *ad libitum*. Standard laboratory conditions of the natural photo period of 12h light-dark cycle were maintained. Animals were denied food for 12 h prior to sacrifice in the morning. They were euthanized by cardiac puncture, and the kidney, liver, pancreas, psoas muscles, small intestines, and heart were harvested for *ex vivo* assays. All animals used were maintained under the guidelines and approval of the animal ethics committee of the University of KwaZulu-Natal, Durban, South Africa (Protocol approval number: AREC/038/019D).

2.10.2 Intestinal glucose absorption study

The effects of extracts on glucose absorption in isolated rat intestinal jejunum were determined using Hassan *et al.*,2010 modified technique. Jejunum isolated from gastro-intestinal-tract (GIT) of rats was sectioned into equal portions (5 cm in length). The partitioned sections were rinsed by injecting 2 ml of Krebs's buffer through the jejunal lumen using a sterile syringe prior to their inversion to expose their inner wall and villi.

Inverted sections were then incubated in tubes containing 8 ml each of GKS solution (control), GKS solution with different concentrations of the plant infusion and GKS solution with 3 mM acarbose solution (positive control) for 2 hours at 37°C, in an atmosphere of 5% CO₂ and 95% Oxygen. Aliquots (1 ml) were collected from each incubation tube before and after incubation with the intestinal tissue. The glucose concentrations were determined using a glucose kit (Thermo Scientific) in a Labmax Plenno Chemistry Analyzer (Labtest Inc., Costa Brava, Brazil). Thereafter the amount of glucose absorption was calculated using the following formula:

$$\text{Intestinal glucose absorption} = \frac{\text{GC1} - \text{GC2}}{5 \text{ cm of jejunum}}$$

Where, “GC1” and “GC2” are glucose concentrations (mg/dl) before and after incubation, respectively.

2.10.3 Measurement of glucose uptake in isolated rat psoas muscles/liver tissues

The effects of the extracts on glucose uptake by excised rat psoas muscle/liver tissues were determined according to the method described by Hassan *et al.*,2010. Fresh psoas muscles/liver hepatocytes were collected and sectioned into 0.5 g pieces. The pieces were then incubated in tubes containing 8 ml each of GKS (control), GKS solution with different concentrations of plant infusion and GKS solution with 100 U/ml insulin solution (positive control) for 1 hour at 37°C, in a humidified atmosphere with 5% CO₂ and 95% Oxygen. Aliquots (1 ml) were collected from each incubation tube before and after incubation with the muscles/liver tissues. The glucose concentrations were determined using a glucose kit (Thermo Scientific) in a Labmax Plenno Chemistry Analyzer (Labtest Inc., Costa Brava, Brazil). The amount of glucose uptake was calculated using the following formula:

$$\text{Muscle glucose uptake} = \frac{\text{GC1} - \text{GC2}}{0.5 \text{ G of muscle/liver tissue}}$$

Where, “GC1” and “GC2” are glucose concentrations (mg/dl) before and after incubation, respectively.

2.10.4 Preparation of tissue homogenates

The harvested tissues were homogenized in a phosphate buffer (0.2 mM, pH 6.9) and centrifuged at 3500 x g for 10 min (4°C). The supernatant was transferred to sample tubes and stored at -20°C until further analysis.

2.10.5 Fructose-1,6-bisphosphatase activity

The effects of the extracts on the activity of fructose-1,6-bisphosphatase were determined using the methods of Balogun and Ashafa, 2017. Liver tissue homogenates (100 µl) were added to 600 µl 0.1M Tris-HCl buffer (pH 7.0), 50 µl of 0.05 M fructose, 125 µl of KCl (0.1 M), 50 µl MgCl₂ (0.1 M) and 125 µl EDTA (1 mM). The mixture was incubated for 15 min at 37°C and terminated by the addition of 10% trichloroacetic acid (50 µl). The resulting solution was centrifuged at 5000 g for 10 min and the supernatant incubated at 37°C for 30 min with freshly prepared 9% ascorbic acid. Absorbance was measured at 680 nm using a microtiter-plate multimode detector (Synergy HTX). Thereafter the fructose-1,6-bisphosphatase enzyme activity was calculated from an inorganic phosphate standard curve.

2.10.6 Glycogen phosphorylase activity

The effects of the extracts on glycogen phosphorylase activity were carried out according to the method outlined by Balogun and Ashafa, 2017 with modification. Tissue homogenate (200 µl) was added to 64 mM glucose-1-phosphate (100 µl) and 4% glycogen (100 µl). The reaction mixture was then incubated at 30°C for 10 min, followed by the addition of 2.5 ml of 20% ammonium molybdate. Then, the mixture was incubated for 45 min at 30°C before the addition of Elon reducer and distilled water. The absorbance was read at 600 nm using a microtiter- plate multimode detector (Synergy HTX). Glycogen phosphorylase activity was expressed as inorganic phosphate (Pi) released/min/mg protein.

2.10.7 ATPase enzyme assay

The ATPase activities of the tissue samples were determined using Mahato *et al.*, 2011 slightly modified protocol. Homogenised tissues (200 µl) were incubated with 0.25 M glucose (100 µl), 5 mM KCl (200 µl), 0.1 M Tris-HCl buffer (1300 µl), and 50 mM ATP (40 µl) at 37°C in a shaker for 30 min. Thereafter, 1 ml of distilled water and 1.25% ammonium molybdate were added respectively to stop the reaction. 1 ml of freshly prepared 9% ascorbic acid was then added to the reaction mixture and allowed to stand for 30 min. Absorbance was read at 660 nm and ATPase activity was calculated as the amount of inorganic phosphate (Pi) released/min/mg protein.

2.11 Oxidative stress biomarkers

2.11.1 Preparation of tissue homogenates and induction of oxidative stress *ex vivo*

Aliquots of tissue homogenates (100 µl) prepared as detailed in Section 2.10.4 were incubated with an equal volume of the plant extracts and the pro-oxidant, 0.1 M FeSO₄ (30 µl) for 30 min at 37°C. The reaction containing no infusion (untreated) served as a negative control, while ascorbic acid served as a positive control. After incubation, the modulatory effects of the plant extracts on the activity of the selected oxidative biomarkers were analysed as detailed below:

2.11.2 Determination of lipid peroxidation level

Lipid peroxidation level of the samples was determined by measuring thiobarbituric acid reactive substances (TBARS), expressed as malondialdehyde (MDA) equivalent (Chowdhury & Soulsby, 2002). Samples (100 µl) were mixed with an equal volume of 8.1% SDS solution, 375 µl of 20% acetic acid, 1 ml of 0.25% thiobarbituric acid (TBA), and 425 µl of distilled water. The reaction mixture was boiled at 95°C for 1 h in a water bath. Thereafter, the boiled mixture (200 µl) was pipetted into 96-well plate and absorbance read at 532 nm. A standard curve was generated from the absorbance of standard MDA, from which the TBARS concentration was extrapolated.

2.11.3 Determination of reduced glutathione (GSH) level

GSH levels in the tissue homogenates were determined according to a method described by Ellman, 1959. Homogenates (200 µl) were mixed with an equal volume of 10% TCA to precipitate proteins. It was gently mixed and centrifuged at 3500 rpm for 5 min (25°C). Thereafter, aliquots of the supernatant (200 µl) were pipetted into a 96 well plate. Ellman reagent (50 µl) was then added, and the plate was allowed to stand for 5 min before absorbance was read at 415 nm. GSH level was then extrapolated from the standard curve of plotted GSH concentrations.

2.11.4 Determination of superoxide dismutase (SOD) activities

Superoxide dismutase activity in the tissue homogenates was determined according to a method described by Gee and Davison, 1989. A 0.1 mM Diethylenetriaminepentaacetic acid (170 µl) was added to the samples (15 µl) in a 96-well plate. Thereafter, 1.6 mM 6-hydroxydopamine (15 µl) was added and mixed by gently tapping all four sides of the plate. Absorbance of the resulting mixture was immediately measured at 492 nm for 5 min at 1min interval.

2.11.5 Determination of catalase (CAT) activities

The catalase enzyme activity in tissue homogenate was determined according to a method described by Hadwan and Abed, 2016. The homogenates (100 µl) were incubated with 65 µM H₂O₂ (1 ml) prepared in 6 mM sodium phosphate buffer (pH 7.4) for 2 min at 37°C. The reaction was terminated by the addition of 32.4 mM ammonium molybdate (5 µL). Absorbance of the resulting chromophore (molybdate/H₂O₂ complex) was measured at 347 nm.

2.12 In vivo animal study

2.12.1 Animals

Seven-week-old male Sprague-Dawley rats (83) were procured from the Biomedical Resource Unit located at the Westville Campus of the University of KwaZulu-Natal, Durban, South Africa. The animals were maintained under the guidelines and approval of the animal ethics committee of the University of KwaZulu-Natal, Durban, South Africa (Protocol approval number: AREC/038/019D).

2.12.2 Animal grouping

Animals were randomly grouped into sixteen (16) groups as follows, with five (5) rats each in the normal or non-diabetic groups and seven (7) rats each in the diabetic groups:

Normal Groups

Normal Control (NC): Normal rats

Normal Toxicological *R. longipes* (NRLTX): Normal rats administered with *R. longipes* (300 mg per kg body weight).

Normal Toxicological *C. africana* (NCATX): Normal rats administered with *C. africana* (300 mg per kg body weight).

Normal Toxicological *S. brachypetala* (NSBTX): Normal rats administered with *S. brachypetala* (300 mg per kg body weight).

Diabetic Groups

Diabetic Control (DBC): Diabetic rats

Diabetic *R. longipes* low dose (DRLL): Diabetic rats administered with low dose of *R. longipes* (150 mg per kg body weight).

Diabetic *R. longipes* high dose (DRLH): Diabetic rats administered with high dose *R. longipes* (300mg per kg body weight).

Diabetic *C. africana* low dose (DCAL): Diabetic rats administered with low dose of *C. africana* (150 mg per kg body weight).

Diabetic *C. africana* high dose (DCAH): Diabetic rats administered with high dose of *C. africana* (300mg per kg body weight).

Diabetic *S. brachypetala* low dose (DSBL): Diabetic rats administered with low dose of *S. brachypetala* (150 mg per kg body weight).

Diabetic *S. brachypetala* high dose (DSBH): Diabetic rats administered with high dose of *S. brachypetala* (300 mg per kg body weight).

Diabetic Metformin (DBM): Diabetic rats administered with metformin (300 mg per kg body weight).

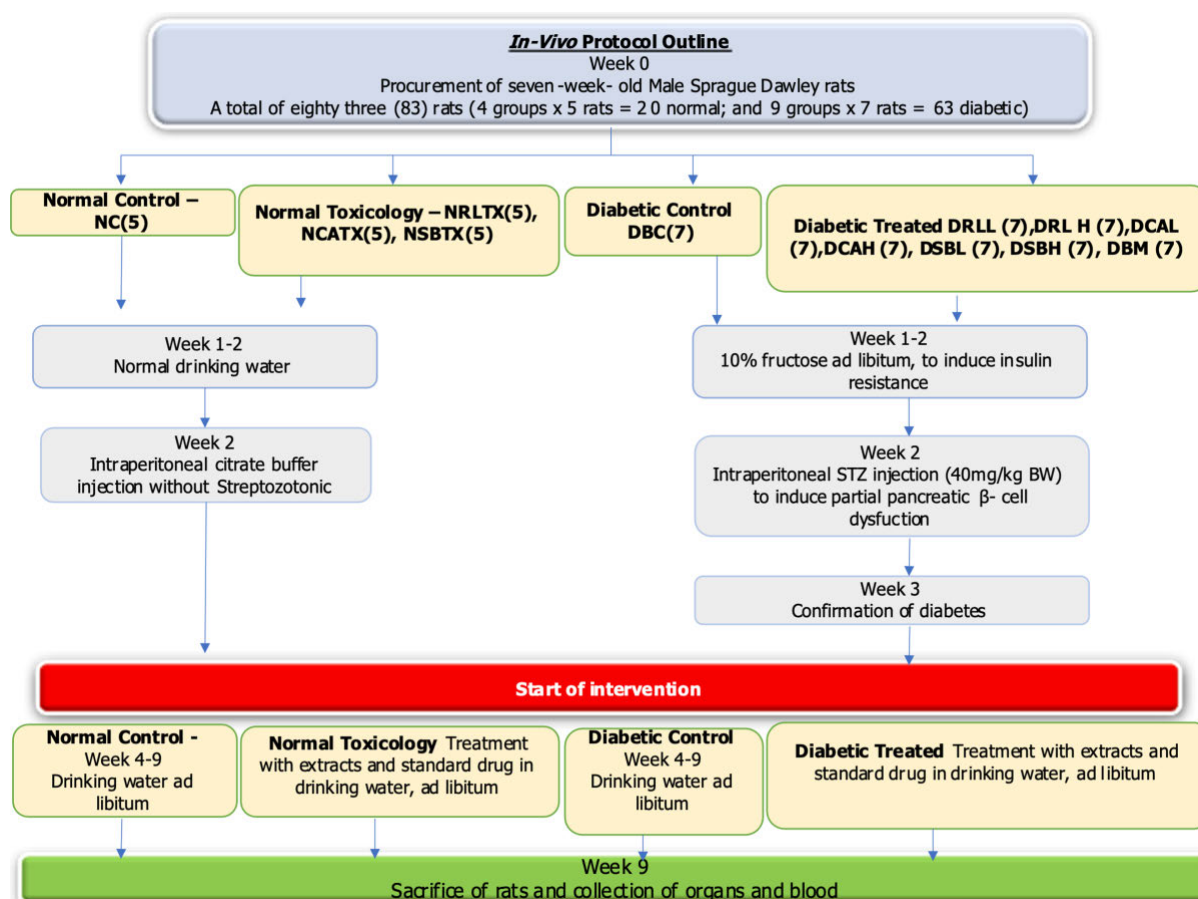


Figure 2.1: Schematic representation of the *in vivo* study protocol.

2.12.3 Induction of diabetes

The procured animals were allowed to acclimate for one week before type 2 diabetes induction (Fig 2.1) using the method described by Wilson and Islam, 2012. During the first two weeks of the experiment, the animals in diabetic groups (DBC, DRLL, DRLH, DCAL, DCAH, DSBL, DSBH and DBM) were given a 10% fructose solution to induce insulin resistance, while the animals in normal or non-diabetic groups (NC, NRLTX, NCATX, NAATX, and NSBTX; 20 animals total) were given normal drinking water. Thereafter, animals in the diabetic groups were injected intraperitoneally with a low dose of streptozotocin (40 mg/kg body weight) dissolved in a citrate buffer (pH 4.5) to induce partial pancreatic β -cell dysfunction. However, animals in the normal or non-diabetic groups were injected with a similar volume of citrate buffer only. The non-fasting blood glucose (NFBG) levels of all the animals were measured via blood collected from the tail vein one week after the streptozotocin injection using a portable glucometer. Animals with NFBG levels greater than 200 mg/dl were considered as diabetic whereas those with NFBG levels < 200 mg/dl were excluded from the experimental trial.

2.12.4 Intervention

After the diabetes induction, all animals were given *ad libitum* access to drinking water and a commercial rat pellet diet. Animals in the plant extract treatment groups (DRLL, DRLH, DCAL, DCAH, DSBL, DSBH, NRLTX, NCATX, NAATX, and NSBTX) were given a single daily oral dose of the respective plant extract for 4 weeks (150 or 300 mg per kg body weight). Animals in the DBM group received a single oral dose of metformin (300 mg per kg body weight). The animals in the NC and DBC groups received the same amount of normal drinking water via the same route. Throughout the experiment, each rat's body weight and blood glucose levels were measured weekly, while food and fluid intake were recorded daily.

2.12.5 Oral Glucose Tolerance Test (OGTT)

The OGTT was carried out in the final week of the 4-week intervention period. Following an overnight fast, glucose was administered to each animal at a dose of 2.0 g/kg body weight, and blood samples were collected from the tail vein at 0, 30, 60, 90, and 120 min. A portable Glucometer was used to measure glucose levels.

2.12.6 Collection of blood and organs

At the end of the intervention period, animals were fasted overnight and euthanized using isoflurane overdose. Blood was collected via cardiac puncture into sterile centrifuge tubes and kept on ice

for two to three hours. The serum was then separated by centrifugation at 3000 rpm for 15 min and stored at -20°C for further analysis. The liver, kidney, heart, brain, and pancreas were immediately collected after the sacrifice. Except for the pancreas and brain, all other organs were washed with normal saline, wiped with filter paper, weighed, and stored at -80°C in separate Ziploc bags. The pancreas and brain were collected, weighed, wrapped in aluminum foil, and placed in a Ziploc bag before being stored at -80°C for biochemical studies. A small portion of each organ was removed and preserved in phosphate buffered formalin solution for histopathological analysis.

2.12.7 Fasting blood glucose and homeostatic model assessment (HOMA)

The fasting blood glucose concentration of all the animals were measured using a portable Glucometer and the serum level of insulin was determined using an ultrasensitive rat insulin enzyme-linked immunoassay ELISA Kit (Elabscience Biotechnology Inc, USA). The HOMA scores for insulin resistance (IR) and pancreatic β -cell function were calculated using the fasting serum insulin and fasting blood glucose concentrations as per the following formulae:

$$\text{HOMA} - \text{IR} = \frac{[\text{Insulin } (\frac{\text{U}}{\text{L}})] \times \text{Blood glucose } (\frac{\text{mMol}}{\text{L}})]}{22.5}$$

$$\text{HOMA} - \beta = \frac{[20 \times \text{Insulin } (\frac{\text{U}}{\text{L}})]}{[\text{Blood glucose } (\frac{\text{mMol}}{\text{L}}) - 3.5]}$$

Conversion factor: Insulin (1 U/L = 7.174 pMol/L) and Blood glucose (1 mMol/L = 18 mg/dL)

2.12.8 Serum lipid profiles

The total cholesterol, HDL-cholesterol and triglycerides were measured by using an Automated Chemistry Analyzer (Labmax Plenno, Lagoa Santa, Brazil) and the LDL-cholesterol was calculated according to Friedewald et al. (1972) equation as shown below:

$$\text{LDL} - \text{Cholestrol } (\frac{\text{mMol}}{\text{L}}) = [\text{TC} - \text{HDL} - (\frac{\text{TG}}{5})]$$

Where, TG/5 is equivalent to the concentration of VLDL-cholesterol.

Atherogenic index of the plasma (AI) was calculated according to the method described by Liu et al. (1999) and expressed as:

$$\text{AIP} = \frac{\text{Total cholestrol} - \text{HDL cholestrol}}{\text{HDL cholestrol}}$$

2.12.9 Serum biochemical analysis

Biochemical parameters linked to kidney and liver function including lactate dehydrogenase (LDH), urea, uric acid, alkaline phosphatase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, creatine kinase-myocardial band (CK-MB) in the serum were quantitatively determined using standard assay kits in an Automated Chemistry Analyzer (Labmax Plenno, Labtest Co. Ltd., Lagoa Santa, Brazil).

2.12.10 *In vivo* antioxidant studies

The modulatory activity of the plant extracts on oxidative stress biomarkers was assessed in the serum and animal tissues. The excised tissues (liver, kidney, heart, and pancreas) were first homogenized in 4000 µl of homogenization buffer (50 mM sodium phosphate buffer with triton X-100, pH 7.5) using a workstation homogenizer (IKA-Works, Staufenim Breisgau, Germany). Soon after, the solution was transferred to a microtube and centrifuged for 15 mins at 15000 rpm. The supernatant was then transferred to another microtube and stored at -80°C for further analysis. The supernatant was used to determine the thiobarbituric acid reactive substances (TBARS) levels, reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), were assessed as previously described in section 2.11.2-2.11.5.

2.12.11 Liver glycogen content

The phenol-sulphuric acid method was used to determine the liver glycogen content in the various treatment groups after the completion of the experiment (Lo *et al.*,1970). About 0.3 g of liver sample was measured and placed on ice in 1.5 ml eppendorf tubes with a forceps. To each liver sample, 0.5 ml of 30% KOH saturated with Na₂SO₄ was added and then placed in a boiling water bath for 30 min. The samples were removed and cooled on ice before being mixed with 670 µl of 95% ethanol. Thereafter, samples were allowed to stand for 30 min before being centrifuged at 840 g (4°C) for 25 min. The supernatant was discarded and 500 µl of 95% ethanol was added to the glycogen precipitate. After vortexing and centrifugation (840 g, 4°C), for 10 min the precipitate was dissolved in 1.0 ml of distilled water. 20 µl of dissolved precipitate solution was transferred to a separate tube containing 180 µl distilled water. Glycogen standards of 10, 20, 40, 80, 160, 320, and 640 g/ml concentrations were prepared from a 1 mg/ml glycogen stock solution (Oyster, Type II, Sigma-Aldrich, USA). To both standards and samples, 0.5 ml of 5% phenol was added, followed by 2.5 ml of 96-98% H₂SO₄. After the reaction set-up stood for 10 min at room temperature (25°C), the absorbance was measured at 490 nm. The concentrations of liver glycogen in triplicate were interpolated from a glycogen standard curve.

2.12.12 Histopathological Analysis

For tissue processing, each organ section was dehydrated in increasing concentrations of ethanol (70% to 90 %) at 1-hour intervals followed by immersion in 100% ethanol overnight. Then, the tissues were cleared in three 1.5-hour xylene changes before embedding in paraffin wax. The paraffin blocks were cut to the desired dimensions. Sections were cut at 3 μ m intervals and immersed in a 40-45°C water bath before being mounted onto slides and air-dried overnight. Dry slides were deparaffinised in two xylene changes for 5 and 2 min, respectively. Slides were then rehydrated by successive immersion in decreasing ethanol concentrations before being placed in distilled water. The slides were stained with haematoxylin as the primary stain for 5 min, and washed with running water then, counterstained with eosin for a further 3 min. The slides were dipped in 90-100% ethanol followed by xylene, wiped and cover slipped using DPX mounting agent cover-slipped and viewed with Leica slide scanner (SCN 4000, Leica Bio-systems, Germany).

2.13 Statistical analysis

The data was represented as mean $\pm$ SEM/SD from 3 independent experiments. One-way analysis of variance (ANOVA) was used to determine statistical significance at $p < 0.05$. Statistical analysis was carried out using IBM SPSS for Windows, version 26.0 (SPSS Inc., Chicago, IL, USA).

CHAPTER 3

***IN VITRO AND EX VIVO STUDIES TO INVESTIGATE THE ANTIOXIDANT AND ANTIDIABETIC ACTIVITIES OF TREMA ORIENTALIS* Linn. Blume.**

3.1 *Trema orientalis*

3.1.1 Background and plant description

Trema orientalis belongs to the family ulmaceae and is commonly known as pigeon wood (English) umvangazi (Zulu) (Smith, 1966). *Trema orientalis* is a deciduous medium-sized tree with drooping branches that bear small, round, green fruits, which become black when ripe (Adinortey *et al.*,2013). *Trema orientalis* irregularly flowers from late winter to autumn, producing inconspicuous small greenish unisexual flowers. It has a smooth and light grey bark with conspicuous lenticels with rough and hairy young leaves that are finely separated at the margins and become smoother as they age (Adinortey *et al.*,2013). The height to which the tree grows is heavily dependent on the environmental conditions, particularly water. *Trema orientalis* can grow up to 18 m on forest margins but only up to 1.5 m in the KwaZulu-Natal bushveld (Smith, 1966). *Trema* contains about 50 species widely distributed in tropical and subtropical regions of the world, including tropical Africa southwards through the Erongo Mountains in Namibia and down to South Africa. *Trema orientalis* is the only species recorded in South Africa (Smith, 1966).



Figure 3.1: *Trema orientalis* Linn. Blume: Common names: pigeon wood (English) umvangazi (Zulu). Adapted without permission from www.sanbi.org.

3.1.2 Ethnobotanical uses

The fruit, leaves, stems, twigs, and seeds of *T. orientalis* are used in traditional medicine in West Africa, Tanzania, East Africa, and elsewhere (Hines and Eckman, 1993; Iwu, 1993). It has previously been reported to be used to treat pneumonia, pleurisy, toothache, haematuria, blood stasis, diabetes, and helminthiasis in dogs, as well as an antidote to general poisoning (Iwu, 1993; Adinortey *et al.*,2013). The Zulu people of South Africa consume young leaves as spinach and use roots and stem bark as traditional medicine (Adinortey *et al.*,2013). In folkloric medicine, the root of *T. orientalis* plants is used to treat trauma, blood stasis, haematuria, and intestine and stomach bleeding (Hines and Eckman, 1993; Ibrahim *et al.*,2020).

3.1.3 Biological activities

A previous study published in 2011 by Abiodun revealed that the aqueous extract of *T. orientalis* stimulated duodenum contractility in a rabbit model, implying its potential use as a laxative. The study also revealed that its laxative properties were due to its cholinomimetic and anticholinesterase activities (Abiodun *et al.*,2011). Laxatives, also known as purgatives, are foods, compounds, or drugs used to induce bowel movements or loosen the stool and are mostly used to treat constipation. The pharmacological importance of *T. orientalis* against diabetes and its complications was established in a previous study that demonstrated its hypoglycemic effects in a diabetic rat model (Dimo *et al.*,2006). The hexane extract of *T. orientalis* was found to have antiplasmodial activity, indicating its potential for use as an antimalarial drug (Abiodun *et al.*,2011). *Plasmodium falciparum* is a protozoan parasite that is one of the *Plasmodium* species that causes malaria in humans (Abiodun *et al.*,2011). *T. orientalis* polyphenols have previously been identified as the most likely diuretic bioactive compounds (Uddin *et al.*,2008). Increased diuresis is one of the body's mechanisms for eliminating water-soluble toxins. This could further explain the reasons behind *T. orientalis* being traditionally used as an antitoxin. Crude methanol and aqueous root extracts of *T. orientalis* showed activity against gram-negative bacteria, insinuating the anti-microbial activity of this plant and further justifying its use as such traditionally (Chowdhury and Islam, 2004).

3.1.4 Phytochemistry

Previous research has described a variety of phytoconstituencies that have been identified in the various sections of *T. orientalis* (He *et al.*,2020). A previous report by Adinortey and colleagues in 2013 revealed the presence of tannins, saponins, flavonoids, and triterpenoids in the leaves of *T. orientalis*. Ayoade and colleagues' 2014 quantitative reports on the leaf extracts revealed that the extracts were higher in steroids, flavonoids, and alkaloids, while saponins,

tannins, glycosides, and terpenoids were moderately present. Several studies have also shown the presence of cardiac glycoside and xanthone derivatives (Ogunkoya *et al.*,1973; Ogunkoya *et al.*,1977). Tremetol, a triterpene alcohol, was derived from its stem bark together with sweroside, scopoletin, (-)-epicatechin, lupeol, p-hydroxybenzoic acid, adian-5-en-3-one, 2 α , and 3 β -dihydroxyurs-12-en-28-oic acid. (-)-ampelopsin F, (-)-epicatechin, (+)-catechin, (+)-syringaresinol, N-(trans-p-coumaroyl) tyramine, N-(trans-p-coumaroyl) octopamin, trans-4-hydroxycinnamic acid, and 3,5-dimethoxy-4-hydroxyphenyl-1-O- β -D-glucoside (Noungoué Tchamo *et al.*,2001; Kuo *et al.*,2007). Investigation of the composition of the root and the bark of the root of *T. orientalis* resulted in the identification of hexacosanoic acid, 23-trihydroxyurs-12-en-28-oic acid, simiarenone, 3,4-dihydroxybenzoic acid, β -sitosterol, and 3-O- β -glucopyranosyl- β -sitosterol. Dijoux-Franca *et al.*,2001; Noungoué Tchamo *et al.*,2001). The decoctions of the *T. orientalis* leaf extract were shown to contain varying amounts of flavonoids, saponins, coumarins, polyphenols, sterols, polyterpenes, and cardiotonic glycosides (Parvez *et al.*,2019).

3.1.5 Aim and objectives

This study investigated the antioxidant and antidiabetic activities of crude *Trema orientalis* extracts of varying polarity from different sections by determining their free radical scavenging, electron-donating capacity, and nitric oxide production inhibiting potential *in vitro*. The potentiating capacity of yeast cell glucose uptake and the inhibitory activities of carbohydrate and lipid-digesting enzymes in the extracts were investigated *in vitro*. While the modulation of hepatic glycogenic enzymes and endogenous antioxidant moieties, which act as biomarkers of oxidative tissue injury, intestinal glucose absorption, and muscular/liver glucose uptake were investigated *ex vivo*.

3.2 *Trema orientalis* leaf extracts regulate glucose metabolism while concomitantly abating oxidative pancreatic tissue injury

¹Brian K. Beseni, ¹Kolawole A. Olofinisan ^{1,2}Veronica F. Salau, ¹Md. Shahidul Islam*

1. Department of Biochemistry, University of KwaZulu-Natal, Westville Campus, Durban 4000, South Africa

2. Department of Pharmacology, University of the Free State, Bloemfontein 9300, South Africa

***Corresponding author:** islamd@ukzn.ac.za

3.2.1 Abstract

The current study investigated the effects of *T. orientalis* crude leaf extracts on diabetes and oxidative stress using *in vitro* and *ex vivo* experimental models. Total polyphenol and flavonoid content, antioxidant, and antidiabetic capacities of aqueous, methanol, ethanol, and ethyl acetate extracts of *T. orientalis* crude leaf extracts were quantified *in vitro*. Further *in vitro* models were performed to determine the effects of these extracts on DPPH, FRAP, and NO- scavenging antioxidant activities, as well as their inhibitory effects against key carbohydrate and lipid hydrolysing enzymes. Their effects on glucogenic enzymes, muscle and liver glucose uptake, intestinal glucose absorption, and modulatory effects on oxidative pancreatic injury were determined *ex vivo*. There was no significant ($p > 0.05$) difference in total phenolic content between the aqueous extracts (15,57 mg/g) and methanolic extracts (16,14 mg/g), which had the highest total phenolic content of all extracts. The extracts exhibited comparable DPPH radical-scavenging activity. The ethanolic extract showed the best ferric reducing antioxidant power (FRAP), while the aqueous extract had the best NO production inhibitory activity. The ethanolic extract has the best α -glucosidase, α -amylase inhibitory activity, and glucose uptake attenuating capabilities. Furthermore, this extract exhibited the best activity against ATPase, glycogen phosphorylase, and 1,6-bisphosphatase. The ethanol extract significantly ($p < 0.05$) reduced lipid peroxidation levels and increased superoxide dismutase and catalase activities in oxidative pancreatic injury. The ethanolic extracts, which showed comparatively the best activities from most of the assayed parameters, may further be studied using *in vivo* models to ascertain their precise modes of action.

Keywords: Antidiabetic, glucose uptake, *Trema orientalis*, antioxidant.

3.2.2 Introduction

The diabetes mellitus (DM) pandemic has reached epidemic proportions, with global prevalence increasing at an alarming rate (Hostalek, 2019; Forouhi and Wareham, 2019). Diabetes mellitus (DM) is a metabolic disorder marked by hyperglycemia (Kerner and Brückel, 2014; Punthakee *et al.*,2018). There are several types of diabetes, but the most common etiologies are type 1 (T1D), type 2 (T2D), and gestational diabetes (ADA, 2014; Punthakee *et al.*,2018). A more concerning finding is that diabetes is a comorbidity of several diseases, including hypertension, obesity, and cardiovascular disease (CVD) (Iglay *et al.*,2016; Rubino *et al.*,2020). DM is one of the leading causes of death worldwide, with an estimated annual mortality rate of more than one million people (Khan *et al.*,2020).

The International Diabetes Federation (IDF) estimated in 2019 that approximately 463 million people worldwide have diabetes, with its prevalence expected to increase by almost 51% to approximately 700 million people by 2045. (IDF, 2019). The steep rise in the incidence of diabetes is exacerbated by its incurable nature and the large number of undiagnosed cases, particularly in Africa, where approximately 46% of cases go undiagnosed (IDF, 2019). In 2015, the direct and indirect global cost of DM was estimated to be around US\$1,31 trillion, a crippling sum for impoverished countries with already stretched smaller GDPs (Herman, 2017; Nazir *et al.*,2018).

Various factors, including genetic predisposition, diet, sedentary lifestyle, age, and ethnicity, have been linked to the pathogenesis and progression of diabetes (Bellou *et al.*,2018). Oxidative stress has been shown to play a critical role in the pathogenesis and progression of diabetes (Asmat *et al.*,2016). Oxidative stress occurs when the body's endogenous antioxidant defense systems are overwhelmed by the production of reactive species (RS) (Oguntibeju, 2019). Under normal physiological conditions, reactive species (RS), such as reactive oxygen species (ROS), reactive nitrogen species (RNS), and reactive chlorine species (RCS), have specific homeostatic functions in the body (Jakubczyk *et al.*,2020). They act as secondary messengers, as lytic constituents in the phagocytic burst, as mediators of cell signaling pathways, and in a variety of other processes. (Asmat *et al.*,2016; Forrester *et al.*,2018; Jakubczyk *et al.*,2020). Due to the highly reactive nature of RS caused by unstable unpaired electrons, under pathological conditions of oxidative stress, RS may react with functional biomolecules to attain a stable octet state (Halim and Halim, 2019). Unfortunately, this concomitantly renders the other participating molecule in the reaction nonfunctional (Forrester *et al.*,2018; Halim and Halim, 2019). This is particularly believed to be one of the several ways in which insulin insensitivity occurs when RS damages insulin cell surface receptors and causes aberrant glucose uptake and metabolism (Asmat *et al.*,2016; Kurdekar and

Sushma, 2020). Under homeostatic conditions, excess amounts of these species that can be produced are effectively dissolved by endogenous antioxidant defense systems, including catalase (CAT), superoxide dismutase (SOD), and glutathione (GSH) (Pisoschi and Pop, 2015). These moieties have been widely documented as oxidative stress biomarkers (Robson *et al.*, 2018; Mistry *et al.*, 2020). Molecules that have an intrinsic capacity to positively attenuate their function are widely sought after as therapeutic agents in the management of DM and its related complications (Matough *et al.*, 2012; Asmat *et al.*, 2016).

Current diabetes management strategies, although effective, have several drawbacks, including undesirable multiple side effects (Capoccia *et al.*, 2016). The side effects of the current DM medication include weight loss or gain, diarrhea, water retention, vomiting or nausea, and some have been reported to cause cancer and kidney and liver damage (Sterrett *et al.*, 2016; Stahnke *et al.*, 2020). Furthermore, some may be out of reach, particularly for people living in impoverished regions of the world, especially in sub-Saharan Africa. It is estimated that about 80% of people in the African region rely on the use of traditional medicinal plants for their primary health care needs or to supplement western medicine regimens (Thomford *et al.*, 2015; Buwa-Komoren *et al.*, 2019). There has been a rise in interest by scientists throughout the world to determine the modes of operation of these medicinal plants in terms of their efficacy and safety (Thomford *et al.*, 2015). The eventual goal is to develop pharmacological agents that have reduced side effects and are affordable to people in impoverished regions of the world. *Trema orientalis* is one such plant that is used in Africa for medicinal purposes. A previous study that demonstrated *T. orientalis*'s hypoglycemic effects in a diabetic rat model established its pharmacological importance against diabetes and its complications (Dimo *et al.*, 2006). The current study investigated the antioxidant and antidiabetic potentials of *T. orientalis* crude leaf extracts of varying polarity. Their antioxidant and enzyme-inhibitory activities were investigated *in vitro*. The attenuating effects of the extracts on oxidative pancreatic injury, small intestinal glucose absorption, and muscle and liver glucose uptake were investigated using *ex vivo* experimental models. The plant extracts' modulatory effects on glucogenic and nucleotide-metabolizing enzymes linked to T2D disorders were also investigated.

3.2.3 Materials and methods

Please refer to Chapter 2, sections 2.4.1 to 2.11.5 for detail materials and methods.

3.2.4 Results

The total phenolic and flavonoid contents of the leaf extracts of *T. orientalis* are depicted (Fig. 3.2.1 A and B). There was no significant ($p > 0.05$) difference in total phenolic content between the aqueous extracts (15,57 mg/g) and methanolic extracts (16,14 mg/g), which had the highest total phenolic content of all extracts. The ethanolic extract (11,73 mg/g) contained significantly ($p < 0.05$) the least total phenolic content. The ethanolic extract (4,49 mg/g) contained the highest flavonoid content, while the ethyl acetate extract (0,75 mg/g) contained significantly ($p < 0.05$) the least flavonoid content.

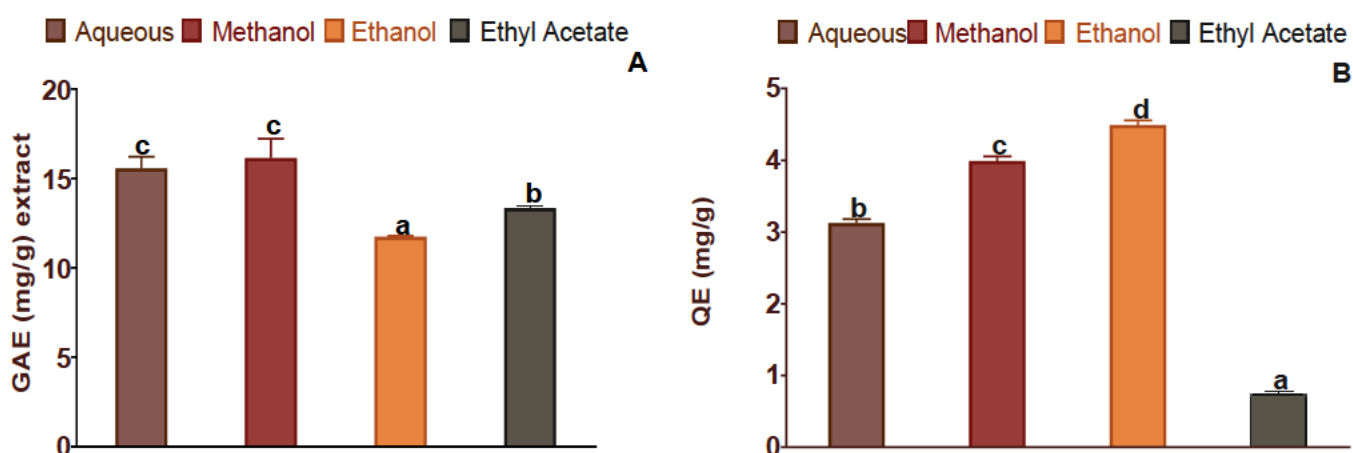


Figure 3.2.1: The total phenolic content (A) and the total flavonoid content (B) of *T. orientalis* crude leaf extracts represented as Gallic acid equivalents (GAE mg/g) and Quercetin equivalents (QE mg/g) respectively. The superscripts a to d represent statistical significance of $p < 0.05$.

The different antioxidant capacities of the *T. orientalis* leaf extracts were determined using the DPPH, FRAP, and NO inhibition *in vitro* assays (Fig. 3.2.2 A, B, and C). Furthermore, the respective IC_{50} values of the extracts were calculated and recorded in Table 3.2.1. The extracts exhibited comparable DPPH radical-scavenging activity. The ethyl acetate extract exhibited the best DPPH radical scavenging activity, resulting in the least IC_{50} value of $9.83 \pm 0.74 \mu\text{g/ml}$, which was comparable to that of the positive control (Vitamin C) ($IC_{50} = 9.21 \pm 0.12 \mu\text{g/ml}$). The ethanolic extract showed the best ferric reducing antioxidant power (FRAP) among all extracts ($IC_{50} = 15,54 \pm 0,55 \mu\text{g/ml}$), while the ethyl acetate extract had the least activity ($IC_{50} = 183,87 \pm 0,13 \mu\text{g/ml}$). The aqueous extract ($IC_{50} = 9,73 \pm 0,66 \mu\text{g/ml}$) outperformed all other extracts in their NO production inhibitory activity. As observed for the FRAP assay, the ethyl acetate extract similarly had the least inhibitory capabilities against NO production.

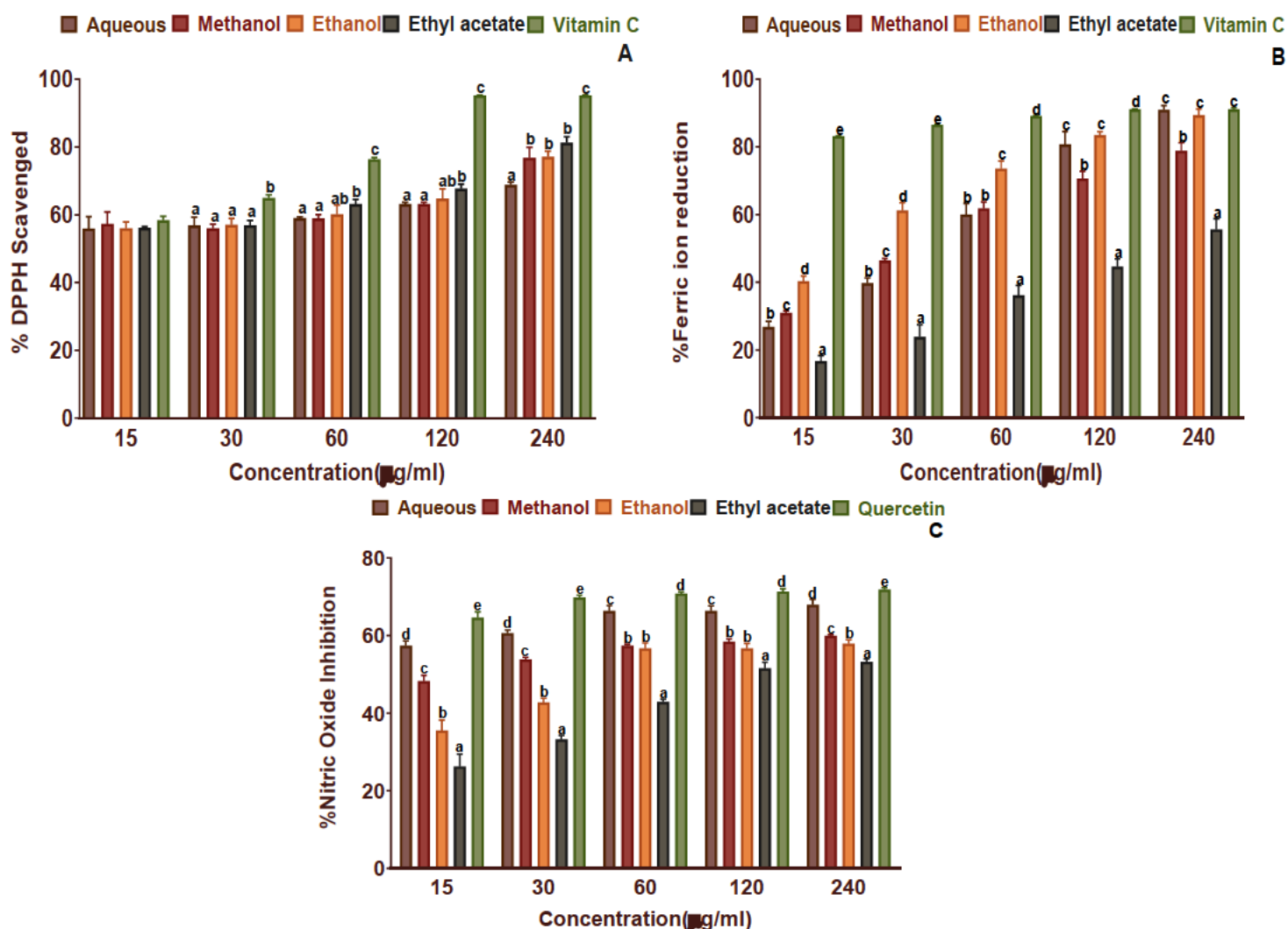


Figure 3.2.2: Effects of *T. orientalis* leaf extracts of extracts on (A) DPPH radical scavenging activity, (B) ferric ion reducing activity, and (C) inhibition of nitric oxide production. Data are presented as mean $\pm$ standard deviation. Different superscripts a-e represent statistical significance of $p < 0.05$.

The inhibitory activities of the leaf extracts of *T. orientalis* against key lipid and carbohydrate- digesting enzymes are shown in Fig. 3.2.3A-C. The extracts showed dose-dependent pancreatic lipase inhibitory activity. Notably, at the highest concentration of 240 µg/ml, all extracts except the ethanol extract exhibited significantly ($p<0.05$) better pancreatic lipase inhibitory capacity than the positive control (orlistat). The methanolic extract had the best pancreatic lipase inhibitory activity, as evidenced by its lowest IC_{50} value of $148,93\pm0,68$ µg/ml, as shown in Table 3.2.1. Furthermore, as seen in Fig 3.2.3B, the methanol and ethanol extracts with IC_{50} values of $87,02\pm0,65$ µg/ml and $89,34\pm0,78$ µg/ml (Table 3.2.1) exhibited the best α glucosidase inhibitory activity among all extracts, including acarbose (the positive control), which had an IC_{50} value of $100,68\pm0,09$ µg/ml. Finally, the extracts similarly showed dose- dependent inhibition of α amylase, with the ethanol extract exhibiting the best inhibitory activity ($IC_{50} = 214,56\pm0,21$ µg/ml) among all the extracts, as shown in Fig. 3.2.3C.

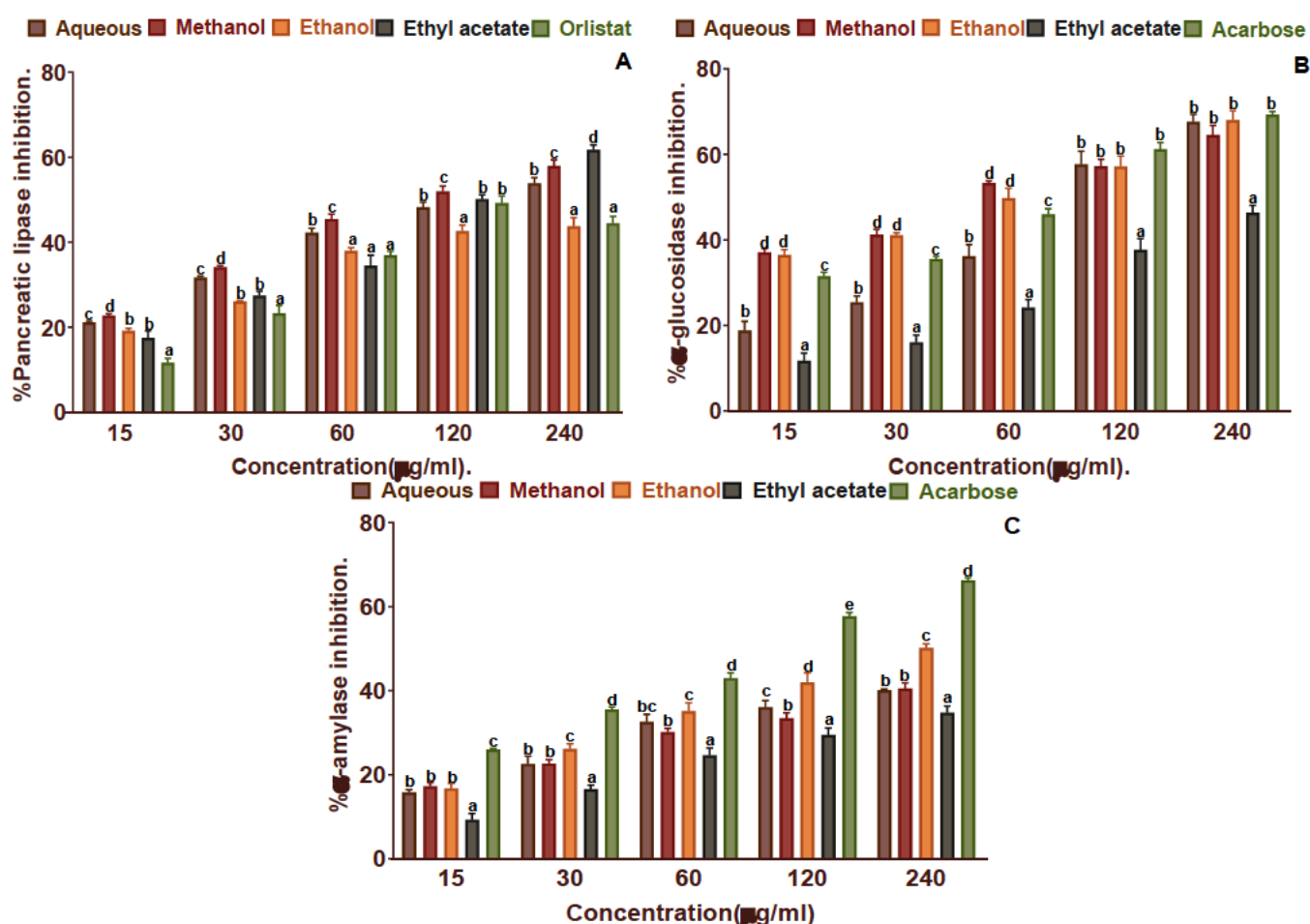


Figure 3.2.3 : Effects of *T. orientalis* bark organic extracts of extracts on (A) Pancreatic lipase inhibition activity, (B) α glucosidase inhibition activity, (C) α amylase inhibition activity. Data is presented as mean $\pm$ standard deviation. Different superscripts a-e represent statistical significance of $p<0.05$.

The glucose uptake potentiating activities of the leaf extracts of *T. orientalis* in the yeast cells, liver, and muscle tissues and their retarding capabilities on glucose absorption by intestinal jejunum tissue are depicted in Figs. 3.2.4 A–D. While the extracts increased glucose uptake in the yeast cell in a dose-dependent manner, the ethanol extract had the overall best activity, as shown in Fig. 3.2.4 A. Furthermore, *T. orientalis* extracts increased glucose uptake more than metformin (the positive control) at all concentrations tested. Notably, at the highest concentration of 240 µg/ml, the ethanol extract resulted in 43.89% glucose uptake, signifying its potency among all other extracts as depicted in Fig. 3.2.4 A. As depicted in Fig. 3.2.4 B, *T. orientalis* extracts exhibited comparable muscle glucose uptake potentiating activity to that of insulin (positive control). Furthermore, treatment with the methanol extract resulted in the overall best glucose uptake in the muscle tissue, especially at the highest concentration of 240 µg/ml, where it resulted in better activity than insulin (positive control). A similar observation was made in which all the extracts, apart from the ethyl acetate extract, demonstrated comparable glucose uptake activity to that of insulin (positive control) in liver tissue, particularly at the highest concentrations of 120–240 µg/ml. Finally, as shown in Fig. 3.2.4 D, the *T. orientalis* extracts exhibited dose-dependent potency to inhibit small intestinal glucose absorption, with the aqueous extract having the highest overall activity. Interestingly, it was shown in Fig. 3.2.4 D that at higher concentrations between 60 and 240 µg/ml, all extracts significantly ($p < 0.05$) reduced absorption better than acarbose (the positive control).

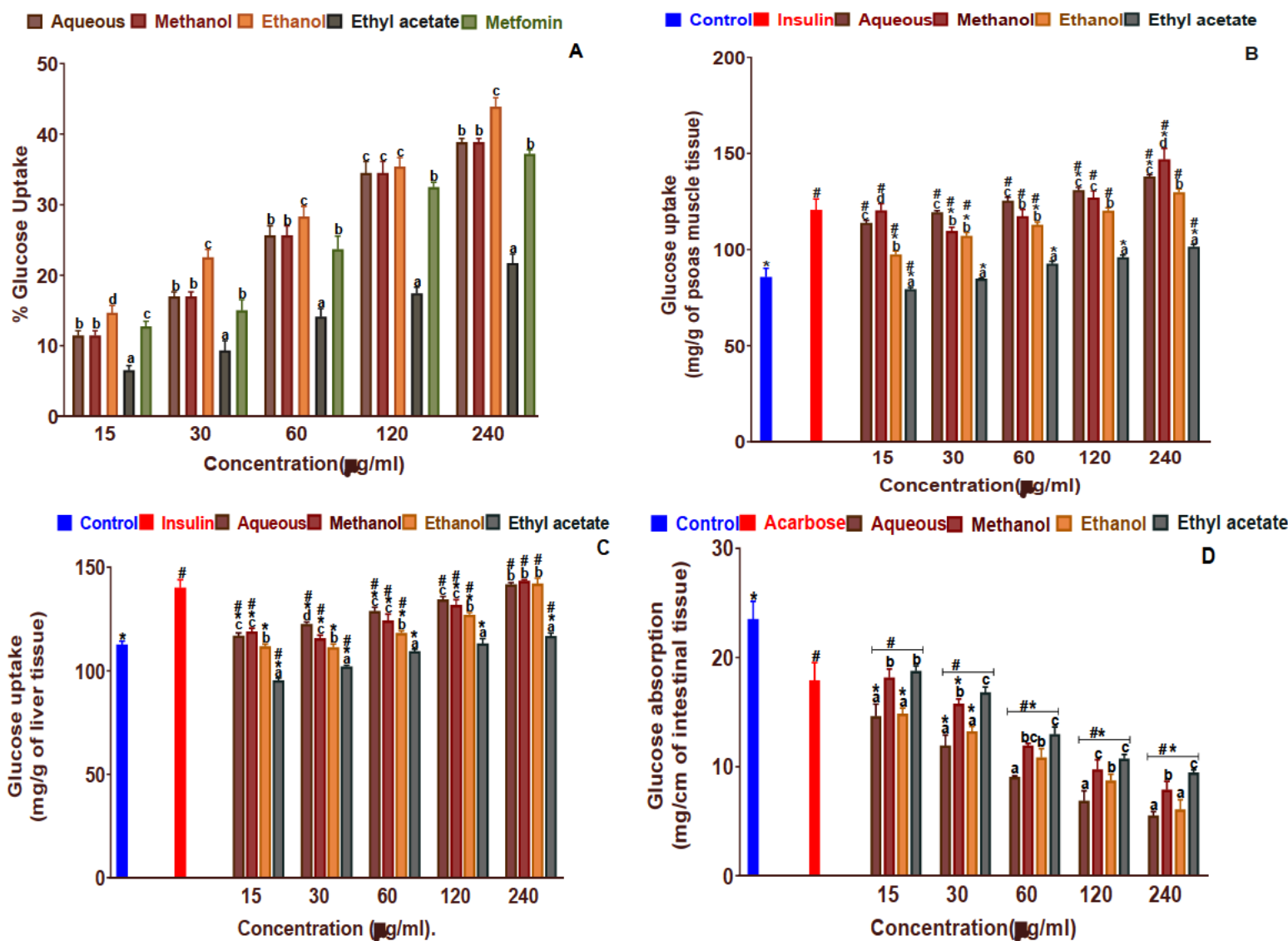


Figure 3.2.4: Modulatory effects of leaf crude extracts of *T. orientalis* on (A) yeast cell glucose uptake, (B) psoas muscle glucose uptake, (C) liver glucose uptake, and (D) intestinal glucose absorption. Data are presented as mean $\pm$ standard deviation. Different superscripts a to d represent statistical significance of $p < 0.05$ among the extracts. *Significantly different from positive control and #Significantly different from normal control ($p < 0.05$).

The effects of *T. orientalis* extracts on oxidative stress biomarkers were analysed *ex vivo* in freshly harvested pancreatic homogenates and depicted in Figs. 3.2.5 A, B, C, and D. Evidently, treatment with the pro-oxidant FeSO_4 induced oxidative pancreatic tissue injury and augmented lipid peroxidation while diminishing the activities of superoxide dismutase (SOD), catalase (CAT), and reduced levels of glutathione (GSH). There was a significant ($p < 0.05$) difference between the untreated tissue and the normal tissue in all the parameters analysed (Fig. 3.2.5 A, B, C, and D). Treatment with leaf extracts of *T. orientalis* reduced MDA (malondialdehyde) products, as shown in Fig. 3.2.5A, indicating a reduction in lipid peroxidation. The ethanolic extract significantly ($p < 0.05$)

reduced lipid peroxidation to a near-normal level, especially at the highest concentration of 240 $\mu\text{g/ml}$. Furthermore, treatment with the *T. orientalis* leaf ethanol extract significantly ($p < 0.05$) increased SOD activity (Fig. 3.2.5B) and CAT activity (Fig. 3.2.5C) better than vitamin C (positive control), especially at higher concentrations of 120–240 $\mu\text{g/ml}$. Finally, as observed in Fig. 3.2.5D, treatment with *T. orientalis* methanol leaf extract significantly ($p < 0.05$) increased GSH levels above all other extracts, including vitamin C, resulting in near-normal levels, especially at the highest concentrations.

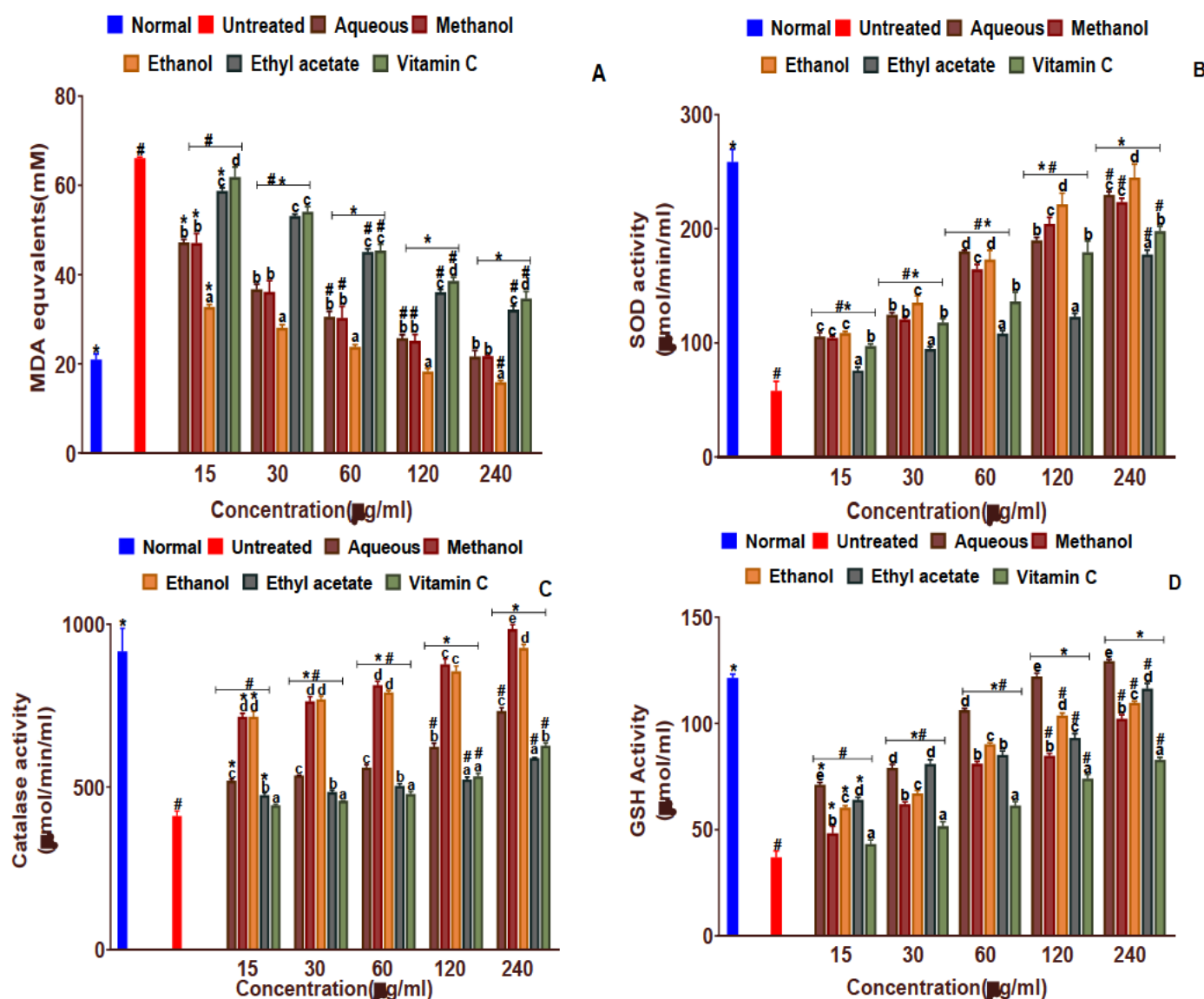


Figure 3.2.5: Effects of *T. orientalis* bark organic extracts of extracts on (A) lipid peroxidation, (B) SOD activity, (C) Catalase activity and (D) GSH activity in oxidative pancreatic injury. Data are presented as mean $\pm$ standard deviation. Different superscripts a, b and c represent statistical significance of $p < 0.05$. *Significantly different from untreated sample and #Significantly different from normal sample ($p < 0.05$, Tukey's- HSD-multiple range post-hoc test, IBM SPSS for Windows).

The untreated tissue exhibited significantly ($p<0.05$) increased ATPase, glycogen phosphorylase, and fructose 1,6-bisphosphatase activity compared to the normal tissue, which was indicative of hyperglycaemic toxicity (Fig. 3.2.6 A, B, and C). Treatment with the leaf extracts of *T. orientalis* reduced ATPase activity in a dose-dependent manner (Fig. 3.2.6 A). Among all the extracts, the aqueous and ethanol extracts had the best ATPase-reducing activity. Their activities were comparable to those resulting from quercetin treatment (positive control). The leaf extracts of *T. orientalis* further reduced the activities of both glycogen phosphorylase activity (Fig. 3.2.6B) and 1,6-bisphosphatase activity (Fig. 3.2.6C) to near-normal levels, especially at the highest concentrations (120–240 $\mu\text{g/ml}$). The ethyl acetate extract exhibited the least activity among all the extracts.

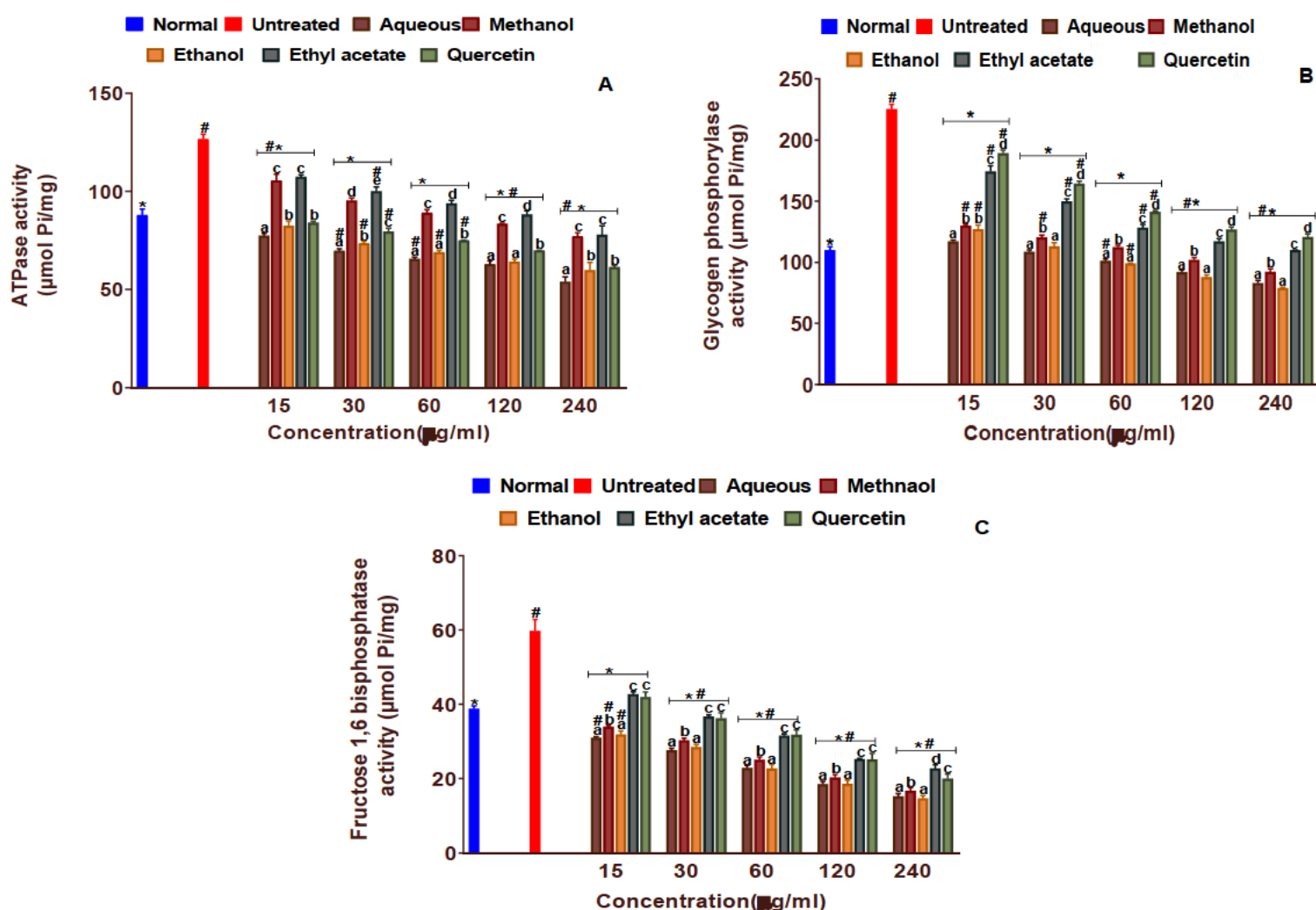


Figure 3.2.6: The modulatory effects of organic bark crude extracts of *T. orientalis* on (A) ATPase activity, (B) Glycogen phosphorylase activity and (C) Fructose 1,6 bisphosphatase activity. Data are presented as mean $\pm$ standard deviation. Different superscripts a, b and c represent statistical significance of $p<0.05$. *Significantly different from untreated sample and #Significantly different from normal sample ($p<0.05$), Normal=normal tissues, Untreated=tissues oxidatively injured but not treated.

Table 3.2.1: The IC₅₀ values of the different leaf extracts of *T. orientalis* under assayed antioxidant and enzyme inhibition parameters.

	Aqueous Extract	Methanol Extract	Ethanol Extract	Ethyl acetate Extract	Vitamin C	Quercetin	Acarbose	Orlistat
DPPH (µg/ml)	14,38±0,61	10,27±0,34	10,10±0,37	9,83±0,74	9,21±0,12	-	-	-
FRAP (µg/ml)	56,97±0,15	50,67±0,97	15,54±0,55	183,87±0,13	6,67±0,88	-	-	-
NO (µg/ml)	9,73±0,66	18,39±0,81	45,62±0,15	106,22±0,23	-	2,49±0,81	-	-
Pancreatic lipase(µg/ml)	177,23±0,72	148,93±0,68	261,45±0,86	156,17±0,92	-	-	-	114,52±0.83
α-glucosidase(µg/ml)	133,95±0,93	87,02±0,65	89,34±0,78	242,47±0,87	-	-	100,68±0,09	-
α-amylase (µg/ml)	312,03±0,41	322,31±0,36	214,56±0,21	365,57±91	-	-	118,57±0,97	-

Data is presented as mean ± standard deviation. Key: DPPH = 2,2-diphenyl-1-picrylhydrazyl. FRAP = Ferric reducing antioxidant power. NO = Nitric oxide assay.

3.2.5 Discussion

Diabetes mellitus (DM) is on the rise globally, prompting researchers all over the world to investigate alternative treatment options. Traditional medicinal plants, such as *T. orientalis*, that are used in folkloric medicine to treat a variety of ailments have thus emerged as a lucrative source of novel antidiabetic pharmacological agent templates (He *et al.*,2020). Recent advances in pharmacognosy document plant-derived secondary metabolites, including phenols, alkaloids, flavonoids, and tannins, as having several therapeutic benefits against several human ailments (Jain *et al.*,2019). As a result, the high concentration of phenolics and flavonoids (Fig. 3.21A and 3.2.1B) in *T. orientalis* extracts further corroborates the folkloric use of this plant in the treatment of various diseases such as diabetes, cancer, and bacterial infections (Jain *et al.*,2019).

Chronic hyperglycaemia-induced oxidative stress has been linked to the worsening of morbidity-causing complications in diabetic patients (Volpe *et al.*,2018). Chronic hyperglycaemia is associated with both an increase in free radical production and a breakdown of antioxidant defense systems (Volpe *et al.*,2018). Polyphenolics found in medicinal plants have been widely reported in previous studies to play an important role in free radical scavenging and thus attenuating the oxidative stress cascade (Xiao and Hogger, 2015). Polyphenols are antioxidants that can scavenge free radicals due to the ability of their aromatic ring structure to accept protons and stabilize the unpaired electrons (Stagos, 2019). Among other secondary metabolites, the polyphenolic compounds in the *T. orientalis* extracts may have been responsible for their enhanced capacity to scavenge free radicals (DPPH and NO) and donate free electrons (FRAP) (Fig. 3.2.2A-C). Previous reports corroborate the therapeutic effects of such activities in diabetic patients as they aid in mopping up excess free radicals under conditions of sustained hyperglycaemia-induced oxidative stress (Anhê *et al.*,2013; Sun *et al.*,2020).

Lipids are among the most abundant macromolecules, forming much of the structural barrier (cell membrane) in tissues, and are thus particularly vulnerable to free radical attack because they are in tissues (Recknagel *et al.*,2020). The irreversible chemical reaction between free radicals and biomolecules such as lipids, proteins, and DNA causes permanent changes that render them non-functional (Recknagel *et al.*,2020). This chemical reaction has been linked to the pathophysiology of degenerative diseases such as diabetes (Newsholme *et al.*,2016). The ability of *T. orientalis* extracts, particularly the ethanolic extract, to reduce lipid peroxidation (Fig. 3.2.5 A) supports their protective role against oxidative tissue injury.

Following iron induction, the diminished endogenous antioxidant defense systems, including levels of reduced glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) activities, are indicative of hyperglycaemia-induced oxidative stress in diabetic conditions (Yaribeygi *et al.*,2020). The ability of the *T. orientalis* extracts to augment the capacity of endogenous antioxidant defense moieties observed in the current study is indicative of their therapeutic effects against oxidative stress. These observations are coherent with previous studies that show plant extracts to show their antioxidant activities through several mechanisms (Asif, 2015; Santos-Sánchez *et al.*,2019). Taken together, the ability of the extracts to scavenge free radicals, donate electrons, and enhance the activity of endogenous antioxidant systems insinuates the beneficial effects of these extracts to help maintain homeostatic redox balance in diabetic patients.

Free fatty acids are high-energy molecules that are metabolized when more preferred carbohydrate sources are depleted, typically during prolonged fasting. Increased ingestion of dietary fatty acids has been linked to dyslipidemia in diabetic patients (Lottenberg *et al.*,2012). Furthermore, increased β oxidation, the process by which lipids are catabolised, has been linked to exacerbated free radical production and hyperinflammation through the overproduction of proinflammatory factors such as cytokines (Eid *et al.*,2019; Poznyak *et al.*,2020). As most diabetic patients are obese, the ability of the *T. orientalis* extracts to inhibit the lipid-digesting action of pancreatic lipase (Fig. 3.2.3A) may aid in weight management. Furthermore, this may reduce low-grade inflammation associated with increased β oxidation and is implicated as a major cause of exacerbated insulin resistance in obese diabetic patients (Poznyak *et al.*,2020). Sustained postprandial blood glucose spikes observed in diabetic patients following carbohydrate-rich meals are indicative of dysregulated glucose metabolic machinery's inability to regulate blood glucose levels (Ojo, 2016). Several site-specific glycosidic bond hydrolysing enzymes break down these complex macromolecular dietary carbohydrates into simpler forms as they pass through the digestive tract. Previous studies have shown the antidiabetic potential of pharmacological agents that can inhibit α -amylase and α -glucosidase, which are key carbohydrate-digesting enzymes (Picot *et al.*,2014; Proença *et al.*,2022). Therefore, the capabilities of the *T. orientalis* extracts to inhibit α -glucosidase (Fig. 3.2.3B) and α -amylase (Fig. 3.2.3C) may help retard the rate of complex carbohydrate digestion and absorption in the small intestine. Reduced rates of glucose influx help the glucose-metabolizing machinery clear the circulatory blood glucose more effectively, thereby abating glucose spikes and hyperglycaemia (Proença *et al.*,2022). *T. orientalis* extracts were shown in the current study to reduce intestinal tissue glucose absorption (Fig. 3.2.4D), implying their ability to further reduce the mismatch between glucose assimilation and utilization in diabetics with dysregulated glucose metabolic pathways. Previous research has shown that inhibiting sodium-dependent glucose cotransporter 1 (SGLT1), which actively transports glucose from the

lumen into the blood, can reduce glucose absorption by the small intestine (Tahrani *et al.*,2013).

Assimilated glucose is transported by the blood to various peripheral tissues, where it is converted to ATP, the metabolic energy source, or stored as fat in adipose tissue or glycogen in the liver and muscle tissue (Alam *et al.*,2022). The utilization of glucose by peripheral tissues is an important mechanism for maintaining blood glucose homeostasis (Alam *et al.*,2022). Several studies have shown the importance of the hypoglycaemic effect of increased peripheral tissue glucose utilisation (Kalsbeek *et al.*,2014). As a result, the enhanced ability of *T. orientalis* extracts to increase glucose uptake in muscle, liver, and yeast cells (Fig. 3.2.4A-C) suggests that they have the potential to reduce chronic hyperglycaemia in diabetic patients (Kalsbeek *et al.*,2014; Alam *et al.*,2022). Increased stimulation of the translocation of glucose transporter 4 (GLUT4) from its vacuolic stores to the cell surface membrane by the *T. orientalis* extracts may account for the observed increased glucose uptake in muscle tissue (Fig. 3.2.4B) in the current study. The fusion of GLUT-4 molecules with the membrane of insulin-responsive tissue is associated with an increase in glucose influx into the tissue of up to 10 to 30-fold (Stöckli *et al.*,2011). In the liver, however, the extracts of *T. orientalis* may work by enhancing glucose flux through the upregulation of the GLUT2 isoform of glucose transporters, which is the main regulatory factor in the hepatocytes (Stöckli *et al.*,2011). Increased ATPase activity under hyperglycaemic conditions, as observed in the current study (Fig. 3.2.6 A), results in dysregulated energy redox due to an increase in the ADP+ Pi:ATP ratio, signifying excessive ATP depletion (Erukainure *et al.*,2021). The ability of *T. orientalis* extracts to reduce ATPase activity suggests that they have the potential to restore energy balance in diabetics, who frequently complain of tiredness because of this imbalance. Similarly, the extracts of *T. orientalis* were shown to reduce the activity of liver glucogenic enzymes, namely glycogen phosphorylase and fructose 1,6-bisphosphate (Fig. 3.2.6 B and C). These enzymes catalyse key steps in liver glycogenolysis and gluconeogenesis (Timson, 2019; Stravodimos *et al.*,2017). Increased activity of these glucogenic enzymes results in increased hepatic glucose output. Increased hepatic output is among the major contributing factors to the chronic hyperglycaemia observed in diabetic patients (van Poelje *et al.*,2007). The ability of the extracts to inhibit the action of glycogen phosphorylase and fructose 1,6-bisphosphate observed in the current study therefore insinuates their capacity to avert hyperglycaemia from endogenous sources. The ability of the extracts to increase glucose uptake and inhibit glucogenic enzymes by the extracts of *T. orientalis* is indicative of their hypoglycaemic potential.

3.2.6 Conclusion

Data from the current study suggests the enhanced antioxidant and antidiabetic potential of the phytoconstituents in the leaf crude extracts. *T. orientalis* crude leaf extracts were shown to promote glucose uptake, inhibit carbohydrate and lipid digesting enzymes, and modulate enzymes involved in carbohydrate metabolism in muscle and liver tissues. The aqueous and ethanolic extracts, which showed comparatively the best activities from most of the assayed parameters, may further be studied using *in vivo* models to ascertain their precise modes of action.

3.3 *Trema orientalis* crude bark extracts inhibit carbohydrate and lipid digesting enzymes, attenuate muscular glucose uptake and ameliorate oxidative cardiac tissue injury

¹Brian K. Beseni, ¹Kolawole A. Olofinisan ^{1,2}Veronica F. Salau, ¹Md. Shahidul Islam*

1. Department of Biochemistry, University of KwaZulu-Natal, Westville Campus, Durban 4000, South Africa

2. Department of Pharmacology, University of the Free State, Bloemfontein 9300, South Africa

***Corresponding author:** islamd@ukzn.ac.za

3.3.1 Abstract

Traditional medicinal plants such as *T. orientalis* have been shown to possess a myriad of secondary metabolites that have potential therapeutic effects against human diseases. The current study investigated the effects of stem bark extracts of *T. orientalis* against diabetes and oxidative stress-linked parameters using *in vitro* and *ex vivo* experimental models. GCMS analysis was used to identify bioactive compounds in the plant extracts of varying polarity, after which their total polyphenol and flavonoid contents were quantified. Thereafter, their antioxidant, carbohydrate, and lipid hydrolysing enzyme inhibitory activities were investigated *in vitro*. The effects of *T. orientalis* extracts on glucogenic enzymes, muscle and liver glucose uptake, intestinal glucose absorption, and modulatory effects on oxidative pancreatic injury were determined *ex vivo*. Pentadecanoic acid (19.67%), phytol (16.56%), and l-(+)-ascorbic acid 2,6- dihexadecanoate (27.59%) were the most abundant compounds in the aqueous, methanol, ethanol, and ethyl acetate extracts, respectively. The ethanolic extract had significantly ($p < 0.05$) the highest amount of total phenolics (15.73 mg/g), while the aqueous (4.39 mg/g) and ethanol (4.44 mg/g) extracts had comparably the highest flavonoid content. The ethanol extract exhibited the best DPPH and NO radical scavenging, while it was the second-best in the FRAP assay. The ethanolic and aqueous extracts exhibited comparably the best carbohydrate hydrolysing enzyme inhibitory capacities. However, the ethanolic extract exhibited significantly ($p < 0.05$) lower intestinal glucose absorption and high glucose uptake activities compared to other extracts. Furthermore, it significantly ($p < 0.05$) reduced lipid peroxidation levels, increased reduced glutathione levels, and superoxide dismutase and catalase activities in oxidative cardiac tissue injury. The ethanolic extract additionally exhibited

the best glucogenic and nucleotide hydrolysing enzyme inhibitory activity among all the extracts. The data indicate that the ethanolic extract demonstrated promising antioxidant and antidiabetic activities among all extracts, which warrants further research in experimental animal models to confirm the findings of this study.

Keywords: Antidiabetic, glucogenic enzymes, glucose uptake, *Trema orientalis*, antioxidant.

3.3.2 Introduction

Diabetes mellitus (DM) is a multifactorial metabolic disorder that culminates in perpetual hyperglycaemia (Petersmann *et al.*,2018; Zheng *et al.*,2018). Signs and symptoms of diabetes vary between individuals in both occurrence and intensity (Zheng *et al.*,2018). Short-term signs include polyuria, polydipsia, uncontrollable weight loss, polyphagia, and blurred vision. Type I, type II, and gestational diabetes are the most common etiopathogenetic types of diabetes, with type II accounting for 90% of known cases (Petersmann *et al.*,2018). The diabetes scourge has reached alarming prevalence rates, with an estimated 451 million people between the ages of 18 and 99 reportedly living with this condition in 2017 (Cho *et al.*,2018). Furthermore, the number is projected to rise to 693 million by the year 2045 (Cho *et al.*,2018). This inevitably translates to a heavy global expenditure on healthcare costs due to diabetes and its related complications, which was estimated to be USD 850 billion in 2017 (Cho *et al.*,2018). The pathogenesis and progression of DM are related to the relative lack of insulin, its aberrant action, or both (Cersosimo *et al.*,2018). Deleterious long-term complications of uncontrolled DM range from blindness, foot ulcers, lower limb amputations, cardiovascular complications, sexual dysfunction, and kidney failure (ADA, 2013). These combined effects drastically reduce the quality of life in diabetic patients and often lead to morbidity and premature mortality. The prevalence of diabetes and its associated complications is increasing worldwide. The severity of this condition is further aggravated by its incurable nature and its co-occurrence with other degenerative, non-communicable conditions, including cardiovascular disease (CVD).

Non-pharmacological and pharmacological diabetes management strategies are available, with the common goal of maintaining homeostatic blood glucose levels (Shrivastava *et al.*,2013). Despite their widespread adoption and success, these strategies have numerous drawbacks (Wu *et al.*,2014). Nonpharmacological strategies, such as increasing physical activity participation, are met with strong opposition, with people reverting to their old habits (Raveendran *et al.*,2018). However, pharmacological strategies are very expensive and have several undesirable side effects, such as gastro-intestinal irritations, weight gain, and vomiting drawbacks (Wu *et al.*,2014). These compounded negative effects have prompted scientists across the globe to sort out novel templates

that may be developed into cost-effective antidiabetic therapeutics with milder side effects (Rizvi and Mishra, 2013). Medicinal plants such as *Trema orientalis* have thus recently emerged as an infinite source of promising antidiabetic agents as they possess multitudes of compounds with known therapeutic bioactivities. Among the groups of compounds that are found to have potential therapeutic potential are alkaloids, terpenoids, phenolics, flavonoids, coumarins, and saponins. *Trema orientalis* is a member of the ulmaceae family and is also known as pigeon wood (English) and umvangazi (Zulu) (Smith, 1966). The pharmacological importance of *T. orientalis* against diabetes and its complications was established in a previous study that demonstrated its hypoglycemic effects in a diabetic rat model (Dimo *et al.*, 2006). The current study sought to identify the phytoconstituencies of cured *T. orientalis* bark and quantify its phenolic and flavonoid content. The antioxidant and antidiabetic potentials of *T. orientalis* crude bark extracts were investigated. *In vitro* studies were conducted to determine the antioxidant and enzyme-inhibitory activities of the crude bark extracts. Furthermore, *ex vivo* experimental models were used to investigate the extracts' attenuating effects on oxidative cardiac tissue injury, small intestinal glucose absorption, and muscle/liver glucose uptake. The modulatory effects of plant extracts on glucogenic and nucleotide-metabolizing enzymes were also investigated *ex vivo*.

3.3.3 Materials and methods

Please refer to Chapter 2, sections 2.4.1 to 2.11.5 for detailed materials and methods.

3.3.4 Results

GC-MS analysis revealed the presence of a myriad of compounds in the different crude extracts of *T. orientalis* bark, as represented in Table 3.3.1.. The most abundant compounds were pentadecanoic acid (19.67%) in the aqueous extract, phytol (16.56%) in the methanol extract, and l-(+)-ascorbic acid 2,6-dihexadecanoate (27.59%) in the ethanol and ethyl acetate extracts. Phytol and phytol acetate were the most commonly identified compounds among all the *T. orientalis* extracts.

Table 3.3.1: Compounds identified in the *T. orientalis* crude bark extracts as revealed by GCMS analysis.

Compounds	Retention time(min)	Molecular Formula	Molecular weight (g/mol)	Relative abundance (%)
Aqueous				
2-Methyl-4-tert-octylphenol	14.825	C ₁₅ H ₂₄ O	220.35	0.82
Hexestrol	14.936	C ₁₈ H ₂₂ O ₂	270.4	1.58
4- (2,2,6-trimethylbicyclo [4.1.0] hept-1-yl) -1-buten-2-yl acetate	15.362	C ₁₆ H ₂₆ O ₂	250.38	1.18
Phytol, acetate	15.692	C ₂₂ H ₄₂ O ₂	338.6	20.10
Paltinol C	16.992	C ₁₆ H ₂₂ O ₄	278.34	3.22
Pentadecanoic acid	17.101	C ₁₅ H ₃₀ O ₂	242.4	6.99
Phytol	18.444	C ₂₀ H ₄₀ O	296.5	6.13
Hexadecatrienal	18.865	C ₁₆ H ₂₆ O	234.38	16.94
2-dimethylamino 3-cyclopentylpropionic acid	21.561	C ₁₂ H ₂₃ NO ₂	213.32	1.47
Methanol				
Methyl hexofuranoside	14.698	C ₇ H ₁₄ O ₆	194.18	8.29
Tetradecanoic acid	14.980	C ₁₄ H ₂₈ O ₂	228.37	0.20
n-Heptadecanol-1	15.216	C ₁₇ H ₃₆ O	256.5	0.09
Phytol, acetate	15.685	C ₂₂ H ₄₂ O ₂	338.6	3.65
Hexadecanoic acid, methyl ester	16.604	C ₁₇ H ₃₄ O ₂	270.5	0.28
Pentadecanoic acid	17.192	C ₁₅ H ₃₀ O ₂	242.4	19.67
Methyl 10-trans,12-cis-octadecadienoate	18.270	C ₁₉ H ₃₄ O ₂	294.5	0.25
Methyl elaidolinolenate	18.330	C ₂₄ H ₄₀ O ₄	392.6	0.65

Phytol	18.453	C ₂₀ H ₄₀ O	296.5	4.35
Octadecanoic acid	19.085	C ₁₈ H ₃₆ O ₂	284.5	4.86
Ethanol				
D-Mannitol	6.638	C ₆ H ₁₄ O ₆	182.17	1.04
3-O-Methyl-d-glucose	14.410	C ₇ H ₁₄ O ₆	194.18	13.73
Phytol, acetate	15.687	C ₂₂ H ₄₂ O ₂	338.6	12.16
Pentadecanoic acid	17.105	C ₁₅ H ₃₀ O ₂	242.4	14.19
Hexadecanoic acid, ethyl ester	17.281	C ₁₈ H ₃₆ O ₂	284.5	1.59
Phytol	18.450	C ₂₀ H ₄₀ O	296.5	16.56
Linoleic acid	18.770	C ₁₈ H ₃₂ O ₂	280.4	5.73
Ethyl linolenate	18.945	C ₂₀ H ₃₄ O ₂	306.5	1.81
Stearic acid	18.995	C ₁₈ H ₃₆ O ₂	284.5	2.69
13-Tetradecen-1-ol acetate	19.174	C ₁₆ H ₃₀ O ₂	254.41	1.02
Ethyl acetate				
D-Mannitol	6.893	C ₆ H ₁₄ O ₆	182.17	0.68
2,3-dihydro-2-methyl-5-phenyl- benzofuran	8.435	C ₁₅ H ₁₄ O	210.27	0.28
Methylsuccinic acid	9.990	C ₅ H ₈ O ₄	132.11	1.45
Dihydroactinidiolide	12.505	C ₁₁ H ₁₆ O ₂	180.24	0.11
Lauric Acid	12.840	C ₁₂ H ₂₄ O ₂	200.32	0.30
2-Methyl-4-Tert-Octylphenol	14.821	C ₁₅ H ₂₄ O	220.35	0.09
4-Tert-Amylphenol	14.933	C ₁₁ H ₁₆ O	164.24	0.12
Tetradecanoic acid	15.061	C ₁₄ H ₂₈ O ₂	228.37	0.54
Ascabiol	15.170	C ₁₄ H ₁₂ O ₂	212.24	0.10
Phytol, acetate	15.716	C ₂₂ H ₄₂ O ₂	338.6	3.66
l-(+)-Ascorbic acid 2,6- dihexadecanoate	17.541	C ₃₈ H ₆₈ O ₈	652.9	27.59
cis-9-Hexadecenal	18.006	C ₁₆ H ₃₀ O	238.41	0.33
Ethyl iso-allocholate	18.208	C ₂₆ H ₄₄ O ₅	436.6	0.71
Phytol	18.540	C ₂₀ H ₄₀ O	296.5	5.50
Arachidonic acid	20.388	C ₂₀ H ₃₂ O ₂	304.5	0.69
Hexadecadien-1-ol	20.727	C ₁₆ H ₃₀ O	238.41	0.66
Squalene	24.904	C ₃₀ H ₅₀	410.7	0.68

The total phenolic and flavonoid contents of *T. orientalis* bark organic extracts are depicted in Figs. 3.3.1A and B. The ethanolic extract had significantly ($p<0.05$) the highest amount of total phenolics (15.73 mg/g). This was followed by the methanol (13.80 mg/g) and aqueous (11.30 mg/g) extracts. Among all extracts, ethyl acetate (9.17 mg/g) had the lowest phenolic content. The aqueous (4.39 mg/g) and ethanol (4.44 mg/g) extracts had the highest flavonoid content, followed by the methanol extract. Similarly, for the analysis of total phenolic content, ethyl acetate extract had significantly ($p<0.05$) the lowest flavonoid content.

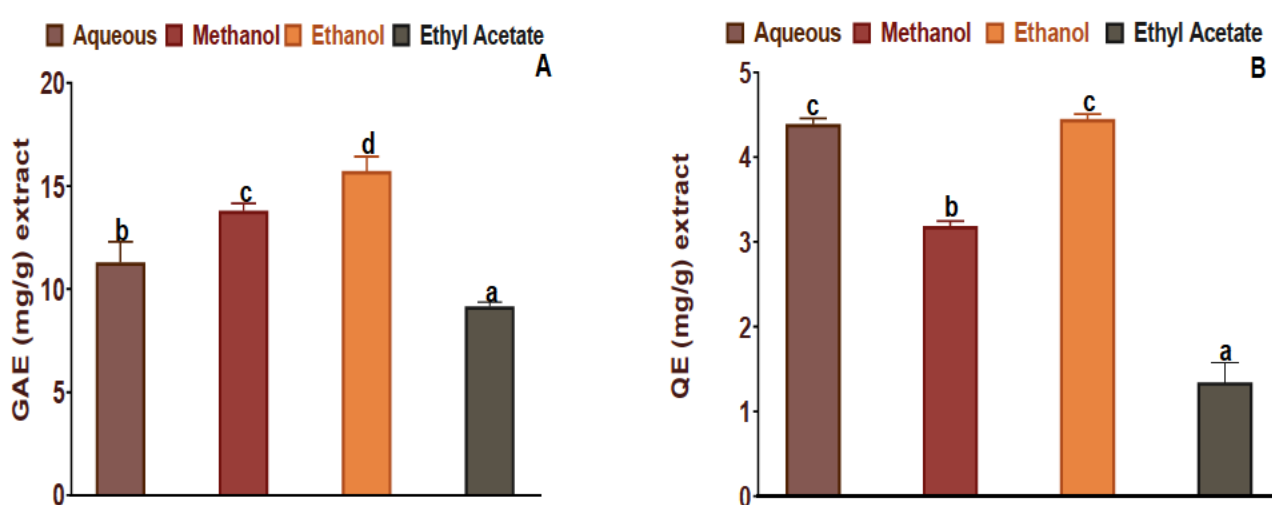


Figure 3.3.1: The total phenolic content (A) and the total flavonoid content (B) of *T. orientalis* crude bark extracts represented as Gallic acid equivalents (GAE mg/g) and Quercetin equivalents (QE mg/g) respectively. The superscripts a to d represent statistical significance of $p<0.05$.

The antioxidant activities of the *T. orientalis* bark extracts were evaluated using DPPH, FRAP, and NO inhibition assays *in vitro* and are depicted in Figs. 3.3.2A–C. As shown in Fig. 3.3.3A, the ethanol extract had the highest DPPH radical scavenging activity with a corresponding IC_{50} value of $164,00 \pm 0,1 \mu\text{g/ml}$, as recorded in Table 3.3.2. Furthermore, as seen in Table 3.3.3, the ethyl acetate extract had the lowest DPPH radical scavenging capacity, as signified by a high IC_{50} value of $453,08 \pm 0,84 \mu\text{g/ml}$. The ability of the extracts to reduce ferric ion analysed by the FRAP assay was polarity dependent, as shown in Figs. 3.3.2B, with the aqueous extract having the highest activity, followed by the methanol, ethanol, and ethyl acetate extracts, respectively. Finally, the *T. orientalis* extracts exhibited comparable NO scavenging capacity at most of the assayed concentrations,

resulting in IC₅₀ values of 9,64±0,76 µg/ml, 10,31±0,77 µg/ml, and 9,37±0,86 µg/ml for aqueous methanol and ethanol extracts, respectively, as shown in Table 3.3.2. However, the ethyl acetate extract exhibited the least NO radical scavenging capability among all the extracts.

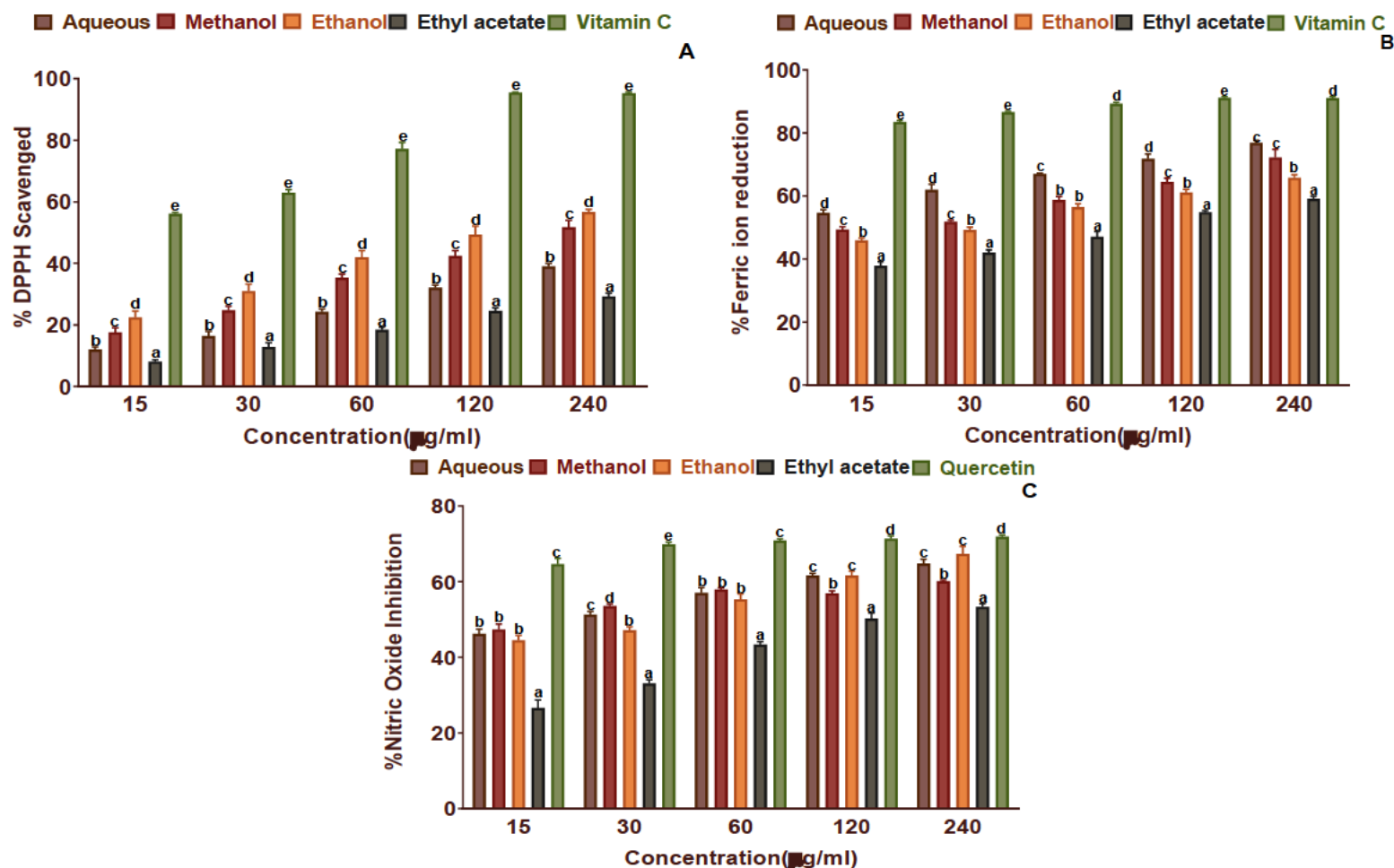


Figure 3.3.2: Effects of *T. orientalis* bark organic extracts of extracts on (A) DPPH radical scavenging activity, (B) Ferric ion reducing activity, (C) Nitric oxide production inhibition. Data are presented as mean ± standard deviation. Different superscripts a-e represent statistical significance of $p < 0.05$.

The inhibitory capacities of the bark crude extracts of *T. orientalis* on key lipid and carbohydrate-digesting enzymes are shown in Fig. 3.3.3A-C. The extracts exhibited a dose- dependent inhibitory capacity against all the enzymes. As shown in Fig. 3.3.3A, the aqueous extract exhibited the best porcine pancreatic lipase inhibitory capabilities, especially at the highest concentration of 240 µg/ml, where it outperformed orlistat (the positive control). However, as shown in Table 3.3.2, orlistat (the positive control) showed comparatively superior pancreatic lipase inhibition activity with an IC₅₀ value of 114,52±0.83 µg/ml. As shown in Fig 3.3.3B, the ethanolic extract exhibited better α

glucosidase inhibitory activity among all extracts at all the assayed concentrations, resulting in an IC_{50} value of $34,42 \pm 0,73 \mu\text{g/ml}$, which was significantly better than acarbose with an IC_{50} value of $100,68 \pm 0,09 \mu\text{g/ml}$ (Table 3.3.2). In contrast, the aqueous extract had the best α amylase inhibitory capabilities, as shown in Fig. 3.3.3C, with a low IC_{50} value of $93,05 \pm 0,43 \mu\text{g/ml}$ depicted in Table 3.3.2. The α amylase inhibitory capabilities of the aqueous extract were closely followed by the ethanolic extract, which had an IC_{50} value of $109,10 \pm 0,66 \mu\text{g/ml}$, as shown in Table 3.3.2. Interestingly, both of these extracts exhibited better inhibitory activity against α amylase in comparison to acarbose, which had an IC_{50} value of $118,57 \pm 0,97$.

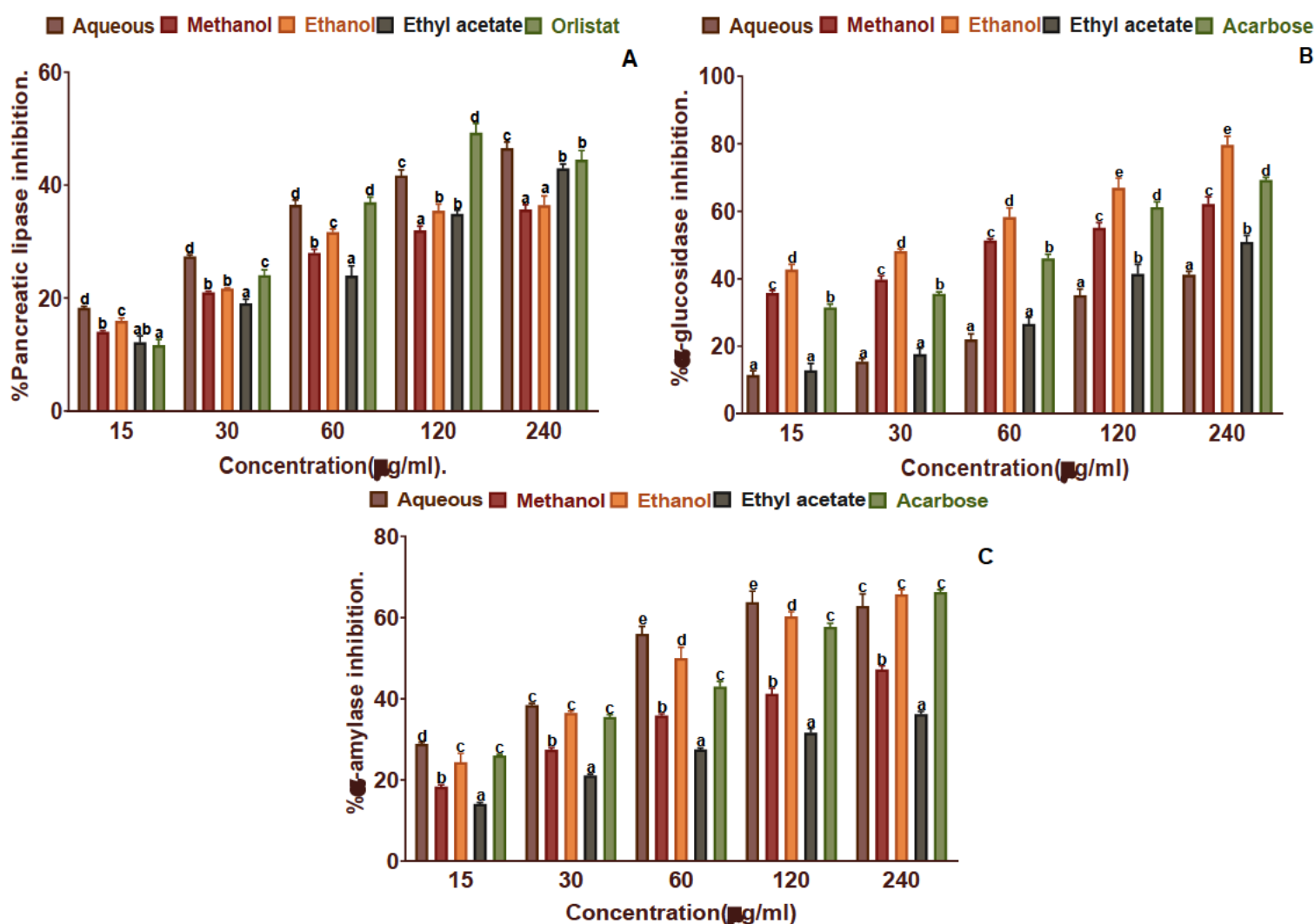


Figure 3.3.3: Effects of *T. orientalis* bark crude extracts on (A) Pancreatic lipase inhibition activity, (B) α glucosidase inhibition activity, and (C) α amylase inhibition activity. Data is presented as mean $\pm$ standard deviation. Different superscripts a-e represent statistical significance of $p < 0.05$.

Table 3.3.2: The IC₅₀ values of different bark extracts of *T. orientalis* under different *in vitro* antioxidant and enzyme inhibitory parameters.

	Aqueous extract	Methanol extract	Ethanol extract	Ethyl acetate extract	Vitamin C	Quercetin	Acarbose	Orlistat
DPPH (µg/ml)	313,21±0,99	205,64±0,43	164,00±0,15	453,08±0,84	9,21±0,12	-	-	-
FRAP (µg/ml)	5,87±0,62	19,26±0,18	24,14±0,25	113,30±0,37	6,67±0,88	-	-	-
NO (µg/ml)	9,64±0,76	10,31±0,77	9,37±0,86	173,84±0,97	-	2,49±0,81	-	-
Pancreatic lipase(µg/ml)	240,65±0,83	381,23±0,19	367,33±0,92	274,54±0,19	-	-	-	114,52±0,83
α-glucosidase(µg/ml)	282,65±0,96	104,10±0,85	34,42±0,738	212,94±0,15	-	-	100,68±0,09	-
α-amylase (µg/ml)	93,05±0,43	236,97±0,93	109,10±0,66	373,70±0,15	-	-	118,57±0,97	-

Data is presented as mean ± standard deviation. Key: DPPH = 2,2-diphenyl-1-picrylhydrazyl. FRAP = Ferric reducing antioxidant power. NO = Nitric oxide inhibition assay.

The effects of *T. orientalis* bark extracts on glucose uptake activity in yeast cells, muscle, and liver tissue are shown (Fig. 3.3.4A-C). Furthermore, the retarding effect of the extracts on glucose absorption by intestinal jejunum tissue are depicted in Fig. 3.3.4D. As shown in Fig. 3.3.4A, the ethanolic and aqueous extracts stimulated the highest glucose uptake activity among all the extracts, including metformin (the positive control), in yeast cells, especially at the highest concentration of 240 µg/ml. Furthermore, the aqueous and ethanol *T. orientalis* bark extracts resulted in the highest glucose uptake activities in both muscle and liver tissue, as observed in Figs. 3.3.4B and 3.3.4C. Interestingly, the extracts stimulated glucose uptake capacities that were comparable to those demonstrated by insulin (the positive control), particularly at the highest concentration of 240 µg/ml. The ethyl acetate extract resulted in the least amount of glucose uptake in muscle tissue, while the same was true for the aqueous extract in liver tissue. Finally, as depicted in Fig. 3.3.4D, the extracts retarded glucose absorption in a dose-dependent manner, with the ethanolic extract resulting in significantly ($p<0.05$) the least absorption rates at all the concentrations assayed. Furthermore, the extracts outperformed acarbose (the positive control) and resulted in significantly ($p<0.05$) lower glucose absorbance at concentrations greater than 60 µg/ml.

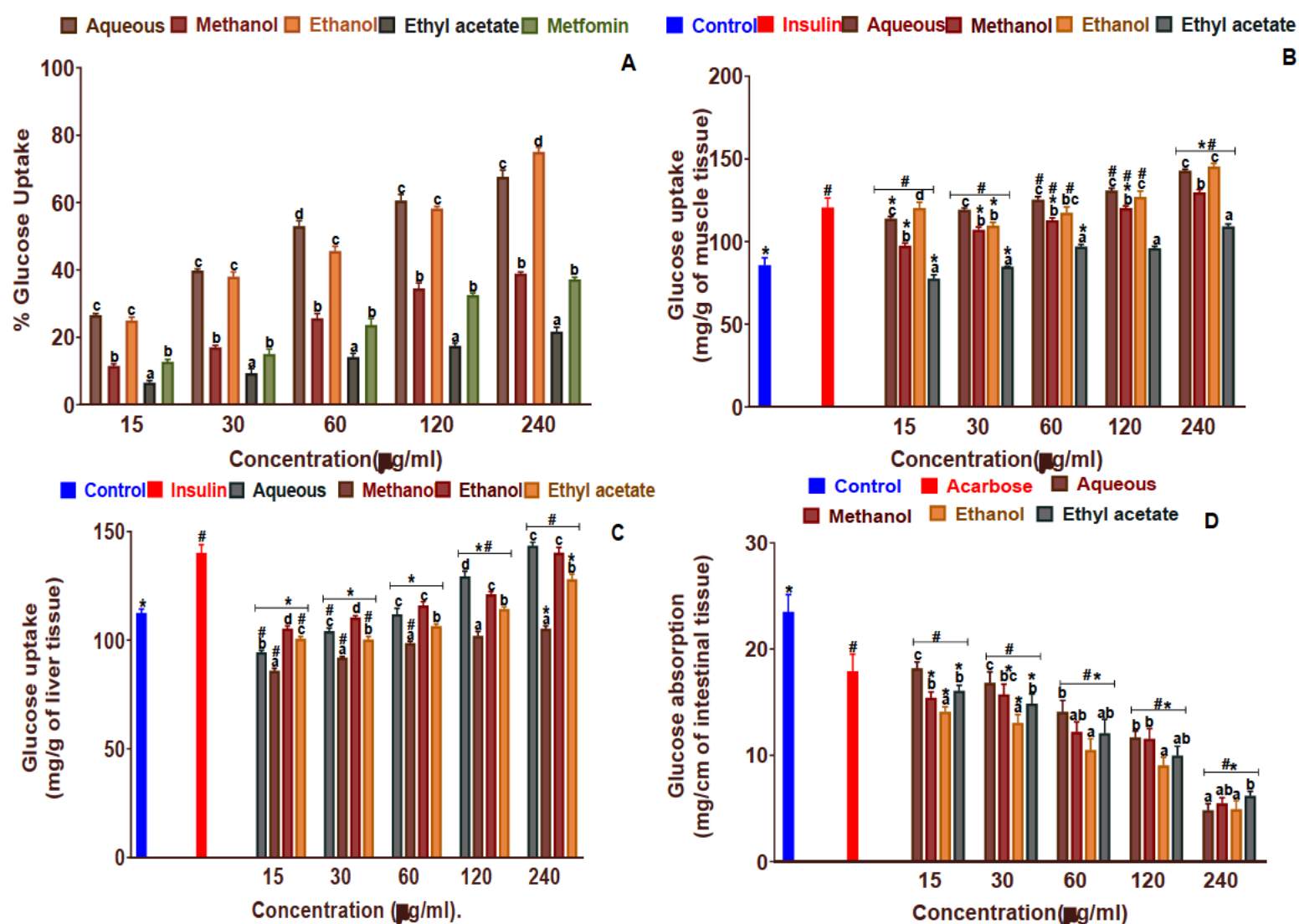


Figure 3.3.4: The modulatory effects of bark crude extracts of *T. orientalis* on (A) yeast cell glucose uptake, (B) psoas muscle glucose uptake, (C) liver glucose uptake and (D) intestinal glucose absorption. Data are presented as mean $\pm$ standard deviation. Different superscripts a, b, c and d represent statistical significance of $p < 0.05$. *Significantly different from positive control group and #Significantly different from normal control group ($p < 0.05$).

The effects of *T. orientalis* leaf extracts on oxidative stress biomarkers were analysed *ex vivo* in freshly harvested cardiac muscle tissue homogenates and graphed in Figs. 3.3.5A–D. A significant increase in lipid peroxidation with a concomitant decrease in superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) levels was observed after treatment with FeSO₄, which was indicative of oxidative tissue damage. There was a significant ($p < 0.05$) difference between the untreated tissue and the normal tissue in all the parameters analysed (Fig. 3.3.5A–D). Treatment with the aqueous and ethanolic extracts of *T. orientalis* bark extracts resulted in significantly ($p < 0.05$) lower MDA equivalents, suggesting their potency to abate lipid peroxidation as observed in Fig. 3.3.5A. Furthermore, these extracts had better activity than vitamin C, resulting in near-normal (malondialdehyde) MDA levels, especially at the highest concentrations. A similar trend was observed in which the ethanol and aqueous extracts stimulated the best SOD activity (Fig. 3.3.5B) activity and GSH levels (Fig. 3.3.5D), especially at concentrations of 120–240 µg/ml. Finally, as shown in Fig. 3.3.5C, the aqueous and methanol extracts stimulated the best CAT activity.

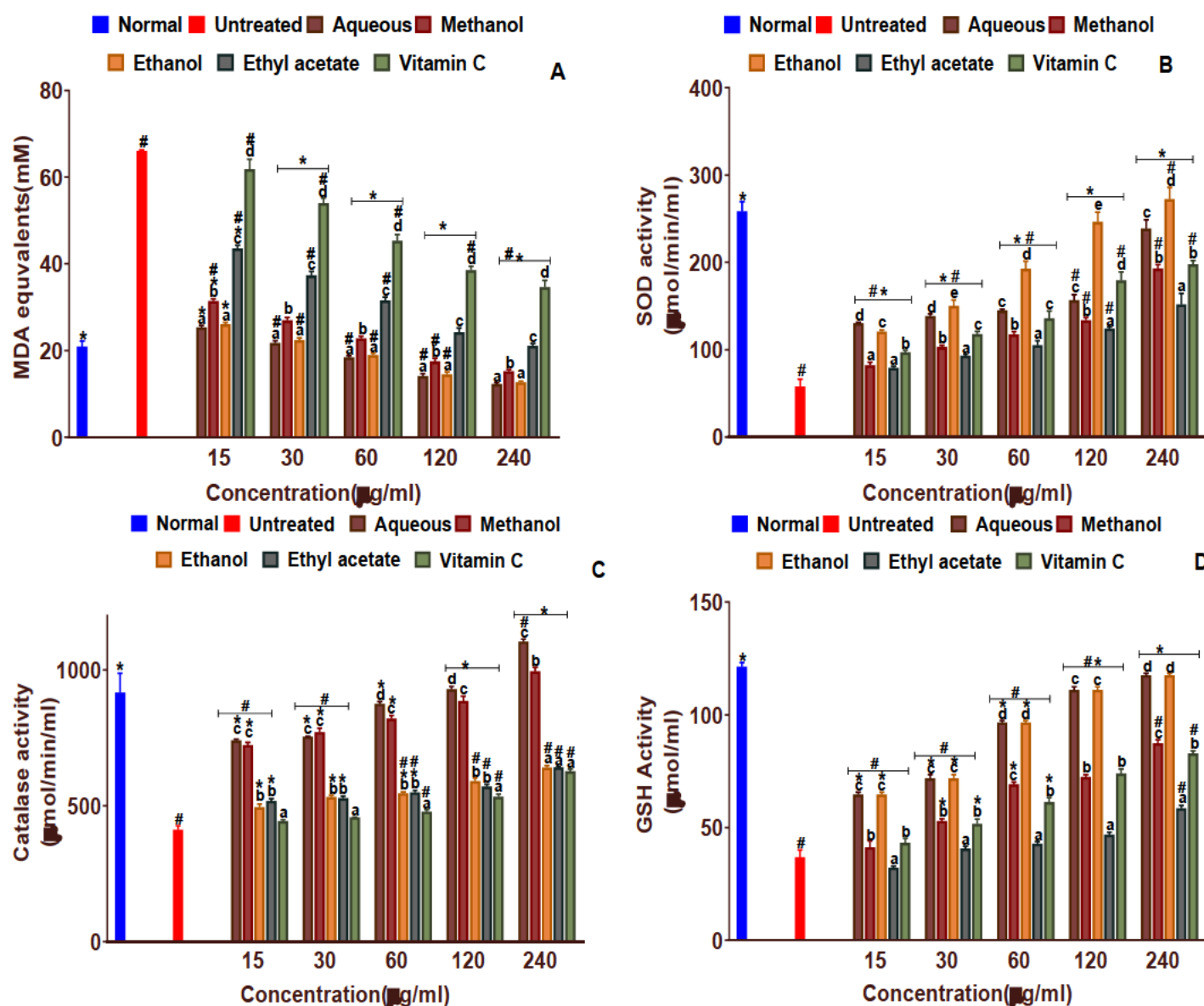


Figure 3.3.5: Effects of *T. orientalis* bark crude extracts on (A) lipid peroxidation, (B) SOD activity, (C) Catalase activity and (D) GSH activity in oxidative cardiac muscle tissue injury. Data are presented as mean $\pm$ standard deviation. Different superscripts a–d represent statistical significance of $p < 0.05$. *Significantly different from untreated sample and #Significantly different from normal sample ($p < 0.05$, Tukey's-HSD-multiple range post-hoc test, IBM SPSS for Windows). Normal=normal tissues, Untreated=tissues oxidatively injured but not treated.

Figs. 3.3.6A-C show that glucotoxicity induced significantly ($p < 0.05$) higher ATPase, glycogen phosphorylase, and fructose 1,6-bisphosphatase activity. The bark extract significantly reduced ATPase (Fig. 3.3.6A), glycogen phosphorylase (Fig. 3.3.6B), and fructose 1,6-bisphosphatase (Fig. 3.3.6C) activity in a dose-dependent manner. Among all extracts, the aqueous and ethanol extracts had the highest ATPase inhibitory activity (Fig. 3.3.6A), which was comparable to that of vitamin C (positive control). The ethanolic extract exhibited the best glycogen phosphorylase inhibitory activity, as shown in Fig. 3.3.7B. The ethyl acetate extract had the lowest glycogen phosphorylase inhibitory capacity but significantly ($p < 0.05$) outperformed quercetin (positive control) at all assayed concentrations. A similar trend was observed in which the ethanol extract exhibited the best fructose 1,6-bisphosphatase inhibitory activity, as shown in Fig. 3.3.6C.

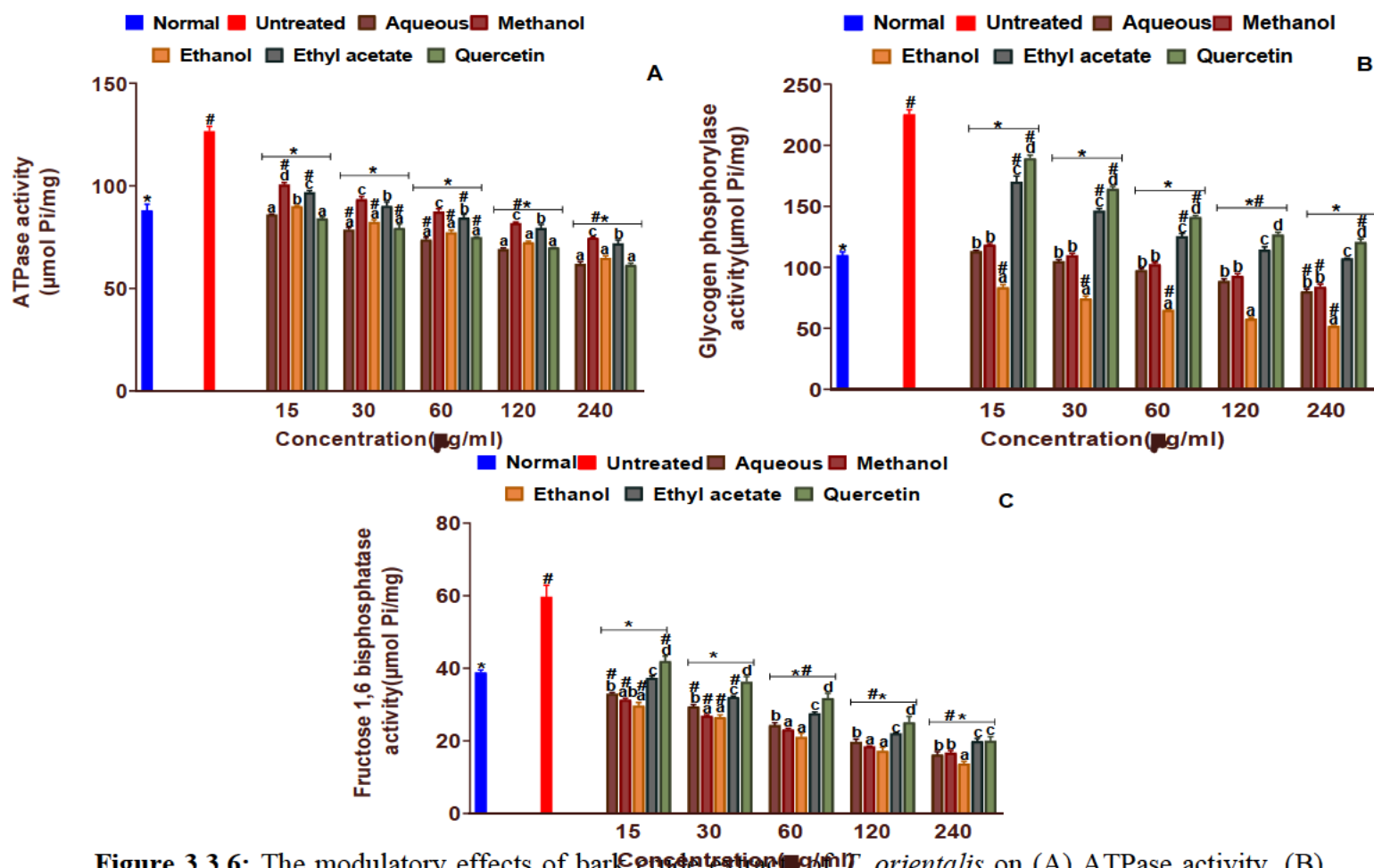


Figure 3.3.6: The modulatory effects of bark crude extracts of *T. orientalis* on (A) ATPase activity, (B) Glycogen phosphorylase activity and (C) Fructose 1,6 bisphosphatase activity. Data are presented as mean $\pm$ standard deviation. Different superscripts a-d represent statistical significance of $p < 0.05$. *Significantly different from untreated sample and #Significantly different from normal sample ($p < 0.05$).

3.3.5 Discussion

The upward trend in the global prevalence of diabetes mellitus (DM) over the years is of concern, as the trend is projected to worsen if current management strategies are not improved (Sun *et al.*, 2022). Consequently, scientists globally have endeavoured to develop for the betterment of DM management. Folkloric traditional medicinal plants have thus emerged as key reservoirs of novel anti-diabetic therapeutic agent templates. Their beneficial potential emanates from secondary metabolites, including alkaloids, terpenoids, saponins, flavonoids, and phenolics, which are among the most well-known therapeutic groups (Twaij and Hasan, 2022).

The myriad of compounds from different secondary metabolic groups identified through GCMS (Table 3.3.1) analysis may be responsible for the bioactivities exhibited by the *T. orientalis* extracts in the current study. Among the identified compounds, hexestrol, a stilbene derivative that can be found in citrus fruit peels, was previously used in the treatment of breast and prostate cancer (Vardanyan and Hruby, 2006; Mihaylova and Popova, 2023). A previous study documented the enzyme inhibitory activities of 2-Methyl-4-tert-octylphenol, which was present in the extracts, to have a strong binding affinity in the active site pocket of α amylase and α glucosidase (Mohamed *et al.*, 2022). Furthermore, plants high in such phenolic (Fig. 3.3.1A) and flavonoids (Fig. 3.3.1B) compounds as observed in the current study have been shown to have potent antioxidant capacities. Thus, such plants may help circumvent the oxidative stress-linked damage caused in diabetic patients. In the current study, extracts of *T. orientalis* had potent radical scavenging activity (Fig. 3.3.2A) and electron-donating capacity (Fig. 3.3.2B). Previous research has found a correlative link between high flavonoid and phenolic content and increased antioxidant capacity (Haminiuk *et al.*, 2012). These ring-structured molecules can chelate, donate delocalized electrons, or directly sacrificially react with free radicals to achieve the octet state they require (Zeb, 2020). Furthermore, several studies have highlighted the potential antioxidant and hypoglycemic activities of phytol and its derivatives, including phytol acetate (Islam *et al.*, 2018; Oyebode *et al.*, 2019). While saturated aliphatic alcohols generally do not exhibit strong antioxidant properties, phytol shows significant antioxidant activity due to the allylic nature of its alcohol group. One proposed mechanism of action involves the double bond in phytol forming an intermediate resonance structure, where the oxygen forms a double bond with the adjacent carbon, causing the double bond to shift to the 3,4 carbons (Islam *et al.*, 2016). This antioxidant activity is advantageous as it prevents oxidative damage to functional biomolecules by excess free radicals under oxidative conditions such as those in DM (Craft *et al.*, 2012b).

Oxidative stress-induced lipid peroxidation is exacerbated in DM patients with uncontrolled chronic hyperglycaemia (Preiser, 2012). Excessive free radicals damage structural or functional lipids in the

bilayer membranes of tissues (Craft *et al.*,2012b) and have been linked to the pathogenesis of several degenerative diseases, including cardiovascular disease (CVD), Alzheimer's disease, and diabetes mellitus (DM) (Liguori *et al.*,2018). In the current study, the *T. orientalis* extracts were shown to reduce lipid peroxidation (Fig. 3.3.5A) induced by FeSO₄, and this may be beneficial for diabetic patients who have chronically exacerbated hyperglycaemia-induced oxidative stress (Craft *et al.*,2012b). Under diabetic conditions, endogenous antioxidant defense systems are diminished. Therefore, the ability of the *T. orientalis* bark extracts to increase superoxide dismutase (SOD) (Fig 3.3.5B) and catalase (CAT) (Fig 3.3.5C) activities and increase the levels of GSH (Fig 3.3.5D) insinuates their therapeutic capacity to restore oxidative homeostatic balance in diabetic patients. The ability of *T. orientalis* bark extracts to enhance these endogenous antioxidant defenses may also be beneficial in protecting against damage mediated by oxidative stress, particularly in cardiac tissue, which has been linked to cardiovascular disease and heart failure (Pitocco *et al.*,2013).

An imbalance between calorie intake and its metabolism is implicated as a major cause of obesity, which is both a risk factor and a comorbidity of DM. Furthermore, excessive intake of fatty foods, which are a high-energy source, and their subsequent assimilation are exacerbating factors in diabetic dyslipidaemia. Regulation of fatty acid digestion thus forms a cornerstone mechanism for lowering diabetic dyslipidaemia. Pancreatic lipase is the enzyme responsible for the release of free fatty acids into the gastrointestinal tract after triglyceride breakdown. Therefore, the ability of the *T. orientalis* bark extract to inhibit pancreatic lipase (Fig. 3.3.3A) may aid in preventing diabetic dyslipidaemia.

Diabetic patients constantly have sustained post-prandial blood glucose spikes due to the relatively slow clearance of circulatory blood glucose caused by dysregulated carbohydrate metabolism (Blake, 2021). In recent years, there has been progress in the development of antidiabetic pharmacological agents that work by inhibiting key carbohydrate-digesting enzymes such as α amylase and α glucosidase (Dirir *et al.*,2022). While α amylase breaks down complex dietary carbohydrates into smaller fragments in the mouth, α glucosidase functions at the small intestinal brush border interface, degrading primarily disaccharides to glucose before its eventual assimilation into the blood stream. In the current study, the *T. orientalis* extracts were observed to inhibit the action of α amylase (Fig. 3.3.3C) and α glucosidase (Fig. 3.3.3B), which function to degrade complex carbohydrates. Inhibiting these enzymes slows the rate of carbohydrate digestion, reducing the rate at which it is absorbed into the blood (Dirir *et al.*,2022). This allows more time for the disposal of circulatory blood glucose, thus circumventing postprandial blood glucose spikes in patients with DM. Retarding the rate of glucose absorption by the intestines has also been similarly employed as an

effective strategy to help manage postprandial glucose spikes. Previous studies have shown that compounds that can retard glucose absorption in the intestinal lumen exert hypoglycaemic activity (Sun and Miao, 2020). As observed in the current study, the *T. orientalis* extracts managed to reduce the amount of glucose absorbed by the small intestine (Fig. 3.3.4D). This, together with the inhibition of α glucosidase and α amylase activity by the *T. orientalis* extracts, shows the potential to delay the digestion and absorption of carbohydrates. After glucose is absorbed by the small intestine, it is taken up by various tissues, where it drives energy-dependent metabolic reactions through the production of ATP via glycolysis and oxidative phosphorylation. Excess glucose is stored as glycogen in liver and muscle tissues or as lipids in adipose tissue for use during prolonged fasting periods. Thus, glucose uptake by peripheral tissues, as observed in the current study (Figs. 3.3.4B and 3.3.4C), has a hypoglycaemic effect as it mediates the reduction of circulatory glucose. The *T. orientalis* extracts were shown to increase the amount of glucose taken up by the muscle tissue and the yeast cells (Fig. 3.3.4A), insinuating their antidiabetic potential.

ATPase is a nucleotide hydrolyzing enzyme that converts ATP to ADP+ Pi (inorganic phosphate) with concurrent energy release. Its increased ATPase activity under hyperglycaemic conditions (Fig. 3.3.6A) results in dysregulated energy redox due to an increase in the ADP+ Pi:ATP ratio, indicating excessive ATP depletion (Erukainure *et al.*,2021). *T. orientalis* extracts' ability to reduce ATPase activity suggests that they may have the potential to restore energy balance in diabetics, who frequently have fatigue because of this imbalance. Glycogenic phosphorylase and fructose 1,6-bisphosphatase, respectively, are enzymes that mediate glycogenolysis and gluconeogenesis. Their excessive activity, as in diabetic conditions (Fig. 3.3.6A and B), is associated with increased hepatic glucose output, exacerbating chronic hyperglycaemia (Timson, 2019; Stravodimos *et al.*,2017). Inhibitors of glycogen phosphorylase and fructose 1,6-bisphosphatase have recently been identified as potential hypoglycaemic agents for T2D treatment. Therefore, the ability of the *T. orientalis* extracts to reduce the activities of these enzymes insinuates their antidiabetic activity through hepatic glucose uptake modulatory activity.

3.3.6 Conclusion

The observed antioxidant and antidiabetic activity of *T. orientalis* bark extracts could be due to the synergistic effects of multiple bioactive compounds, including flavonoids and phenolics. This study suggests that *T. orientalis* extracts contain potent antioxidant agents coupled with α glucosidase, α amylase, and intestinal glucose absorption inhibitors, as well as moieties that modulate peripheral tissue glucose uptake. These may be harnessed for the development of a therapeutic strategy for the management and control of postprandial hyperglycaemia in T2D and its associated complications.

CHAPTER 4

IN VITRO AND EX VIVO STUDIES TO INVESTIGATE THE ANTIOXIDANT AND ANTIDIABETIC ACTIVITIES OF *ALBIZIA ADIANTHIFOLIA* (SCHUMACH.) W.F.Wight.

4.1 *Albizia adianthifolia* (Schumach.) W.F.Wight

4.1.1 Background and plant description

Albizia is a large plant genus with approximately 120 to 140 species widely distributed in tropical African regions, including Rwanda, Tanzania, Angola, the Southern Democratic Republic of the Congo, Zambia, Mozambique, Namibia, Zimbabwe, and Botswana (Lewis and Rico Arce, 2005). *Albizia adianthifolia* (Schumach) W.F.Wight of the family Fabaceae and subfamily Mimosoideae is commonly known as rough-barked flat-top (Maroyi, 2018). This small to medium-sized tree has a flattened umbrella-like crown with pinnate oblong leaves. The plant also bears an oblong fruit pod, and the inflorescence possesses an axillary head with small bisexual flowers with colours ranging from red to greenish white. Moreover, when the fruit is ripe, it becomes pubescent, transversely veined, and pale brown in colour. The plant has brown seeds, which are globose in shape, whereas the bark of the plant, which is either smooth or rough in texture, has a creamy inner side (Maroyi, 2018). Depending on the geographical vegetation, *Albizia adianthifolia*, as a deciduous tree plant, can grow to heights ranging from 18 m to 35 m (Palmer and Pitman, 1972).



Figure: 4.1: *Albizia adianthifolia* (Schumach.) W.F.Wight: Common names: flat-crown albizia, (English.); Mucherenje (Shona); platkroon (African.); umgadankawu, (Zulu); umHlandlothi (Xhosa); mchani-mbawe (Swahili). Adapted without permission from www.sanbi.org.

4.1.2 Ethnobotanical uses

There are reports that different parts of *Albizia adianthifolia* are usually harvested from the wild for use as wood, food, and medicine (Maroyi, 2018). The bark of *A. adianthifolia* is employed in ethnoveterinary medicine, whereas in South Africa's traditional medicine markets, it is commonly prescribed as a purgative and remedy for diabetes, eye problems, and sexually transmitted infections (Pujol, 1990; Lebbie and Guries, 1995; Grace *et al.*, 2003). According to Maroyi (2018), *A. adianthifolia* is also used by the Murehwa and Mutoko people in Zimbabwe for treating sore eyes, cuts, ulcers, sore throat, tonsillitis, tuberculosis, gonorrhoea, diabetes, and heart diseases. The plant bark, leaves, and roots were also employed in folkloric medicine for managing stomach-related ailments in several other African countries, including South Africa (Pujol, 1990; Mabogo *et al.*, 1990; Ramathal and Ngassapa, 2001; Senkoro *et al.*, 2014). Furthermore, studies by Gbekley *et al.*, (2018) indicate *A. adianthifolia* application in the management of hypertension in the Togo maritime region (Gbekley *et al.*, 2018).

4.1.3 Biological activities

Several scientific studies have been conducted to verify the folkloric applications of *A. adianthifolia*. A previous study by Aderibigbe (2018) revealed the ability of *A. adianthifolia* ethanol extract to act as an antidepressant in mice. Furthermore, *A. adianthifolia* was shown to inhibit acetylcholinesterase and, hence, have the capacity to prevent mental illnesses. The plant's bark ethanol extract also showed antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Micrococcus luteus*, *Escherichia coli*, and *Klebsiella pneumoniae*, with minimum inhibitory concentration (MIC) values ranging from 3.13 mg/ml to 6.25 mg/ml (Eldeen *et al.*, 2005). In another study, a combination of *A. adianthifolia* and *Trichilia dregeana* displayed synergistic effects against selected sexually transmitted infections (De Wet *et al.*, 2012). The antioxidant activities of various extracts of *A. adianthifolia* from different parts have also been documented using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay (Sonibare *et al.*, 2017), Trolox equivalent antioxidant capacity (TEAC) (Tamokou *et al.*, 2012), and their ability to modulate the activity of endogenous antioxidant enzymes (Beppe *et al.*, 2015).

4.1.4 Phytochemistry

To date, about 90 secondary metabolites have been isolated from the leaves, roots, and stem bark of *A. adianthifolia* (Maroyi, 2018). The isolated, identified, and characterized phytochemical compounds from *A. adianthifolia* include apocarotenoids, dipeptides, essential oils, fatty acids, flavonoids, histamines, imidazolyl carboxylic acids, steroids, triterpene saponins, and triterpenoids (Akande *et al.*, 2018). The essential oils, fatty acids, triterpene saponins, flavonoids, and

phenolics are considered the most prominent family of phytochemical compounds occurring in *A. adianthifolia* (Akande *et al.*, 2018). Moreover, Akande *et al.*, (2018) revealed that *A. adianthifolia* oil contained β -caryophyllene, e-geranyl acetone, acorenone, viridiflorol, α -zingiberene, and ar-curcumene. The same study further showed selin-11-en-4- α -ol, caryophyllene oxide, and α -humulene as the major constituents in the stem bark oil, whereas the root bark oil contained predominantly pentadecanal (Akande *et al.*, 2018).

4.1.5 Aim and objectives

This study aims to investigate the antioxidant and antidiabetic activities of different *Albizia adianthifolia* crude extracts by determining their *in vitro* free radical scavenging, electron- donating capacity, and nitric oxide mop-up potential. Furthermore, it investigated the carbohydrate and lipid digestive enzymes' inhibitory activities of the extracts *in vitro*. The plant extracts modulation of hepatic glucogenic enzyme activities, intestinal glucose absorption, muscle glucose uptake and endogenous antioxidant proteins, which are biomarkers of oxidative tissue injury, were also investigated *ex vivo*.

4.2 *Albizia adianthifolia* crude leaf extracts regulate glucose metabolism and ameliorate oxidative stress-linked cardiac tissue injury

¹Brian K. Beseni, ¹Kolawole A. Olofinisan ^{1,2}Veronica F. Salau, ¹Md. Shahidul Islam*

1. Department of Biochemistry, University of KwaZulu-Natal, Westville Campus, Durban 4000, South Africa

2. Department of Pharmacology, University of the Free State, Bloemfontein 9300, South Africa

***Corresponding author:** islamd@ukzn.ac.za

4.2.1 Abstract

Traditional medicine has been practiced since time immemorial to manage several ailments, including diabetes. *Albizia adianthifolia*, of the family Fabaceae, is a folkloric medicinal plant that is used to remedy several ailing conditions. The total phenolic and flavonoid content of the different *A. adianthifolia* extracts were quantified prior to determining their antioxidant activities. Thereafter, the ability of crude extracts to inhibit the activities of key carbohydrate and lipid-digesting enzymes was determined *in vitro*. The ability of the *A. adianthifolia* extracts to retard intestinal glucose absorption, modulate glucogenic enzymes, and enhance muscular glucose uptake was investigated *ex vivo*. Moreover, their modulatory effects on lipid peroxidation, superoxide dismutase (SOD) activity, catalase (CAT) activity, and reduced glutathione (GSH) levels in cardiac tissue were also determined *ex vivo*. The leaf ethanol extract exhibited the best DPPH ($IC_{50} = 96,11 \pm 0,45 \mu\text{g/ml}$), FRAP ($IC_{50} = 21,12 \pm 0,17 \mu\text{g/ml}$), and nitric oxide scavenging capacity. The aqueous extracts showed the best pancreatic lipase inhibition, while the plant methanol extract had better α -glucosidase and α -amylase inhibitory activities. The *A. adianthifolia* extracts ameliorated heart tissue oxidative injury by lowering lipid peroxidation products while increasing the activities of SOD and CAT with a simultaneous reduction of GSH levels. Further molecular and *in vivo* analyses are required to substantiate these preliminary findings.

Keywords: Antidiabetic, glucose uptake, *Albizia adianthifolia*, antioxidant.

4.2.2 Introduction

Diabetes mellitus (DM) is becoming increasingly prevalent worldwide, with an estimated 536 million adults currently affected by the condition, making it one of the leading global causes of morbidity and premature mortality (Lovic *et al.*,2020; Sun *et al.*,2022). DM is a multifactorial metabolic disorder characterized by glucose metabolism dysregulation and chronic hyperglycaemia. The events leading to this metabolic alteration may emanate from defective insulin secretion or insensitivity in target tissues (Lovic *et al.*,2020). DM risk factors include age, unhealthy diet, ethnicity, genetic predisposition, and physical inactivity (Roglic, 2016). Despite these factors, oxidative stress due to the imbalance between intrinsic antioxidant defense systems and the production of reactive species (RS) has been described in the pathogenesis of the disease (Oguntibeju, 2019). Under oxidative stress conditions, several biochemical pathways are dysregulated. These include the polyol pathway, the hexosamine pathway, and glycation end-product production (AGEs) (Nowotny *et al.*,2015; Jud and Sourij, 2019).

Glycation end-products (AGEs) are a group of modified proteins and/or lipids with damaging potential (Nowotny *et al.*,2015). Reports have shown AGEs further result in the development of new pathological AGE receptors (RAGEs) that further exacerbate the effects of hyperglycaemic-induced oxidative stress (Jud and Sourij, 2019). This has been implicated as one of the biochemical reactions leading to insulin resistance linked with type II diabetes, which represents 90% of all diabetic cases (Iglay *et al.*,2016). With uncontrolled hyperglycaemia in diabetic conditions resulting in microvascular and macrovascular complications, including neuropathy, nephropathy, and stroke, there is a need for effective therapeutic strategies to mitigate the menace of the pathology (Harding *et al.*,2019).

According to Thrasher (2017), current diabetes management strategies involving oral pharmacological agents such as biguanides, sulfonylureas, and thiazolidinediones focus on glycaemic control to exert their antidiabetic action. Despite the reported effectiveness of these medications, their utilization is associated with several drawbacks, such as high cost and other side effects, including weight gain, nausea, and flatulence (Marín-Peñalver *et al.*,2016). Consequently, there is a search for cost-effective novel antidiabetic agent templates with reduced side effects (Marín-Peñalver *et al.*,2016). In this regard, folkloric medicinal plants have emerged as a source of bioactive compounds that may be investigated and developed into novel therapeutic agents (Malviya *et al.*,2010; Chinsembu, 2019).

Albizia adianthifolia of the family Fabaceae is among such medicinal plants that have long been employed as a traditional remedy for managing diabetes, eye problems, and sexually transmitted infections (Maroyi, 2018). Several compounds, such as acorenone, viridiflorol, α - zingiberene, and curcumin, have been identified and characterized from *A. adianthifolia* (Akande *et al.*,2018). Previous reports have shown that *A. adianthifolia* has antioxidant, antibacterial, and hypoglycemic pharmacological activities (Tamokou *et al.*,2012; Beppe *et al.*,2015; Amuri *et al.*,2017). However, the precise therapeutic mechanism of action of *A. adianthifolia* phytoconstituencies remains poorly understood and documented. This study aimed to ascertain the antioxidant and hypoglycaemic activities of *A. adianthifolia* by determining its extract modulatory effects on glucose uptake/absorption as well as inhibition of digestive and glucogenic enzyme activities using *in vitro* and *ex vivo* models.

4.4.3 Materials and methods

Please refer to Chapter 2, sections 2.4.1 to 2.11.5 for detailed materials and methods.

4.2.4 Results

The total phenolic and flavonoid contents of *A. adianthifolia* leaf extracts are depicted in Figs.

4.2.1A and B. The ethanol (12.13 mg/g) and ethyl acetate (12.13 mg/g) extracts contained comparably higher total phenolic content than those in the aqueous (6.98 mg/g) and methanol extracts (Fig. 4.2.1A). The total flavonoid content (Fig 4.2.1B) was observed to be polarity- dependent, as the ethyl acetate extract contained the highest amount (30.15 mg/g) of flavonoids, while the aqueous extract (most polar) contained significantly ($p<0.05$) the least content (1.32 mg/g). All extracts contained significantly ($p<0.05$) different amounts of total flavonoids from each other.

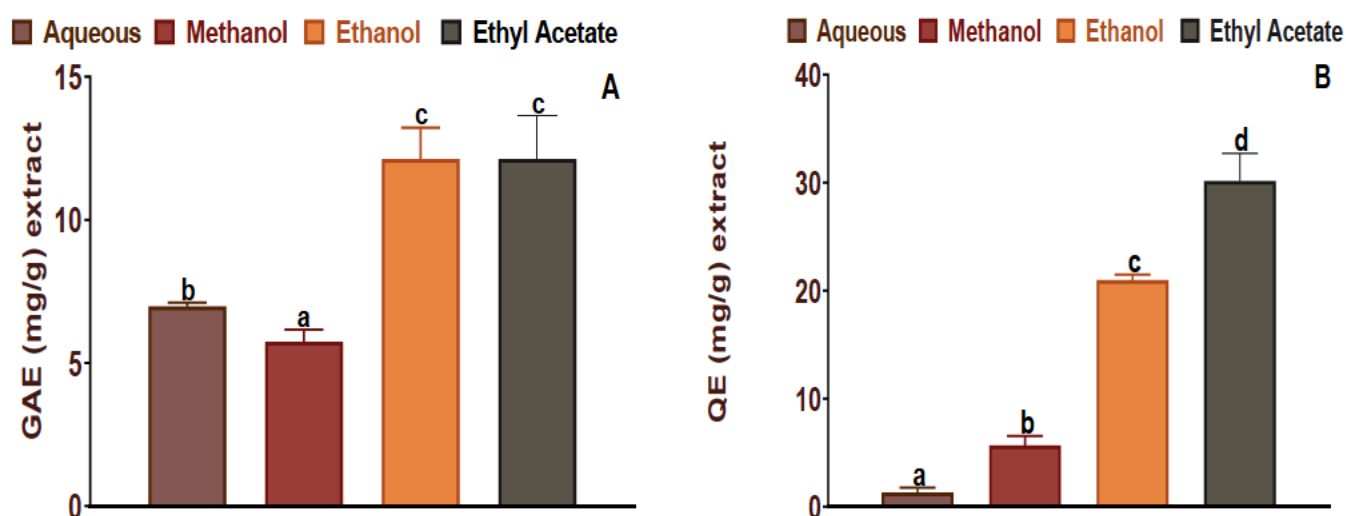


Figure: 4.2.1: *A. adianthifolia* leaf extracts (A) total phenolic content represented as gallic acid equivalents (GAE mg/g) and (B) the total flavonoid content represented as quercetin equivalents (QE mg/g). Bars with superscripts a-d are statistically different at $p<0.05$.

The antioxidant activities of the *A. adianthifolia* leaf crude extracts were determined using the DPPH radical scavenging, ferric reducing antioxidant power (FRAP) (, and NO inhibition assays, as depicted in Fig. 4.2.2A–4.2.2C, respectively. The extracts showed dose-dependent DPPH radical- scavenging activity. The ethanol extract exhibited the best DPPH scavenging activity (Fig. 4.2.2A) at all the assayed concentrations except at the highest concentration (240 $\mu\text{g/ml}$), where the aqueous extract outperformed it. In Table 4.6.1, the ethanol extract also exhibited the least IC_{50} value ($21,12\pm0,17$ $\mu\text{g/ml}$) for the FRAP assay (Fig. 4.2.2B). The ability of the ethanol extract to reduce the ferric ion was closely followed by that of the methanol extract, while the ethyl acetate extract showed the least activity. At all concentrations, the methanol, compared with the other extracts, significantly ($p<0.05$) inhibited nitric oxide production (Fig. 4.2.2C) except at 15 $\mu\text{g/ml}$, where its activity was not different

from that of the aqueous extract. Despite the methanol extracts' nitric oxide production inhibition capacity, their overall activities did not surpass those of the positive control (quercetin) for all the test concentrations (Fig. 4.2.2C).

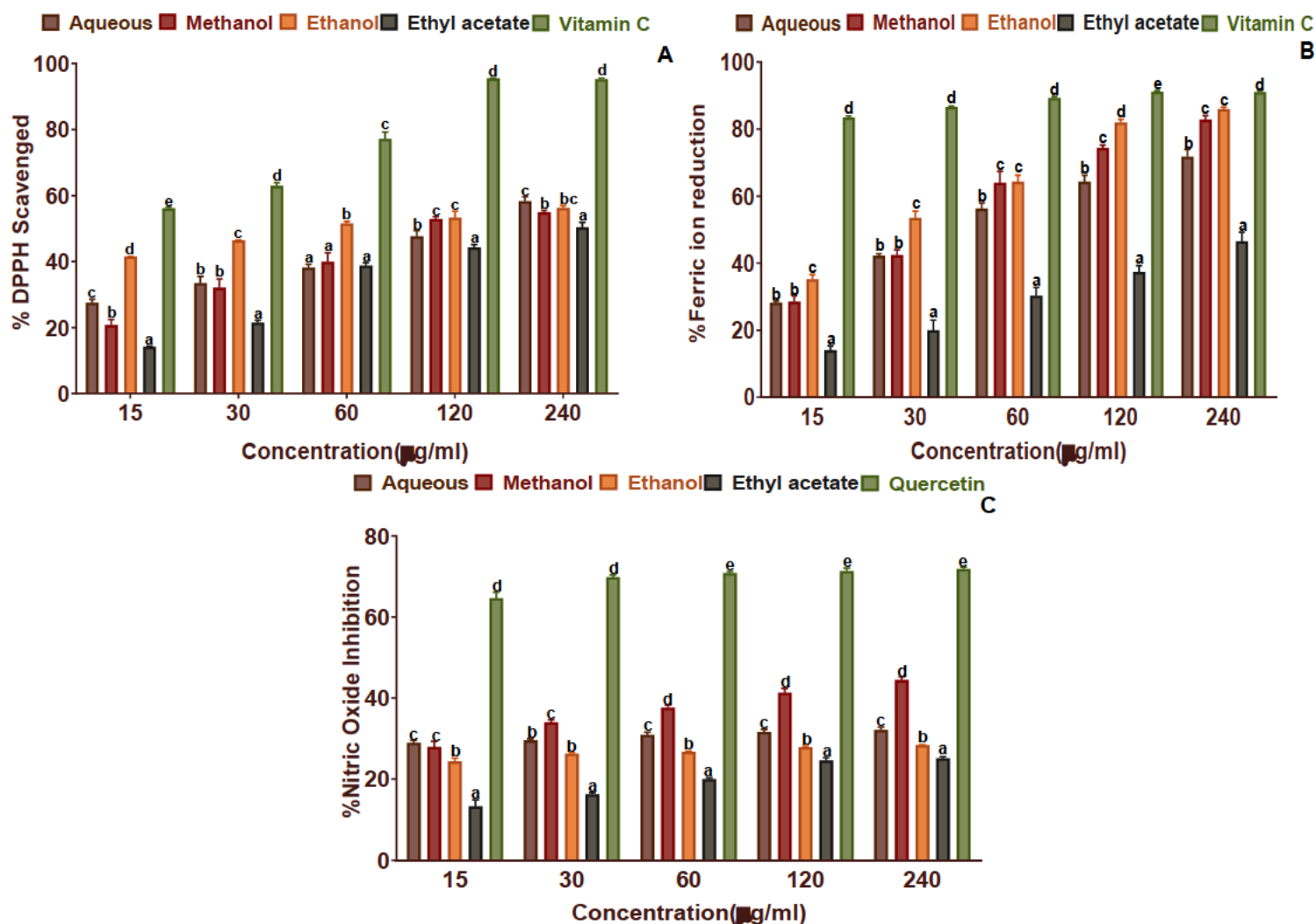


Figure: 4.2.2: The effects of *A. adianthifolia* leaf extracts on (A) DPPH radical scavenging activity, (B) Ferric ion reducing activity, (C) Nitric oxide production inhibition. Data are presented as mean $\pm$ standard deviation. Different superscripts a-e represent statistical significance of $p < 0.05$.

The inhibition activities of *A. adianthifolia* leaf extracts on lipid and carbohydrate digestive enzymes are depicted in Figs. 4.2.3A–4.2.3C. Among the crude extracts tested, the aqueous extracts showed the best pancreatic lipase inhibition (Fig. 4.2.3A), particularly at the highest concentration (240 µg/ml). This better activity of the aqueous extract is also indicated by its IC_{50} value (112, $48 \pm 0, 21$ µg/ml), which is lower when compared with 114, 52 ± 0.83 µg/ml of the orlistat positive control (Table 4.2.1). In Fig. 4.2.3B, the methanol extract exhibited significantly ($p < 0.05$) better α -glucosidase inhibitory activity among all the extracts, with an IC_{50} value of $184,43 \pm 0,83$ µg/ml

(Table 4.2.1), which suggests superior efficacy than the positive control (acarbose). All the extracts showed a dose-dependent inhibition of α -amylase. The methanol extract had the best α -amylase inhibition activity ($IC_{50} = 126,23 \pm 0,75 \mu\text{g/ml}$) among all the *A. adianthifolia* leaf extracts (Fig. 4.2.3C).

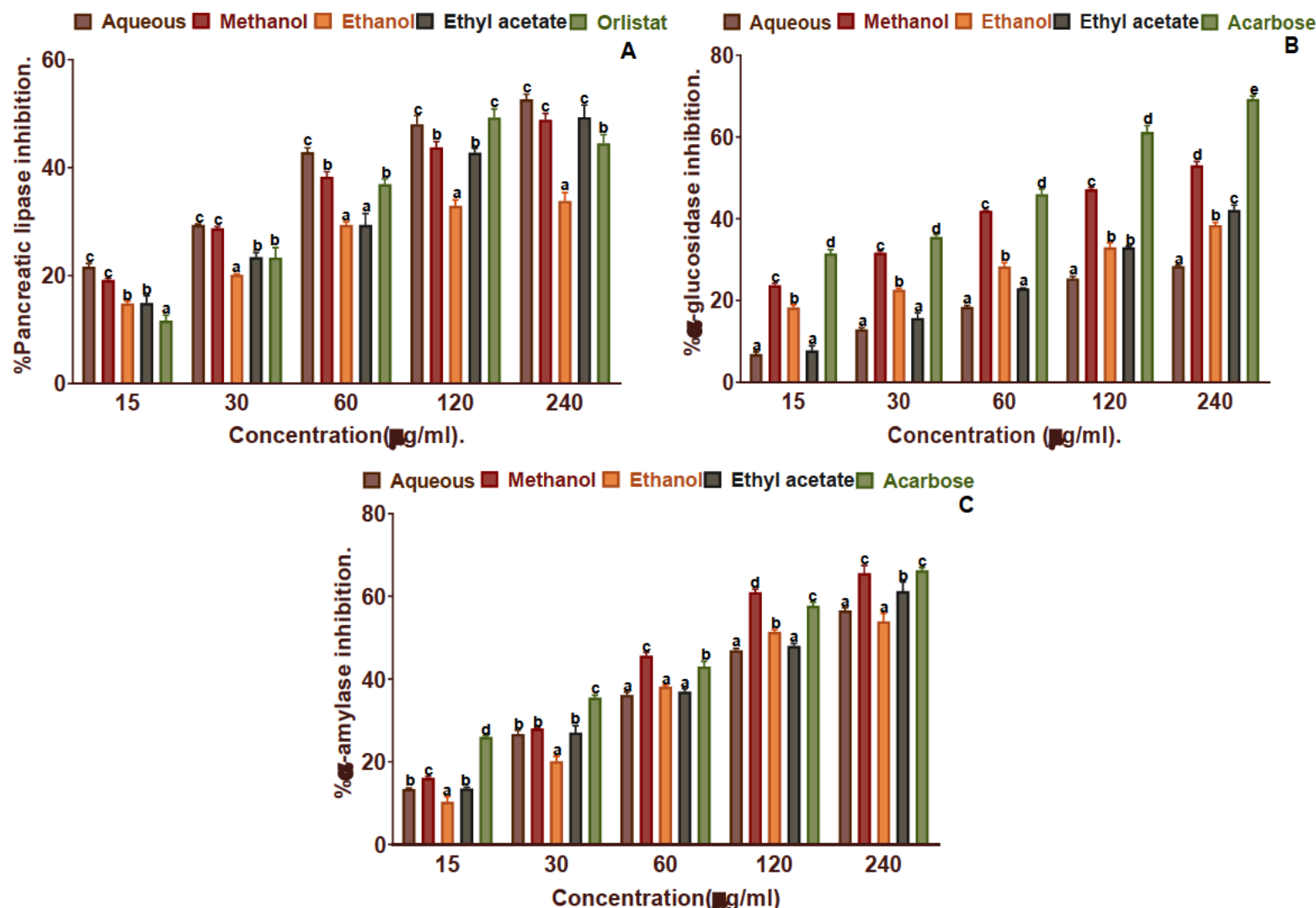


Figure: 4.2.3: *A. adianthifolia* leaf extracts on (A) pancreatic lipase inhibition activity, (B) α -glucosidase inhibition activity, (C) α -amylase inhibition activity. Data is presented as mean $\pm$ standard deviation. Different superscripts a-d represent statistical significance of $p < 0.05$.

Table 4.2.1: The IC₅₀ values of *A. adianthifolia* leaf crude extracts *in vitro* assays.

	Aqueous Extract	Methanol Extract	Ethanol Extract	Ethyl acetate Extract	Vitamin C	Quercetin	Acarbose	Orlistat
DPPH (µg/ml)	162,23±0,43	165,89±0,37	96,11±0,45	204,54±0,76	9,21±0,12	-	-	-
FRAP (µg/ml)	77,692±0,81	53,733±0,36	21,123±0,17	244,29±0,37	6,67±0,88	-	-	-
NO (µg/ml)	1579±0,41	300,13±0,22	1707±0,39	705,37±0,23	-	2,49±0,81	-	-
Pancreatic lipase (µg/ml)	112,48±0,21	137,81±0,37	211,51±0,07	146,29±0,47	-	-	-	114,52±0,83
α-glucosidase(µg/ml)	460,93±0,33	184,43±0,83	359,19±0,17	274,8±0,20	-	-	100,68±0,09	-
α-amylase (µg/ml)	175,99±0,44	126,23±0,75	179,02±0,62	159,89±0,75	-	-	118,57±0,97	-

Data is presented as mean ± standard deviation. Key: DPPH = 2,2-diphenyl-1-picrylhydrazyl. FRAP = Ferric reducing antioxidant power. NO = Nitric oxide assay.

The effects of *A. adianthifolia* leaf extracts on glucose uptake and absorption are depicted (Fig. 4.2.4A–4.2.4D). The extracts exhibited a dose-dependent effect on yeast cells' glucose uptake (Fig. 4.2.4A). The ethanol extract exhibited the best glucose uptake effect on yeast cells, particularly at the highest concentration (240 µg/ml), where it increased the sugar uptake by 46,88%. This value was higher than that of metformin, which caused a 37.19% increase in glucose uptake at the former concentration (Fig. 4.2.4A). Conversely, in Fig. 4.2.4A, the least glucose uptake effect was observed by the ethyl acetate extract, which at the highest concentration increased glucose uptake by 21,92% in the yeast cells. Treatment of the psoas muscle (Fig. 4.2.4B) and liver (Fig. 4.2.4C) tissues with insulin resulted in significantly higher glucose uptake than the normal control. A dose-dependent glucose uptake trend was observed when both the muscle and liver tissues were treated with *A. adianthifolia* leaf extracts, as depicted in Figs. 4.2.4B and 4.2.4C. Except for the ethyl acetate extract, the muscular glucose uptake effect of all the other extracts surpasses that of insulin, as observed in Fig. 4.2.4B. Moreover, in Fig. 4.2.4D, the ethyl acetate extract had significantly ($p<0.05$) low glucose absorption ability in the intestinal jejunum tissue, even at the highest concentration (240 µg/ml). Interestingly, all the other extracts exhibited better suppression of glucose absorption with respect to the positive control (acarbose).

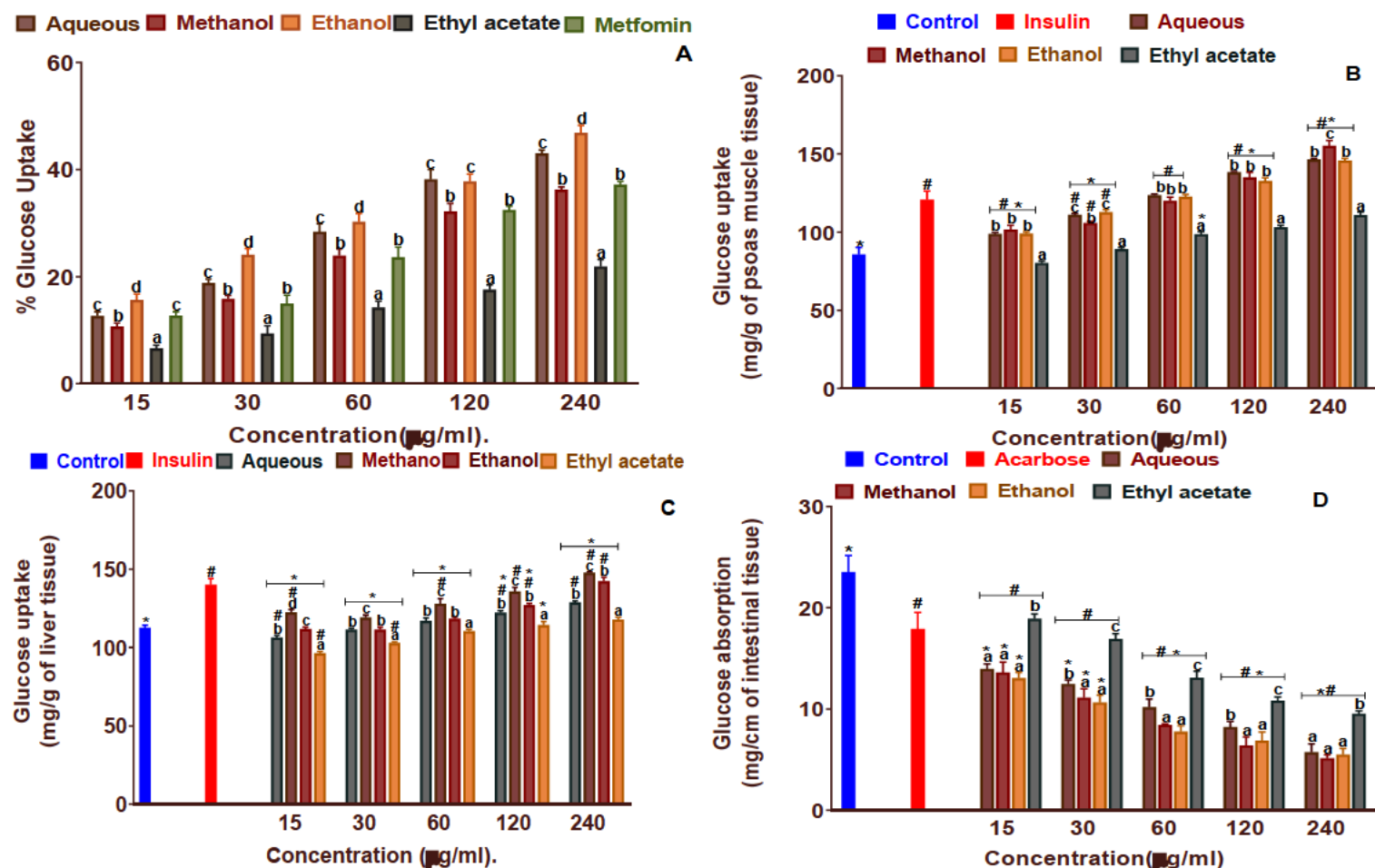


Figure: 4.2.4: *A. adianthifolia* leaf crude extracts effects on (A) yeast cell glucose uptake, (B) psoas muscle glucose uptake, (C) liver glucose uptake and (D) intestinal glucose absorption. Data are presented as mean $\pm$ standard deviation. Different superscripts a, b and c represent statistical significance of $p < 0.05$. *Significantly different from the positive control and #significantly different from the control.

The effects of *A. adianthifolia* extracts on oxidative stress biomarkers in oxidant-treated heart tissue homogenates are depicted in Figs. 4.2.5A–4.2.5D. Treatment with FeSO_4 induced oxidative injury, thus augmenting lipid peroxidation while diminishing the activities of superoxide dismutase (SOD), catalase (CAT), and levels of reduced glutathione (GSH). There was a significant ($p < 0.05$) difference between the untreated tissue and the normal tissue in all the biochemical markers analysed (Fig. 4.2.5A–4.2.5D). Treatment with the *A. adianthifolia* leaf extract resulted in a significant ($p < 0.05$) reduction in lipid peroxidation (Fig. 4.2.5A), particularly with the aqueous and ethanol extracts. In Fig. 4.2.5B, *A. adianthifolia* leaf extracts significantly ($p < 0.05$) increased SOD activity in heart tissue (Fig. 4.2.5B) after FeSO_4 treatment. The ethyl acetate extract displayed the best SOD-enhancing activity and even outperformed vitamin C, especially at the highest concentration (240 $\mu\text{g/ml}$). As

depicted in Fig. 4.2.5C, the *A. adianthifolia* extracts dose-dependently increased catalase activity. The ethanol and methanol extracts' catalase-enhancing abilities were comparable at all the assayed concentrations, which were significantly ($p < 0.05$) higher than those of vitamin C and the other extracts. Data presented in Fig. 4.2.5D indicate that GSH levels increased significantly ($p < 0.05$) in the aqueous and methanol treatment groups. At the highest concentrations, the aqueous and leaf extracts' GSH levels were near normal.

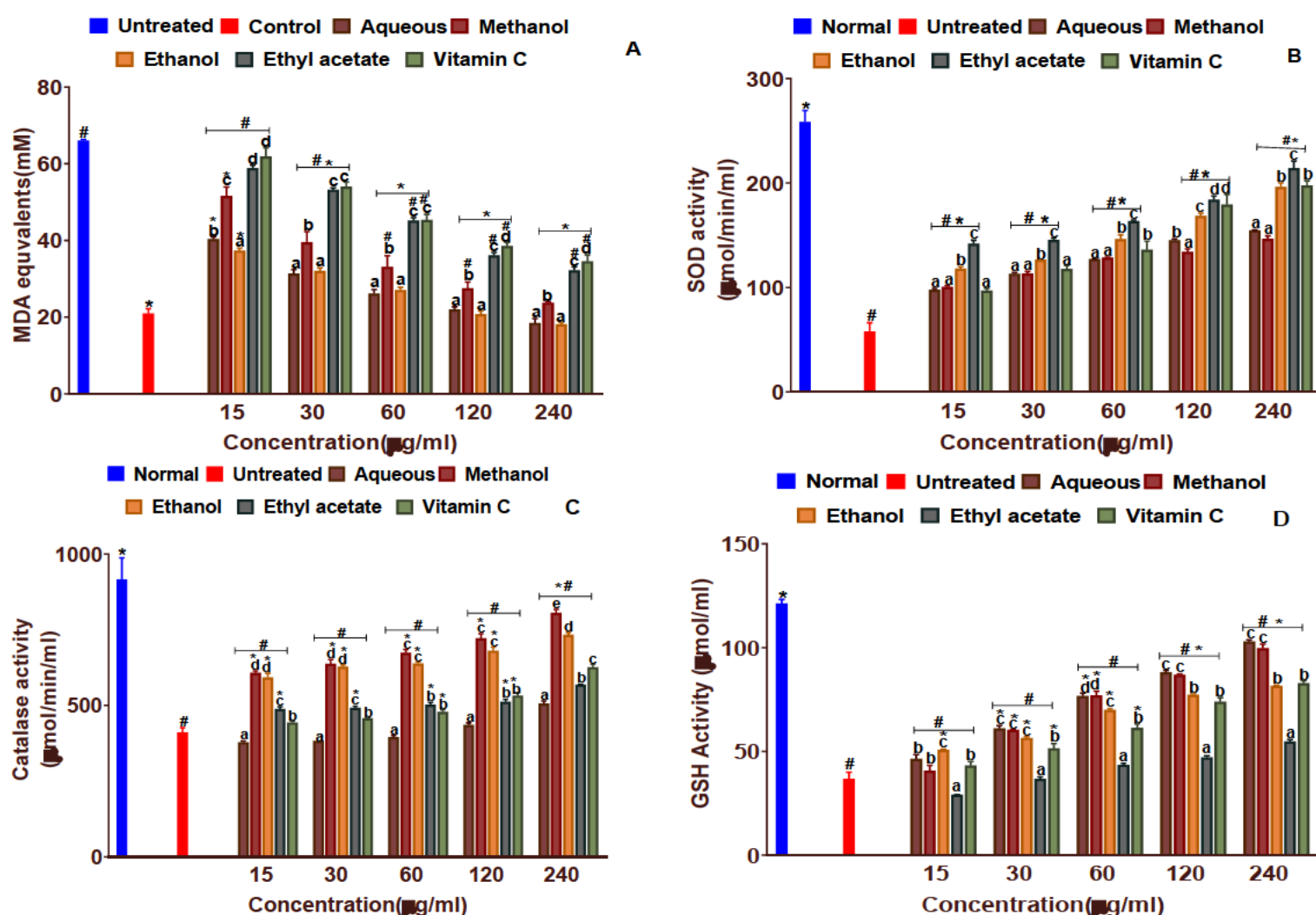


Figure: 4.2.5: Effects of *A. adianthifolia* leaf extracts on (A) lipid peroxidation, (B) SOD activity, (C) catalase activity and (D) GSH level in oxidative cardiac tissue injury. Data are presented as mean $\pm$ standard deviation. Different superscripts a, b and c represent statistical significance of $p < 0.05$. *Significantly different from the untreated sample and #significantly different from the normal sample.

The effects of *A. adianthifolia* leaf crude extracts on ATPase, glycogen phosphorylase, and fructose-1,6-bisphosphatase activities in liver tissue homogenate are represented in Fig. 4.2.6A–6C. Glucotoxicity resulted in significantly ($p<0.05$) higher ATPase activity (Fig. 4.2.6A), glycogen phosphorylase activity (Fig. 4.2.6B), and fructose 1,6-bisphosphatase activity (Fig. 4.2.6C) in the untreated treatment group compared with the normal. ATPase activity was significantly reduced ($p<0.05$) by *A. adianthifolia* leaf extract treatment at all the assayed concentrations (Fig. 4.2.6A). While the aqueous and methanol extracts had similar ATPase inhibitory activities at all concentrations, their potencies were better than those of the vitamin C treatment group. Moreover, in Fig. 4.2.6B, *A. adianthifolia* aqueous and ethanol extracts significantly reduced glycogen phosphorylase activity better than the other treatment and the positive control (vitamin C), particularly at 240 $\mu\text{g/ml}$. Interestingly, the leaf ethanol extracts, amongst other treatments, significantly reduced ($p<0.05$) fructose 1,6-bisphosphatase activity when compared with the untreated tissue at the test concentrations (Fig. 4.2.6C).

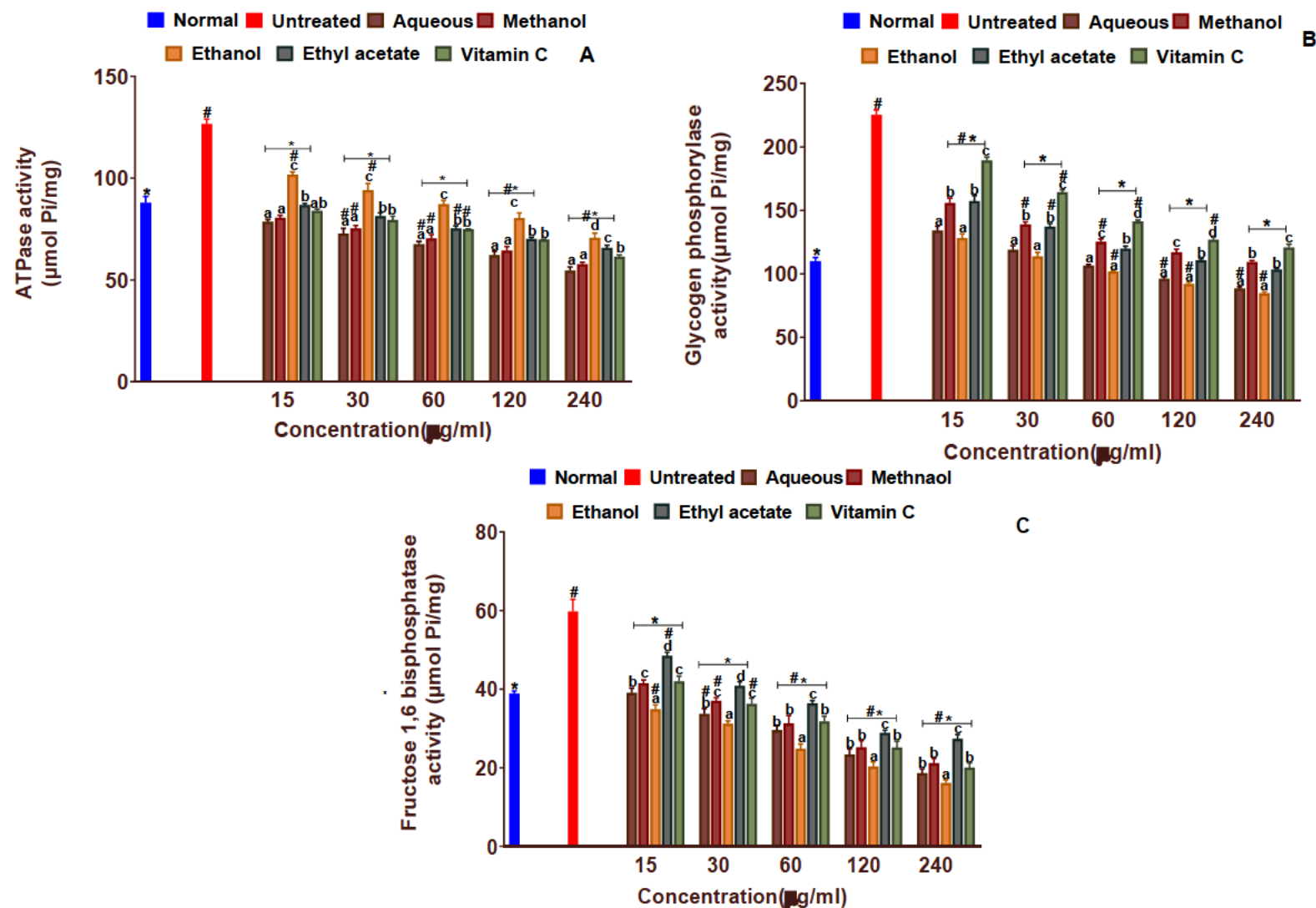


Figure: 4.2.6: The effects of *A. adianthifolia* crude extracts on (A) ATPase activity, (B) Glycogen phosphorylase activity and (C) Fructose 1,6 biphosphatase activity. Data are presented as mean $\pm$ standard deviation. Different superscripts a, b and c represent statistical significance of $p < 0.05$. *Significantly different from the untreated sample and #significantly different from the normal sample.

4.2.5 Discussion

The continuous rise in the prevalence of diabetes mellitus (DM) has adversely impacted the quality of life of individuals and placed an enormous burden on the global healthcare system (Bommer *et al.*,2018). Hyperglycaemia-induced oxidative stress, which results in dysregulated polyol and hexosamine pathways and increased protein kinase C activation, has been linked to developing long-term DM complications (Ighodaro, 2018). Conversely, previous studies have shown that traditional medicinal plants are endowed with secondary metabolites that act as antioxidants and may be beneficial in mitigating these pathological conditions' progression (Kooti *et al.*,2016). In the current study, *A. adianthifolia* extracts were shown to have considerable amounts of total phenolic (Fig. 4.2.1A) and flavonoid content (Fig. 4.2.1B), which reports have correlated with potent antioxidant activities (Zhang and Tsao, 2016). These properties, as exhibited by phenolic compounds, have been linked with delocalized π electrons or H-atoms from their OH functional groups and inherent ring structures. Consequently, dietary intake of foods with high amounts of phenolic compounds has been associated with the delayed onset of several degenerative diseases, including diabetes (Zhang and Tsao, 2016).

Dietary exogenous and endogenous antioxidants in the living cell system circumvent potential damage caused by excess reactive species (Huyut *et al.*,2017). In Fig. 4.2.2, *A. adianthifolia* extracts showed their antioxidant activity by their ability to scavenge DPPH free radicals and donate lone electron pairs to reduce the ferric ion. Uncontrolled hyperglycaemic induced oxidative stress by increasing reactive species concentration and hence their reaction with tissue membrane lipids in a process known as lipid peroxidation (Newsholme *et al.*,2016). An increase in this process has been recognized as a cause of oxidative stress and an exacerbating factor in DM pathophysiology and complications. The ability of the *A. adianthifolia* extracts to reduce lipid peroxidation in the heart homogenate (Fig. 4.2.5A) could insinuate their beneficial role in preventing damage to tissues of the circulatory system. Some findings suggest that coronary tissue damage via lipid peroxidation and atherosclerosis is implicated in the development of several cardiovascular diseases (CVD) (Matheus *et al.*,2013; Almourani *et al.*,2019).

Medicinal plants have also been known to ameliorate the damaging effects of free radicals by modulating the levels and activities of endogenous antioxidant defense systems (Adwas *et al.*,2019). In the current study, treatment with *A. adianthifolia* extracts increased the activities of superoxide dismutase (SOD) and catalase (CAT) while simultaneously increasing the level of reduced glutathione (GSH), as shown in Fig. 4.2.5B-D. These enzymatic and non-enzymatic antioxidant defense systems work in tandem and play critical roles in maintaining a homeostatic redox balance in the body (Adwas *et al.*,2019). SOD is a group of metalloenzymes that scavenges superoxide

radicals and converts them to hydrogen peroxide (H_2O_2), which is then immediately neutralized into water and oxygen by CAT before it can cause any tissue damage (Bratovic, 2020). On the other hand, the reduced glutathione (GSH) system is a major intracellular thiol-disulfide redox buffer dependent on NADPH, which aids in the amelioration of excess reactive species (Bratovic, 2020). Improved activity and capacity of antioxidant defense systems are particularly beneficial to diabetic patients who face the challenge of oxidative imbalance due to chronic hyperglycaemia.

As complex dietary carbohydrates pass down the digestive tract, they are successively broken down into glucose, which is eventually absorbed into the bloodstream via the intestinal membrane (Feinman *et al.*, 2015). Interestingly, previous studies have shown the importance of some oral pharmacological agents capable of inhibiting key enzymes involved in this catabolic process (Ali Asgar, 2013; Sanni *et al.*, 2020). They exert their antidiabetic properties by delaying the overall rate of carbohydrate digestion with a consequent reduction in glucose absorption rate, which ultimately blunts the postprandial plasma glucose spikes. Some of these target carbohydrate digestive enzymes include α -glucosidase found on the brush border interface of the small intestine and α -amylase in the saliva and pancreatic juice. As observed in this study, the *A. adianthifolia* extracts inhibition of α -glucosidase (Fig. 4.2.3B) and α -amylase (Fig. 4.2.3C) could suggest their capacity as an alternative therapy for controlling hyperglycaemia with delayed progression of DM-linked complications.

Glucose transportation across the brush border membrane is regulated by the sodium- dependent glucose transporter (SGLT1) and the facilitated-transporter (glucose transporter 2)(GLUT2) (Pico and Martínez, 2019). While the apical membrane constitutive SGLT1 is mainly active at a low luminal glucose concentration, GLUT2 is recruited from the basolateral membrane at higher glucose concentrations (Pico and Martínez, 2019). Consequently, phytoconstituents capable of reducing intestinal glucose absorption, as observed with the *A. adianthifolia* extracts in Fig. 4.6.4C, may function by inhibiting the actions of SGLT1 and GLUT2 (Pico and Martínez, 2019). After glucose is in the bloodstream, it is taken up by peripheral tissues such as the brain, muscle, or adipose tissue, which either utilize it immediately for energy production or store it for subsequent use. Glucose disposal by peripheral tissues clears blood glucose from circulation, thus aiding glycaemic regulation. While *A. adianthifolia* extracts unregulated glucose uptake in yeast cell suspension in Fig. 4.2.4A, there is the possibility that the product from the plant may exert a similar effect in different peripheral tissues. Previous studies have shown the close resemblance of the yeast cells to the mammalian cells, thereby making this model effective in assessing tissue glucose utilization processes. As observed in the current study (Fig. 4.2.4B), pharmacological therapeutic agents that enhance muscular glucose uptake may be beneficial for maintaining euglycemic levels in diabetic patients.

Under normal conditions, excess glucose is stored in the liver and muscles as glycogen. Conversely, in times of extended fasting, these glycogen stores are broken down into glucose to meet the body's metabolic needs (Nagy *et al.*,2013). Glycogen phosphorylase is among the various enzymes involved in the metabolic pathway leading to the breakdown of stored glycogen (Nagy *et al.*,2013). Inhibition of muscular glycogen phosphorylase enhances glycogen build-up in skeletal muscle, thereby upregulating its clearance from the blood. Glycogen phosphorylase enzymes and fructose-1,6-bisphosphatase also play vital functions in circulating blood glucose homeostasis (Somsaki *et al.*,2013). In the muscular tissue, fructose- 1,6-bisphosphatase replenishes the intramuscular glycogen pool through gluconeogenesis. However, increased fructose-1,6-bisphosphatase activity in the liver increases hepatic glucose release, which could further exacerbate chronic hyperglycaemia in diabetic patients. The consequent ability of the *A. adianthifolia* extracts to suppress glycogen phosphorylase and fructose-1, 6-bisphosphatase activities in Fig. 4.2.6B and Fig. 4.2.6C, respectively, may support the antidiabetic potency of the *A. adianthifolia* leaf extracts as observed previously in Fig. 4.2.3. Taken together, the ability of the plant extracts under the current study to increase muscular glucose uptake and inhibit the action of key glucogenic enzymes shows their potential as antidiabetic agent templates.

4.2.6 Conclusion

The results of this study suggest the antioxidant and antidiabetic activities of *A. adianthifolia* leaf extracts could be due to their inherent bioactive compounds, including flavonoids and phenolics. The extracts were shown to inhibit carbohydrate and lipid-digesting enzymes, promote muscular and hepatic glucose uptake, and retard its absorption by the small intestinal jejunum. The ethanol extract exhibited the overall best pharmacological activities compared to all other extracts, so it can be further studied using an experimental animal model to ascertain the results of this study.

4.3 *Albizia adianthifolia* crude bark extracts modulate glucose metabolism and ameliorate oxidative (FeSO₄) pancreatic tissue injury

¹Brian K. Beseni, ¹Kolawole A. Olofinson ^{1,2}Veronica F. Salau, ¹Md. Shahidul Islam*

1. Department of Biochemistry, University of KwaZulu-Natal, Westville Campus, Durban 4000, South Africa

2. Department of Pharmacology, University of the Free State, Bloemfontein 9300, South Africa

***Corresponding author:** islamd@ukzn.ac.za

4.3.1 Abstract

Traditional medicinal plants possess a multitude of secondary metabolites that are known to have therapeutic benefits for humans. *Albizia adianthifolia*, of the family Fabaceae, is a folkloric medicinal plant that is used to remedy several afflictions. The antioxidant capacity of the extracts was determined by the DPPH, FRAP, and NO inhibition assays following the quantification of their total phenolic and flavonoid content *in vitro*. Thereafter, the ability of the extracts to inhibit key carbohydrate and lipid digestive enzymes was investigated *in vitro*. The ability of the extracts to modulate muscular glucose uptake, retard intestinal glucose absorption, and regulate several glucogenic enzymes was determined *ex vivo*. Furthermore, the modulatory effects of *Albizia adianthifolia* on pancreatic FeSO₄-induced oxidative stress- linked parameters were investigated *ex vivo*. The ethanolic bark extract contained significantly ($p < 0.05$) higher concentrations of phenolics and showed enhanced antioxidant activity among all the extracts. *Albizia adianthifolia* extracts were shown to have pancreatic lipase, α glucosidase, and α amylase inhibitory activity. Furthermore, the extracts increased glucose uptake in muscle and liver tissues while reducing its absorption in the intestinal jejunum. The extracts improved pancreatic oxidative stress biomarkers with a concomitant reduction of glucogenic enzymes. The ethanol extract of *A. adianthifolia* bark extract exhibited the best overall pharmacological activities compared to all others, warranting further study using an experimental animal model to ascertain the results of this study.

Keywords: Antidiabetic, glucose uptake, *Albizia adianthifolia*, antioxidant, glucose absorption.

4.3.2 Introduction

The rampant increase in the global prevalence of diabetes mellitus (DM) is a growing concern, as an estimated 536.6 million individuals between the ages of 20 and 79 were reportedly living with the condition in 2022 (Sun *et al.*,2022). The global incidence of DM is projected to rise to 12.2% (783.2 million) by 2045 if the disease's current trajectory remains unchanged (Sun *et al.*,2022). Diabetes is characterized by metabolic dysfunction leading to chronic hyperglycaemia. Several heterogeneous aetiologies of DM are recognized based on their underlying biochemical characteristics and onset (Venkatesan, 2021). The most prevalent sub- types of diabetes include type 1, type 2, and gestational (Venkatesan, 2021). Despite these classifications, type 2 diabetes (T2D), the most common form of diabetes, stems from defects in insulin secretion, insulin action, or both and thus results in disturbances of carbohydrate, fat, and protein metabolism (Adler *et al.*,2021). Other forms of diabetes, such as those that are drug-induced and emanate as side effects of other ailments, including viral infections and cancer, are also known to exist (Banday *et al.*,2020).

Although the multifaceted biochemical triggers for the pathogenesis of diabetes remain poorly understood (Banday *et al.*,2020; Venkatesan, 2021), oxidative stress due to an imbalance between the cellular antioxidant defense systems and reactive species production has been majorly implicated in the pathogenesis and progression of the disease (Zhang *et al.*,2020). Interestingly, some reactive oxygen species (ROS), reactive nitrogen species (RNS), and reactive chloride species (RCS) may perform several important biochemical functions as secondary messengers, activators, and immune response modulators (Zhang *et al.*,2020). However, under homeostatic conditions, overproduction of these molecules can be deleterious and lead to dysregulated β pancreatic cell function and peripheral tissue insulin resistance (Yaribeygi *et al.*,2020; Eguchi *et al.*,2021).

Diabetes occurs as a comorbidity with several other conditions, such as hypertension, obesity, hyperlipidaemia, chronic kidney disease (CKD), and cardiovascular disease (CVD) (Igley *et al.*,2016). Moreover, according to the findings of Igley *et al.*, (2016), DM comorbidity burden increased with age and was higher in men than women. The scourge of disease is aggravated by the ineffectiveness of some of the current antidiabetic pharmacological agents, which are sub-categorized based on their modes of action into sulfonylureas, biguanides, and α -glucosidase inhibitors (Meneses *et al.*,2015). Additionally, there are concerns over the side effects, including contraindications, bowel irritations, weight gain, and hypoglycaemic episodes, of these current pharmacological agents (Meneses *et al.*,2015).

In this regard, scientists across the globe have thus sought to look for antidiabetic pharmacological templates to create novel drugs with higher efficacy and reduced undesirable side effects (Yatoo *et al.*,2017). Traditional medicinal plants, which inherently produce an array of secondary metabolites with therapeutic effects, have thus emerged as good choices for developing novel antidiabetic agents. *Albizia adianthifolia* of the family Fabaceae has long been recognized for its traditional use against several degenerative conditions, such as diabetes (Maroyi, 2018). Despite the wide use of *Albizia adianthifolia* and other local medicinal plants, their full clinical potential remains poorly documented. This study was thus carried out to determine the antioxidant and antidiabetic capabilities of the *A. adianthifolia* bark extracts using *in vitro* and *ex vivo* models.

4.3.3 Materials and methods

Please refer to Chapter 2, sections 2.4.1 to 2.11.5 for detailed materials and methods.

4.3.4 Results

The total phenolic and flavonoid contents of *A. adianthifolia* bark extracts are depicted in Figs.4.3.1A and 4.3.1B, respectively. The ethanolic bark extract contained a significantly ($p<0.05$) higher concentration of phenolics (16.37 mg/g) among all the extracts. It was followed by the aqueous extract (11.31 mg/g), while the ethyl acetate contained significantly ($p<0.05$) the least amount (6.44 mg/g) of phenol phytochemicals. In Fig. 4.3.1B, the bark ethanol extract (18.28 mg/g) contained significantly ($p<0.05$) the highest content of total flavonoids, whereas the aqueous extract (11.84 mg/g) had the lowest concentration. There was no significant difference between the flavonoid content of the methanol (14.45 mg/g) and ethyl acetate extracts (14.47 mg/g).

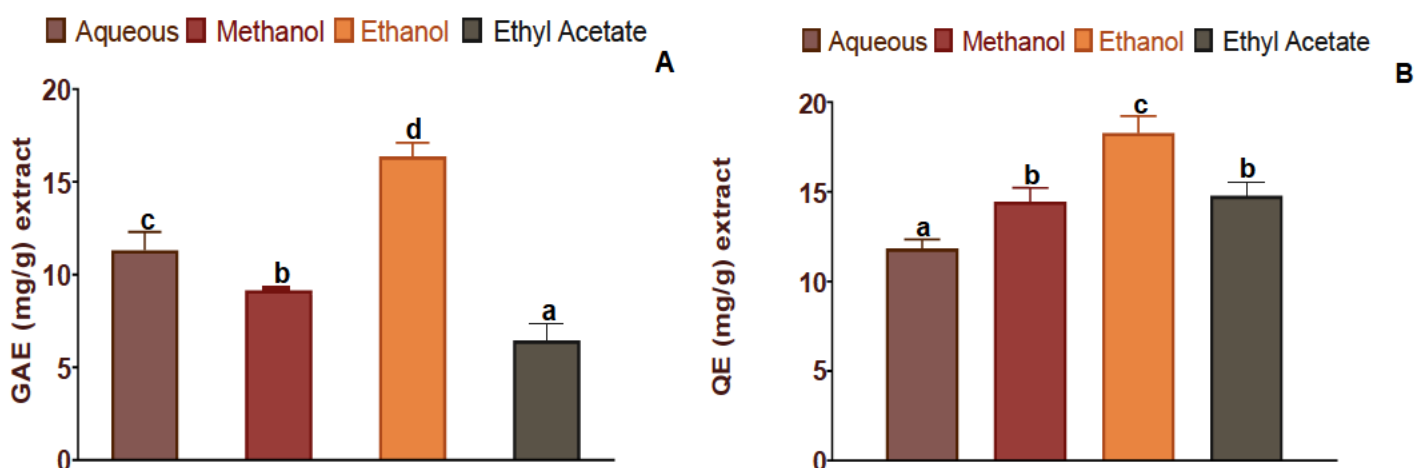


Figure: 4.3.1: The total phenolic content (A) of the *A. adianthifolia* crude bark extracts represented as gallic acid equivalent (GAE mg/g), and the total flavonoid content (B) represented as quercetin equivalent (QE mg/g). The superscripts a - d represent the statistical significance of $p<0.05$.

The antioxidant capabilities of *A. adianthifolia* bark extracts are presented in Figs. 4.3.2A-4.3.2C. The ethanol extract significantly ($p<0.05$) outperformed all the other extracts at all assayed concentrations except at 240 $\mu\text{g/ml}$. Moreover, the same extract ($\text{IC}_{50} = 10.53 \pm 0.15$) had comparable DPPH scavenging activity as the positive control (Vitamin C) with an IC_{50} value of 9.21 ± 0.12 $\mu\text{g/ml}$, as shown in Table 4.2. In the Fe^{3+} reducing antioxidant power assay, the ethanol extract ($\text{IC}_{50} = 48,76 \pm 0.93$ $\mu\text{g/ml}$) also showed the best activity, and this was closely followed by that of the aqueous extract with a $72,56 \pm 0.29$ $\mu\text{g/ml}$ IC_{50} value as shown in Table 4.3.1. The aqueous extract showed significantly ($p<0.05$) better nitric oxide inhibition capabilities than the other extracts except at 240 $\mu\text{g/ml}$, which displayed similar activities with the ethanol extract.

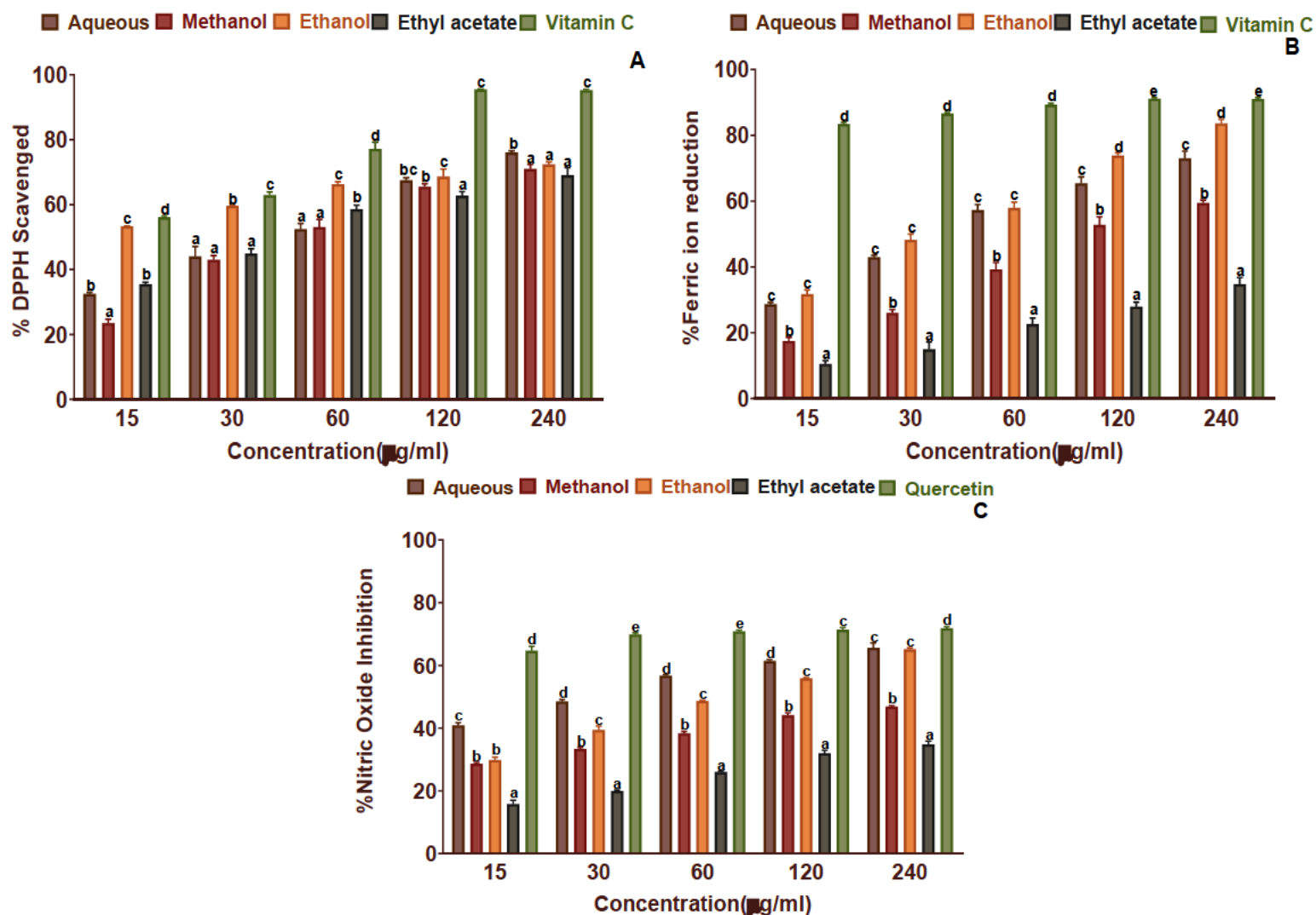


Figure: 4.3.2: *A. adianthifolia* bark organic extracts (A) DPPH radical scavenging activity, (B) Ferric ion reducing activity, (C) Nitric oxide production inhibition. Data are presented as mean $\pm$ standard deviation. Different superscripts a, b and c represent statistical significance of $p < 0.05$.

The lipid and carbohydrate digesting enzyme inhibitory capacities of *A. adianthifolia* bark organic extracts are shown in Figs. 4.3.3A–4.3.3C. In Table 4.3.1, the aqueous extract showed the best pancreatic lipase inhibition activity ($IC_{50} = 179,71 \pm 0,23 \mu\text{g/ml}$) among all the tested crude extracts. The *A. adianthifolia* bark ethanol extract showed significantly ($p < 0.05$) the least pancreatic lipase inhibition at the test concentrations. In Fig. 4.3.3B, the ethyl acetate extract had the best α -glucosidase inhibition with significantly higher activity than all the other extracts, especially at 120 $\mu\text{g/ml}$ –240 $\mu\text{g/ml}$ (Fig. 4.3.3B). However, the ethyl acetate extract's α -glucosidase inhibitory capacity did not surpass that of the acarbose-positive control. Although the extracts showed dose-dependent inhibition of α -amylase in Fig. 4.3.3C, the ethyl acetate extract exhibited the best activity with IC_{50} value of $151,46 \pm 0,89 \mu\text{g/ml}$, indicating higher potency relative to the other extracts (Table 4.3.1).

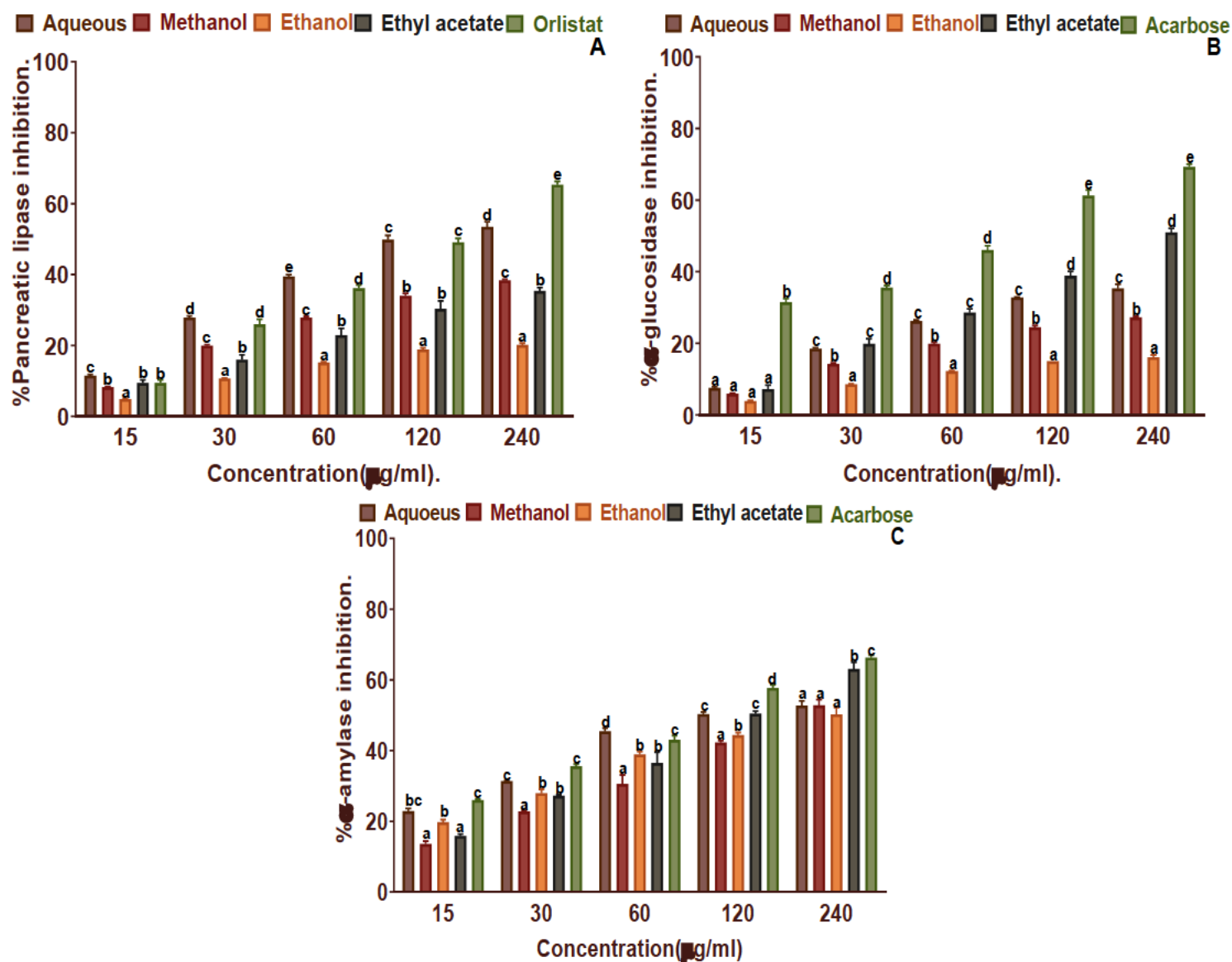


Figure: 4.3.3: *A. adianthifolia* bark extracts (A) Pancreatic lipase inhibitory activity, (B) α -glucosidase inhibitory activity, (C) α -amylase inhibitory activity. Data are presented as mean $\pm$ standard deviation. Different superscripts a, b and c represent statistical significance of $p < 0.05$.

Table 4.3.1: The IC₅₀ values of *A. adianthifolia* bark extracts under different antioxidant and enzyme inhibitory *in vitro* assays.

	Aqueous Extract	Methanol Extract	Ethanol Extract	Ethyl acetate Extract	Vitamin C	Quercetin	Acarbose	Orlistat
DPPH (µg/ml)	67,66±0,48	86,22±0,52	10,53±0,15	60,57±0,76	9,21±0,12	-	-	-
FRAP (µg/ml)	72,56±0,29	155,82±0,46	48,76±0,93	370,95±0,27	6,67±0,88	-	-	-
NO (µg/ml)	44,51±0,37	251,76±0,73	108,91±0,22	401,71±0,43	-	2,49±0,81	-	-
Pancreatic lipase(µg/ml)	179,71±0,23	310,52±0,71	725,66±0,89	228,35±0,47	-	-	-	114,52±0,83
α-glucosidase(µg/ml)	343,91±0,67	492,74±011	946,58±0,04	213,91±0,90	-	-	100,68±0,09	-
α-amylase (µg/ml)	175,18±0,23	202,52±0,54	208,47±0,69	151,46±0,89	-	-	118,57±0,97	-

Data are presented as mean ± standard deviation. Key: DPPH = 2,2-diphenyl-1-picrylhydrazyl. FRAP = Ferric reducing antioxidant power. NO = Nitric oxide assay.

The data for the effect of *A. adianthifolia* bark extracts on glucose uptake in yeast cells incubated with increasing concentrations of the plant is depicted in Fig. 4.3.4A. The bark extracts all displayed glucose uptake activities that were superior to those of the standard metformin drug. Amongst the extracts, the *A. adianthifolia* bark ethanol extract had significantly ($p<0.05$) the highest glucose uptake effect in yeast cells (Fig. 4.3.4A), with a 69.98% uptake increase at 240 $\mu\text{g/ml}$. On the other hand, the bark methanol extract had a significantly ($p<0.05$) higher percentage of glucose uptake in the muscle tissue (Fig. 4.3.4B) at the test concentration. The extract glucose uptake activity was also better when compared with insulin at 60–240 $\mu\text{g/ml}$. The retarding effect of the bark extracts of *A. adianthifolia* on glucose absorption by intestinal jejunum tissue is depicted in Fig. 4.3.4C. The bark ethanol extract considerably lowered intestinal glucose absorption when compared with the other extracts at all assayed concentrations. However, the ethyl acetate bark extract had the least inhibitory effect. All the *A. adianthifolia* bark extracts displayed better suppression of glucose absorption relative to the standard acarbose compound, especially at 60–240 $\mu\text{g/ml}$.

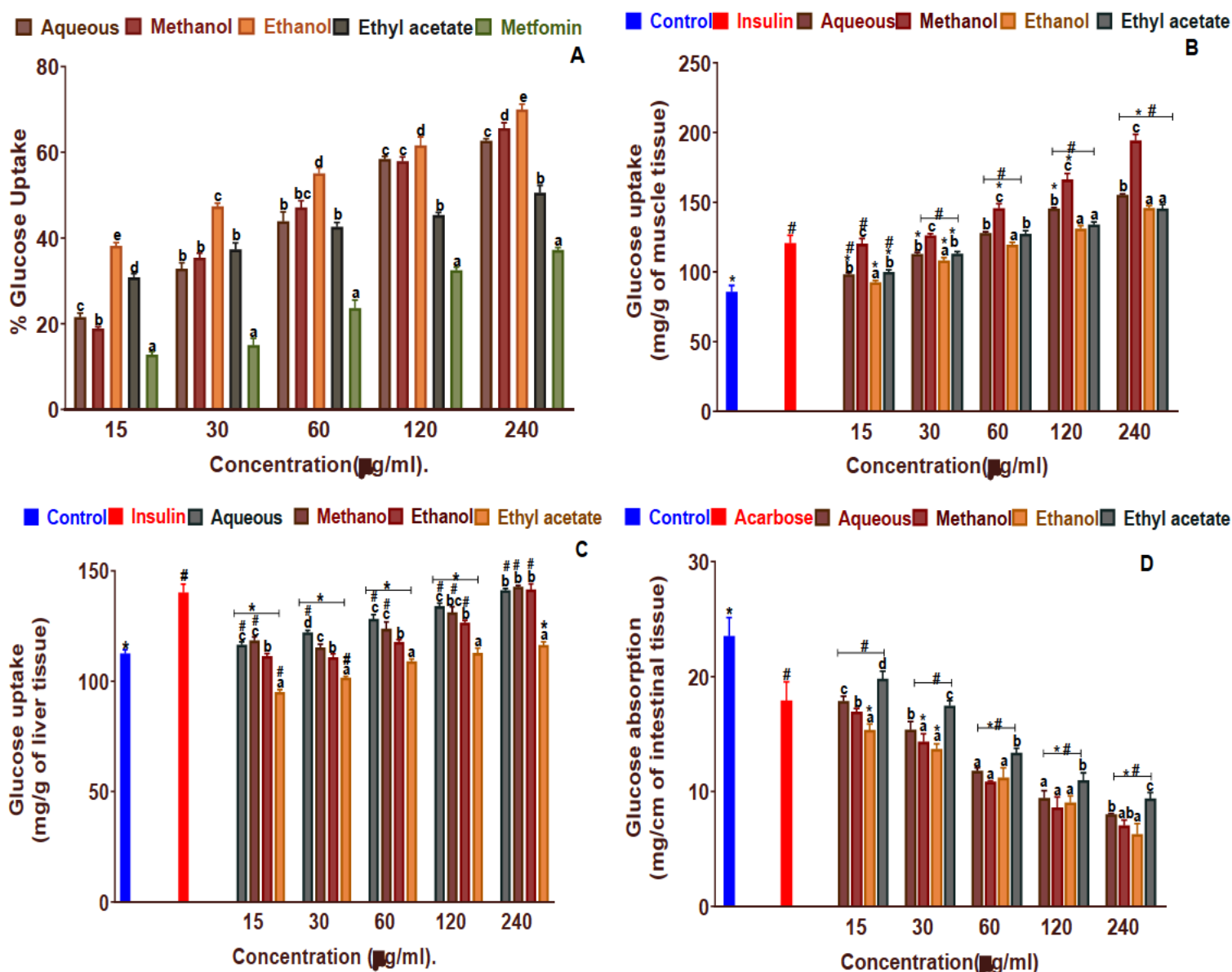


Figure: 4.3.4: The effects of *A. adianthifolia* bark extracts on (A) yeast cell glucose uptake, (B) psoas muscle glucose uptake and (C) intestinal glucose absorption. Data are presented as mean $\pm$ standard deviation. Different superscripts a, b and c represent statistical significance of $p < 0.05$. *Significantly different from untreated sample and #significantly different from normal sample ($p < 0.05$).

The results of the *A. adianthifolia* extracts effect on oxidative stress biomarkers analyzed in freshly harvested pancreatic homogenates *ex vivo* are depicted in Figs. 4.3.5A–4.3.5D. Oxidative pancreatic tissue damage due to FeSO_4 treatment resulted in elevated lipid peroxidation with diminished activities of superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) levels. There was a significant ($p < 0.05$) difference between the untreated tissue and the normal tissue in all the parameters analyzed (Fig. 4.3.5A–4.3.5D). Treatment with the *A. adianthifolia* bark extracts significantly ($p < 0.05$) reduced lipid peroxidation in the pancreatic tissue, as depicted in Fig. 4.3.5A.

The aqueous and methanol extracts had comparable activities at the test concentrations while reducing malondialdehyde (MDA) to near-normal levels at 120–240 $\mu\text{g/ml}$. In Fig. 4.3.5B, the bark ethanol extract significantly ($p<0.05$) enhanced SOD activity better than the other extracts and higher than vitamin C at 240 $\mu\text{g/ml}$. Though the bark extracts, as observed in Fig. 4.3.5C, increased catalase activity dose-dependently, the aqueous extracts significantly had the best catalase-enhancing abilities ($p<0.05$) higher than those of other extracts and vitamin C. However, in Fig. 4.3.5D, the *A. adianthifolia* bark ethanol extract significantly ($p<0.05$) increased GSH levels better than the other treatments.

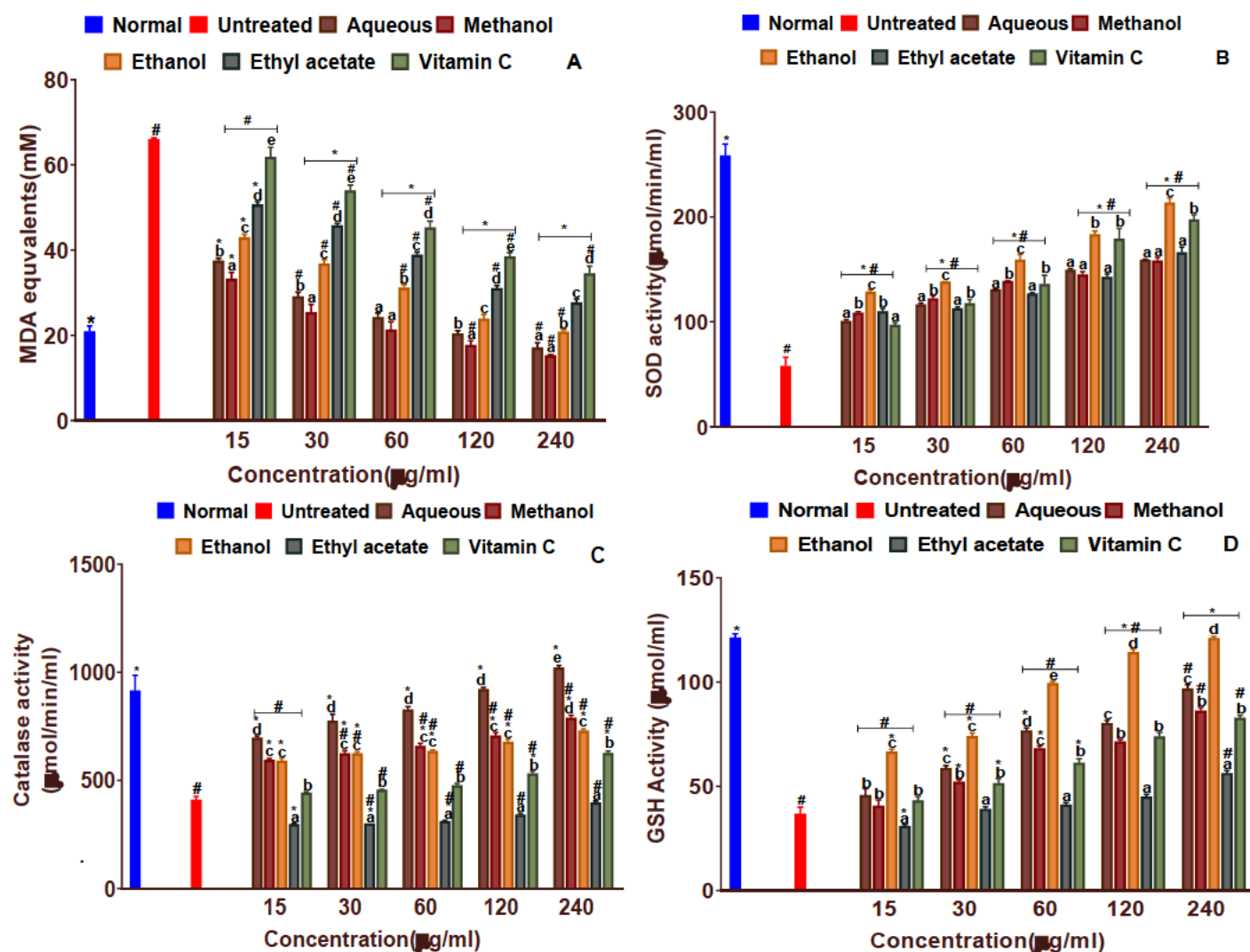


Figure: 4.3.5: Effects of *A. adianthifolia* bark extracts on (A) lipid peroxidation, (B) SOD activity, (C) Catalase activity and (D) GSH activity in oxidative pancreatic injury. Data are presented as mean $\pm$ standard deviation. Different superscripts a, b and c represent statistical significance of $p<0.05$. *Significantly different from untreated sample and #significantly different from normal sample.

The effects of *A. adianthifolia* bark crude extracts on ATPase, glycogen phosphorylase, and fructose 1,6-bisphosphatase activities in pancreatic tissue oxidative injury are represented (Fig. 4.3.6A–4.3.6C). Oxidative tissue injury after treatment with FeSO₄ resulted in a significant ($p<0.05$) increase in ATPase activity (Fig. 4.3.6A), glycogen phosphorylase activity (Fig. 4.7.6B), and fructose 1,6-bisphosphatase activity (Fig. 4.3.6C) compared to normal tissue. Treatment with the *A. adianthifolia* bark extracts resulted in a significant ($p<0.05$) reduction of ATPase activity in the untreated tissue at the test concentrations (Fig. 4.3.6A). The ethyl acetate extract of *A. adianthifolia* showed the least to lowest ATPase activity, while the other extracts showed activity comparable to vitamin C (Fig. 4.3.6A). There was a significant ($p<0.05$) reduction in the glycogen phosphorylase activity upon treatment with the bark extracts of *A. adianthifolia* (Fig. 4.3.6B). At the highest test concentrations, the glycogen phosphorylase activity in the extract-treated group decreased to a near-normal level. However, the result in Fig. 4.3.6B indicates that the ethanol extracts outperformed all the other extracts. In Fig. 4.3.6C, treatment with the *A. adianthifolia* bark extract significantly reduced ($p<0.05$) fructose 1,6-bisphosphatase activity below the untreated group level. The *A. adianthifolia* bark aqueous and ethanol extracts had comparable enzyme inhibitory activity, surpassing the methanol and ethyl acetate extracts, particularly at 240 µg/ml.

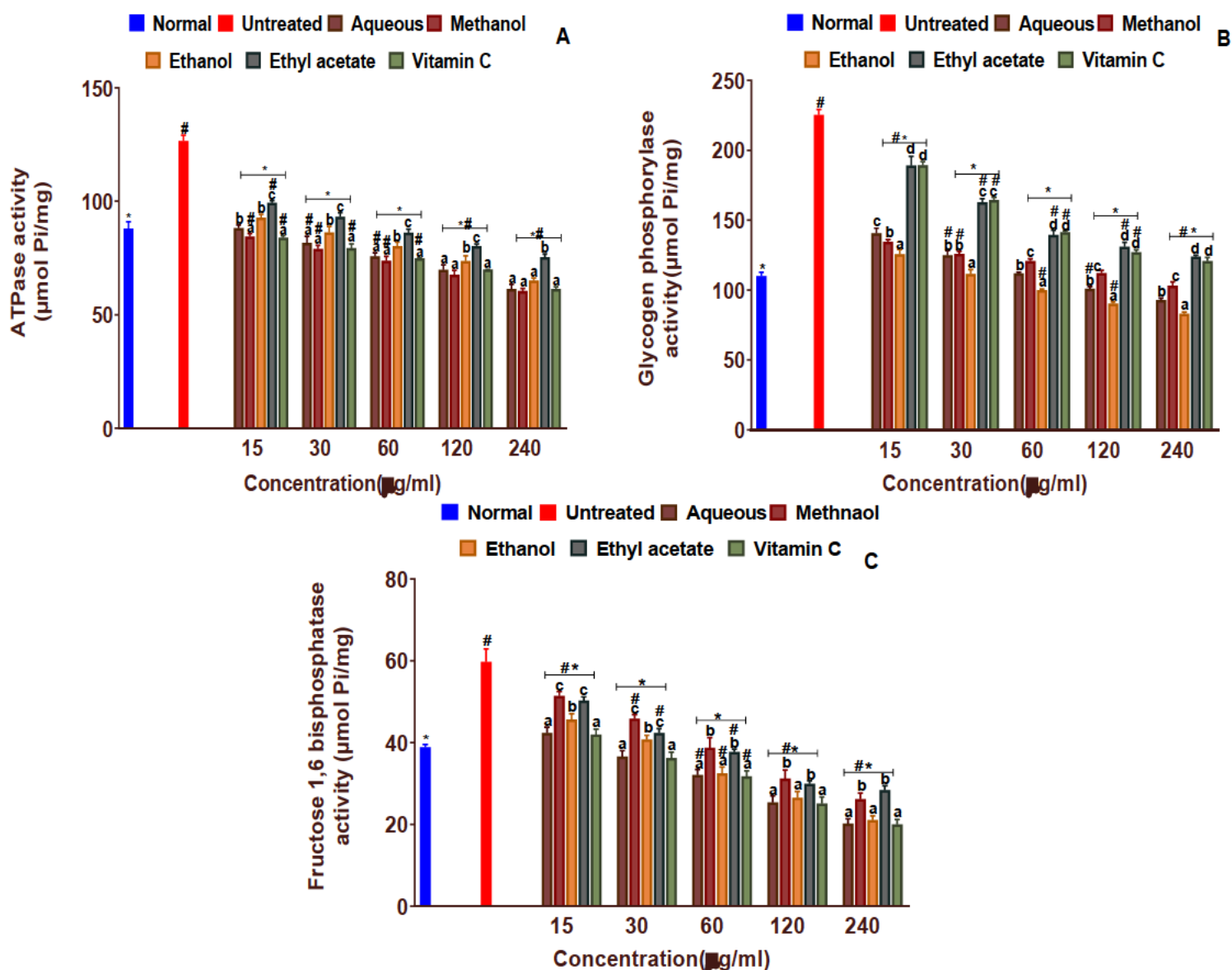


Figure: 4.3.6: *A. adianthifolia* bark crude extracts effect on (A) ATPase activity, (B) Glycogen phosphorylase activity and (C) Fructose 1,6 bisphosphatase activity. Data are presented as mean $\pm$ standard deviation. Different superscripts a, b and c represent statistical significance of $p < 0.05$. *Significantly different from untreated sample and #significantly different from normal sample ($p < 0.05$).

4.3.5 Discussion

The pathogenesis and progression of diabetes mellitus (DM) are linked to dysregulated insulin production or action emanating from oxidative stress (Eguchi *et al.*,2021). Therefore, plants with high antioxidant properties may have therapeutic effects against DM and its associated long-term complications. *A. adianthifolia*, which has been reported to contain a high antioxidant potential, was investigated for its antioxidant and antidiabetic activities to ascertain its folkloric use in managing diabetes. The therapeutic benefits of traditional medicinal plants stem from the bioactive secondary metabolites they produce, including saponins, terpenes, alkaloids, flavonoids, and phenolics (Mushtaq *et al.*,2018).

Plants rich in total phenolics and flavonoids (Fig. 4.3.1A and 4.3.1B), such as *A. adianthifolia* used in the current study, have been reported to have enhanced antioxidant biological activities (Kumar and Goel, 2019). *A. adianthifolia* in the current study demonstrated antioxidant capacity by scavenging free radicals (Fig. 4.3.2A) and donating their delocalized π electrons (Fig. 4.3.2B). Exogenous dietary antioxidants have been reported to maintain redox homeostasis and counter the effects of reactive species (RS) produced due to hyperglycemia- induced oxidative stress in diabetic patients (Jain *et al.*,2019). Increased oxidative stress is known to mediate tissue damage through lipid peroxidation, in which excess reactive species react with structural and functional lipids, rendering them non-functional. As observed in the current study under conditions of increased oxidative stress after FeSO₄ treatment, there is an increase in lipid peroxidation (Fig. 4.3.5A), which was reduced by the extracts of *A. adianthifolia*. Plant-derived secondary metabolites may aid in restoring oxidative balance by modulating the activities of endogenous antioxidant defense systems (Mushtaq *et al.*,2018). Consequently, the increased SOD and CAT activities and GSH levels in the *A. adianthifolia* extracts' treatment groups (Fig. 4.3.5B–4.3.5D) could insinuate the potential antioxidant activities of the plant, which may be beneficial for regulating a balanced redox in DM patients.

Maintenance of euglycemic glucose levels is central to the effectiveness of any DM management strategy, and this may be achieved through the reduction of postprandial hyperglycaemic spikes (Alam *et al.*,2019; Bailey and Day, 2019). Several approaches exist to achieve this, including inhibiting key carbohydrate-digesting enzymes such as α -glucosidase and α -amylase (Alam *et al.*,2019). The current study shows that the *A. adianthifolia* extracts inhibited the action of α glucosidase (Fig. 4.3.3B) and α amylase (Fig. 4.3.3C), which is beneficial to DM patients who are glucose intolerant (Alam *et al.*,2019). Inhibiting these enzymes retards the rate of glucose assimilation into the bloodstream, thereby allowing the circulatory insulin to offset the glucose load before it results in postprandial hyperglycaemic spikes (Chinsembu, K.C., 2019). These inhibitory activities of the plant extract correlate with their suppression of glucose uptake in the intestinal tissue, as observed in Fig. 4.3.4C.

Studies have shown that some antidiabetic agents modulate glycaemic control by reducing carbohydrate digestion rates and absorption with subsequent prevention of postprandial hyperglycaemic spikes (Alam *et al.*, 2019; Bailey and Day, 2019; Chinsembu, K.C., 2019).

The term diabetes has been coined to highlight the increasing comorbidity of DM and obesity (Farag and Gaballa, 2011). Obesity results when the amount of dietary calorie intake exceeds the amount utilized by the body for energy needs. Studies have shown that the increased β oxidation of excess lipids exacerbates inflammation due to enhanced proinflammatory cytokine activation. Therefore, the pancreatic lipase enzyme controls blood lipid levels, which mediates the digestion and eventual assimilation of dietary fatty acids. Phyto-constituencies that can inhibit the action of pancreatic lipase, as observed in the current study with the *A. adianthifolia* extracts (Fig. 4.3.3A), may thus help reduce the risk of obesity by delaying the rate of lipid absorption from the intestines. Moreover, the plant could also aid in weight control in diabetic patients with dyslipidaemia and other cardiovascular complications.

Glucose molecules from dietary carbohydrates absorbed into the blood are eventually assimilated by the brain, liver, and muscle tissues for energy metabolism. The overall effect of glucose uptake into these tissues is a reduction in circulatory blood glucose levels. Previous studies have shown that molecules that upregulate glucose uptake into peripheral tissues provide an avenue for glycaemic control. In the current study, the *A. adianthifolia* extracts increased glucose uptake in the yeast cells (Fig. 4.3.4A) and psoas muscle tissue (Fig. 4.3.4B). These findings are corroborated by Ho and colleagues' (2017) previous study, where some medicinal plants displayed hypoglycemic effects via promoting glucose uptake. Thus, *A. adianthifolia* may be beneficial in regulating blood glucose levels in diabetic patients by improving peripheral tissue glucose utilization while averting hyperglycaemia and its associated health issues.

Apart from exogenously acquired dietary glucose, it can be produced endogenously through the process of glycogenolysis by the liver. In diabetic patients, it has been observed that they have increased hepatic glucose output. Hepatic glucose output is mediated by a series of enzymes, including glycogen phosphorylase and fructose 1,6-bisphosphatase. In the current study, the extracts of *A. adianthifolia* were observed to reduce glycogen phosphorylase activities (Fig. 4.3.6A) and fructose 1,6-bisphosphatase (Fig. 4.3.6B), which are key to glucose output by the liver. Previous studies have shown the lucrative nature of glycogen phosphorylase and fructose 1,6-bisphosphatase inhibitors as antidiabetic pharmacological agents. These exert their hypoglycaemic activity by reducing the degradation of stored hepatic glycogen to glucose, which causes hyperglycaemia. These results show the potential of extracts of *A. adianthifolia* as potent hypoglycaemic agents that mediate their antidiabetic activity through the reduction of hepatic glucose output.

4.3.6 Conclusion

The results of this study indicate enhanced antioxidant and antidiabetic activities of *A. adianthifolia* through several mechanisms. The extracts' capability to scavenge free radicals and bolster endogenous antioxidant defense moieties points to their protective potential against oxidative stress. Furthermore, the extracts' antihyperglycemic potential was observed by their capacity to inhibit key carbohydrate and lipid-digesting enzymes as well as modulating enzymes linked to carbohydrate metabolism in the muscle and liver tissues. The ethanol extract of *A. adianthifolia* bark extract exhibited the best overall pharmacological activity compared to all other extracts, so it can be further studied using an experimental animal model to ascertain the results of this study.

CHAPTER 5

***IN VITRO*, *EX VIVO* AND *IN VIVO* STUDIES FOR INVESTIGATING THE ANTIOXIDANT AND ANTIDIABETIC ACTIVITIES OF *RHUS LONGIPES* (Engl.)**

5.1 *Rhus longipes* (Engl.)

5.1.1 Background and plant description

Rhus longipes (Engl.) synonym *Searsia longipes* (Engl.) Moffett of the family anacardiaceae is a wild small to medium tree commonly found in open woodlands and margins of forest/thickets (Moffett, 2007). It has distinct drooping stems and branches that bear trifoliate obovate pale green leaves with conspicuous net-veining. *Rhus longipes* branches are brownish grey and slightly furrowed with a cylindrical shape. The branchlets at the end of the main branches are of a similar colour although they appear angular, with a sparse to densely fulvous pilose. During the flowering season, it bears small yellowish-green axillary and terminal inflorescences. These flowers give rise to small fleshy round clustered fruits that ripen to give a distinct raisin-like taste. *R. longipes* and other related species are widely spread geographically throughout tropical regions of Africa from Burkina Faso, eastern Democratic Republic of Congo through Zimbabwe, Malawi, to South Africa (Olabanji, et al 2006).



Figure 5.1: *Rhus longipes* (Engl.): Common names: *Inhlokotshiyane* (Ndebele) Large-leaved rhus (English) *Mufokosiana* (Shona). Adapted without permission from www.zimbabweflora.co.zw.

5.1.2 Ethnobotanical uses

Maroyi in 2011 reported the traditional use of the root extract as a remedy for infertility in women and to dilate the birth canal by the people in the Nhema communal area in Zimbabwe. Furthermore, root and leaf sap extracts of the plant have been reported as laxatives and used for abortion (Burkill, 1995). The root decoction is drunk as a treatment for malaria (Burkill, 1995). In Ogbomoso, Southwest Nigeria, *R. longipes* is frequently used to cure malaria and asthma (Olorunnisola *et al.*, 2017). *R. longipes* has traditionally been documented to remedy stomachache and diarrhea (Mashimbye, 2019). Leaf and root decoctions of *Rhus natalensis* and *Rhus vulgaris*, close relatives of *R. longipes*, have also been similarly reported to remedy stomach aches and diarrhea (Degu *et al.*, 2022). Nyagumbo and colleagues in 2022 reported the use of the leaf decoction that is taken orally to remedy cough in Zimbabwe.

5.1.3 Biological activities

A previous study reported the effects of *R. longipes* extracts against the adult stage of *Schistosoma mansoni* and thus its beneficial capacity against schistosomiasis (Chacha and Mbugi, 2019). This study was further corroborated by Balahbib and colleagues in 2020 who reported the schistosomicidal activity of the methanolic stem bark extracts against several stages of the disease life cycle. Olasunkanmi in 2022 reported the antioxidant activity of *R. longipes* ethanolic extracts against 2, 2'-azino-bis 3-ethylbenzthiazoline-6- sulfonic acid (ABTS), 2, 2'-diphenyl-1-picrylhydrazyl (DPPH), superoxide (O_2^-), and nitric oxide (NO) radicals.

5.1.4 Phytochemistry

The identification of bioactive phytochemicals present in *R. longipes* remains scanty. However, a previous study conducted by Olasunkanmi in 2022 reported the presence of tannins, flavonoids, phenolics, terpenoids, steroids, alkaloids, and saponins in ethanol and aqueous extracts of *R. longipes*. Furthermore, this pioneer study identified the presence of compounds such as neophytadiene, octahydro-carotene, and 28-heptatriaconten-2-one, silane, aziridine, 1-(2-buten-2-yl), trimethyl-cholest-4-ene-3,6-diyl, tetramethyl tricyclo-undec-9-ene (Olasunkanmi *et al.*, 2022).

5.2 *Rhus longipes* (Engl.) infusions improve glucose metabolism and mitigate oxidative biomarkers in ferrous sulfate induced renal injury

¹Brian K. Beseni, ¹Kolawole A. Olofinsan, ²Veronica F. Salau, ^{1,2}Erukainure L. Ochuko, ¹Md. Shahidul Islam*

1. Department of Biochemistry, School of Life Sciences, University of Kwazulu-Natal, Westville Campus, Durban 4001, South Africa
2. Department of Pharmacology, University of the Free State, Bloemfontein 9300, South Africa

***Corresponding author:** islamd@ukzn.ac.za; Telephone: +27312608717

Preface: The study highlights potential bioactivities that scientifically justify the use of *Rhus longipes* in the traditional management of diabetes. The infusions' capacity to inhibit carbohydrate digesting enzymes, retard intestinal glucose absorption and increase muscular glucose uptake make them a promising pharmacological template for diabetic management. Additionally, this study highlights the antioxidant properties of the plant infusions vis-à-vis their protective effects on oxidative renal damage. Although the *Rhus longipes* fruits are edible, the study further established the low cytotoxicity of the bark and leaf, thus insinuating the relative safety of their intrinsic phyto-constituents. It has been published in the Asian Pacific Journal of Tropical. (Beseni, B.K., Olofinsan, K.A., Salau, V.F., Erukainure, O.L. and Islam, M.S., 2022. *Rhus longipes* (Engl.) infusions improve glucose metabolism and mitigate oxidative biomarkers in ferrous sulfate-induced renal injury. *Asian Pacific Journal of Tropical Biomedicine*, 12(11), p.453.<https://doi.org/10.4103/2221-1691.360561>)

5.2.1 Abstract

The current study investigated the antioxidant, antidiabetic, and potential cytotoxic activities of *R. longipes* leaf and bark aqueous infusions via *in vitro* and *ex vivo* studies. 100 g of dried *R. longipes* plant materials were infused in 1000 ml of boiling distilled water after an overnight defatting in n-hexane. The recovered extracts were characterized via Gas-Chromatography Mass Spectrometry (GC-MS) analysis and then used for *in vitro* antioxidant and digestive enzyme inhibitory activity studies. The modulatory effects of the *R. longipes* infusions on muscular glucose uptake, intestinal glucose absorption and biomarkers of renal oxidative injury were also investigated *ex vivo*. The binding affinities of the leaf and bark identified compounds on carbohydrate and lipid digestive enzymes were also screened via *in silico* computational analysis. The GC-MS result revealed the presence of several phyto-compounds, including

palmitoleic acid, octadecanamide, 24,25-dihydroxyvitamin D and L-ascorbic acid. The bark infusion had significantly ($p<0.05$) higher total phenolic contents than those of the leaf infusion while also demonstrating better DPPH scavenging ($IC_{50}=10.5\pm1.03\text{ }\mu\text{g/ml}$) and ferric reducing ($IC_{50}=9.85\pm0.32\text{ }\mu\text{g/ml}$) activities. Both *R. longipes* infusions at their highest concentrations significantly ($p<0.05$) increased glucose uptake in yeast suspension and rat psoas muscle with concomitant suppression of glucose absorption in the jejunum of the small intestine. With no cytotoxicity on normal Vero cells, the infusions lowered lipid peroxidation, increased cellular reduced glutathione (GSH) concentration and the activities of superoxide dismutase (SOD) and catalase (CAT) in renal homogenate treated with pro-oxidant (FeSO_4). *R. longipes* demonstrates great potential as a source of phyto-based antioxidant and hypoglycaemic pharmacological agents. Nonetheless, further molecular, and *in vivo* analyses are still required to ascertain these preliminary findings.

Keywords: Antioxidant, Oxidative biomarkers, Enzyme inhibition, Antidiabetic, *Rhus longipes*.

5.2.2 Introduction

Diabetes mellitus (DM) is an enervating metabolic disorder characterized by chronic hyperglycaemia, with type I, type II and gestational diabetes being the most prevalent types of the condition (Zimmet *et al.*,2016, ADA, 2015). Risk factors for DM include genetic predisposition, age, ethnicity, and unhealthy lifestyle (Zimmet *et al.*,2016). Similarly, high-calorie diets and lack of physical activity, which progressively develop into obesity, have also been implicated as a major growing risk factor and comorbidity of diabetes pathology (ADA, 2015).

In obese individuals, the elevated amounts of pro-inflammatory factors, non-esterified fatty acids, and cytokines lead to an increased cellular concentration of reactive free radicals, which ultimately suppress the activities of the body's intrinsic antioxidant enzymes, such as superoxide dismutase and catalase (Al-Goblan *et al.*,2014). Then, the redox imbalance or oxidative stress that emerges allows the excess free radicals to react with DNA, proteins, and lipids biomolecules, which gradually lose their biological structure and function (Ghasemi-Dehnoo *et al.*,2020). Another critical biochemical process associated with diabetes is insulin resistance. The development and progression of this condition at the cellular level occur when there is systemic loss of function of the cell surface receptors on insulin-responsive tissues. Besides the insulin resistance in diabetes, there is also the impairment of pancreatic β -cells function, resulting in reduced insulin production. These two situations synergistically will lead to aberrant glucose metabolism, which is proceeded by hyperglycaemia.

Chronic hyperglycaemia has been implicated as an underlying cause of increased morbidity and premature mortality in diabetes-associated complications (Gregg *et al.*,2016). These complications, which include cardiovascular and cerebrovascular diseases, retinopathy, nephropathy, and neuropathy, have been well documented in previous studies (Papatheodorou *et al.*,2016). As part of the complications, findings have indicated that diabetes patients have higher odds of developing diabetic kidney disease (DKD) than those without diabetes (Gheith *et al.*,2016). Interestingly, hyperglycaemia-mediated oxidative damage to renal blood vessels has been linked to dysfunctional glomerular hemodynamics in diabetic nephropathy (Fernandes *et al.*,2016). While there are many commercially available drugs for diabetes and its complications, available reports have shown that their utilization, especially by people living in the low to middle-income countries of the sub-Saharan African region, is limited by undesirable side effects, whereas for some others, it is the cost (Fernandes *et al.*,2018). In South Africa, up to 80% of people rely on or have consulted traditional medicine practitioners for their primary health care needs (Van Wyk and Prinsloo, 2018). Consequently, the efficacies of these medicinal plants must be scrutinized since there is no documented evidence of their standardized mode of action (Fernandes *et al.*,2018).

Rhus longipes, also referred to as *Searsia longipes* is a small tree with long drooping stems and branches of the family Anacardiaceae that bears small fleshy raisin-tasting fruits (Moffett, 2007). It is commonly known as Inhlokotshiyane (Zulu), large-leaved rhus (English) or Mufokosiana (Shona). *R. longipes* roots are traditionally used to treat infertility in women, while Zimbabweans in Mashonaland used leaves to alleviate diabetes-related symptoms (Maroyi, 2011). Moreover, in Southwest Nigeria, the plant has been reported to manage asthma and malaria (Olorunnisola *et al.*,2017).

To the best of our knowledge, the antidiabetic potentials of *R. longipes* remain poorly investigated. *R. longipes* use in managing diabetes and its complications in traditional medicine have been established. Therefore, this study aims to evaluate and scientifically document the bioactivities of the plant by determining its carbohydrate and lipid digestive enzymes inhibitory capabilities, tissue glucose absorption and uptake modulatory effects, and antioxidative protective potentials in oxidative renal injury using *in vitro*, *in silico* and *ex vivo* models.

5.2.3 Materials and methods

Please refer to Chapter 2, sections 2.4.1 to 2.11.5 for detailed materials and methods.

5.2.4 Results

As shown in Fig. 5.2.1, both infusions contained significantly ($p < 0.05$) higher amounts of phenolics than flavonoids. The bark infusion's total polyphenol (60.16 mg/g gallic acid equivalent), however, was significantly ($p < 0.05$) higher than those of the leaf infusion.

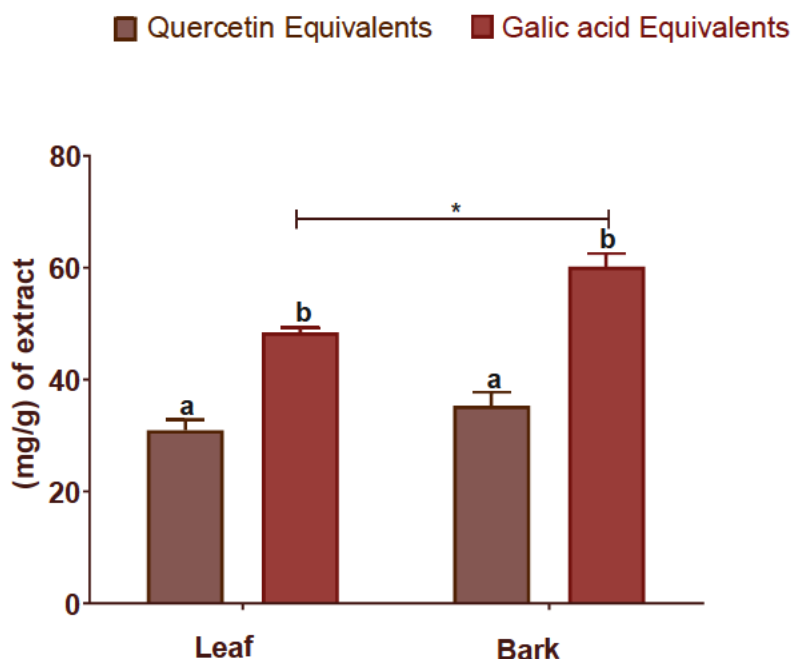


Figure 5.2.1: *R. longipes* leaf and bark infusions total phenolic and flavonoid contents measured in GAE mg/g and QE mg/g respectively. Superscripts a-b represent statistical significance of $p < 0.05$ within the infusion's groups. * Represents statistical significance of $p < 0.05$ between the infusion's groups.

GC-MS analysis of *R. longipes* leaf and bark aqueous infusion (Table 5.2.1) revealed the presence of n-Pentadecanol, 2-Ethyl-1-dodecanol, 1-(+)-Ascorbic acid 2,6-dihexadecanoate, Palmitoleic acid, Octadecanamide, 24,25-dihydroxyvitamin D, cis-11-Eicosenamide, β -D-Mannofuranoside, 1-O-(10-undecenyl)-, 2-Amino-1,3-propanediol, 1-Heneicosanol, Phytol, acetate, 5,15-Dimethylnonadecane, 1-(+)-Ascorbic acid 2,6-dihexadecanoate, n-Nonadecanol-1, cis-Vaccenic acid, L-Ascorbic acid, 6-octadecanoic, as depicted in Table 2. Palmitoleic acid had the highest % relative abundance (1.04%) in the leaf infusion, while 2-Amino-1,3-propanediol was the most abundant (7.35%) in the bark infusion. The compounds in the bark infusion had a higher percentage relative abundance than those in the leaf infusion of *R. longipes*. Although the two parts contained different phyto-

compounds, some compounds such as l-(+)-Ascorbic acid 2,6-dihexadecanoate, octadecanamide, cis-11- eicosenamide were present in both parts.

Table 5.2.1: Phyto-compounds in *R. longipes* leaf and bark infusions.

Compounds	Retention time (min)	Molecular Formula	Molecular weight (g/mol)	Relative abundance (%)
Leaf				
n-Pentadecanol	13.05	C ₁₅ H ₃₂ O	228.41	0.63
2-Ethyl-1-dodecanol	16.3	C ₁₄ H ₃₀ O	214.39	0.59
l-(+)-Ascorbic acid 2,6- dihexadecanoate	17.17	C ₃₈ H ₆₈ O ₈	652.9	0.86
Palmitoleic acid	18.91	C ₁₆ H ₃₀ O ₂	254.41	1.04
Octadecanamide	19.22	C ₁₈ H ₃₇ NO	283.5	0.88
24,25-Dihydroxyvitamin D	20.48	C ₂₇ H ₄₄ O ₃	416.6	0.59
cis-11-Eicosenamide	20.83	C ₂₀ H ₃₉ NO	309.5	0.51
β-D-Mannofuranoside, 1-O- (10-undecenyl)-	22.4	C ₁₇ H ₃₂ O ₆	332.4	0.68
Bark				
2-Amino-1,3-propanediol	4.07	C ₃ H ₉ NO ₂	91.11	7.35
1-Heneicosanol	15.267	C ₂₁ H ₄₄ O	312.6	1.24
Phytol, acetate	15.721	C ₂₂ H ₄₂ O ₂	338.6	1.93
5,15-Dimethylnonadecane	16.899	C ₂₁ H ₄₄	296.6	1
l-(+)-Ascorbic acid 2,6- dihexadecanoate	17.092	C ₃₈ H ₆₈ O ₈	652.9	5.49
n-Nonadecanol-1	18.252	C ₁₉ H ₄₀ O	284.5	4.91
cis-Vaccenic acid	18.871	C ₁₈ H ₃₄ O ₂	282.5	3.91
L-Ascorbic acid, 6- octadecanoate	19.042	C ₂₄ H ₄₂ O ₇	442.6	1.82
Octadecanamide	19.185	C ₁₈ H ₃₇ NO	283.5	1.47
cis-11-Eicosenamide	20.885	C ₂₀ H ₃₉ NO	309.5	1.56

As indicated in Figs. 5.2.2A and 5.2.2B, the infusions' DPPH scavenging and electron donating capabilities were dose-dependent. At lower concentrations (15-30 µg/ml), there was no significant ($p < 0.05$) difference in the radical scavenging activities of both the leaf and bark infusions (Fig. 5.2.2A). The bark infusion, however, had significantly higher activity at concentrations of 60-240 µg/ml with a lower IC_{50} value (10.5 ± 1.03 µg/ml), suggesting better antioxidant potency relative to those of the leaf infusion ($IC_{50} = 12.86 \pm 0.22$ µg/ml) as depicted Table 5.2.2. Although the bark infusion electron donating capacities ($IC_{50} = 9.85 \pm 0.32$) in Table 5.2.2 were higher compared to that of the vitamin C ($IC_{50} = 6.67 \pm 0.88$), the activity of the former was better than that of the leaf infusion ($IC_{50} = 11.00 \pm 0.10$). In Fig. 5.2.2C, the leaf and the bark infusions have significantly ($p < 0.05$) higher nitric oxide scavenging activities than the positive control (vitamin C) at all the test concentrations. Despite this observation, the NO activities of both infusions were not significantly ($p > 0.05$) different from each other at 60- 240 µg/ml (Fig. 5.2.2C).

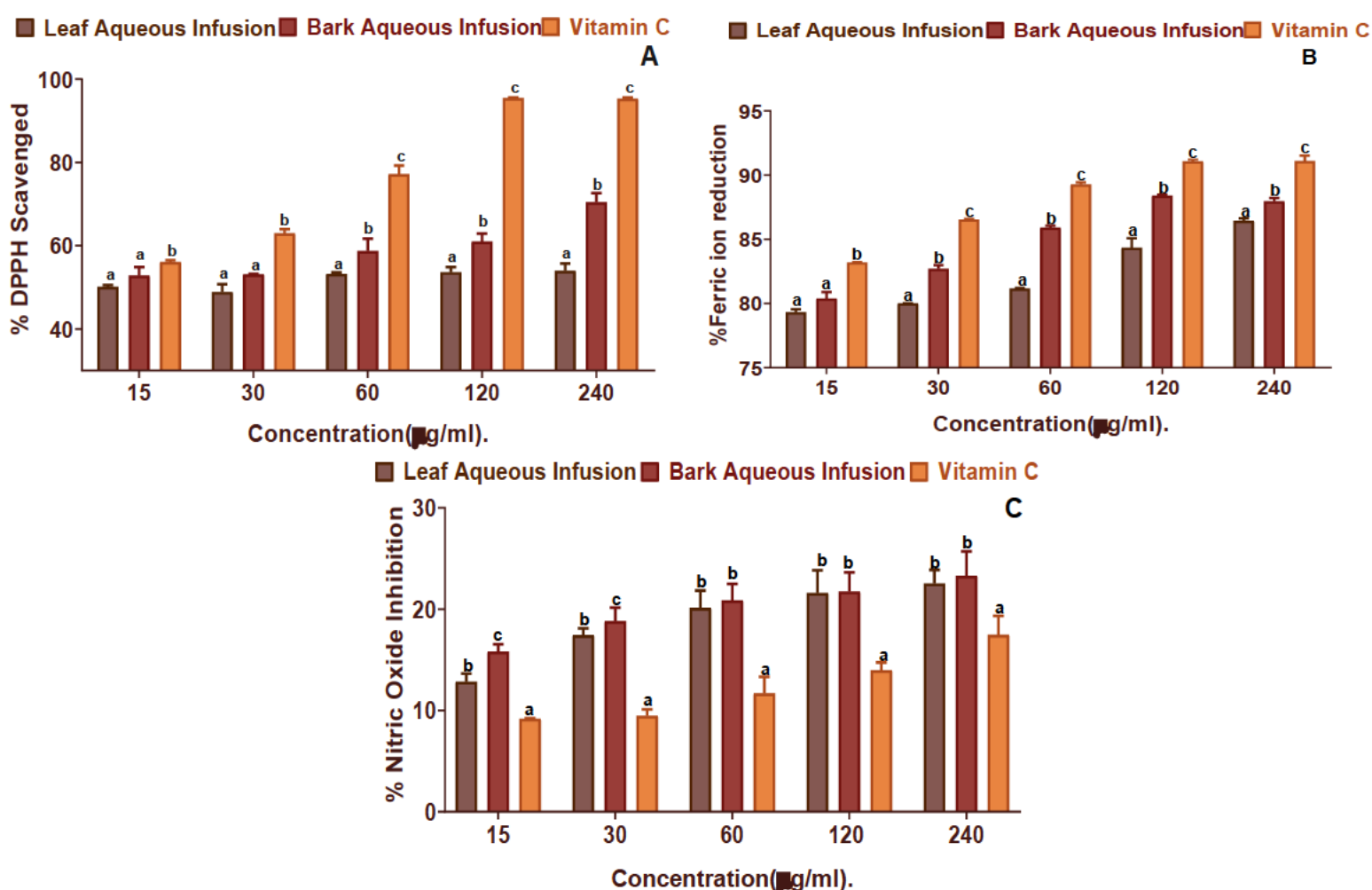


Figure 5.2.2: *R. longipes* leaf and bark infusions (A) DPPH scavenging (B) ferric reduction and (C) nitric oxide scavenging activities. Bars with superscripts a-c for each concentration are statistically different at $p < 0.05$ level of significance.

The leaf infusion significantly ($p<0.05$) outperformed the bark infusion in inhibiting α -amylase at all the assayed concentrations, as indicated in Fig. 5.2.3A with an IC_{50} value of $135.62 \pm 0.01 \mu\text{g/ml}$ (Table 5.2.2). At $240 \mu\text{g/ml}$, there was no significant difference in the inhibitory activity of the leaf infusion and the positive control (acarbose). In Fig. 5.2.3B, there was no significant ($p<0.05$) difference in the α -glucosidase inhibitory activities between the *R. longipes* leaf and the bark infusions at the test concentrations except at $60 \mu\text{g/ml}$. Moreover, in Fig. 5.2.3C, the *R. longipes* bark infusion had significantly ($p<0.05$) higher pancreatic lipase inhibitory activities with a lower IC_{50} value ($48.37 \pm 0.27 \mu\text{g/ml}$) in Table 5.2.2, indicating superior activity that surpasses those of the leaf infusion ($IC_{50} = 62.52 \pm 0.31 \mu\text{g/ml}$) and the orlistat ($IC_{50} = 123.55 \pm 0.81 \mu\text{g/ml}$) positive control.

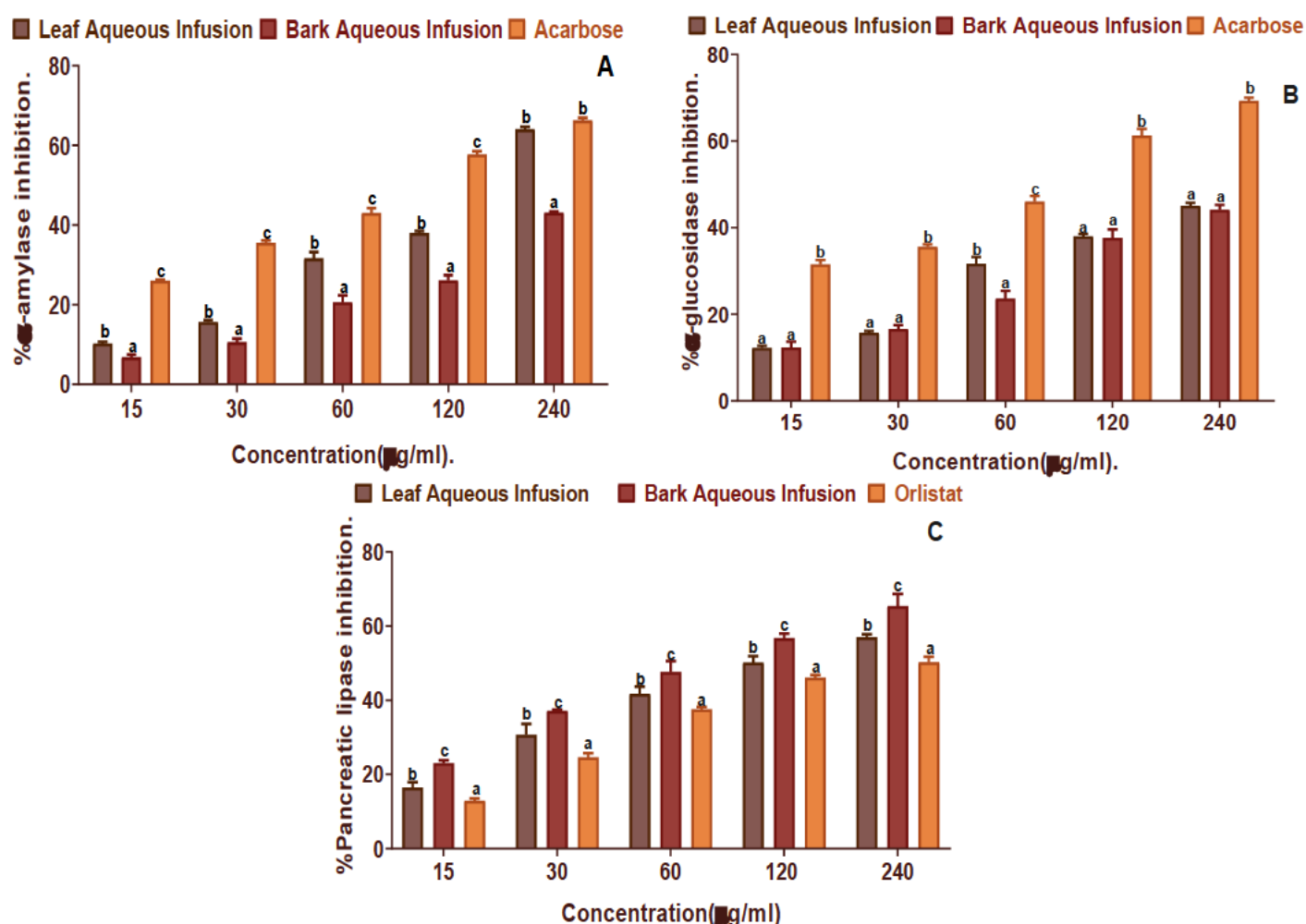


Figure 5.2.3: *R. longipes* leaf and bark infusions (A) α -amylase (B) α -glucosidase (C) pancreatic lipase inhibitory activities. Bars with superscripts a-c for each concentration are statistically different at $p<0.05$ level of significance.

Table 5.2.2: IC₅₀ values of *in vitro* biological activities of *R. longipes* leaf and bark aqueous infusions.

Activities	Leaf Infusion	Bark Infusion	Vitamin C	Acarbose	Orlistat
	(µg/ml)	(µg/ml)	(µg/ml)	(µg/ml)	(µg/ml)
DPPH	12.86 ± 0.22	10.5 ± 1.03	9.21 ± 0.12	-	-
FRAP	11.00 ± 0.10	9.85 ± 0.32	6.67 ± 0.88	-	-
NO	485.47 ± 0.12	472.01 ± 0.36	552.22 ± 0.41	-	-
α-Glucosidase	238.67 ± 0.45	242.32 ± 0.73	-	100.68 ± 0.09	-
α-Amylase	135.62 ± 0.01	311.38 ± 0.02	-	118.57 ± 0.97	-
Pancreatic lipase	62.52 ± 0.31	48.37 ± 0.27		-	123.55 ± 0.81

DPPH = 2,2-diphenyl-1-picrylhydrazyl. FRAP = Ferric reducing antioxidant power. NO = nitric oxide.

The leaf infusion showed competitive inhibition for α -amylase with K_m value = 2.0750 mM and V_{max} value = 0.1096 μ M/min (Fig. 5.2.4A), while the bark infusion showed mixed inhibition for α -amylase with K_m value = 0.9915 mM and V_{max} value = 0.0984 μ M/min. (Fig. 5.2.4B). The leaf infusion showed competitive inhibition for α -glucosidase with K_m value = 1.8383 mM and V_{max} value = 0.3799 μ M/min (Fig. 5.2.4C), while the bark infusion showed mixed inhibition for α -glucosidase with K_m value = 4.053 and V_{max} value = 0.5309 μ M/min. (Fig. 5.2.4D). Both the leaf and bark infusions of *R. longipes* showed competitive inhibition of pancreatic lipase. The K_m and V_{max} values for the leaf infusion were 6.9826 mM and 1.6507 μ M/min (Fig. 5.2.4E), while the bark K_m and V_{max} values were 8.7504 mM and 1.6507 μ M/min, respectively as indicated in Fig. 5.2.4F.

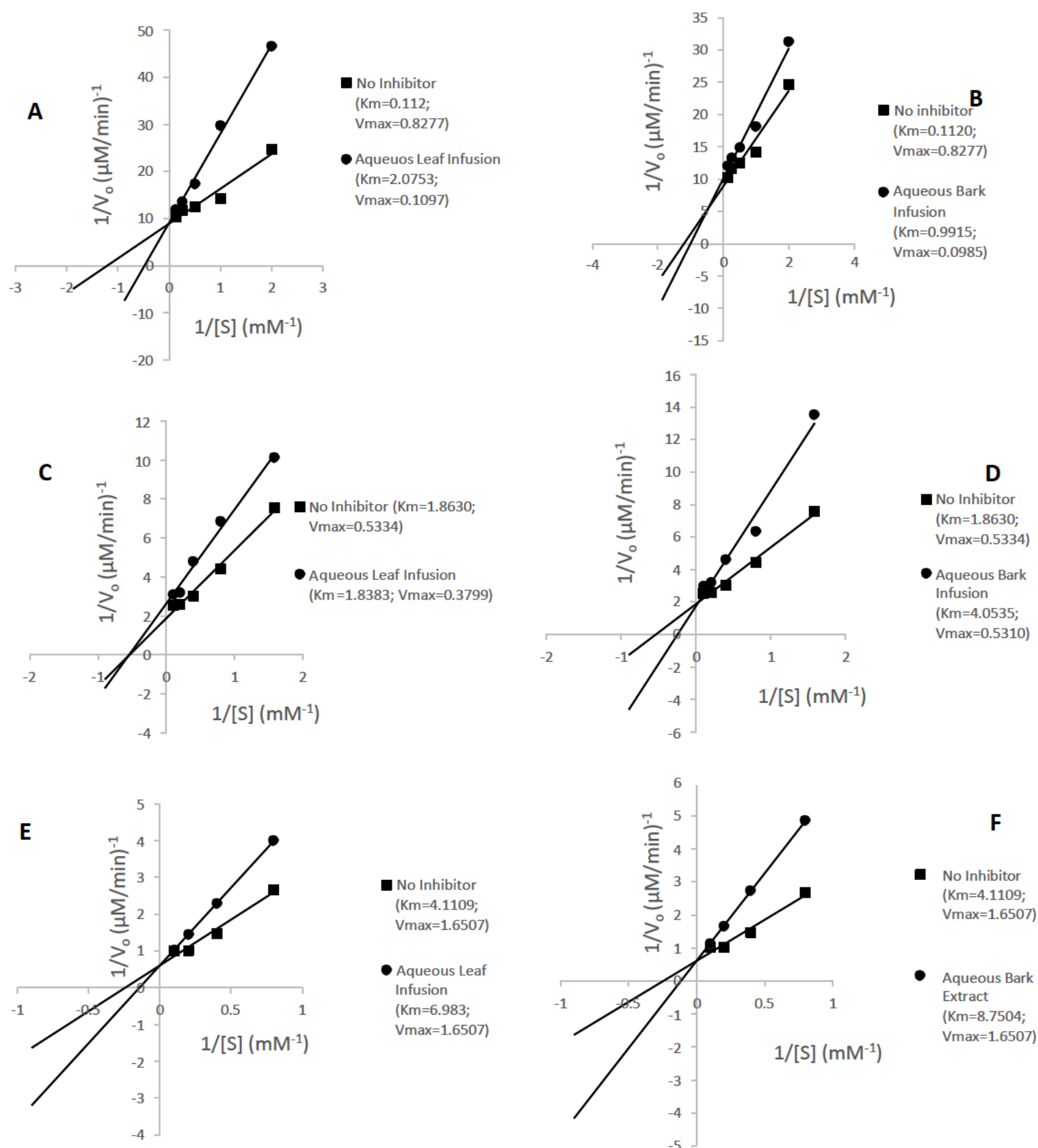


Fig. 5.2.4: The double reciprocal plots of α -amylase (A-B) α -glucosidase (C-D) and pancreatic lipase (E-F) inhibition in the presence or absence of *R. longipes* leaf and bark infusions.

After the 24 hours treatment period, the result of the MTT cytotoxicity test, as depicted in Fig. 5.2.5, indicates that there was no significant ($p < 0.05$) difference between the % cell viability between the leaf and bark infusion at 15 - 120 $\mu\text{g/ml}$ assay concentrations. Interestingly, there was no significant difference between the % cell viability of the normal Vero cells and those treated with the leaf and bark infusions. Treatment with H_2O_2 , however, significantly ($p < 0.05$) reduced the cell viability to 57.27% in relation to the normal control.

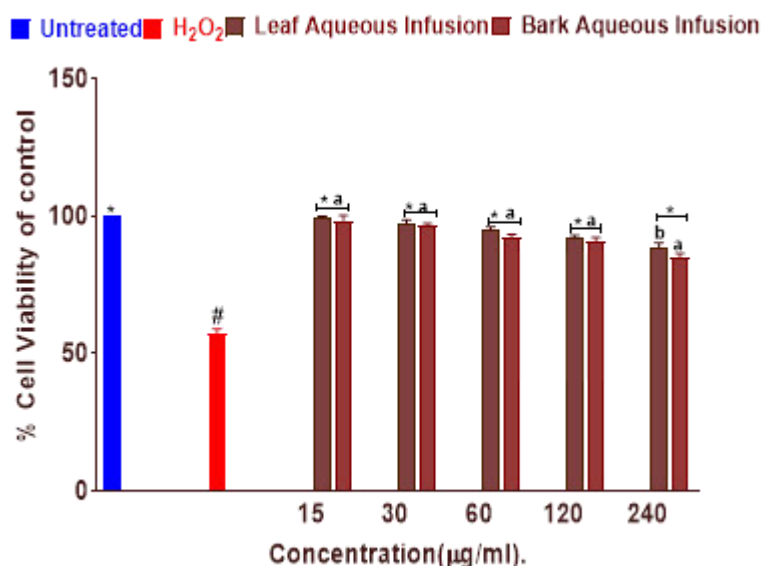


Figure 5.2.5: Cytotoxic activities of *R. longipes* leaf and bark infusions against Vero cells. Leaf and bark infusions bars with superscripts a-b at each concentration are statistically different at $p < 0.05$ while those with * and # are significantly different from H_2O_2 and normal control treatment, respectively.

As depicted in Fig. 5.2.6A, there was a dose-dependent increase in glucose uptake in yeast cells incubated with the plant infusions. At the assayed concentrations, the bark infusion significantly ($p < 0.05$) increased glucose uptake to an extent better than the leaf infusion. Similarly, in Fig 5.2.6B, there was an upward trend in the amount of glucose taken up by the rat psoas muscle tissue with increasing concentrations of the leaf and bark infusions. However, the bark infusion only at 240 $\mu\text{g/ml}$ had significantly higher glucose uptake activity exceeding those of the positive control treatment (insulin). The infusions also in Fig. 5.2.6C suppressed intestinal glucose absorption dose-dependently. At the higher concentrations (120 – 240 $\mu\text{g/ml}$), both the leaf and bark infusions significantly ($p < 0.05$) outperformed acarbose which served as the positive control.

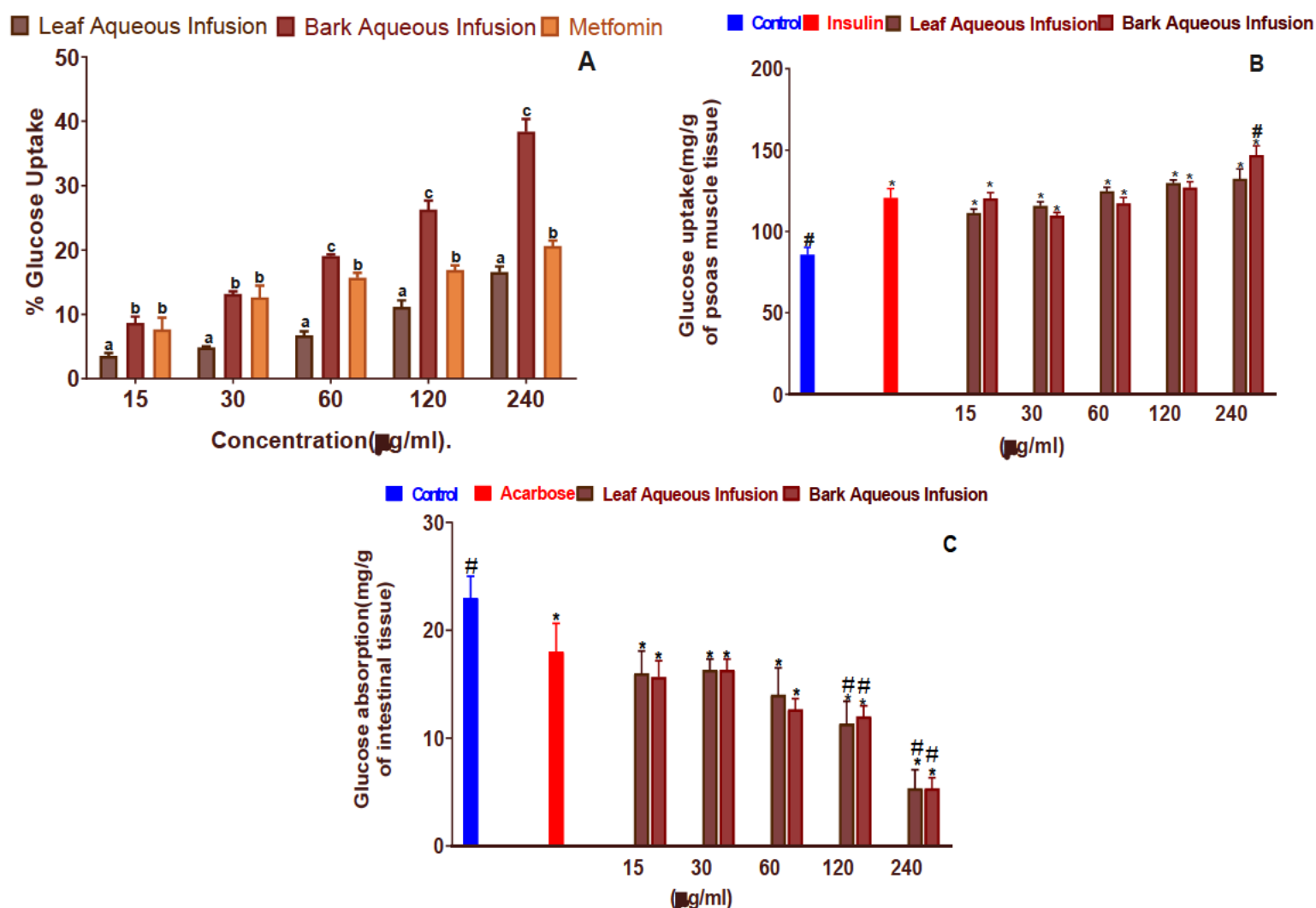


Figure 5.2.6: The effects of *R. longipes* leaf and bark infusion on (A) glucose uptake in yeast suspension (B) glucose uptake in excised rat psoas muscle (C) glucose absorption in the intestinal jejunum. Bars with superscripts a-b are statistically different at $p < 0.05$ whereas those with * and # are different from the control and acarbose treatment, respectively.

As shown in Figs. 5.2.7A – 5.2.7D, FeSO_4 induction led to significantly ($p < 0.05$) increased MDA levels while reducing the level of GSH as well as SOD and catalase activities. Treatment with the leaf and bark aqueous infusions of *R. longipes* resulted in a dose-dependent reduction in MDA to near normal levels (Fig. 5.2.7A). Treatment with the infusions at all concentrations produced activities that were better than those of vitamin C. While all the infusions gradually increased the GSH concentration above the untreated control (Fig. 5.2.7B), the bark displayed potency exceeding the leaf infusion and the antioxidant standard. Moreover, in Fig. 5.2.7C, the leaf and bark increased SOD activity in their treatment groups. The leaf infusion at 60-240 µg/ml, however, portrayed activities that were better than those of the bark infusion and

vitamin C. Both infusions increased the catalase activity to proportions significantly higher than the positive control at all concentrations (Fig. 5.2.7D). When comparing the effects of the plant infusions on the enzyme, the leaf infusion displayed activity better than the bark infusion.

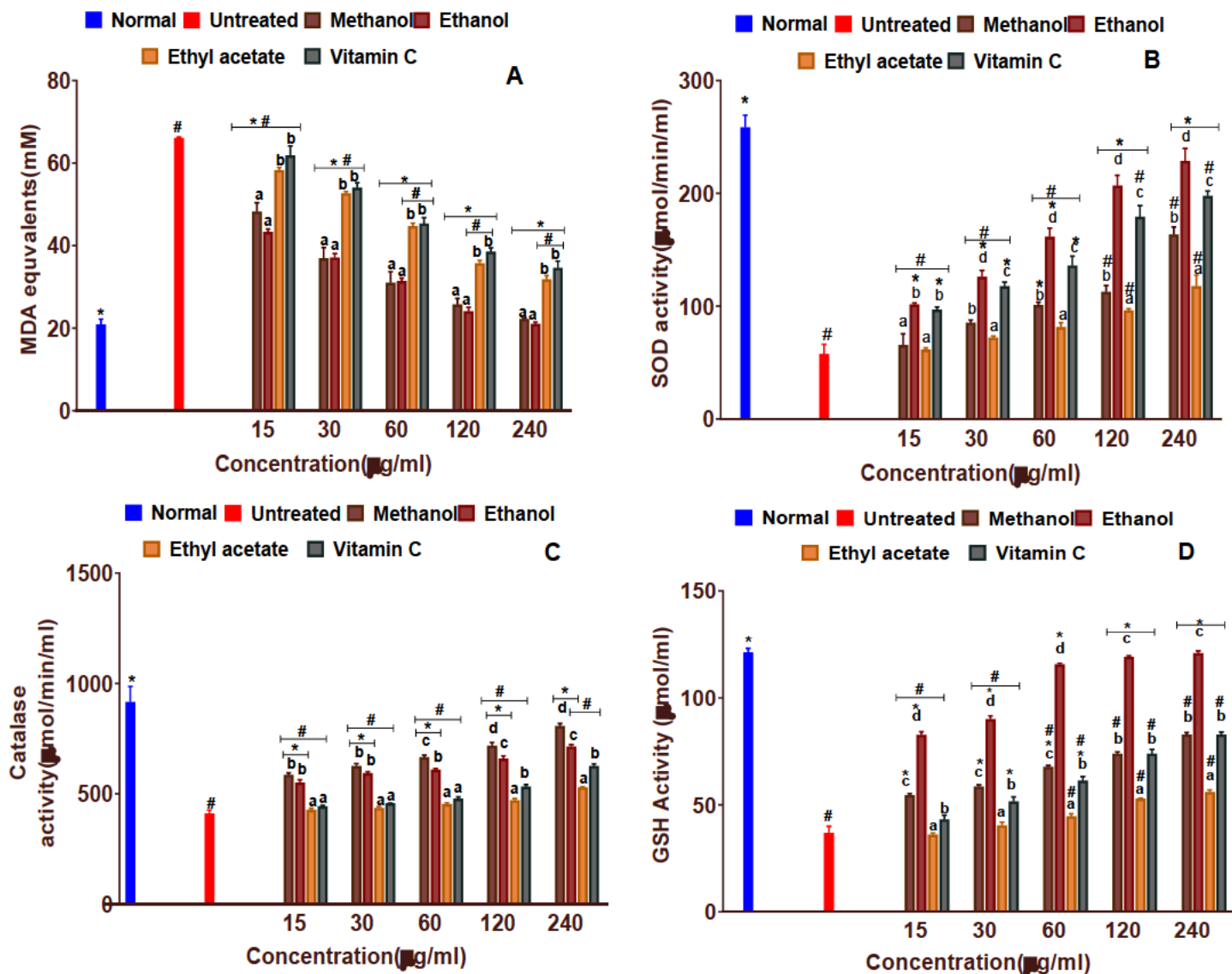


Figure 5.2.7: The effects of *R. longipes* leaf and bark infusion on (A) lipid peroxidation (B) GSH level (C) SOD activity (D) CAT activities on oxidative induced kidney injury. Bars with superscripts a-b are statistically different at $p < 0.05$ whereas those with * and # are different from the untreated and normal control treatment, respectively.

The molecular docking scores in Table 5.2.3 indicate that 24,25-Dihydroxyvitamin D had the lowest free binding energy with the digestive enzymes. The respective -8.6 kcal/mol, -9.4 kcal/mol and -9.0 kcal/mol binding energy that the compound produced with α -glucosidase, α -amylase and pancreatic lipase were comparatively lower than those of the protein standard inhibitors (Table 5.2.3). The 24,25-Dihydroxyvitamin D best 2D ligand-receptor interaction poses with α glucosidase, α amylase, and pancreatic lipase are shown in Figs. 5.2.8A – 5.2.8C, respectively, suggest that the compound formed multiple interactions with the pocket protein residues of enzymes. These include a conventional H-bond with HIS 1584 and Pi-Alkyl-bonds with TRP1369, TRP1355 and PHE1427 amino residues of α -glucosidase (Fig. 5.2.8A). While forming only Pi-Sigma Pi-Alkyl with α -amylase active site residues, it was able to interact via stronger hydrogen bonds amongst other forces with amino acids found at the pancreatic lipase catalytic pocket (Fig. 5.2.8C).

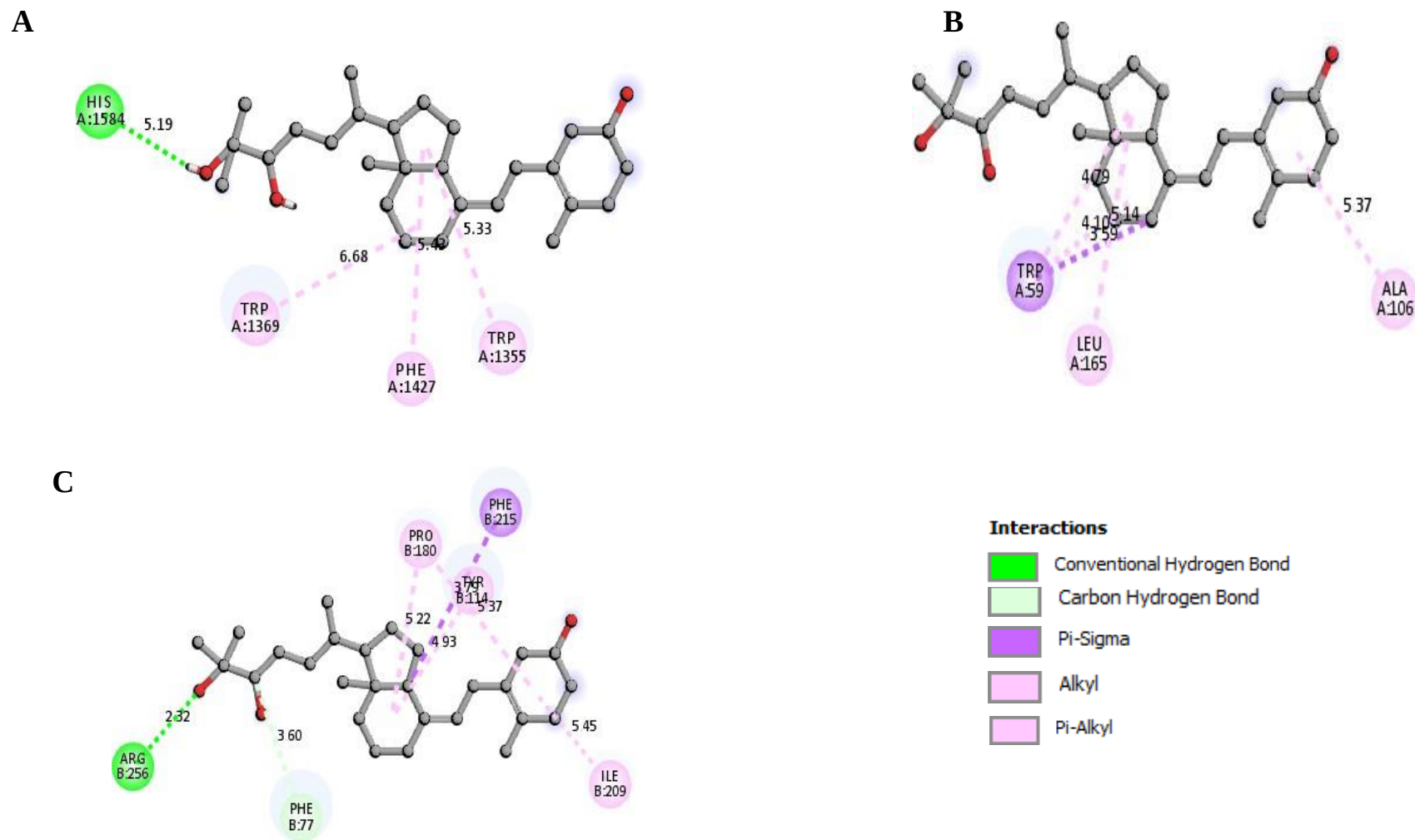


Figure 5.2.8: 2D images of the molecular interactions between 24,25-Dihydroxyvitamin D and active site amino acid residues of (A) α -glucosidase (B) α -amylase (C) pancreatic-lipase.

Table 5.2.3: Free binding energy of *R. longipes* leaf and bark infusions secondary metabolites with carbohydrate and lipid digestive enzymes.

Ligand	Free energy of binding (kcal/mol)		
	α -Glucosidase	α -Amylase	Pancreatic lipase
Acarbose	-7.5	-7.8	-
Orlistat	-	-	-6.7
n-Pentadecanol	-4.6	-5.1	-5.8
2-Ethyl-1-dodecanol	-5.7	-5	-5.4
l-(+)-Ascorbic acid 2,6-dihexadecanoate	-7.0	-5.8	-6.6
Palmitoleic acid	-5.3	-5.0	-5.1
Octadecanamide	-5.9	-5.4	-6.2
24,25-Dihydroxyvitamin D	-8.6	-9.4	-9.0
cis-11-Eicosenamide	-5.6	-5.0	-6.4
β -D-Mannofuranoside, 1-O-(10-undecenyl)-	-6.7	-5.9	-6.5
2-Amino-1,3-propanediol	-3.8	-3.9	-3.7
1-Heneicosanol	-5.4	-5.0	-5.7
Phytol, acetate	-5.9	-6.0	-6.9
5,15-Dimethylnonadecane	-5.9	-5.6	-5.8
n-Nonadecanol-1	-5.8	-4.9	-5.0
cis-Vaccenic acid	-5.2	-5.3	-6.2
L-Ascorbic acid, 6-octadecanoate	-7.0	-6.0	-6.4

5.2.5 Discussion

The global upsurge in the prevalence of diabetes in the last decade has prompted the immediate need for novel intervention strategies (Gaonkar and Hullatti, 2020). Plants produce various secondary metabolites, constituting a reservoir of beneficial pharmacological agents. Reports have indicated that about 70% to 80% of the rural African population still employ traditional medicine for their primary healthcare needs (Van Wyk and Prinsloo, 2018, Gaonkar and Hullatti, 2020). The current study investigates the potential cytotoxicity, antidiabetic and antioxidant activities of the leaf and bark infusions of the folkloric medicinal plant *R. longipes*.

Innate and dietary acquired exogenous antioxidants counteract the harmful effects of excessive reactive species produced during the normal metabolic activities of the body (Schieber and Chandel, 2014). When the protective capacities of these antioxidant systems become insufficient in the body, it causes oxidative stress. This biochemical process has been implicated in the aetiology of many degenerative conditions, including diabetes (Cabello-Verrugio *et al.*, 2016). Indeed, studies have described chronic hyperglycaemia-induced oxidative stress as a factor promoting the development and progression of nephropathy. While representing one of the most common symptoms of end-stage diabetes complications, this renal tissues-associated pathology is characterized by severe impairment of kidney functions. The abilities of the *R. longipes* stem bark and leaf infusions to donate free electrons (FRAP) (Fig. 5.2.2B) and scavenge free radicals (DPPH and NO) in Figs. 5.2.2A and 5.2.2C could be evidence of their potent antioxidant pharmacological properties. These observations are further supported by the results in Fig 5.2.7, where the infusions reduced the cellular concentration of lipid peroxidation product (MDA) while enhancing antioxidant protein level and activities in kidney tissues treated with FeSO₄ pro-oxidant. Plants contain a wide variety of phytoconstituents, including phenols, flavonoids, carotenoids, and tannins with excellent antioxidant properties (Palipoch, 2013). Findings have shown that the aromatic ring on phenolic compounds is endowed with hydrogen and electron-donating abilities in addition to transition metal ion chelating capabilities (Malam *et al.*, 2012). Therefore, the observed antioxidative effects of these infusions may be attributed to their total phytochemical composition (Fig. 5.2.1) and the presence of several phyto-compounds in the plant, as revealed by the GC-MS analysis (Table 5.2.2). This observation is coherent with previous studies, which show a positive correlation between high total phenolic and flavonoid content and antioxidant capacity (Malam *et al.*, 2012).

When complex dietary carbohydrates are ingested, they pass through the digestive tract and are broken down into simple sugars, which are subsequently absorbed by the small intestines. Consequently, one of the modes of action employed by some antidiabetic agents in restoring homeostatic blood glucose levels in a diabetic state is retarding the absorption of glucose in the small intestine (Dabur *et al.*,2018). These pharmacological agents perform these functions by competing with substrate molecules at the active site of carbohydrate digestive enzymes such as α -amylase and α -glucosidase. Thus, these inhibitors stall carbohydrate polymer hydrolysis into monosaccharide sugar units and decrease the latter biomolecule's absorption into the bloodstream (Dabur *et al.*,2018). In the current study, the potency of the infusions, more importantly, those of the leaf in inhibiting α -amylase (Fig. 5.2.3A) and α -glucosidase enzymes (Fig. 5.2.3B) may suggest the plant as a viable source of alternative antidiabetic therapy. Interestingly, these findings are also supported by the kinetic inhibitory studies (Fig. 5.2.4), which indicated the leaf infusion competitive inhibition of the enzymes. Additionally, the infusion's ability to reduce glucose absorption in intestinal tissue (Fig. 5.2.6C) could further evidence the *R. longipes* parts' antidiabetic properties.

Studies have shown that increased dietary lipid intake and assimilation lead to obesity, a significant risk factor and comorbidity of diabetes. In this regard, compounds from plants with pancreatic lipase inhibitory activities are described as better alternatives to synthetic chemicals (Liu *et al.*,2020). Indeed, in Fig 5.2.3C and Table 5.2.1, the *R. longipes* leaf and bark infusions displayed lipase inhibitory properties that exceed that of the orlistat clinical drug. Moreover, the result of the enzyme mode of inhibition studies in Fig. 4 suggests that phyto-compounds in the infusion may compete with lipid substrate at the active site of the digestive enzyme. Among the compounds in the infusions that could be attributed to this antilipase bioactivity is 24,25-dihydroxyvitamin D, which had the lowest binding affinity with the enzyme (Table 5.2.3). As could be observed in Fig. 5.2.8C, the ability of the infusions to competitively inhibit lipase may be associated with the strong hydrogen bond and other molecular interactions formed between the compound and the enzyme's active site amino residues. Previous investigations have shown that dihydroxyvitamin D and some of the other compounds in Table 5.2.2 exert their inhibitory activity by acting as competitive, mixed, non-competitive, or un-competitive inhibitors, as seen in Figs. 5.2.4 (Bansode *et al.*,2016).

Cytotoxicity studies, in which the toxic effects of plant-derived compounds are tested on normal cells, are an important first step in determining the success of a pharmaceutical therapeutic agent's development. In this regard, plant extracts with minimal or no significant

adverse effect on the viability of normal cell lines (Fig. 5.2.5) are sought after in the development of oral pharmacological agents because of their low toxicological effects (McGaw *et al.*,2014). In a previous study, *R. longipes* was shown to be non-toxic to female Swiss albino mice at concentrations of up to 2000 mg/kg body weight (Chacha and Mbugi, 2019). Plant extracts with low toxicity additionally have reduced undesirable side effects as they work harmoniously with the body's natural biochemical pathways (Kooti *et al.*,2016). The reduced cytotoxic effects of *R. longipes* infusions demonstrated in this study at the tested doses may therefore justify its use in traditional diabetes management decoctions.

Another mechanism employed by antidiabetic agents in restoring the normoglycemic state in diabetic conditions is by promoting glucose uptake in peripheral tissues (Olofinisan *et al.*,2022, Salau *et al.*,2020). Under normal physiological conditions, insulin signals facilitate glucose transport in skeletal muscle tissues via activating their glucose transporter 4 (GLUT4) cell surface receptor. However, in diabetes, this glucose metabolism signalling pathway is impaired due to insulin resistance. The ability of the *R. longipes* infusions to increase glucose uptake in yeast cells in Fig. 5.2.6A and, more importantly, psoas muscle tissues (Fig. 6C) may justify the antidiabetic efficacies of the plants used in traditional diabetic management. In support of this study, Woldemariam and Van Winkle's previous findings demonstrate that plant extracts that increase glucose uptake in yeast models may encourage increased glucose utilization by peripheral tissues in diabetic patients.

5.2.6 Conclusion

This study evidenced *R. longipes* leaf and bark infusions' remarkable antioxidant potential through their abilities to scavenge free radicals while enhancing endogenous antioxidant systems in oxidative renal injury. The efficacies of the infusions in suppressing intestinal glucose absorption while increasing the sugar uptake in isolated rat psoas muscle also indicate the plant's antidiabetic properties. As the plant did not show much cytotoxicity, further *in vivo* findings are still required to decipher the biological activities of the infusions under physiological conditions in live animals.

5.3 *Rhus longipes* (Engl.) bark extracts inhibit carbohydrate digesting enzymes activity, promote psoas muscle and hepatic glucose uptake and mitigate FeSO₄-induced pancreatic oxidative injury.

¹Brian.K. Beseni, ¹Almahi. I. Mohamed, ¹Kolawole A. Olofinisan, ²Veronica F Salau,

¹Nontokozo. Z. Msomi, ¹Ochuko L. Erukainure ¹Md. Shahidul Islam*

1. Department of Biochemistry, School of Life Sciences, University of Kwazulu-Natal, Westville Campus, Durban 4001, South Africa.
2. Department of Pharmacology, University of the Free State, Bloemfontein 9300, South Africa.

*Corresponding author: Md. Shahidul Islam: islamd@ukzn.ac.za

5.3.1 Abstract

Rhus longipes leaf and stem bark aqueous infusions have been shown to have antioxidant and some antidiabetic activities *in vitro*. The current study investigated the effects of its stem bark extracts on diabetes and oxidative stress using *in vitro*, *ex vivo*, and *in silico* experimental models, as well as identified its bioactive compounds. Total polyphenol and flavonoid contents, antioxidant, and antidiabetic capacities of the methanol, ethanol, and ethyl acetate extracts of *R. longipes* stem bark were investigated *in vitro*. The effects of *R. longipes* extracts on glucogenic enzymes, muscle and liver glucose uptake, intestinal glucose absorption, and modulatory effects on oxidative pancreatic injury were determined *ex vivo*. The bioactive phytochemicals were identified by Gas Chromatography-Mass Spectrometry (GC-MS), and their binding affinities with various enzymes were determined *in silico*. The ethanolic extract exhibited significantly ($p < 0.05$) higher amount of total polyphenol and exhibited the best antioxidant and α -glucosidase inhibitory activities, lower intestinal glucose absorption and high glucose uptake activities compared to other extracts. Furthermore, it significantly ($p < 0.05$) reduced lipid peroxidation levels and increased reduced glutathione level, and superoxide dismutase and catalase activities in oxidative pancreatic injury. Among the GC-MS identified bioactive compounds, 1,3-Benzenediol, o-(2-furoyl)-o'-(2-methoxybenzoyl) and phenol, 3-pentadecyl showed the best interactions in the active sites of the target enzymes with lowest binding affinities compared to all other compounds. The data indicate that the ethanolic extract demonstrated promising antioxidant and antidiabetic activities, among all extracts, which warrant further research in experimental animal models to confirm the findings of this study.

Keywords: *Rhus longipes*, Stem bark extract, Glucose absorption, Glucose uptake, Antidiabetic, Antioxidant.

5.3.2 Introduction

The prevalence of Diabetes mellitus (DM) has surpassed all previous global records and is expected to soar further. Though an estimated 463 million people globally were affected by diabetes in 2019, these statistics are projected to rise by 51% by 2045 (Thomas, 2019). While this disease accounted for 5.1% of the total mortality in South Africa in 2014, deaths due to the pathology have been predicted to rise to 5.5% by 2017 (Thomas, 2019). DM is a chronic metabolic disorder triggered by aberrant glucose metabolism, which ultimately culminates in hyperglycaemia (Kerner and Brückel 2014). Risk factors associated with DM include age, diet, ethnicity, lack of physical activity and genetic predisposition (Pan *et al.*, 2016). Based on aetiologies, DM can be classified as Type I, type II, and gestational diabetes. Despite these classifications, the precise pathogenesis of DM remains poorly understood due to the multifactorial nature of factors that could contribute to the disease's emergence (Ma *et al.*, 2018). However, oxidative stress has been implicated as a major contributing factor in the development and progression of DM (Asmat *et al.*, 2016).

Oxidative stress is an imbalance between the production of reactive oxygen species (ROS) with other free radicals and endogenous antioxidant defence systems of the body (Pamplona and Costantini, 2011). Though homeostatic levels of some ROS are essential as secondary messenger molecules in several pathways, excess production of ROS, reactive nitrogen species (RNS), and reactive chloride species (RCS) could cause damage to cell surface receptor proteins, integral lipids, and DNA functional biomolecules (Ma *et al.*, 2018; Tiwari *et al.*, 2013). Interestingly, studies have described oxidative stress to mediate hyperglycemia in diabetes and, consequently, aberrant glucose metabolism regulation via dysfunctional insulin synthesis in the pancreas or hormone insensitivity in the muscle or liver tissues (Ghasemi-Dehnoo *et al.*, 2020).

Morbidity and premature mortality associated with DM emanate from the disease's complications, broadly categorized as either microvascular or macrovascular complications (Long and Dagogo-Jack, 2011). Indeed, the management of these complications, which include retinopathy, nephropathy, neuropathy, coronary heart disease, cerebrovascular disease, and peripheral vascular diseases, has been linked to substantial global economic loss (Iglay *et al.*, 2016; Long and Dagogo-Jack, 2011). Consequently, the rising costs of DM medication have prompted about 80% of people in rural Africa to rely on traditional medicinal herbs for their primary healthcare needs (Maroyi, 2017).

The use of traditional medicinal plants, which has been practiced for generations, is increasingly gaining more attention around the world (Maroyi 2017). Unlike existing synthetic pharmacological treatments, novel plant-derived therapeutics have the added benefit of being readily available with relatively reduced adverse side effects (Manukumar *et al.*, 2017). *Rhus longipes* is a folkloric traditional medicinal plant of the family anacardaceae. Locally, the plant has been reported to remedy infertility and as a dilating agent for the birth canal during childbirth (Maroyi 2011). In several areas of Nigeria, different parts of the plant have been described to alleviate malaria and asthma (Olorunnisola *et al.*, 2017). Pharmacologically, Olorunnisola *et al* (2017) found that *R. longipes* has high antioxidant capabilities and modulating selected endogenous antioxidants may prove effective in preventing paracetamol-induced hepatic toxicity. Very recently, we also reported the antioxidant and antidiabetic activities of the aqueous infusions of leaf and stem bark on oxidative renal injury using some *in vitro* and *ex vivo* experimental models (Beseni *et al.*, 2022).

The current study aimed to evaluate the antioxidant and antidiabetic potentials of the various organic extracts of *R. longipes* stem bark via measuring their effects on carbohydrate digesting enzymes inhibitory activities *in vitro* as well as on oxidative pancreatic injury, small intestinal glucose absorption and, muscle and liver glucose uptake using *ex vivo* experimental models. The modulatory effects of the plant extracts on glucogenic, lipid and nucleotide metabolizing enzymes linked with T2D disorder were also investigated along with the identification of bioactive compounds using GC-MS analysis and their binding affinities were measured *in silico* against various target enzymes.

5.3.3 Materials and methods

Please refer to Chapter 2, sections 2.4.1 to 2.11.5 for detailed materials and methods.

5.3.4 Results

Table 5.3.1 shows the compounds identified in the *R. longipes* bark extracts based on the retention time. While phytol acetate, phytene-2 and phytol represent the most common phytoconstituents in the different extracts, the relative abundance of the first compound was more than those of the others in the methanol and ethanol extracts. Methyl behenate (3.83%), a natural fatty acid, was the least abundant constituent in the methanolic extract. Phenol, 4,4'-methylenebis (1.21%) and 2-Butanone, 4-(4-methoxyphenyl) (0.58%) were the least abundant compounds in the ethanolic and ethyl acetate extracts, respectively. The ethyl acetate extract had the most characterized compounds, including phenol, 2-[(4-hydroxyphenyl) methyl], phenol, 3-pentadecyl and diisooctyl phthalate, which contain phenolic rings. However, the phenol, 3-pentadecyl percentage abundance in the ethanol extract (1.26%) was higher than in the ethyl acetate extract (1.02%).

Table 5.3.1: Phyto-compounds identified by Gas Chromatography-Mass Spectroscopy (GC-MS) in *R. longipes* bark extracts.

Compounds	Retention time (min)	Molecular Formula	Molecular weight (g/mol)	Relative abundance (%)
Methanol				
Phytol, acetate	15.71	C ₂₂ H ₄₂ O ₂	338.60	40.27
Phytol	16.15	C ₂₀ H ₄₀ O	296.50	15.00
Tetra tetracontane	19.54	C ₄₄ H ₉₀	619.20	15.92
Methyl behenate	22.05	C ₂₃ H ₄₆ O ₂	354.60	3.83
Ethanol				
Phytol, acetate	15.69	C ₂₂ H ₄₂ O ₂	338.60	25.32
Phytol	16.14	C ₂₀ H ₄₀ O	296.50	8.16
Phenol, 4,4'-methylenebis	18.65	C ₂₉ H ₄₄ O ₂	424.70	1.21
2-Methyl-7-Octadecyne	18.73	C ₁₉ H ₃₆	264.50	1.36
7,10,13-Hexadecatrienal	18.79	C ₁₆ H ₂₆ O	234.38	1.92
Ethyl behenate	19.18	C ₂₄ H ₄₈ O ₂	368.60	6.28
Toly acetate	20.17	C ₉ H ₁₀ O ₂	150.17	2.13
6,27-Hexatriactontadien-2-one	20.76	C ₃₆ H ₆₈ O	516.90	1.39
Phenol, 3-pentadecyl-	21.96	C ₂₁ H ₃₆ O	304.50	1.26

Docosanoic acid, ethyl ester	22.59	C ₂₄ H ₄₈ O ₂	368.60	12.42
Ethyl acetate				
2-Butanone, 4-(4-methoxyphenyl)	14.29	C ₁₁ H ₁₄ O ₂	178.23	0.58
Phenol, 4-(1,1,3,3-tetramethylbutyl)	14.41	C ₁₄ H ₂₂ O	206.32	1.92
Spiro-1-(cyclohex-2-ene)-2'-(5'-oxabicyclo [2.1.0]pentane)	14.49	C ₁₄ H ₂₂ O	206.32	1.80
Phenol, 2-methyl-4-(1,1,3,3-tetramethylbutyl)-	14.55	C ₁₅ H ₂₄ O	220.35	1.26
1,3-Benzenediol,o-(2-furoyl)-o'-(2-methoxybenzoyl)	14.59	C ₁₉ H ₁₄ O ₅	322.30	1.37
4-Methyl-2-tert-octylphenol	14.62	C ₁₅ H ₂₄ O	220.35	1.46
Hexestrol, O-acetyl-	14.72	C ₂₀ H ₂₄ O ₃	312.40	1.01
Phthalic acid	14.82	C ₈ H ₆ O ₄	166.13	1.61
Phenol, 4-(1,1-dimethylpropyl)	14.94	C ₁₁ H ₁₆ O	164.24	3.04
Hexestrol dimethyl ether	15.02	C ₂₀ H ₂₆ O ₂	298.40	1.49
Phytol, acetate	15.68	C ₂₂ H ₄₂ O ₂	338.60	7.05
Phytol	16.14	C ₂₀ H ₄₀ O	296.50	2.22
7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-dien	16.54	C ₁₇ H ₂₄ O ₃	276.40	5.96
Dibutyl phthalate	16.99	C ₁₆ H ₂₂ O ₄	278.34	0.60
3,5-Di-tert-butyl-4-hydroxyphenylpropionic acid	17.34	C ₁₇ H ₂₆ O ₃	278.40	1.04
Phenol, 2-[(4-hydroxyphenyl)methyl]	18.62	C ₁₃ H ₁₂ O ₂	200.23	1.14
Benzene, 1-(1,1-dimethylpropoxy)-4-methyl	20.17	C ₁₂ H ₁₈ O	178.27	1.75
Phenol, 3-pentadecyl	21.95	C ₂₁ H ₃₆ O	304.50	1.02
Docosanoic acid, ethyl ester	22.59	C ₂₃ H ₄₆ O ₂	354.60	7.61
Octacosyl acetate	22.72	C ₃₀ H ₆₀ O ₂	452.80	2.14

The results of FRAP, DPPH and NO scavenging activity assays to determine the antioxidant properties of the bark extracts are shown in (Fig. 5.3.1A-5.3.1C). As indicated by its lowest IC₅₀ value (12.41±0.57 µg/ml) in Table 5.3.2, the ethanol extract exhibited better DPPH scavenging activity than the other extracts. Despite this result, it failed to outperform the positive control (Vitamin C) with IC₅₀ = 9.21±0.12 µg/ml. There was no significant difference in the ferric-reducing power of all the extracts (Fig. 5.3.1B). However, in Fig. 5.3.1C the methanol extract showed significantly (p<0.05) better nitric oxide mopping capacity than the other extracts and the positive control (Quercetin) at the test concentrations used.

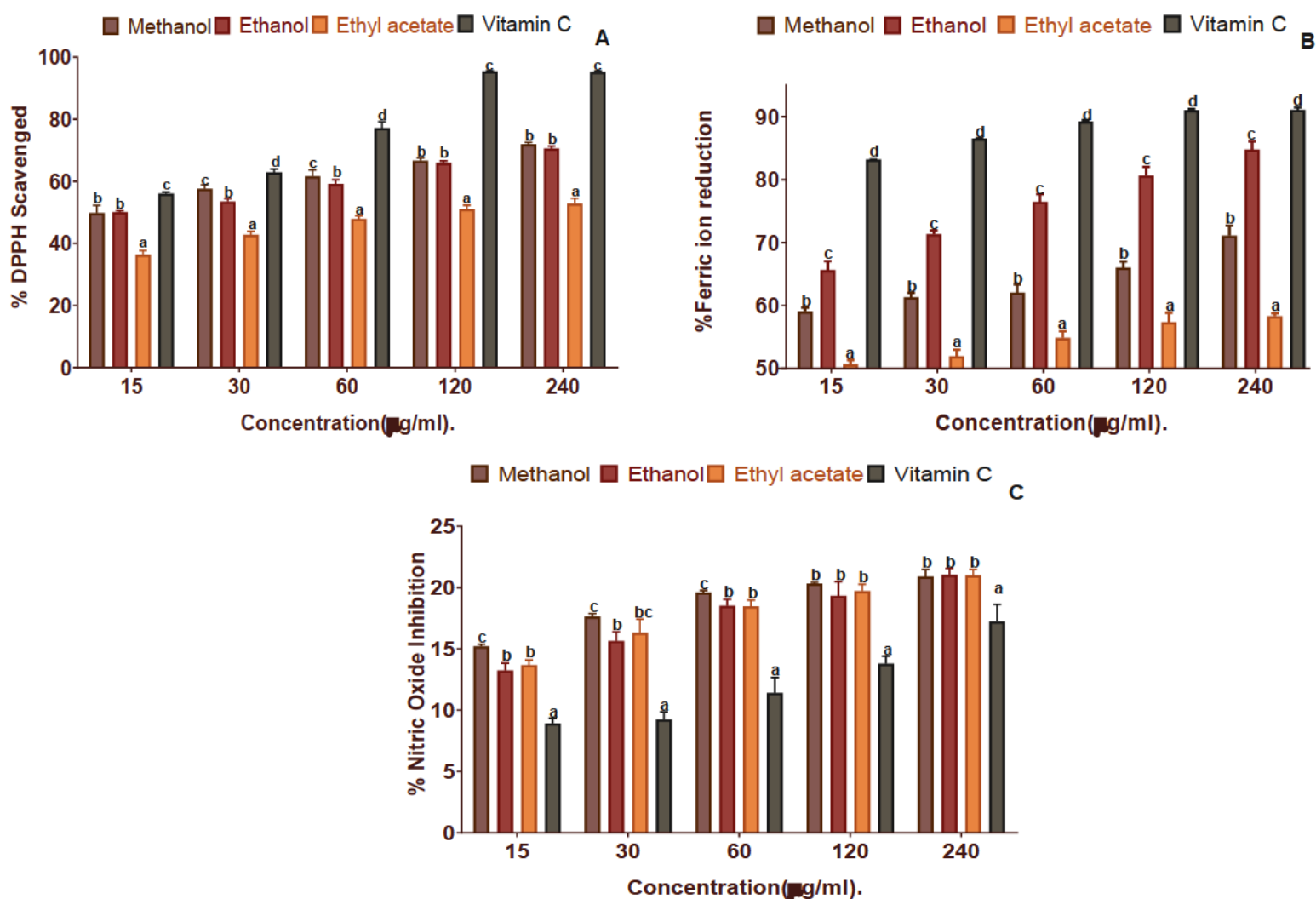


Figure 5.3.1: Effects of *R. longipes* bark organic extracts of extracts on (A) DPPH radical scavenging activity, (B) Ferric ion reducing activity, (C) Nitric oxide production inhibition. Data are presented as mean ± standard deviation. Different superscripts a, b and c represent statistical significance of p<0.05.

Data for *R. longipes* bark extracts inhibitory activities on lipid and carbohydrate-digesting enzymes are shown in Fig. 5.3.2A-5.3.2C. With significantly higher inhibitory activity at 240 $\mu\text{g/ml}$ (Fig. 5.3.2A), the ethanol extract with $\text{IC}_{50} = 551.74 \pm 0.47 \mu\text{g/ml}$ (Table 5.3.2) showed the best lipase enzyme inhibition among the test extracts. However, this extract potency was lower than those of orlistat (positive control) which showed pancreatic lipase inhibition activity with $\text{IC}_{50} = 114.52 \pm 0.83 \mu\text{g/ml}$. While the α -glucosidase inhibitory activity of ethanol extract was not significantly different from those of methanol extract at 15-60 $\mu\text{g/ml}$, the former showed statistically better activity than the latter and the positive control (acarbose) at 120 - 240 $\mu\text{g/ml}$ (Fig. 5.3.2B). Although the ethanol extract had lower α -amylase inhibition relative to acarbose at 15-60 $\mu\text{g/ml}$, its smaller IC_{50} value ($151.76 \pm 0.28 \mu\text{g/ml}$) in Table 5.3.2 suggests the best inhibitory activity compared to the other extracts (Fig. 5.3.2C).

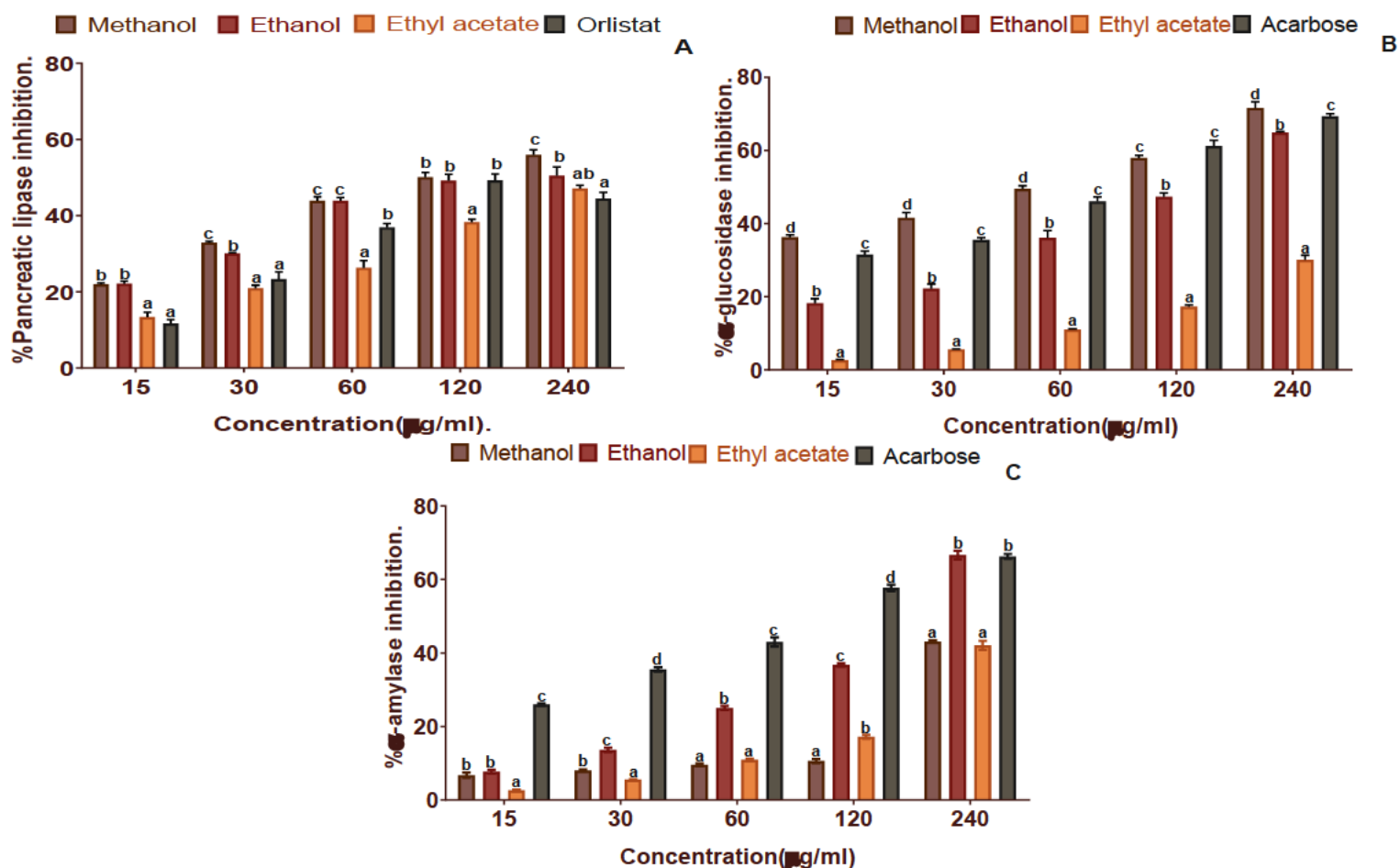


Figure 5.3.2: Effects of *R. longipes* bark extracts on (A) Pancreatic lipase inhibition (B) α -Glucosidase inhibition (C) α -Amylase inhibition. Data is presented as mean $\pm$ standard deviation. Different superscripts a, b and c represent statistical significance of $p < 0.05$.

The total phenolics and flavonoid contents of *R. longipes* bark extracts are depicted in Table 5.3.2. The ethanol extract total phenolic concentration (36.89 mg/g GAE) was significantly ($p < 0.05$) higher relative to those in both the methanol and ethyl acetate extracts. However, the ethyl acetate extract was observed to have the highest total flavonoid concentration (26.68 mg/g QE), which was not significantly ($p > 0.05$) different from that of the ethanol extract (23.17 mg/g QE). The methanol extract has the least total flavonoid concentration (12.67 mg/g QE) among the bark extract samples.

Table 5.3.2: Total phytochemical contents of *R. longipes* bark extracts and IC₅₀ values for *in vitro* bioactivity assays.

Parameters analysed	Methanol extract	Ethanol extract	Ethyl acetate extract	Vitamin C	Quercetin	Acarbose	Orlistat
Total phenolic content (mg GAE/g)	20.71±2.31	36.89±1.55	18.53±2.01	-	-	-	-
Total flavonoid content (mg QE/g)	12.66±1.53	23.17±0.55	26.68±0.80	-	-	-	-
DPPH (µg/ml)	13.84±0.39	12.41±0.57	273.72±0.91	9.21±0.12	-	-	-
FRAP (µg/ml)	8.22±0.42	7.83±0.17	8.31±0.65	6.67±0.88	-	-	-
NO (µg/ml)	471.84±0.31	544.54±0.87	964.01±0.46	-	989.83±0.27	-	-
Pancreatic lipase (µg/ml)	>1000	551.74±0.47	806.26±0.71	-	-	-	114.52 ± 0.83
α-Glucosidase (µg/ml)	57.70±0.42	41.87±0.37	275.61±0.61	-	-	100.68 ± 0.09	-
α-Amylase (µg/ml)	171.90±0.93	151.76±0.28	290.81±0.45	-	-	118.57 ± 0.97	-

Data is presented as mean ± standard deviation. DPPH = 2,2-diphenyl-1-picrylhydrazyl. FRAP = Ferric reducing antioxidant power. NO = Nitric oxide assay. GAE= Gallic acid equivalents (mg/g), QE=Quercetin equivalents (mg/g).

The effect of *R. longipes* bark extracts on glucose absorption in rat small intestinal jejunum is depicted in Fig. 5.3.3A. While all the extracts showed a dose-dependent suppression of glucose absorption in the tissue, the ethanol extract had significantly ($p < 0.05$) lower absorption at assayed concentrations. Interestingly in Fig. 5.3.3B, the ethanol extract at 60 $\mu\text{g/ml}$ and 240 $\mu\text{g/ml}$ also displayed better glucose uptake activity in yeast cell suspension relative to the other extract and metformin. Moreover, in the muscle tissue (Fig. 5.3.3C), the ethanol extract significantly enhanced glucose uptake to levels that were not statistically different from the positive control (insulin). Findings in Fig. 5.3.3D also revealed that the ethanol extract at 240 $\mu\text{g/ml}$ has significantly ($p < 0.05$) higher glucose uptake activity that exceeds the control liver tissue and is comparable to the experimental insulin group

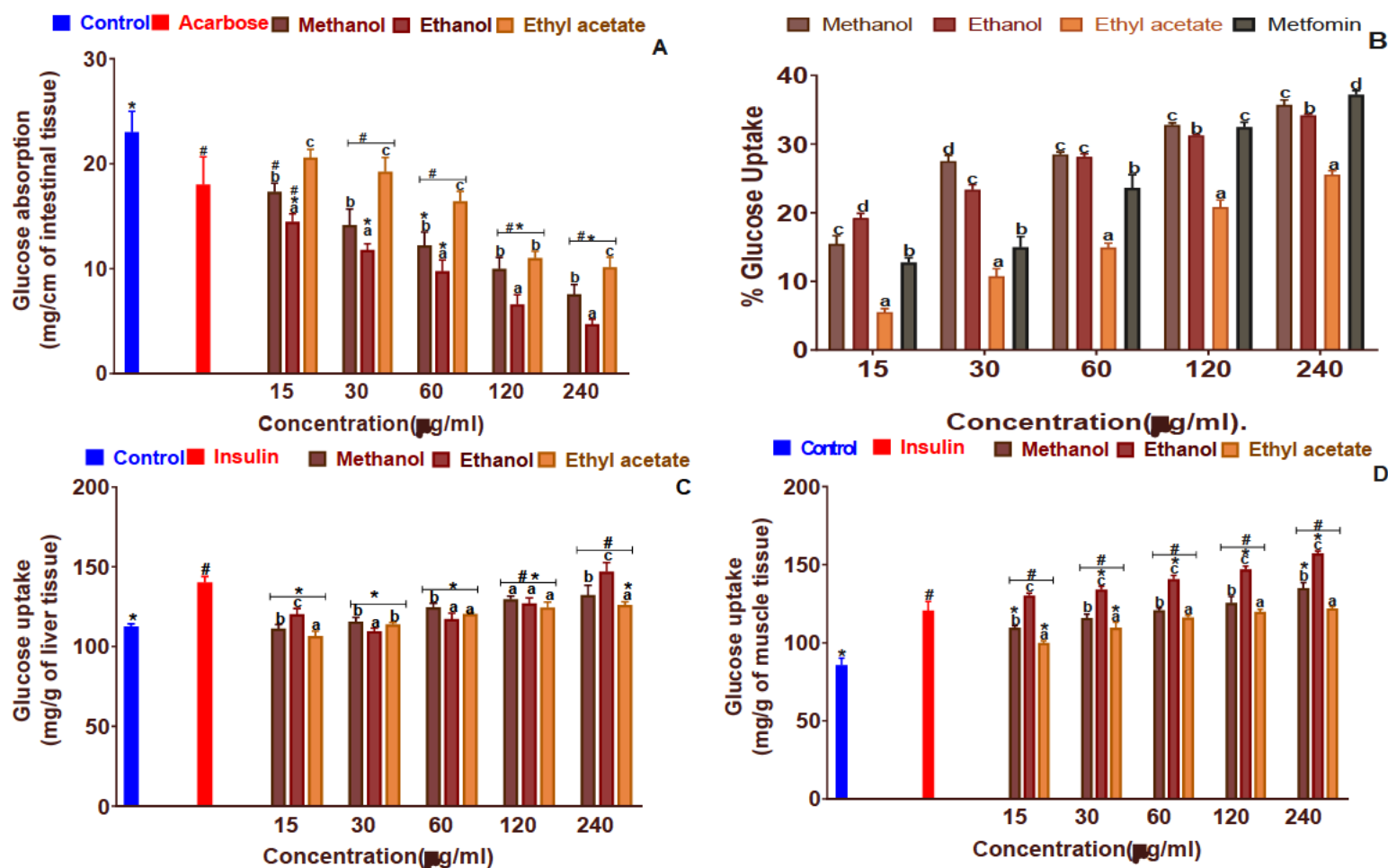


Figure 5.3.3: Effects of *R. longipes* bark extracts of on (A) Intestinal glucose absorption, (B) Liver glucose uptake (C) Muscle glucose uptake, and (D) Yeast cell glucose uptake. Data are presented as mean $\pm$ standard deviation of triplicate determinations. Different letters (a-c) above the bars for a given concentration are significantly different from each other ($p < 0.05$). *Significantly different from untreated sample and #significantly different from normal sample ($p < 0.05$).

The bark methanol and ethanol extract considerably lowered glycogen phosphorylase activity better than vitamin C in Fig. 5.3.4A. Enzyme activity in the latter group, more specifically, was not statistically different from the normal tissue group at 60-240 $\mu\text{g/ml}$. Compared to the untreated group, incubation with the extracts also lowered fructose 1,6 bisphosphatase activity in the treatment groups (Fig. 5.3.4B). At the assay concentrations, the ethanol extract displayed significantly lower inhibitory effects that suggest better modulation of the gluconeogenic enzymes.

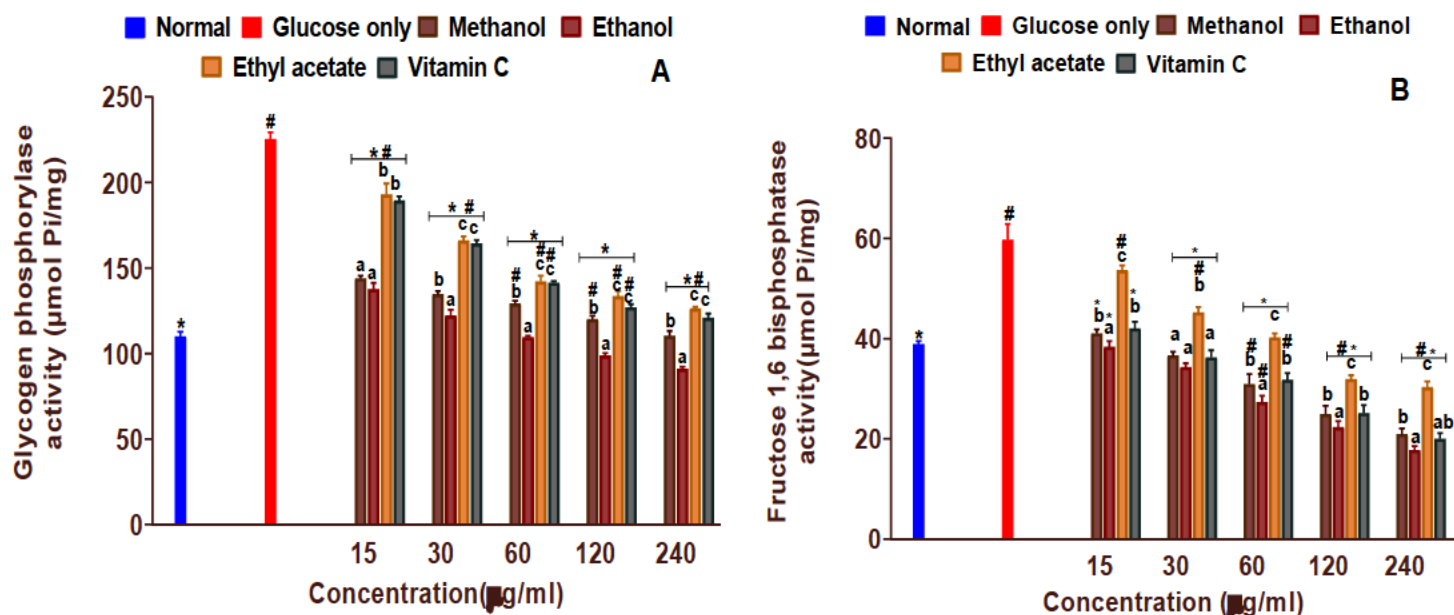


Figure 5.3.4: Effects of *R. longipes* bark extracts on the activities of liver (A) Glycogen phosphorylase and (B) Fructose 1,6 bisphosphatase enzymes. Data are presented as mean $\pm$ standard deviation of triplicate determinations. Different letters (a-c) above the bars for a given concentration are significantly different from each other ($p < 0.05$). *Significantly different from untreated sample and #significantly different from normal sample ($p < 0.05$).

The FeSO_4 -induced oxidative injury augmented lipid peroxidation, diminished superoxide dismutase (SOD) and catalase (CAT) activities, as well as reduced glutathione (GSH) levels in the untreated sample group (Fig. 5.3.5). As shown in Fig. 5.3.5A, treatment with the methanol and ethanol extracts significant ($p < 0.05$) reduced MDA to near normal levels at 240 $\mu\text{g/ml}$. While the ethanol extract-treated group SOD activity was significantly higher than those of the other treatments at 120-240 $\mu\text{g/ml}$, which was comparable to the normal tissue sample in Fig. 5.3.5B. Also, in Fig. 5.3.5D, a similar trend was observed in which the GSH level in the previously mentioned group exceeded those of vitamin C, methanol, and ethyl acetate extracts at the used concentrations. Although CAT activities in the methanol and ethanol extract groups

were not statistically different at 15-240 $\mu\text{g/ml}$, treatment with bark samples increased the enzyme activity to a comparable level with the normal sample (Fig. 5.3.5C).

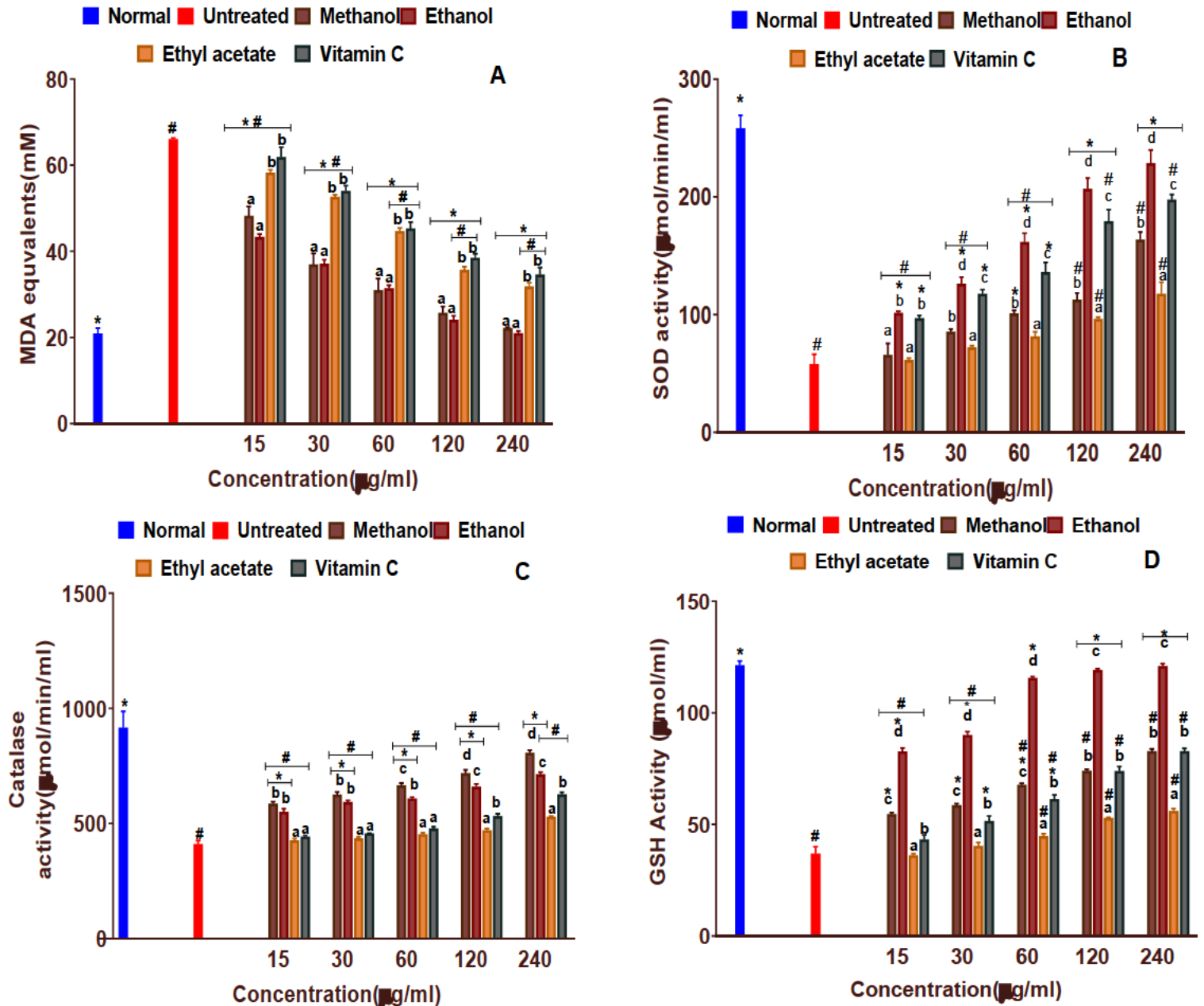


Figure 5.3.5: Effects of *R. longipes* bark extracts on (A) lipid peroxidation, (B) SOD activity, (C) Catalase activity and (D) GSH activity in oxidative pancreatic injury. Data are presented as mean $\pm$ standard deviation of triplicate determinations. Different letters (a-c) above the bars for a given concentration are significantly different from each other ($p < 0.05$). *Significantly different from untreated sample and #significantly different from normal sample.

The data from the *in silico* analysis is presented in Fig. 5.3.6 and Table 5.3.3. Among the phytochemicals in the bark extract, diisooctyl phthalate had the lowest binding affinities with α -amylase (-7.99 kcal/mol), α -glucosidase (-6.21 kcal/mol) and glycogen phosphorylase (-7.82 kcal/mol). However, 1,3-Benzenediol, o-(2-furoyl)-o'-(2-methoxybenzoyl) and phenol, 3-pentadecyl showed stronger attraction to fructose 1,6 bisphosphatase (-6.72 kcal/mol) and pancreatic lipase (-8.98 kcal/mol) proteins, respectively. Two-dimensional images result in Fig. 5.3.6A revealed that diisooctyl phthalate formed H-donor and pi-H interactions with TRY 65 and PHE 166 active site amino acids of α -glucosidase. This compound also had H- acceptor and H- donor bonds with the hydrophobic TRP 342 amino acid at the catalytic cleft of α -amylase (Fig. 5.3.6B). However, Pi-H and H-donor bonds with LYS 544 and GLU 120 stabilized the glycogen phosphorylase and diisooctyl phthalate complex in Fig. 5.3.6E.

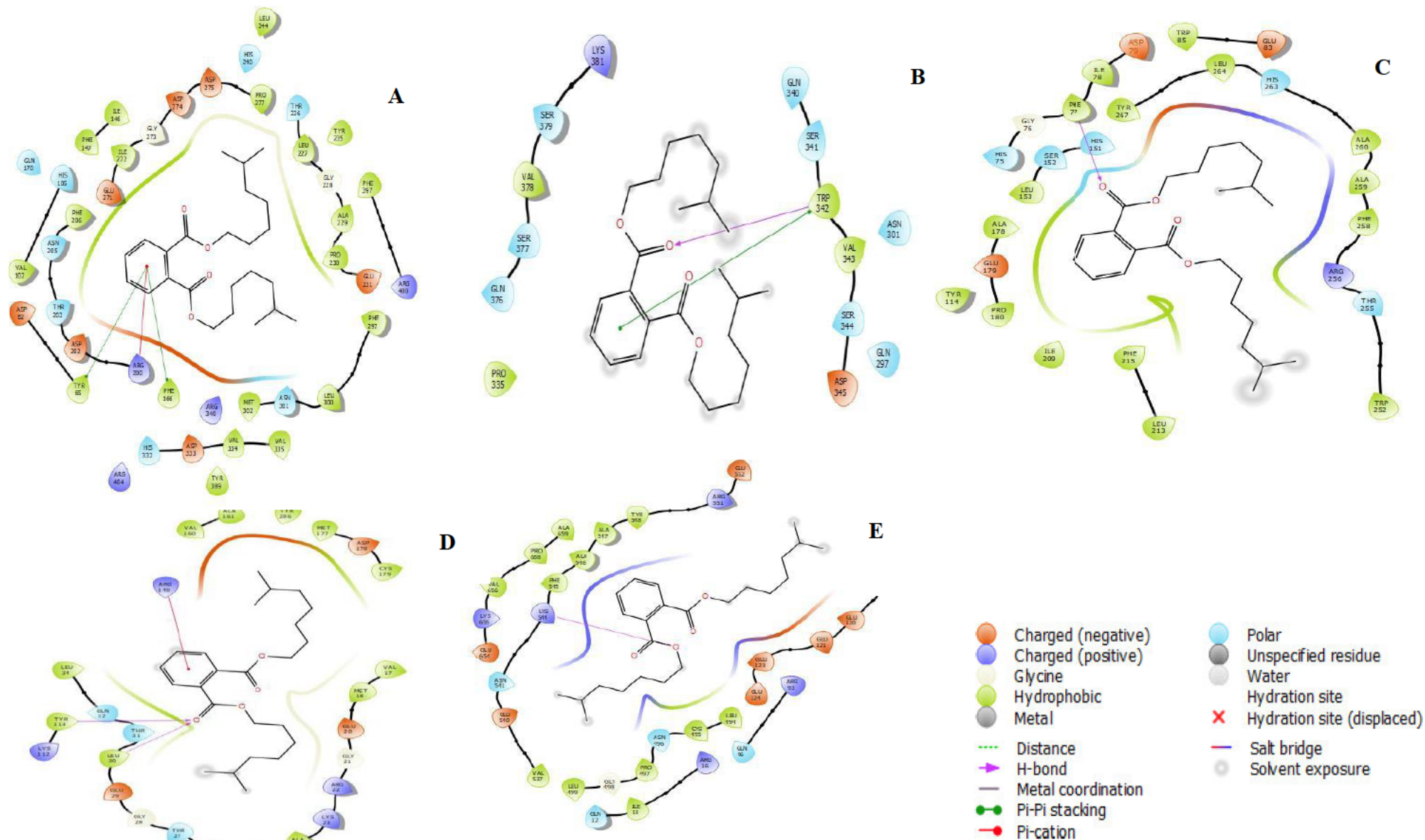


Table 5.3.3: Free binding energy of *R. longipes* bark secondary metabolites with different protein targets.

	Compound	Amino acids			Distance	Interaction	Docking score
α-Glucosidase	Diisooctyl phthalate	OE2	GLU	271	2.98	H-donor	-7.99
	1,3-Benzenediol,o-(2-furoyl)-o'-(2-methoxybenzoyl)-	OD1	ASP	275	3.17	H-donor	-7.05
		CE2	TYR	235	3.85	pi-H	
	Phenol, 3-pentadecyl-Acarbose	OD2	ASP	62	2.80	H-donor	-7.52
		OD2	ASP	333	2.85	H-donor	-3.25
		O	GLY	228	2.56	H-donor	
		OD2	ASP	202	3.16	H-donor	
		O	GLY	228	2.67	H-donor	
		NH2	ARG	200	2.64	H-acceptor	
		6-ring	TYR	65	3.86	H-pi	
α-Amylase	Diisooctyl phthalate	NE1	TRP	342	3.07	H-acceptor	-6.21
	1,3-Benzenediol,o-(2-furoyl)-o'-(2-methoxybenzoyl)-	SER	341		4.29	pi-H	-5.64
		ASP	345		3.93	pi-H	
	Phenol, 3-pentadecyl-Acarbose	O	SER	377	2.78	H-donor	-5.74
		OD1	ASN	5	2.86	H-donor	-6.57
		O	SER	3	3.01	H-donor	
		OD1	ASP	212	2.87	H-donor	
Porcine pancreatic lipase	Diisooctyl phthalate	N	SER	37	2.89	H-acceptor	-7.98

Fructose 1,6 biphosphatase	1,3-Benzenediol,o-(2-furoyl)-o'-(2-methoxybenzoyl)-	CD2	PHE	77	4.09	pi-H	-6.79
	Phenol, 3-pentadecyl-	CD2	LEU	16	4.33	pi-H	-8.98
	Orlistat	6-ring	TYR	114	3.86	H-pi	-8.50
	Diisooctyl phthalate	N	GLY	28	2.92	H-acceptor	-7.19
		CA	GLY	21	3.66	pi-H	
	1,3-Benzenediol,o-(2-furoyl)-o'-(2-methoxybenzoyl)-	OG1	THR	31	2.89	H-acceptor	-6.72
	Phenol, 3-pentadecyl-	NE	ARG	81	2.83	H-acceptor	-6.20
	Ascorbic acid	OD2	ASP	103	H-donor	2.82	-6.20
		OD1	ASP	273	H-donor	2.92	
		OD1	ASP	273	H-donor	3.01	
Glycogen phosphorylase		OG	SER	109	H-acceptor	2.83	
		NH2	ARG	112	H-acceptor	3.10	
		OD2	ASP	255	H-acceptor	2.82	
		NZ	LYS	269	H-acceptor	3.32	
	Diisooctyl phthalate	OE1	GLU	120	H-donor		-7.82
		CD	LYS	544		pi-H	
	1,3-Benzenediol,o-(2-furoyl)-o'-(2-methoxybenzoyl)-	NZ	LYS	544	3.02	H-acceptor	-7.52

Phenol, 3-pentadecyl-	OE1	GLN	96	2.92	H-donor	-7.63
	NZ	LYS	544	4.89	pi-cation	
Ascorbic acid	OE2	GLU	654	3.48	H-donor	-5.26
	OE1	GLU	124	2.95	H-donor	
	NZ	LYS	655	3.43	H-acceptor	
	NH1	ARG	93	3.13	H-acceptor	

5.3.5 Discussion

The surge in the prevalence of diabetes and its associated comorbidities has prompted the search for novel management strategies. Traditional medicinal plants have secondary metabolites, including phenolics, alkaloids and terpenes, that can be harnessed as sources of alternative therapy in disease management including diabetes.

Oxidative stress, characterized by the imbalance between the bodies' antioxidant defence systems, and reactive species (RS) concentration, has been linked with the pathogenesis of several degenerative conditions, including diabetes. Before the development of these pathologies, unstable, reactive species molecules caused damage to cellular components, including lipids, receptor proteins and DNA (Ghasemi-Dehnoo *et al.*, 2020). Consequently, phytochemicals with abundant delocalized π electrons could help alleviate oxidative stress by countering the detrimental effects of these highly reactive free radicals with unpaired electrons (Saranraj *et al.*, 2019). The ability of the *R. longipes* ethanol extract to scavenge DPPH radicals (Fig. 5.3.1A), donate electrons (Fig. 5.3.1B) and suppress lipid peroxidation in Fe^{2+} treated pancreatic homogenate (Fig. 5.3.5A) may suggest antioxidant therapeutic properties. This pharmacology property of the plant is further evidenced in Fig. 5.3.5B-C, where treatment with the extract also improved SOD and CAT activities as well as GSH level in the tissue sample. These proteins, which are components of endogenous antioxidant defence systems, counter the effects of reactive species (Benzie 2000; Rizzo *et al.*, 2010). Moreover, the ability of the extracts to mop-up nitric oxide (Fig 5.3.1C), a potent pro-inflammatory factor, could emphasise the plant's beneficial effect in reducing hyperglycaemia-linked inflammation in diabetic patients (Ghasemi-Dehnoo *et al.*, 2020). While the antioxidant properties of phenol, 3-pentadecyl and diisooctyl phthalate have been described in previous studies (Huang *et al.*, 2016; Shahidi and Ambigaipalan 2015), the presence of the compounds in the *R. longipes* bark extracts, as indicated in Table 5.3.1, may explain this observed pharmacological property.

During the passage through the gastrointestinal tract, several enzymes gradually breakdown complex dietary carbohydrates into simple sugars before they are absorbed into the bloodstream. Amongst these enzymes α -amylase is released from the pancreas and α -glucosidase is present at the brush border of the small intestine. Studies have shown that inhibiting these carbohydrate digestive enzyme activities may help to curb dysregulated glucose metabolism and glucose intolerance associated with hyperglycaemia in diabetic patients (Assefa *et al.*, 2019). Interestingly, the reduction in jejunal glucose absorption,

especially by the bark ethanol extract, as observed in Fig. 5.3.3A, could be attributed to the plant extract suppression of α -glucosidase and α -amylase enzyme activities (Fig. 5.3.2B-C). The fact that diisooctyl phthalate had the highest binding affinity with these enzymes' active site amino acids (Table 5.3.3) may reflect its role in how the plant was able to elicit these modulatory effects on the digestive enzymes. Indeed, *in silico* studies by Lawal *et al.*, (2020) described the diisooctyl phthalate and some compounds in *Pistia strateotes* as potential antidiabetic molecules.

Glucose utilization by peripheral tissues such as the muscle and peripheral adipose and storage in the liver provides an avenue for regulating postprandial blood glucose levels (Sargsyan and Herman 2019). In this regard, the *R. longipes* bark extracts' ability to increase glucose uptake in yeast cells and the muscle and liver tissues (Fig. 5.3.3B-D) may portray the plant's capacity to enhance glucose utilization under the insulin resistant condition linked with T2D. Consequently, these findings suggest that *R. longipes* bark extract could serve as a source of alternative pharmacological agents that could assist diabetic patients in maintaining normal blood glucose levels. In support of this study, intestinal suppression of glucose absorption in the intestine with simultaneously increased uptake of hepatic glucose by the extracts from another medicinal plant which has been documented in one of our previous studies (Olofinson *et al.*, 2022).

Fatty acids are a rich source of energy. However, dysregulation of fatty acids metabolism has been linked to obesity which also represents a comorbidity and risk factor for T2D. Furthermore, excessive catabolism of fatty acid via the β -oxidation pathway has been reported to exacerbate free radical production with consequent perturbation of the body's redox homoeostasis. Hence, the regulation of fatty acid breakdown is key to reduce diabetic dyslipidaemia. In the current study, *R. longipes* bark crude extract inhibited pancreatic lipase (Fig. 5.3.2A), the enzyme responsible for the release of free fatty acids after the breakdown of triglycerides in the gastrointestinal tract. This lipase inhibitory activity of *R. longipes* crude extract may be associated with its bioactive phytochemicals such as phenol-3-pentadecyl, which modulated the enzyme's activity after binding strongly to its active site (Table 5.3.3). Interestingly, the presence of this compound with other secondary metabolites have been shown to have other biological activities of *Rhus* species in a recent study (Nemkul *et al.*, 2021).

In type 2 diabetic patients, glycogen phosphorylase and fructose 1,6 bisphosphatase exacerbate hyperglycaemia by enhancing hepatic glucose output via glycogenolysis and

gluconeogenesis, respectively (Donnier-Maréchal and Vidal 2016). Therefore, pharmacological therapeutic agents that inhibit the function of these enzymes may alleviate hyperglycaemia by regulating hepatic glucose release. Indeed, glycogen phosphorylase and 1,6 biphosphatase inhibitors have been described as potential hypoglycaemic agents for T2D treatment (Donnier-Maréchal, and Vidal 2016; Kaur *et al.*, 2017). The reduction of these enzymes' activities in hepatic tissue treated with the bark extracts (Fig. 5.3.4A-B), as seen in the current study, may further support the antidiabetic efficacies of the plant in modulating hepatic glucose uptake, as observed in Fig. 5.3.3B. Pharmacological agents that inhibit fructose 1,6 biphosphatase have the added advantage of inhibiting gluconeogenesis from all gluconeogenesis substrates while avoiding direct effects on glycogenolysis, glycolysis, and the tricarboxylic acid cycle (Kaur *et al.*, 2017).

5.3.6 Conclusion

The results of this study suggest the antioxidant and antidiabetic activities of *R. longipes* by its ability to promote glucose uptake and by inhibiting carbohydrate and lipid digesting enzymes as well as modulating enzymes linked to carbohydrate metabolism in the muscle and liver tissues. The ethanol extract of *R. longipes* bark exhibited the overall best pharmacological activities compared to all other extracts so it can be further studied using an experimental animal model to ascertain the results of this study. This is currently underway in our laboratory.

5.4 *Rhus longipes* organic leaf extracts regulate glucose metabolism and abate oxidative injury in cardiac tissues

Brian K. Beseni¹, Ochuko L. Erukainure¹, *Md Shahidul Islam¹

1.Department of Biochemistry, University of KwaZulu-Natal, Westville Campus, Durban 4000, South Africa.

***Corresponding author:** islamd@ukzn.ac.za

5.4.1 Abstract

Rhus longipes is a folkloric traditional medicinal plant with pharmacological therapeutic benefits against several ailments including T2D. The current study investigated the effects of organic leaf extracts of *R. longipes* on glucose metabolism and oxidative stress using *in vitro*, *ex vivo*, and *in silico* experimental models. The bioactive compounds in the *R. longipes* were identified through Gas Chromatography-Mass Spectrometry (GC-MS) after which their total polyphenol and flavonoid contents, antioxidant, and antidiabetic capacities were determined *in vitro*. The effects of *R. longipes* extracts on glucogenic enzymes, muscle and liver glucose uptake, intestinal glucose absorption, and modulatory effects on oxidative cardiac tissue injury were determined *ex vivo*. The binding affinities of identified phytochemicals in *R. longipes* against various enzymes were determined *in silico*. Phytol, phytol acetate, diisooctyl phthalate and 3-pentadecylphenol were among the compounds identified in the *R. longipes* extracts. The high phenolics and flavonoids in the extracts may be responsible for their enhanced electron donating (FRAP), DPPH and NO radical scavenging capabilities. Furthermore, the extracts demonstrated heightened α glucosidase and α amylase inhibitory activities coupled with increased capabilities to delay glucose absorption in rat jejunum while enhancing its uptake in the yeast suspension, liver and muscle tissue. The leaf extracts reduced hepatic ATPase and fructose-1,6-bisphosphatase to near normal proportions. *R. longipes* leaf extracts significantly ($p < 0.05$) reduced lipid peroxidation while concomitantly augmenting superoxide dismutase (SOD) and catalase (CAT) activities, as well as elevating glutathione (GSH) levels in the cardiac muscle tissue. These pharmacological activities warrant further molecular and *in vivo* studies to elucidate the precise mechanisms of action of *R. longipes* leaf extracts.

Key words: *R. longipes*, *S. longipes*, Antioxidant, Antidiabetic, Cardiomyopathy, Glucose metabolism

5.4.2 Introduction

Diabetes mellitus (DM) is a multifactorial metabolic disorder characterised by chronic hyperglycaemia whose prevalence rates have reached epidemic levels globally (Petersmann *et al.*,2018; Zheng *et al.*,2018). Although the underlying mechanism is scantily understood, major risk factors associated with the pathogenesis and progression of DM include genetic predisposition, sedentary lifestyles, unhealthy diets, and old age. These multifactorial risk factors contribute to relative insulin deficiency, its aberrant action, or both which are implicated as the major underlying causes of DM (Cersosimo *et al.*,2018). An estimated 451 million adults were reportedly living with DM in 2017 with the statistics projected to rise to about 693 million by the year 2045 (Cho *et al.*,2018). Several DM aetiologies of DM are known to exist but type I, type II, and gestational diabetes are the most prevalent. Epidemiological data further suggests that type II diabetes (T2D) accounts for over 80% of all the reported cases globally (Petersmann *et al.*,2018). Acute signs of T2D which are indicative of chronic hyperglycaemia include polyuria, polydipsia, uncontrollable weight loss, polyphagia, and blurred vision. Evidently, uncontrolled hyperglycaemia in T2D precedes the onset of its associated long-term complications.

Long-term complications linked T2D are broadly classified as microvascular or macrovascular complications. These deleterious long-term complications evidently observed in diabetic patients range from blindness, foot ulcers, lower limb amputations, cardio-vascular complications, sexual dysfunction, and kidney failure (ADA, 2013). Chronic T2D-linked complications weigh heavily on global healthcare systems, drastically reduce the quality of life and lead to morbidity and premature mortality (Cho *et al.*,2018). Furthermore, T2D aggravates comorbidities including hypertension, obesity, and cardiovascular diseases (CVD). Diabetic cardiomyopathy which alters the myocardium structure and function among DM patients is attributed to the global increase in the incidence of heart failure (Boudina, and Abel, 2010). Therefore, proper management of DM through the regulation of glucose metabolism remains a cornerstone in circumventing the onset and effects of its long-term complications.

Currently, there are several strategies for the management of T2D including non-pharmacological and pharmacological strategies levelled at establishing and maintaining homeostatic blood glucose levels. Non-pharmacological T2D management strategies include medical nutritional therapy and increased physical activity engagement (Raveendran *et al.*,2018) while pharmacological strategies include oral agents broadly categorised as

sulfonylureas, thiazolidinediones, α -glucosidase inhibitors, and glinides, (Hu, and Jia, 2019). Despite the wide-spread implementation and success of the aforementioned T2D management strategies several impeding extensive drawbacks have been alluded to their use (Ahola, and Groop, 2013). While non-pharmacological strategies effectively manage mild T2D cases, they are ineffective against more advanced cases and are often met with considerable patient resistance most often reverting to their previous unhealthy dietary and sedentary habits (Ahola, and Groop, 2013). Relatedly, pharmacological strategies are very costly and have a myriad of undesirable side effects including gastro-intestinal irritations, weight gain and vomiting (Chaudhury *et al.*, 2017). The collective cons of both management strategies have prompted scientists across the globe to sort out novel drug templates that may be developed into cost effective antidiabetic therapeutic with high efficacy, bioavailability and less undesirable side effects. Traditional medicinal plants such as *R. longipes* that produce alkaloids, terpenoids, phenolics, flavonoids, coumarins and saponins are an infinite source of bioactive compounds with an assortment of therapeutic capabilities (Nazarian-Samani *et al.*, 2018, Jain *et al.*, 2019). In comparison to existing synthetic pharmacological agents, traditional medicinal plants provide a lucrative source of novel therapeutic agents that are readily available and cost effective (Nazarian-Samani *et al.*, 2018).

Rhus longipes of the family anacardiaceae is a folkloric traditional medicinal plant that has previously been used to remedy infertility and to dilate the birth canal during childbirth (Maroyi 2011). *Rhus longipes* is also used against malaria and to alleviate asthma in several regions of Nigeria (Olorunnisola *et al.*, 2017). Olorunnisola *et al* (2017) furthermore reported on the antioxidant capabilities of *R. longipes* and its efficacy against paracetamol-induced hepatic toxicity. A previous study in our laboratory corroborated these findings as it reported the antioxidant and antidiabetic activities of the aqueous infusions of leaf and stem bark of *Rhus longipes* (Beseni *et al.*, 2022). The study further reported the abilities of the infusions to abate oxidative renal tissue injury (Beseni *et al.*, 2022).

In light of this, the current study is aimed at evaluating the antioxidant and antidiabetic capabilities of several organic extracts of *R. longipes* leaves with varying polarities. Following the identification and quantification of bioactive constituents in the sequential extracts of *R. longipes* leaves using GC-MS, their antioxidant capabilities and inhibitory effects on carbohydrate and lipid digesting enzymes were investigated *in vitro*. The protective effects of the extracts on oxidative cardiac tissue injury, small intestinal glucose absorption, and muscle and liver glucose uptake were determined using *ex vivo* experimental models. Similarly, *ex*

vivo experimental models were employed to evaluate the modulatory effects of the plant extracts on key hepatic glucogenic and nucleotide metabolizing enzymes after which the binding affinities of bioactive compounds against various target enzymes were evaluated *in silico*.

5.4.3 Materials and methods

Please refer to Chapter 2, sections 2.4.1 to 2.11.5 for detailed materials and methods.

5.4.4 Results

Gas Chromatography Mass Spectrometry (GC-MS) analysis of the different leaf extracts of *R. longipes* revealed the presence of a myriad of phytoconstituents as shown in Table 5.4. 1. - (+)-Ascorbic acid 2,6-dihexadecanoate was shown to be the most abundant compound in the methanolic extract (28.05 %). Conversely, 9-methyl-nonadecane and tetradecanoic acid were the least abundant in the methanolic extract with 0.25% abundance each. Finally, squalene was eluted last in the methanolic extract with a retention time of 24.845 min. Phytol (21.48%) was the most abundant compound in both the ethanolic and ethyl acetate extracts with relative abundances of 21.48% and 4.38% respectively. Tributyl acetyl citrate (0.40 %) was the least abundant compound in the ethanolic extract, while tetradecanoic acid had the least relative abundance of 0.48 % in the ethyl acetate extract. Varying proportions of phytol, phytol acetate, diisooctyl phthalate and 3-pentadecylphenol were common among all the extracts. Squalene and tetradecanoic acid were present in the methanolic and ethyl acetate extracts but absent in the ethanolic extract.

Table 5.4.1: The phyto-compounds identified by Gas Chromatography Mass Spectrometry (GC-MS) in the different leaf *R.longipes* extracts.

Compounds	Retention time (min)	Molecular Formula	Molecular weight (g/mol)	Relative abundance (%)
Methanol				
9-methyl-nonadecane,	13.017	C ₂₀ H ₄₂	282.5	0.25
Tetradecanoic acid	14.985	C ₁₄ H ₂₈ O ₂	228.37	0.25
Nonadecanol	15.213	C ₁₉ H ₄₀ O	284.5	0.46
6-Methyl-cyclodec-5-enol	15.354	C ₁₁ H ₂₀ O	168.28	0.51
Dibutyl phthalate	16.985	C ₁₆ H ₂₂ O ₄	278.34	0.44
l-(+)-Ascorbic acid 2,6-dihexadecanoate	17.265	C ₃₈ H ₆₈ O ₈	652.9	28.05
Phytol	18.478	C ₂₀ H ₄₀ O	296.5	13.35
Phytol acetate	19.360	C ₂₂ H ₄₂ O ₂	338.6	1.92
3-pentadecylphenol	21.956	C ₂₁ H ₃₆ O	304.5	2.24
Squalene	24.845	C ₃₀ H ₅₀	410.7	1.44

Ethanol

1-Methylpyrrole	3.546	C ₅ H ₇ N	81.12	0.51
Caryophyllene oxide	13.069	C ₁₅ H ₂₄ O	220.35	0.45
Heptadecanol	15.208	C ₁₇ H ₃₆ O	256.5	0.86
Phytol acetate	15.673	C ₂₂ H ₄₂ O ₂	338.6	3.00
10-Methylundec-2-en-4-olide	16.558	C ₁₂ H ₂₀ O ₂	196.29	0.51
Hexadecanoic acid, ethyl ester	17.271	C ₁₇ H ₃₄ O ₂	270.5	5.69
Phytol	18.443	C ₂₀ H ₄₀ O	296.5	21.48
Ethyl 14-methyl-hexadecanoate	19.159	C ₁₉ H ₃₈ O ₂	298.5	1.02
Tributyl acetyl citrate	19.641	C ₂₀ H ₃₄ O ₈	402.5	0.40
3-pentadecylphenol	21.930	C ₂₁ H ₃₆ O	304.5	1.19
2-Isoamyl-6-methylpyrazine	23.955	C ₁₀ H ₁₆ N ₂	164.25	6.09

Ethyl acetate

Tetradecanoic acid	14.999	C ₁₄ H ₂₈ O ₂	228.37	0.48
Phytol acetate	15.686	C ₂₂ H ₄₂ O ₂	338.6	2.95
2-Pentadecanone, 6,10,14-trimethyl-	15.758	C ₁₈ H ₃₆ O	268.5	0.68
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-dien	16.550	C ₁₇ H ₂₄ O ₃	276.4	1.94
Phytol	18.458	C ₂₀ H ₄₀ O	296.5	4.38
n-Tetracosanol-1	19.190	C ₂₄ H ₅₀ O	354.7	4.17
3-pentadecylphenol	21.958	C ₂₁ H ₃₆ O	304.5	1.28
2-Isoamyl-6-methylpyrazine	24.022	C ₁₀ H ₁₆ N ₂	164.25	3.74
Squalene	24.851	C ₃₀ H ₅₀	410.7	0.80

The total phenolics and flavonoid contents of *R. longipes* leaf extracts are depicted in Fig 5.4.1. The ethanol extract had significantly ($p < 0.05$) the highest total phenolic concentration of 29.41 mg/g GAE among all the extract, while there was no significant difference between the methanol (16.05 mg/g GAE) and ethyl acetate (17.83 mg/g GAE) extracts. Indeed, the total flavonoid concentration appeared polarity dependent with the ethyl acetate extract showing significantly ($p < 0.05$) the highest amount of 24.33 mg/g QE, followed by the ethanol (19.59 mg/g QE) extract and the methanol (8.93 mg/g QE) extracts, respectively.

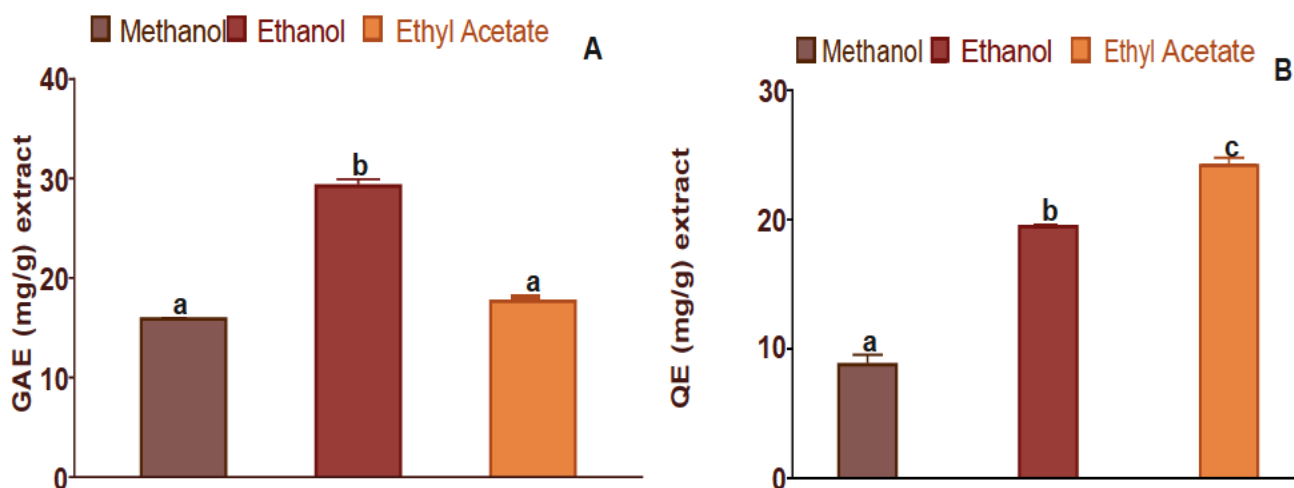


Figure 5.4.1: The total phenolic content of the *R. longipes* crude leaf organic extracts represented as A) Gallic acid equivalents (GAE mg/g) and the total flavonoid content represented as B) Quercetin equivalents (QE mg/g). Different superscripts a-c represents statistical significance of $p < 0.05$.

The methanol and ethanol extracts of *R. longipes* showed comparable DPPH radical scavenging activities at all the tested concentrations with no significant difference between them. The ethyl acetate extract exhibited significantly ($p < 0.05$) the least DPPH radical scavenging activity among all the extracts as seen in Fig 5.4.2 A. Furthermore, as observed in Fig 5.4.2 B, the ethanol extract had the highest FRAP activity, as indicated by the low IC_{50} of $0.66 \pm 0.83 \mu\text{g/ml}$ in Table 5.4.2. While there was no significant ($p > 0.05$) difference among them especially at concentrations greater than $60 \mu\text{g/ml}$, the *R. longipes* leaf extracts demonstrated significantly ($p < 0.05$) better the NO mopng activity of vitamin C (positive control) at all concentrations tested as seen in Fig 5.4.2 A. At concentrations greater than $60 \mu\text{g/ml}$, the *R. longipes* leaf extracts showed better the NO mopng activity of vitamin C (positive control) as seen in Fig 5.4.2A.

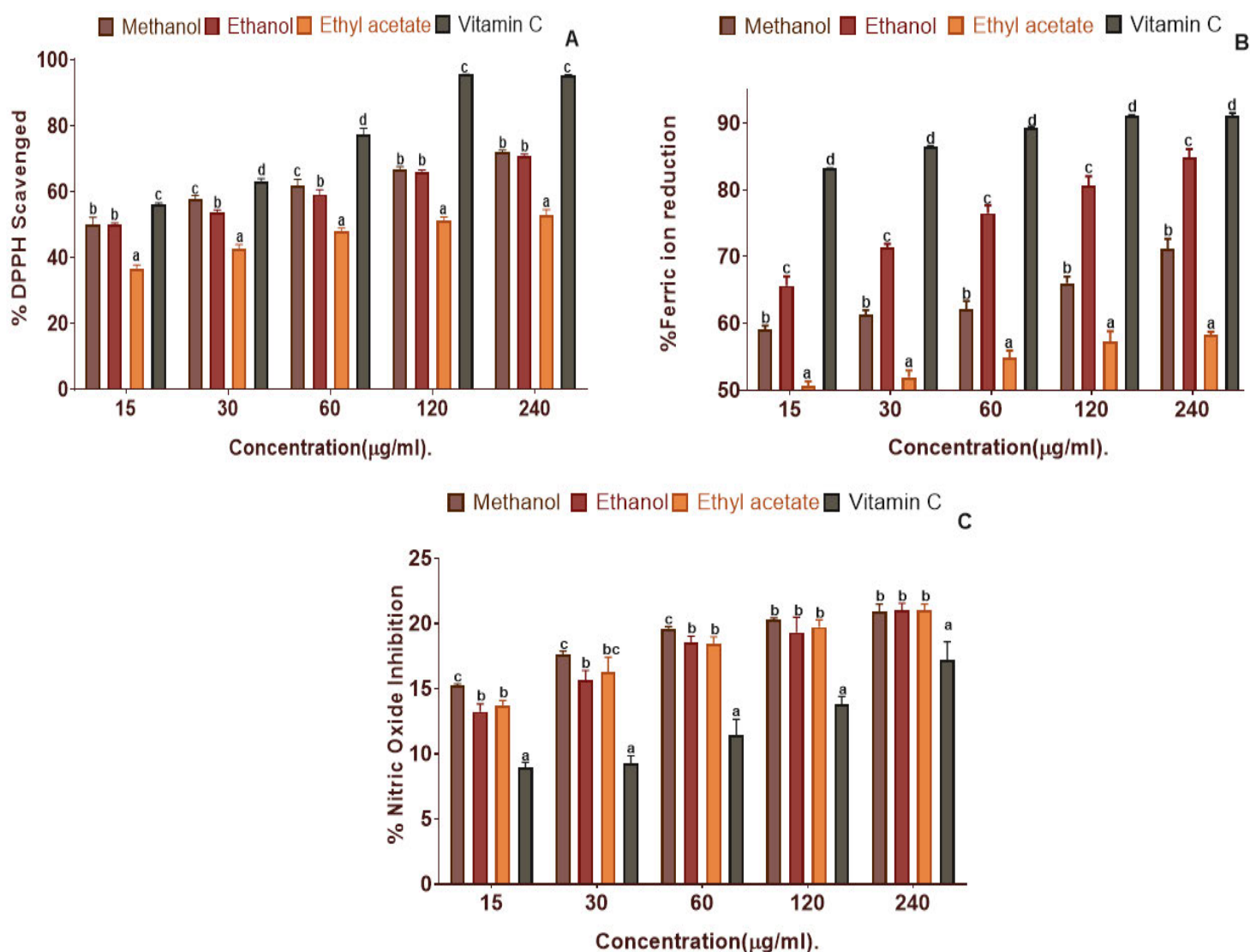


Figure 5.4.2: Effects of *R. longipes* leaf organic extracts of extracts on (A) DPPH radical scavenging activity, (B) Ferric ion reducing activity, (C) Nitric oxide production inhibition. Data are presented as mean $\pm$ standard deviation. Different superscripts a-d represent statistical significance of $p < 0.05$.

The *in vitro* inhibitory activities of *R. longipes* leaf extracts against key lipid and carbohydrate digesting enzymes are depicted in Figs. 5.4.3A-C. As seen in Fig. 5.4.3, the methanol and ethanol extracts had comparable pancreatic lipase inhibitory activities at most of the tested concentrations, while at the highest concentration of 240 µg/ml the methanol extract showed superior capabilities among other extracts including orlistat (positive control). Indeed, inhibitory activities of *R. longipes* leaf extracts against α glucosidase (Fig 5.4.3B) and α amylase Fig 5.4.3C were dose dependent. The methanolic extract had the best inhibitory activity against α glucosidase, with a low IC_{50} value of $92,88 \pm 0,68$ µg/ml (Table 5.4.2). The ethanol extract had the highest inhibitory activity against α amylase.

Furthermore, at 240 $\mu\text{g/ml}$ there was no significant difference in the α amylase inhibitory capabilities of the ethanol *R. longipes* leaf extract and acarbose as depicted in Fig 5.4.3C.

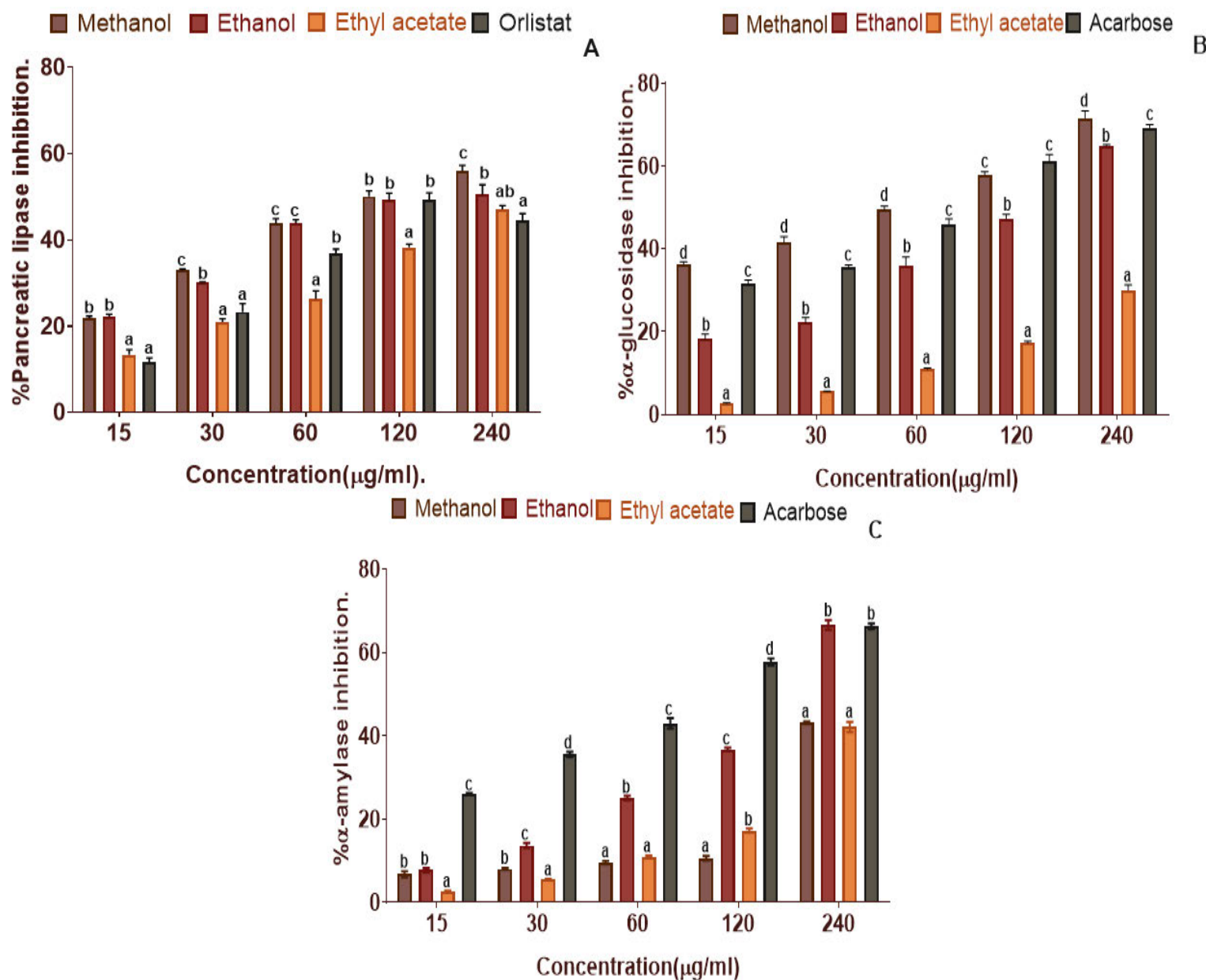


Figure 5.4.3: Effects of *R. longipes* leaf organic extracts of extracts on (A) Pancreatic lipase inhibition activity, (B) α -Glucosidase inhibition activity, (C) α -Amylase inhibition activity. Data is presented as mean $\pm$ standard deviation. Different superscripts a, b and c represent statistical significance of $p < 0.05$.

Table 5.4.2: The IC₅₀ values of *R. longipes* leaf extracts under different assayed parameters.

Activities	Methanol extract (µg/ml)	Ethanol extract (µg/ml)	Ethyl acetate extract (µg/ml)	Vitamin C (µg/ml)	Quercetin (µg/ml)	Acarbose (µg/ml)	Orlistat (µg/ml)
DPPH	12,42±0,16	15,85±0,74	115,74±0,26	9,21±0,12	-	-	-
FRAP	1,34±0,10	0,66±0,83	12,26±0,61	6,67±0,88	-	-	-
NO	640,84±0,2	696,667	671,172	-	989,83±0,27	-	-
Pancreatic lipase	162,50±0,39	191,71±0,97	240±0,15	-	-	-	114,52±0,83
α-glucosidase	92,88±0,68	139,91±0,41	402,65±0,41	-	-	100,68±0,09	-
α-amylase	311,30±0,88	171,89±0,91	293,30±0,09	-	-	118,57±0,97	-

Data is presented as mean ± standard deviation. Key: DPPH = 2,2-diphenyl-1-picrylhydrazyl. FRAP = Ferric reducing antioxidant power. NO = Nitric oxide assay.

The effects of *R. longipes* leaf extracts on glucose absorption in the rat small intestinal jejunum, glucose uptake in yeast suspension, liver and muscle tissues, are depicted in Figs.5.4.4A-D, respectively. All extracts significantly ($p<0.05$) delayed glucose absorption better than acarbose as seen in Fig.5.4.4 A. The extracts further increased glucose uptake in the yeast suspension in a dose dependant manner, with the methanol and ethanol extracts significantly ($p<0.05$) outperforming the ethyl acetate extract as seen in Fig.5.4.B. Interestingly, as observed in Figs.5.4.4 C-D, the ethanol extract demonstrated significantly ($p<0.05$) the highest glucose uptake capabilities in both the liver and muscle tissue especially at 240 $\mu\text{g/ml}$ where it exhibited better activity than insulin (positive control).

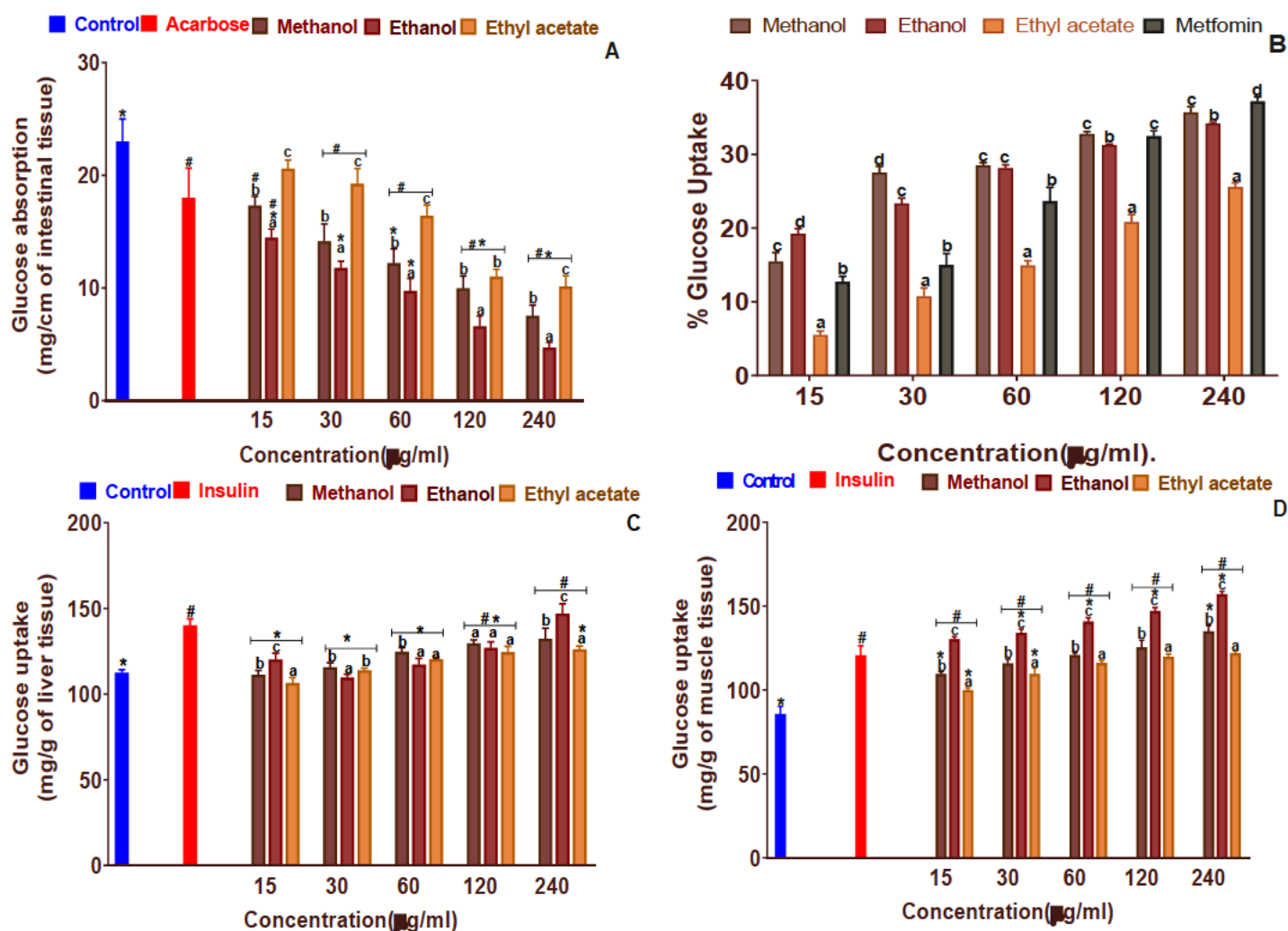


Figure 5.4.4: The modulatory effects of organic leaf crude extracts of *R. longipes* on (A) intestinal glucose absorption, (B) yeast cell glucose uptake (C) liver glucose uptake and (D) psoas muscle glucose uptake. Data are presented as mean $\pm$ standard deviation of triplicate determinations. Different letters (a-c) above the bars for a given concentration are significantly different from each other ($p<0.05$). *Significantly different from untreated sample and #significantly different from normal sample ($p < 0.05$).

As shown in Figs.5.5.4 A-C, hyperglycaemic toxicity significantly ($p<0.05$) elevated hepatic ATPase, glycogen phosphorylase, and fructose 1,6 bisphosphatase activities in the untreated group when compared to the normal group. Treatment with the ethanolic extract at concentrations above 60 $\mu\text{g/ml}$ significantly ($p<0.05$) reduced hepatic ATPase activities resulting in significantly ($p<0.05$) better ATPase inhibitory capabilities than vitamin C as shown in Fig.5.5.4 A. Furthermore, the extracts showed comparable glycogen phosphorylase inhibitory capabilities which are significantly ($p<0.05$) lower than those observed in the untreated group (Fig.5.5.4 B). Finally, except ethyl acetate, the extracts significantly ($p<0.05$) reduced the hepatic fructose 1,6 bisphosphatase to near normal comparable to that of quercetin (positive control) as seen in Fig.5.5.4 C.

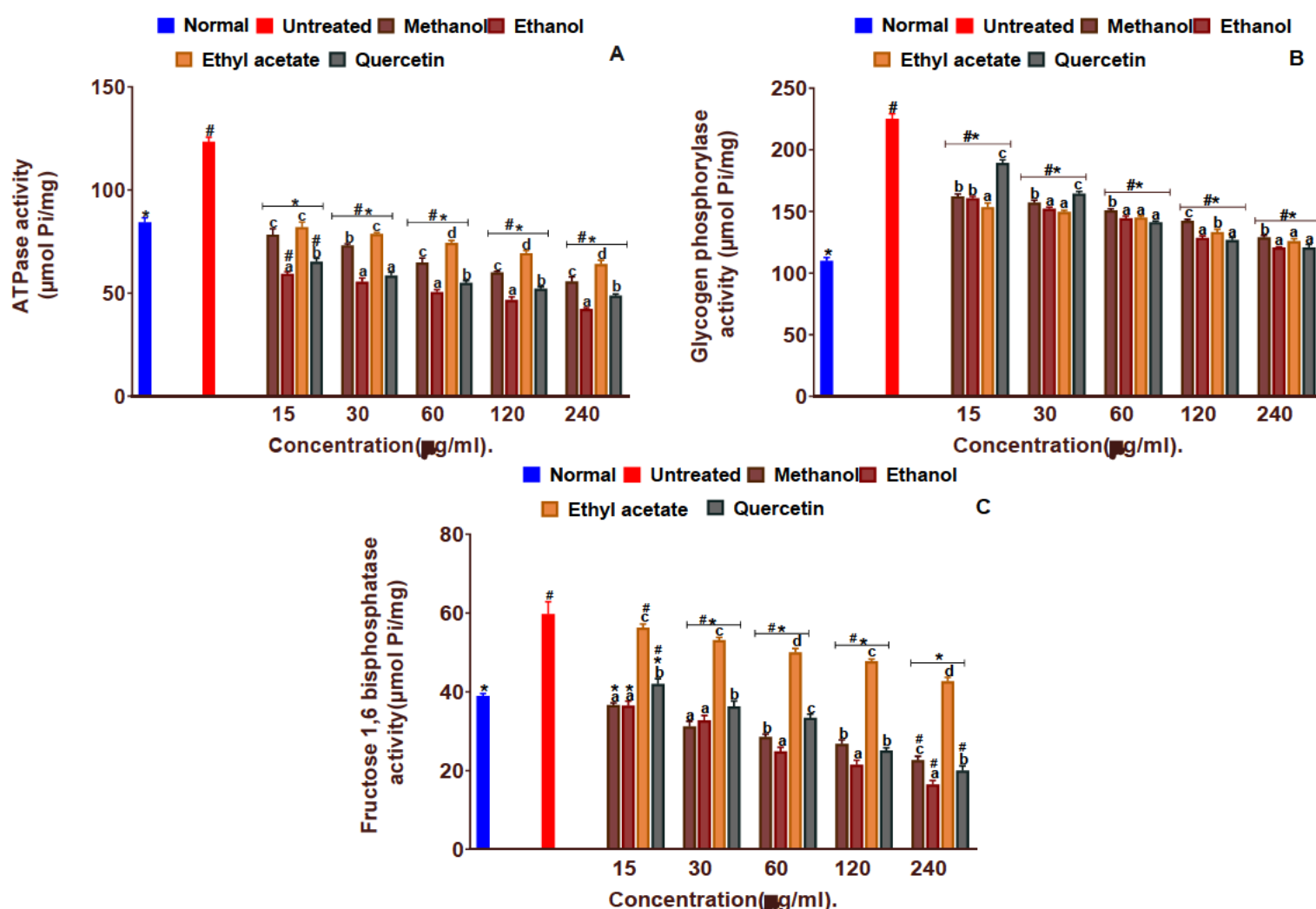


Figure 5.4.5: The modulatory effects of organic leaf crude extracts of *R. longipes* on (A) ATPase activity, (B) Glycogen phosphorylase activity and (C) Fructose 1,6 bisphosphatase activity. Data are presented as mean $\pm$ standard deviation. Different superscripts a, b and c represent statistical significance of $p<0.05$. *Significantly different from untreated sample and #Significantly different from normal sample ($p < 0.05$).

FeSO₄ induced oxidative cardiac tissue injury significantly ($p<0.05$) elevated lipid peroxidation while concomitantly suppressing superoxide dismutase (SOD) and catalase (CAT) activities, as well as glutathione (GSH) levels in the untreated control group as shown in Fig. 5.4.5A-D. Treatment with the methanol and ethanol extracts of significantly ($p<0.05$) reduced MDA levels (Fig.5.4.5A), implying an effective reduced lipid peroxidation particularly at 240 μ g/ml. Concomitantly, at the highest concentration of 240 g/ml, the ethanol extract significantly increased SOD activity compared to the other extracts which showed comparable SOD attenuating capacities, with no significant difference between them across all concentrations tested as depicted in Fig.5.4.5B. In contrast, the methanol extract resulted in significantly ($p<0.05$) the highest CAT augmenting capacity among all the extracts as seen in Fig.5.4.5C. Finally, as observed in Fig.5.4.5D, the ethanol extract significantly ($p<0.05$) increased GSH levels beyond vitamin C and all other extracts.

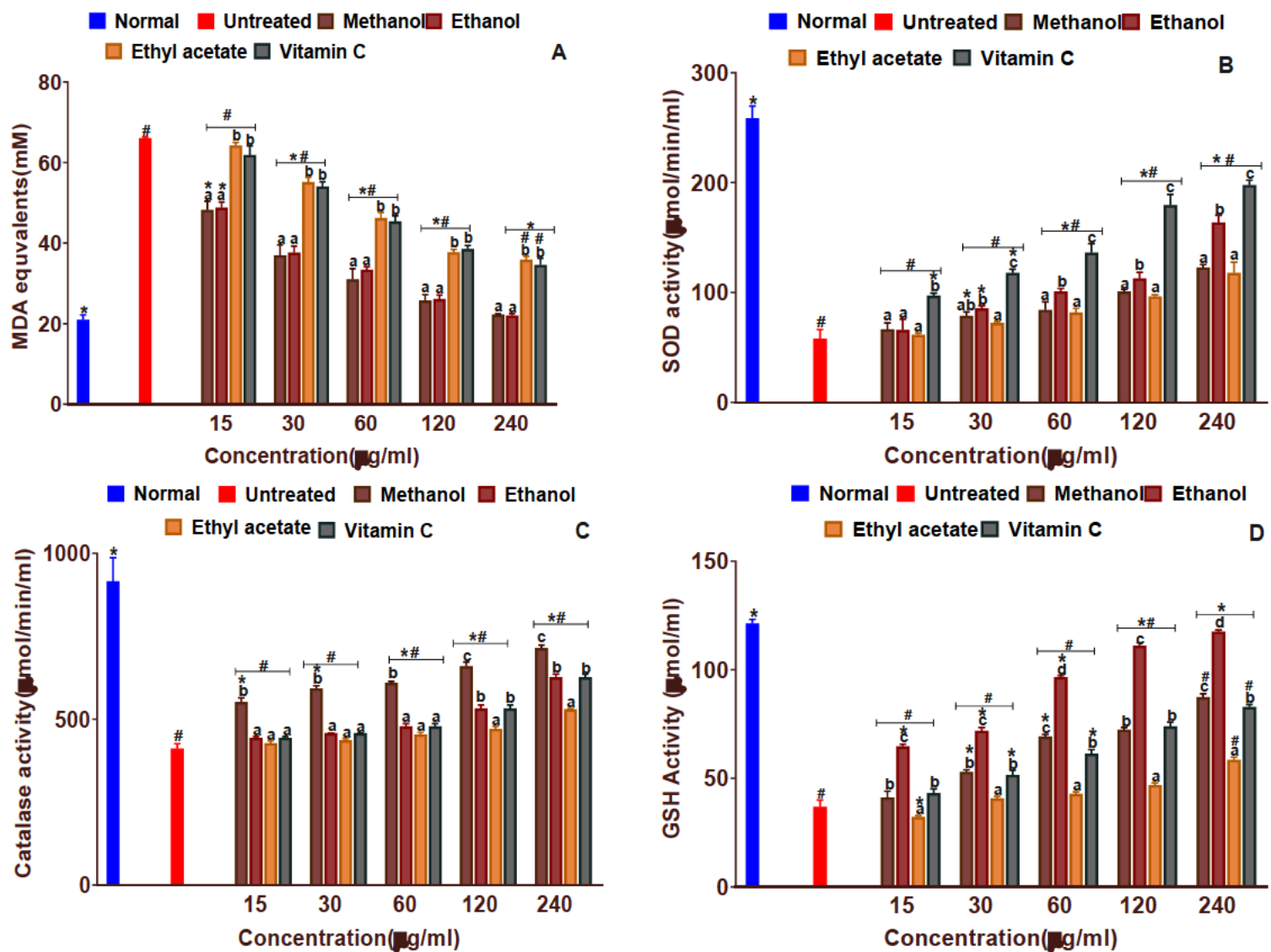


Figure 5.4.6: Effects of *R. longipes* leaf organic extracts on (A) lipid peroxidation, (B) SOD activity, (C) Catalase activity and (D) GSH activity in oxidative cardiac muscle tissue. Data are presented as mean $\pm$ standard deviation of triplicate determinations. Different letters (a-c) above the bars for a given concentration are significantly different from each other ($p < 0.05$). *Significantly different from untreated sample and #significantly different from normal sample.

5.4.5 Discussion

The global rise in the incidence of T2D continues to strain health-care systems, substantially increasing morbidity and premature mortality among patients (Mapa-Tassou et al., 2019). Consequently, there is an urgent need to develop novel antidiabetic therapeutic agents that are inexpensive, widely available, and have a high efficacy with few undesirable side effects (Misra et al., 2019; Artasensi et al., 2020). Traditional medicinal plants have thus emerged as lucrative potential reservoirs of pharmacological agent development templates (Artasensi et al., 2020). Indeed, previous data corroborates the immense value of traditional medicinal plants against several ailments including cancer, cardiovascular diseases (CVD), tuberculosis (TB), and T2D (Kooti, et al., 2016; Necyk, and Zubach-Cassano, 2017; Artasensi et al., 2020). Nutraceutical phytotherapies have an edge over their synthetic counterparts because they interact harmoniously with the body's natural metabolic processes to function synergistically on a variety of targets to provide a wholistic multifaceted therapeutic impact (Sonkamble et al., 2019). Indeed, the therapeutic agents from traditional medicinal plants stem from their inherent groups of secondary metabolites including phenolics, alkaloids, coumarin, tannins, and flavonoid derivatives. (Necyk, and Zubach-Cassano, 2017; Sonkamble et al., 2019).

Interestingly, some synthetic pharmacological antidiabetic medicines currently in the market such as metformin were developed from plant derived secondary metabolites (Abbas et al., 2016). Similarly, the presence of high concentrations of phenolics and flavonoids in Fig. 5.4.1 together with other bioactive compounds as revealed by GCMS analysis in Table 5.4.1 found in the leaf organic extracts of *R. longipes* suggests the plants' potential as a source of therapeutic pharmacological agents. Indeed, previous reports show the anticancer, cholesterol and triglyceride-lowering effects of squalene which was present in all the extracts of *R. longipes* (Table 5.4.1) (Kelly, 1999; Reddy, and Couvreur, 2009). l-(+)-ascorbic acid 2,6-dihexadecanoate a derivative of ascorbic acid has previously been reported as an antioxidant with wound healing properties (Ramya et al., 2015; Jayachitra and Janani, 2019). According to a study by Sakai in 2022, tetradecanoic acid (myristic acid), a fatty acid (14:0), has been shown to increase diacylglycerol kinase (DGK) δ protein levels and enhance glucose uptake in myotubes in a DGK δ -dependent manner. This suggests that myristic acid selectively affects β -tubulin protein levels in C2C12 myotubes through DGK δ , potentially improving skeletal muscle mass and increasing glucose uptake activity per cell. Evidently, these previous findings corroborate the plethora of health benefits associated with the phytoconstituencies of the leaf organic extracts of *R. longipes* including their ability to attenuate

and maintain homeostatic redox balance (Sonkamble *et al.*,2019).

Under homeostatic conditions reactive species (RS) mediate energy redox, act as secondary messengers, and form part of the inflammatory immune response system (Yaribeygi *et al.*,2020). However, under T2D conditions whereby there are excessive RS, intrinsic endogenous antioxidant defense systems are overwhelmed and oxidative stress ensues (Yaribeygi *et al.*,2020). Consequently, excessive RS including reactive oxygen species (ROS), reactive chloride species (RCS), and reactive nitrogen species (RNS) exert deleterious effects against functional biomolecules such as lipids, proteins, and nucleic acids. (Gaschler and Stockwell, 2017; Sonkamble *et al.*,2019; Yaribeygi *et al.*,2020). The disruptive nature of RS on the precise molecular structure and conformation of these molecules impedes their function and is thus implicated in pathogenesis and progression of several degenerative diseases including cancer, Alzheimer's, and T2D (Maiese, K., 2015). However, *R. longipes* leaf organic extracts' abilities to scavenge excessive free radicals (DPPH and NO), donate electrons (FRAP) observed in Fig. 5.4.2A-C together with their capabilities to reduce lipid peroxidation in Fig. 5.4.1 A is indicative of their antioxidant capacity and therapeutic benefits against oxidative cardiac tissue damage. Intriguingly, 3-pentadecylphenol present in *R. longipes* (Table 5.4.1) has previously been detected in cashew nuts (Ike *et al.*,2021), and in the fruit juice of *Bouea macrophylla* of the same family (Trifani *et al.*, 2022) is endowed with several health benefits including enhanced antioxidant activity. Additionally, as suggested in previous reports *R.longipes* leaf organic extracts may alternatively confer therapeutic antioxidant protective abilities in tissues by bolstering their innate endogenous antioxidant defense systems (Catalá, and Díaz, 2016; Gaschler, and Stockwell, 2017).

Undeniably, endogenous antioxidant defense systems including reduced glutathione (GSH), super oxide dismutase (SOD) and catalase (CAT) form the innate protective response against excessive RS (Aguilar *et al.*,2016). GSH is a non-enzymatic protein that forms the first line of defense against RS (Mironczuk-Chodakowska *et al.*,2018). SOD dismutates superoxide to produce hydrogen peroxide which is further neutralized to water by CAT (Neha *et al.*,2019). As evident in Fig. 5.4.6B-D the induction of oxidative cardiac tissue injury undeniably led to diminished antioxidant defense systems. In the absence of coronary artery disease, damage to cardiac muscle tissue by excessive RS is a major cause of diabetic cardiomyopathy which predisposes in T2D patients to increased risks of heart failure (Liu *et al.*,2014). Consequently, *R. longipes* leaf organic extracts which indeed increased the levels of GSH and augmented the activities of SOD and CAT may help to combat the deleterious effects of excessive RS in T2D abating the development of cardiovascular diseases (CVD) such as diabetic cardiomyopathy

(Pisoschi, and Pop, 2015; Neha *et al.*,2019). Indeed, the current data shows that *R. longipes* leaf organic extracts contain bioactive compounds with immense potential against multifaceted conditions that are aggravated by hyperglycemic induced oxidative stress in T2D (Sonkamble *et al.*,2019).

In obese patients with T2D, oxidative stress is exacerbated as the two conditions aggravate each other due to shared genetic risk factors. (Andolfi, and Fisichella, 2018; Blüher, and Stumvoll, 2020). Obesity is characterized by an abnormal buildup of fatty acids in adipose tissue, which is especially prevalent in T2D patients with dysregulated lipid metabolic pathways and hyperactivity of lipid hydrolyzing enzymes, such as pancreatic lipase. Indeed, the capabilities of *R. longipes* leaf organic extracts to inhibit pancreatic lipase activity observed in Fig. 5.4.3A insinuates their potential to curb excessive fatty acid metabolism therefore abate T2D linked dyslipidaemia and obesity. Evidently, previous reports indicate that T2D patients with healthy body weight have improved glycemic levels, glucose tolerance, peripheral tissue insulin sensitivity, abdominal fat distribution and inflammatory responses as opposed to those that are obese. (McMurray *et al.*,2016; Andolfi, and Fisichella, 2018; Blüher, and Stumvoll, 2020).

Dietary carbohydrates drive energy dependent pathways through their conversion to adenosine triphosphate (ATP), any surplus is then stored as glycogen in muscle and liver or as fatty acids in the adipose tissue (Ahmadovna and Sharipovna, 2021). Complex carbohydrates are broken down to monosaccharides such as glucose by key hydrolytic enzymes including α amylase and α glucosidase. Indeed, the rapid absorption of monosaccharides including glucose by the small intestine mediated through facilitated diffusion by the sodium-glucose linked transporter 1 (SGLT1) is implicated in postprandial blood glucose spikes in T2D patients. Therefore, the ability of *R. longipes* to inhibit α amylase and α glucosidase (Fig. 5.4.3B & C) coupled with their ability to retard glucose absorption in the small intestine as shown in Figure 5.4.4A, insinuates the extracts' capabilities to reduce hyperglycemia in T2D patients. This observation is coherent with previous studies that allude to the antihyperglycemic capacity of some traditional medicinal plants used in T2D management to their inhibitory capabilities against key carbohydrate-digesting enzymes (Alam *et al.*,2019; Bhatnagar, and Mishra, 2022). Similarly, acarbose and voglibose which function by inhibiting α glucosidase are among the prominent T2D management drugs currently being prescribed which were derived from natural pharmacological templates (Alam *et al.*,2019; Bhatnagar and Mishra, 2022).

Peripheral tissues including the brain, adipose, liver, and muscles require copious amounts of glucose to drive their constant high energy-demanding biochemical pathways and therefore

constitute the major glucose disposal mechanisms in the body (Lin *et al.*,2021). Augmented glucose clearance by peripheral tissues is a key regulatory mechanism to reduce hyperglycemia in T2D patients (Thilagam *et al.*,2013; Han *et al.*,2016). Consequently, data in the current study which revealed the *R. longipes* extracts' ability to increase glucose uptake in yeast cell suspension (Figure 5.4.4B), liver (Figure 5.4.4 C), and muscle tissue Figure 5.4.4 D), infers their capacity to alleviate chronic hyperglycemia in T2D patients by increasing blood glucose clearance (Thilagam *et al.*,2013). Indeed, improved glucose disposal especially in the adipose and muscle tissues is due to increased insulin-mediated GLUT4 translocation (Chadt, and Al-Hasani, 2020). Therefore, the *R. longipes* extracts' may increase glucose uptake in these tissues by upregulating GLUT4 translocation presumably by binding to several proteins in the insulin transduction pathway. While GLUT4 is the major insulin-responsive glucose transporter in the adipose and muscle tissue, other isoforms including the GLUT2 perform similar functions in the liver (Chadt, and Al-Hasani, 2020).

Increased calorie influx under fed conditions stimulates glycogenesis in the muscle and liver (Zhang *et al.*,2021). Indeed, hepatic glycogen stores are exploited to maintain homeostatic blood glucose levels through glycogenolysis during intermittent fasting (Zhang *et al.*,2021). Consequently, the depletion of hepatic glycogen stores prompts gluconeogenesis in which glucose is synthesized from non-carbohydrate sources including amino acids, glycerol, and lactate (Han *et al.*,2016). Glycogen phosphorylase and fructose 1,6 bisphosphatase play pivotal roles in glycogenolysis and gluconeogenesis (Bano, 2013; Han *et al.*,2016). Therefore, the overactivity of hepatic glycogen phosphorylase (Figure 5.4.5B) and fructose 1,6 bisphosphatase (Figure 5.4.5C) observed insinuate elevated glycogenolysis and gluconeogenesis leading to increased hepatic glucose output and exacerbated hyperglycaemia in T2D. Indeed, the inhibitory capabilities of the *R. longipes* extracts on both hepatic glycogen phosphorylase and fructose 1,6 bisphosphatase may therefore aid in abating exacerbated chronic hyperglycemia in T2D patients. Furthermore, inhibiting glycogen phosphorylase increases glycogen store build-up (Hayes *et al.*,2014), whereas therapeutic drugs that inhibit fructose 1,6 bisphosphatase have been demonstrated to diminish protein breakdown and hence may alleviate weight loss in T2D patients (Kaur *et al.*,2017). Indeed, the data shown in this study is coherent with previous studies that highlight the promising capacity of agents showing glucogenic hepatic enzyme inhibitory activities as antidiabetic therapeutic agents (Donnier-Maréchal *et al.*,2015; Han *et al.*,2016).

ATPase is a nucleotide metabolizing enzyme responsible for energy production through the conversion of ATP to ADP and an inorganic phosphate molecule (Radosinska, and Vrbjar,

2016). Indeed, ATPase is crucial in regulating the energy redox in the body by maintaining homeostatic ATP:ADP ratios in the body. Consequently, the hyperactivity of hepatic ATPase as observed in Figure 5.4.5A insinuates ATP depletion signifying a shift in the homeostatic ATP:ADP ratio. Depletion of ATP in T2D is linked to perpetual fatigue as tissues are deprived of energy to drive energy requiring reactions including GLUT4 translocation and muscle contraction (Radosinska and Vrbjar, 2016). Therefore, the ability of the *R.longipes* extracts to inhibit hepatic ATPase activity is indicative of their potential to regulate energy redox in T2D patients with dysregulated ATP:ADP ratios.

5.4.6 Conclusion

These results indicate the antioxidant and antidiabetic potential of *R. longipes* as depicted by the extracts' capacity to increase glucose uptake in peripheral liver and muscle tissues while inhibiting key glycogenic enzymes and retarding glucose absorption in rat intestinal jejunum. Furthermore, the notable protective effects of *R. longipes* against oxidative damage in cardiac muscle tissue through reduced lipid peroxidation and an increase in glutathione level, as well as enhanced activities of SOD and catalase suggest its potential to reduce cardiovascular complications associated with T2D. However, these pharmacological activities exhibited by *R. longipes* warrant further investigation through molecular and *in vivo* studies to elucidate their precise mechanisms of action.

5.5 *Rhus longipes* (Engl.) improves atherogenic indices, glucose metabolism and ameliorates oxidative stress in streptozotocin-induced type 2 diabetic rat model

Brian K Beseni¹, Kolawole A Olofinisan¹, Erukainure L Ochuko¹ * Shahidul Md Islam¹

1. Department of Biochemistry, University of KwaZulu-Natal, Westville Campus, Durban 4000, South Africa

***Corresponding author:** islamd@ukzn.ac.za

5.5.1 Abstract

The rampant upsurge in the global incidence of type 2 diabetes mellitus (T2D) has reached epidemic levels prompting scientists to probe the potential of the development of novel antidiabetic drugs from traditional medicinal plants such as *Rhus longipes*. The current study investigated the antioxidant, antihyperglycemic and antihyperlipidemic capabilities of *R. longipes* in a T2D rat model. T2D induction was achieved by an intraperitoneal injection of streptozotocin (40 mg/kg bodyweight (bw)) to rats fed with 10% fructose solution *ad libitum* for two weeks. Preceding T2D confirmation, intervention was carried out by daily administration of low dose (150 mg/kg bw) and high dose (300 mg/kg bw) of the *R. longipes* extract 5 times a week during the trial period. Mean food and fluid intake were recorded daily, body weight and blood glucose levels were measured weekly while the oral glucose tolerance test (OGTT) was performed in the last week of treatment. The high dose of *R. longipes* (DRLH) restored euglycemic levels, improved glucose tolerance, and mitigated dyslipidaemia with a concomitant reduction in the atherogenic index. Furthermore, the extracts ameliorated redox imbalance while restoring hepatic glycogen stores through the modulation of hepatic glucogenic enzymes. *R. longipes* reversed oxidative injury on pancreatic tissue as revealed by histopathological examinations potentially by augmenting the antioxidant defence system. Current data suggests that the ameliorative potential of *R. longipes* on T2D-linked pathologies may occur through several mechanisms including upregulated insulin sensitivity and secretion through the regeneration of pancreatic β -cells. Further molecular analyses and clinical trials are necessary to completely elucidate the precise mode of antidiabetic action shown in the current study.

Keywords: Antioxidant, T2D animal model, Dyslipidaemia, Antidiabetic, *Rhus longipes*.

5.5.2 Introduction

The rampant rise in the global incidence of diabetes mellitus (DM) continues to immensely burden healthcare systems (Forouhi and Wareham, 2019). Current epidemiological data shows that DM has reached pandemic proportions and its prevalence is a record high with an estimated

536.6 million adults currently living with the condition (Ogurtsova *et al.*,2022; Sun *et al.*,2022). While several etiological classifications of DM exist, type 1, type 2, and gestational diabetes are the most prevalent, with type 2 diabetes (T2D) accounting for over 90% of all DM cases globally (Ogurtsova *et al.*,2022). While the precise pathogenesis triggers leading to the development and progression of T2D remain scantily understood, ongoing scientific research implicates aberrant insulin metabolism as a major culprit.

Insulin is a pivotal anabolic endocrine hormone that regulates several interconnected physiological pathways including glycogen synthesis, protein synthesis, glucose uptake, lipid metabolism, cellular growth, and differentiation (Yaribeygi *et al* 2019). Disturbances in insulin production, action or both thus contribute to the development of T2D (Yaribeygi *et al* 2019). Indeed, DeFronzo (2009) summed up the major dysregulated pathways that stem from aberrant insulin metabolism as the ominous octet of T2D. These include hepatic and muscular insulin resistance, increased hepatic glucose uptake, increased renal glucose reabsorption, lipotoxicity, neurotransmitter, pancreatic β and α cell dysfunction and incretin deficiency (DeFronzo, 2009; Kelly and Neary, 2020). Furthermore, oxidative stress has recently emerged as the most prominent exacerbating factor linking the ominous octet pathways of T2D, chronic hyperglycemia and associated long-term complications (Pitocco *et al.*,2013).

Oxidative stress is a redox imbalance between reactive species (RS) and endogenous antioxidant defense systems (Pitocco *et al.*,2013). Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are among the most encountered electron-deficient RS which either mediate essential pathways or are by-products of such pathways under homeostatic conditions. Oxidative tissue damage is also mediated through other pathways including polyol and hexosamine pathway activation, increase in intracellular advanced glycation end-products (AGEs) formation and their receptors and increased protein kinase C (PKC) activation. (Pitocco *et al.*,2013; Asmat *et al.*,2016). Consequently, chronic hyperglycaemia-induced oxidative stress is implicated in the development of end stage T2D related macrovascular and microvascular complications (Nellaiappan *et al.*,2022).

T2D microvascular complications include neuropathy, nephropathy and retinopathy, while the macrovascular complications include diabetic heart disease and stroke (Nellaippan *et al.*,2022). Complications resulting from DM are among the leading causes of morbidity and premature mortality with an estimated 6.7 million adults between the age of 20–79 reported to have succumbed to DM in 2021(IDF, 2022). While a cure for T2D is currently not available there are several strategies for its management.

Management strategies for T2D which are aimed at achieving and maintaining homeostatic glycaemic levels are broadly categorised as either non-pharmacological or pharmacological interventions. Non-pharmacological interventions are the first line of therapy and involve medical nutrition therapy, change of lifestyles and bariatric surgery (Raveendran *et al.*,2018). Conversely, pharmacological DM management strategies include insulin injections particularly for type I patients (Hu and Jia, 2019). While T2D oral pharmacological management agents are broadly classified as sulfonylureas, thiazolidinediones, α -glucosidase inhibitors and sodium-glucose cotransporter 2 (SGLT-2) inhibitors (Hu and Jia, 2019). However pharmacological T2D management strategies are marred with undesirable side effects including acute hypoglycaemic shock, weight gain, gastro-intestinal upsets, and flatulence (Salehi *et al.*,2019; Shelke, and Zilate, 2021). Chronic use of these medications may cause contraindications with other medications and have been implicated in renal failure and hepatotoxicity (Padhi *et al.*,2020; Shelke, and Zilate, 2021). Therefore, there is continued research to develop novel antidiabetic agents with high efficacy, increased bioavailability, reduced dosing frequency and reduced side effects (Hu, and Jia, 2019).

Folkloric medicinal plants, endowed with bountiful secondary metabolites, have thus emerged as a lucrative source of novel antidiabetic pharmacological drug development templates (Salehi *et al.*,2019). Traditional medicinal plants have been employed to remedy a multitude of ailments due to their availability, affordability, and low side effects (Sam, 2019). Several secondary metabolite groups including phenols, flavonoids, conjugated glycosides, saponins, carotenoids, alkaloids, and terpenoids confer the beneficial pharmacological activities observed in plants (Sam, 2019). Previous research has shown that these secondary metabolites act as antidiabetic, antihyperglycemic, hypoglycaemic, antihyperlipidemic, and insulin-mimetic properties α -amylase and α -glucosidase inhibition (Salehi *et al.*,2019). Metformin is among the most prominent marketed antidiabetic drugs that was derived from the medicinal plant known as *Galega officinalis* (Abbas *et al.*,2016). Similarly, *R. longipes* currently under study is a folkloric traditional medicinal plant of the family anacardiaceae with

several reported bioactivities. *R. longipes* has previously been used to remedy infertility in Zimbabwe (Maroyi 2011) while certain regions in Nigeria use the plant against asthma and malaria (Olorunnisola *et al.*, 2017). A previous study by Olorunnisola *et al* (2017) documented the antioxidant capabilities of *R. longipes* and its efficacy against paracetamol-induced hepatic toxicity. This was furthermore corroborated by previous studies in our laboratory that reported the antioxidant and antidiabetic capabilities of the aqueous infusions of leaf and stem bark on oxidative renal injury using some *in vitro* and *ex vivo* experimental models (Beseni *et al.*, 2022). Therefore, the current study was aimed at investigating the antidiabetic and antioxidant capabilities of *R. longipes in vivo* using a T2D rat model.

5.5.3 Materials and methods

The materials and methods for this study are detailed in Chapter 2 section 2.12

5.5.4 Results

As depicted in Fig.5.5.1, the diabetic control group (DBC) consumed significantly ($p<0.05$) more feed and fluid when compared to the normal control (NC) group. Furthermore, there was no significant difference between the NC and NRLTX groups for either of these parameters. Treatment with the high dose of *R. longipes* (DRLH) significantly ($p<0.05$) reduced fluid intake. When compared to the DBC group, both the DRLH and DRLH reduced food intake, however the impact was not statistically significant ($p>0.05$).

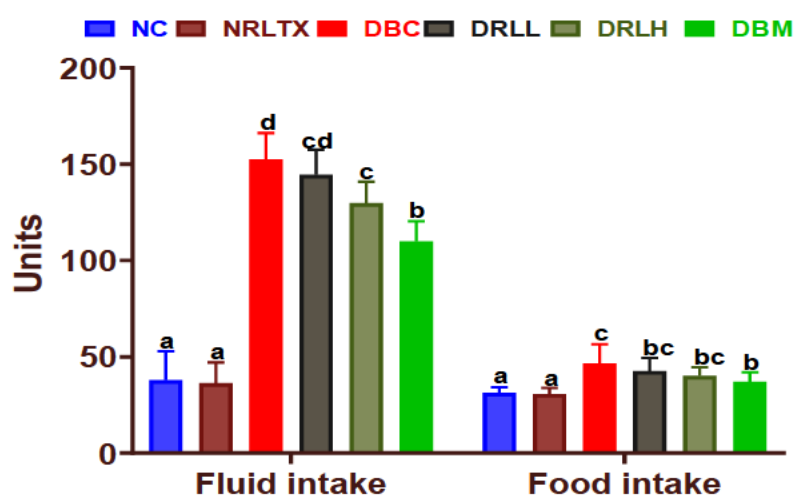


Figure 5.5.1: Food and Fluid intake in different animal groups during the intervention period. NC= Normal Control, NRLTX = Normal rats + *R. longipes* high dose, DBC= Diabetic Control, DRLH= Diabetic rats + *R. longipes* low dose, DRLH =Diabetic rats + *R. longipes* high dose, DBM: Diabetic rats + Metformin (positive control). Different superscripts a-d represent statistical significance of $p<0.05$ (Tukey's multiple range *post hoc* test at $p < 0.05$).

As depicted in Figure 5.5.2, there was no significant ($p>0.05$) difference in body weight among all the groups during the initial week of the trial. However, starting from the third week, the progression of Type 2 Diabetes (T2D) resulted in a significant ($p<0.05$) difference in body weight between the Normal Control (NC) and diabetic groups. Notably, the Diabetic Control (DBC) group displayed clear signs of malaise, leading to a significant ($p<0.05$) reduction in body weight compared to all other groups by the fifth week. Interestingly, treatment with the high dose of *R. longipes* (DRLH) significantly ($p<0.05$) improved body weight gain when compared to the animals in the DBC group, even though it remained significantly ($p<0.05$) lower than the NC group's body weight. Importantly, there were no significant differences observed between the body weight changes of the animals in the DRLH group and those treated with metformin (DBM).

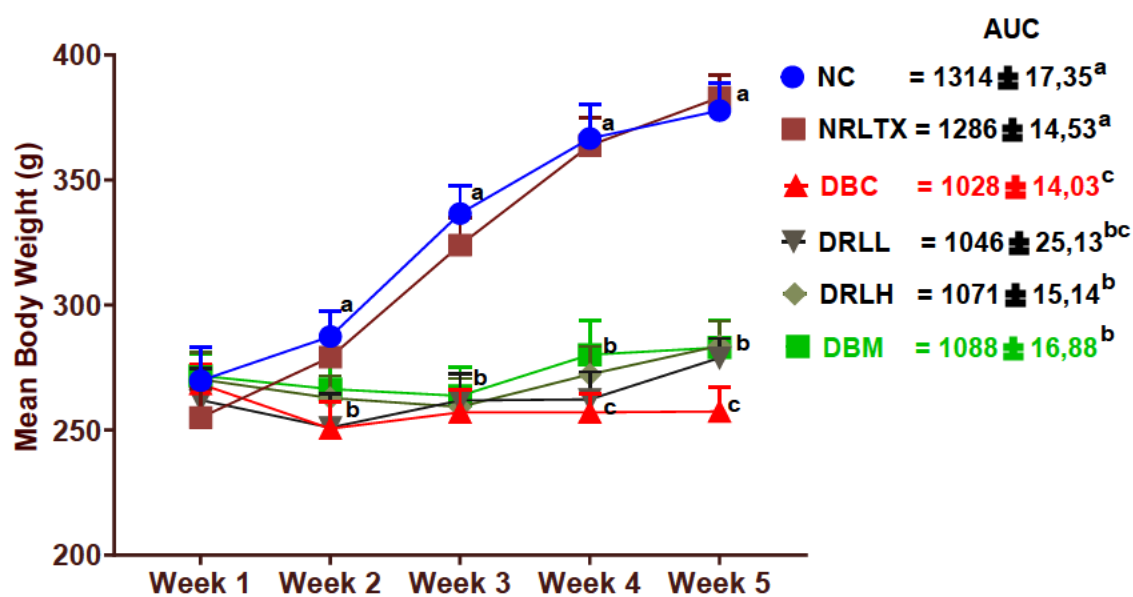


Figure 5.5.2: Effect of oral treatment on mean body weight in different animal groups during the 5-week intervention period. NC= Normal Control, NRLTX = Normal rats + *R. longipes* high dose, DBC= Diabetic Control, DRLL= Diabetic rats + *R. longipes* low dose, DRLH =Diabetic rats + *R. longipes* high dose, DBM: Diabetic rats + Metformin (positive control). Different superscripts a-d represent statistical significance of $p < 0.05$.

The diabetic groups (DBC, DRLL, DRLH and DBM) exhibited significantly ($p < 0.05$) elevated blood glucose levels in comparison to the normal control groups (NC and NRLTX) at the commencement of the trial as observed in Figure 5.5.3. As indicated by the AUC, the DBC group exhibited significantly ($p < 0.05$) the highest blood glucose levels among all the groups. It is noteworthy that, during the initial two weeks of treatment, only the DBM group demonstrated a significant ($p < 0.05$) reduction in blood glucose levels compared to the other diabetic groups. However, starting from the third week onward, both the DRLL and DRLH groups displayed markedly significantly ($p < 0.05$) lower blood glucose levels in comparison to the DBC group. Furthermore, as the trial concluded, these treatments led to blood glucose levels that approached normalcy.

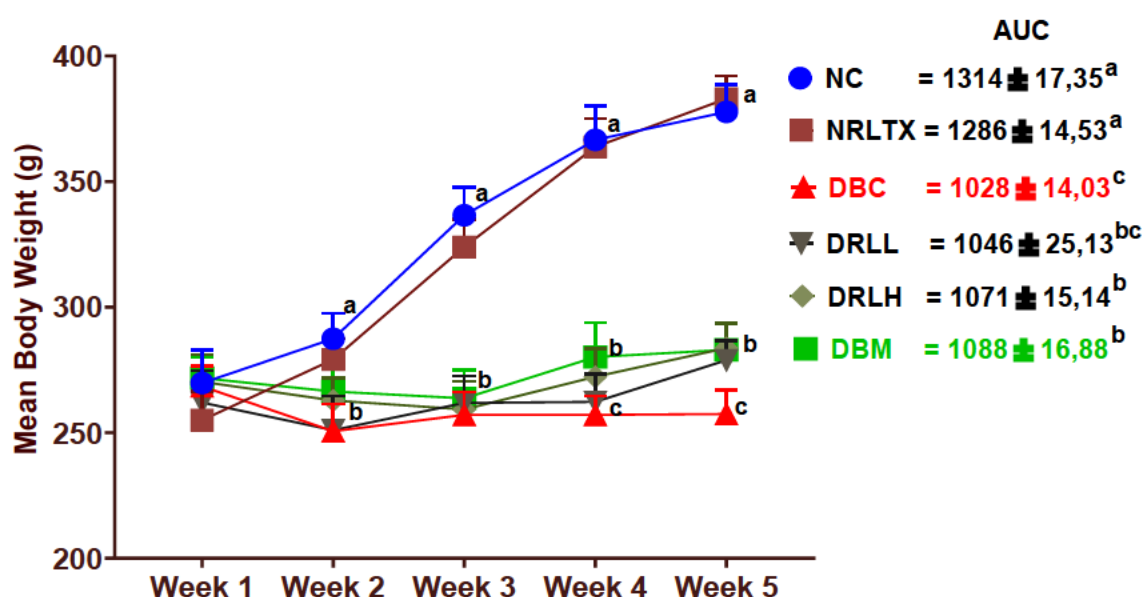


Figure 5.5.3: Effect of oral treatment on blood glucose in different animal groups during the intervention period. **NC**= Normal Control, **NRLTX** = Normal rats + *R. longipes* high dose, **DBC**= Diabetic Control, **DRLL**= Diabetic rats + *R. longipes* low dose, **DRLH** =Diabetic rats + *R. longipes* high dose, **DBM**: Diabetic rats + Metformin (positive control). Different superscripts a-e represent statistical significance of $p < 0.05$ (Tukey's multiple range *post hoc* test at $p < 0.05$).

The oral glucose tolerance test (OGTT) is depicted in Fig. 5.5.4. The administration of glucose load significantly ($p < 0.05$) elevated the blood glucose levels after 30 minutes in all diabetic groups (DBC, DRLL, DRLH and DBM) when compared to the normal groups (NC and NRLTX), while rats in the DBC groups had significantly the highest blood glucose levels among all the groups at this time point, no significant increases were observed in the NC and NRLTX at any of the time points recorded. Evidently, treatment with DRLH significantly

improved glucose tolerance resulting in the lowest blood glucose spike among all groups as signified by the AUC and as observed after 120 minutes in Fig. 5.5.4. Furthermore, no significant difference was observed in glucose tolerance improving capabilities of the *R. longipes* low dose (DRLL) and DBM which also lead blood glucose levels significantly ($p<0.05$) lower than the DBC.

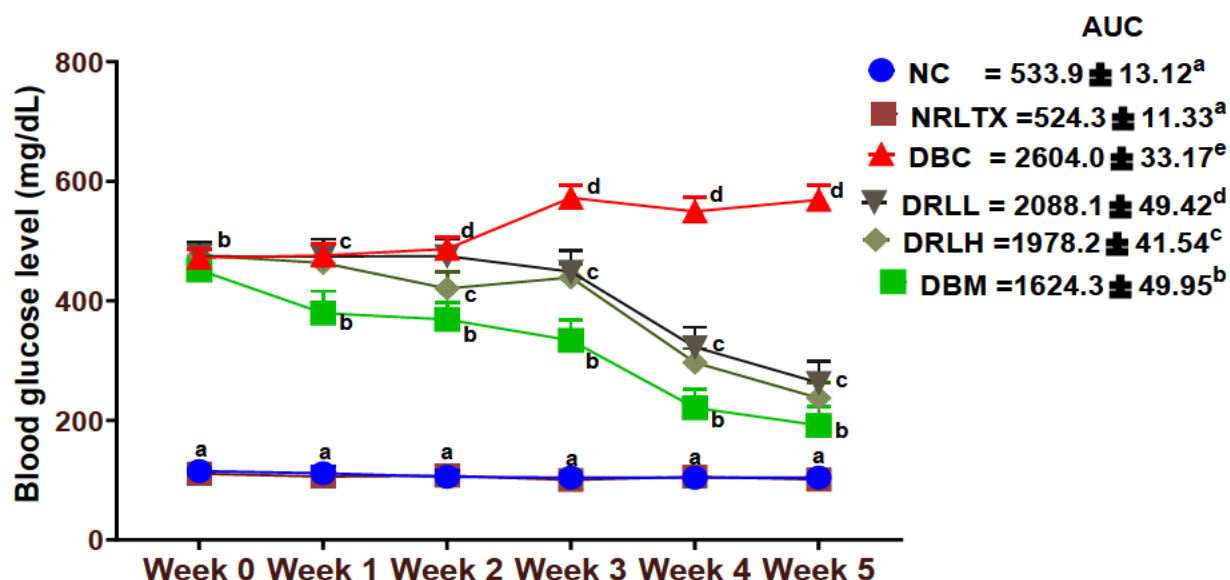


Figure 5.5.4: Oral glucose tolerance test (OGTT) of different animal groups in the last week of experimental period. NC= Normal Control, NRLTX = Normal rats + *R. longipes* high dose, DBC= Diabetic Control, DRLL= Diabetic rats + *R. longipes* low dose, DRLH =Diabetic rats + *R. longipes* high dose, DBM: Diabetic rats + Metformin (positive control). Different superscripts a-d represent statistical significance of $p<0.05$ (Tukey's multiple range *post hoc* test at $p < 0.05$).

As depicted in Fig 5.5.5 A, diabetes induction significantly ($p<0.05$) diminished serum insulin levels in the DBC group when compared to the NC group whose insulin levels were not statistically different from those in the NRLTX. Treatment with DRLH significantly ($p<0.05$) increased serum insulin levels higher than all the diabetic groups, while the low dose of *R. longipes* increased serum insulin levels, the difference was not statistically significant from the DBC group. Furthermore, the homeostasis model assessment of β -cell function (HOMA- β) and insulin resistance (HOMA-IR) scores shown in Fig 5.5.5 B indicate a significant ($p<0.05$) decrease in β pancreatic cell function with a concomitant increase in peripheral tissue function in the DBC group when compared to the normal groups (NC and NRLTX). Treatment with the high dose of *R. longipes* (DRLH) significantly($p<0.05$) increased Homa- β scores while reducing Homa-IR scores signifying improved peripheral tissue insulin resistance.

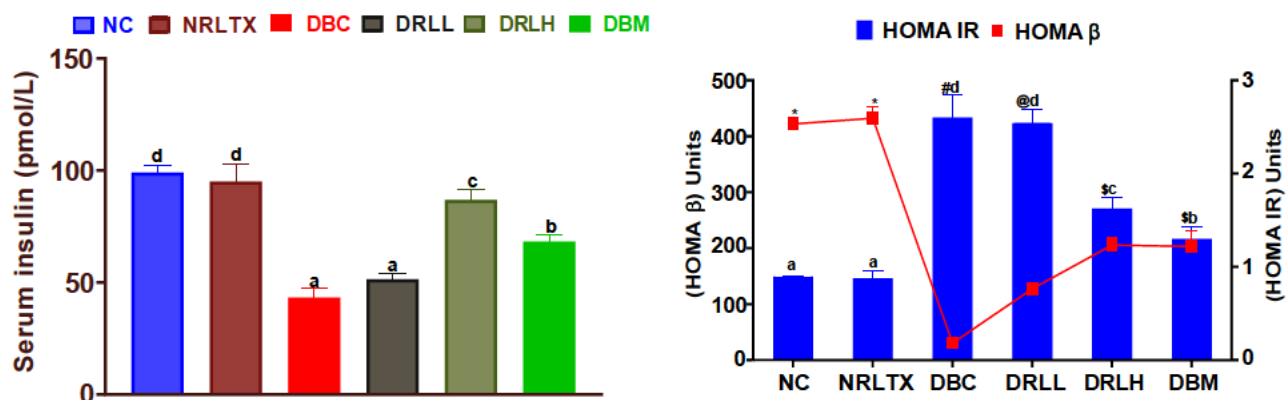


Figure 5.5.5: Effect of oral treatment on A) serum insulin levels B) homeostatic assessment model (HOMA-IR and HOMA-β) in different animal groups during the experimental period. **NC**= Normal Control, **NRLTX** = Normal rats + *R. longipes* high dose, **DBC**= Diabetic Control, **DRLL**= Diabetic rats + *R. longipes* low dose, **DRLH** =Diabetic rats + *R. longipes* high dose, **DBM**: Diabetic rats + Metformin (positive control). Different superscripts a-d above the bars represent $p<0.05$ level of statistical significance in insulin levels and HOMA IR scores while symbols *#@\$ represent statistical significance of $p<0.05$ in HOMA β scores.

As shown in Fig. 5.5.6, the DBC group exhibited significantly ($p<0.05$) elevated relative liver weights with significantly diminished liver glycogen reserves among all test groups including the NC. Treatment with both the *R. longipes* high (DRLH) and low (DRLL) dose and significantly ($p<0.05$) reduced relative liver weights while boosting their liver glycogen reserves. Evidently, the *R. longipes* high (DRLH) dose, which was not significantly($p>0.05$) different from DBM, resulted in better capabilities to reduce relative liver weights and fortify their glycogen reserves than the low dose.

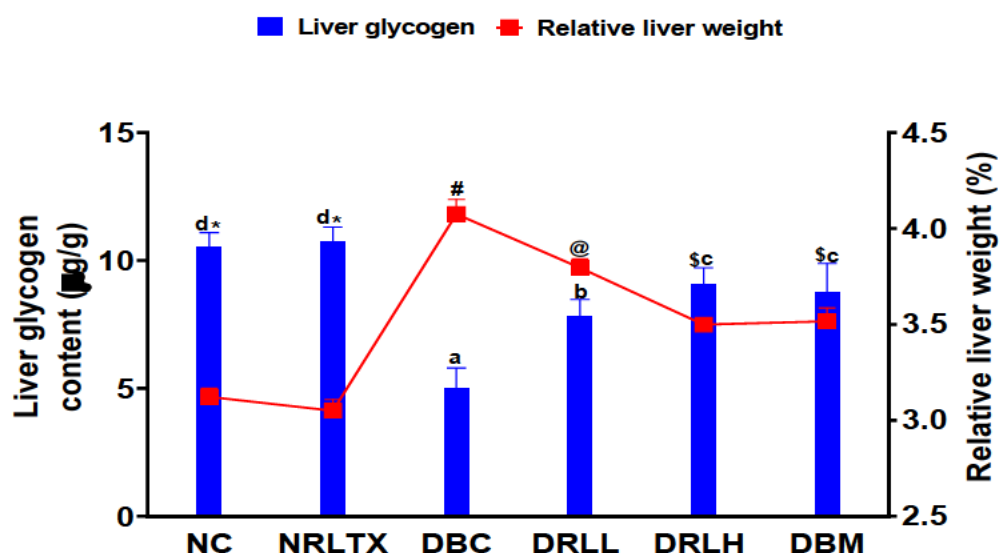


Figure 5.5.6: Effect of oral treatment on relative liver weight and glycogen content in different animal groups during the experimental period. **NC**= Normal Control, **NRLTX** = Normal rats + *R. longipes* high dose, **DBC**= Diabetic Control, **DRLL**= Diabetic rats + *R. longipes* low dose, **DRLH** =Diabetic rats + *R. longipes* high dose, **DBM**: Diabetic rats + Metformin (positive control). Different superscripts a-d above the bars represent $p < 0.05$ level of statistical significance in liver glycogen content while symbols *#@\$ represent $p < 0.05$ level of statistical significance in relative liver weights.

As seen in Table 5.5.1, induction of diabetes significantly ($p<0.05$) elevated the activities of hepatic glycogen phosphorylase, glucose 6 phosphatase and fructose 1,6 bisphosphatase while concomitantly diminishing ATPase activity in the DBC group when compared to the NC group. Treatment with DRLH, which outperformed DRLL, significantly ($p<0.05$) reduced hepatic glycogen phosphorylase, glucose 6-phosphatase and fructose-1,6-bisphosphatase activities while significantly ($p<0.05$) increasing that of ATPase alluding to better glucose metabolism and energy redox balance. Furthermore, as shown in Table 5.5.1 no significant differences ($p<0.05$) were observed in the activities of these enzymes between the NC and NRLTX group.

Table 5.5.1: The effects of oral treatment of hepatic glucogenic and nucleotide hydrolysing enzymes.

	NC	NRLTX	DBC	DRLL	DRLH	DBM
Glycogen phosphorylase*	153,673±9,65 ^a	152,87±8,37 ^a	218,367±11,21 ^e	163,55±7,76 ^c	158,37±8,63 ^b	176,37±8,11 ^d
Glucose 6 phosphatase*	148,76±5,81 ^a	145,76±7,10 ^a	228,71±10,37 ^d	160,76±11,07 ^c	152,12±8,89 ^b	150,97±5,67 ^b
Fructose 1,6 Bisphosphatase*	180,21±7,31 ^a	176,04±9,76 ^a	253,67±12,35 ^d	195,31±9,72 ^c	188,73±9,31 ^b	189,07±7,92 ^b
ATPase*	301,68±10,36 ^d	298,1±10,31 ^d	210,32±10,37 ^a	265,73±8,60 ^b	283,27±12,12 ^c	268,13±8,36 ^b

*Units = μ mol Pi/mg. **NC**= Normal Control, **NRLTX** = Normal rats + *R. longipes* high dose, **DBC**= Diabetic Control, **DRLL**= Diabetic rats + *R. longipes* low dose, **DRLH** =Diabetic rats + *R. longipes* high dose, **DBM**: Diabetic rats + Metformin (positive control). Different superscripts a-d represent statistical significance of $p<0.05$.

As shown in Table 5.5.2, in comparison to the NC group, both α amylase and lipase in the serum and pancreas were significantly ($p<0.05$) elevated in the diabetic control animal group (DBC). Evidently, treatment with the high dose of the extract significantly ($p<0.05$) reduced both α amylase and lipase in the serum and pancreas activities compared to the lower dose.

Table 5.5.2: Effect of oral treatment on carbohydrate and lipid hydrolysing enzymes.

	NC	NRLTX	DBC	DRLL	DRLH	DBM
Serum α -amylase U/L	22,37 $\pm$ 1,08 ^a	23,49 $\pm$ 3,41 ^a	76,49 $\pm$ 3,71 ^e	38,97 $\pm$ 1,96 ^c	28,17 $\pm$ 2,11 ^b	46,37 $\pm$ 2,48 ^d
Pancreatic α -amylase U/L	30,21 $\pm$ 0,37 ^a	31,22 $\pm$ 0,53 ^a	83,57 $\pm$ 2,55 ^e	59,17 $\pm$ 0,98 ^d	38,76 $\pm$ 1,66 ^b	43,16 $\pm$ 1,54 ^c
Serum lipase U/L	21,45 $\pm$ 1,11 ^a	23,17 $\pm$ 1,10 ^a	66,97 $\pm$ 2,82 ^d	41,94 $\pm$ 1,06 ^c	35,63 $\pm$ 1,45 ^b	33,89 $\pm$ 1,23 ^b
Pancreatic lipase U/L	37,367 $\pm$ 2,33 ^a	35,21 $\pm$ 2,87 ^a	92,74 $\pm$ 5,34 ^e	48,73 $\pm$ 3,76 ^c	42,09 $\pm$ 3,74 ^b	53,67 $\pm$ 3,53 ^d

NC= Normal Control, **NRLTX** = Normal rats + *R. longipes* high dose, **DBC**= Diabetic Control, **DRLL**= Diabetic rats + *R. longipes* low dose, **DRLH** =Diabetic rats + *R. longipes* high dose, **DBM**: Diabetic rats + Metformin (positive control). Different superscripts a-d represent statistical significance of $p<0.05$.

The serum lipid profiles of the different animal groups are depicted in Fig 5.5.7. Evidently, the DBC group had significantly ($p<0.05$) elevated total cholesterol, triglyceride, and LDL-cholesterol with concomitantly significantly ($p<0.05$) reduced HDL-cholesterol levels. Consequently, the DBC group had a significantly ($p<0.05$) increased atherogenic index. Treatment with the high dose resulted in a significant ($p<0.05$) decrease in total cholesterol, triacyl glycerides, and LDL cholesterol while a significant ($p<0.05$) increase in HDL levels. Treatment with the high dose also increased HDL levels to levels comparable to those of the NC showing insignificant differences. Furthermore, while DRLH showed significantly($p<0.05$) better activity than DRLL, no significant difference was observed between NC and NRLTX groups in all the lipid parameters analyzed. Treatment with the high dose significantly ($p<0.05$) reduced the atherogenic index as shown in Fig 5.5.7.

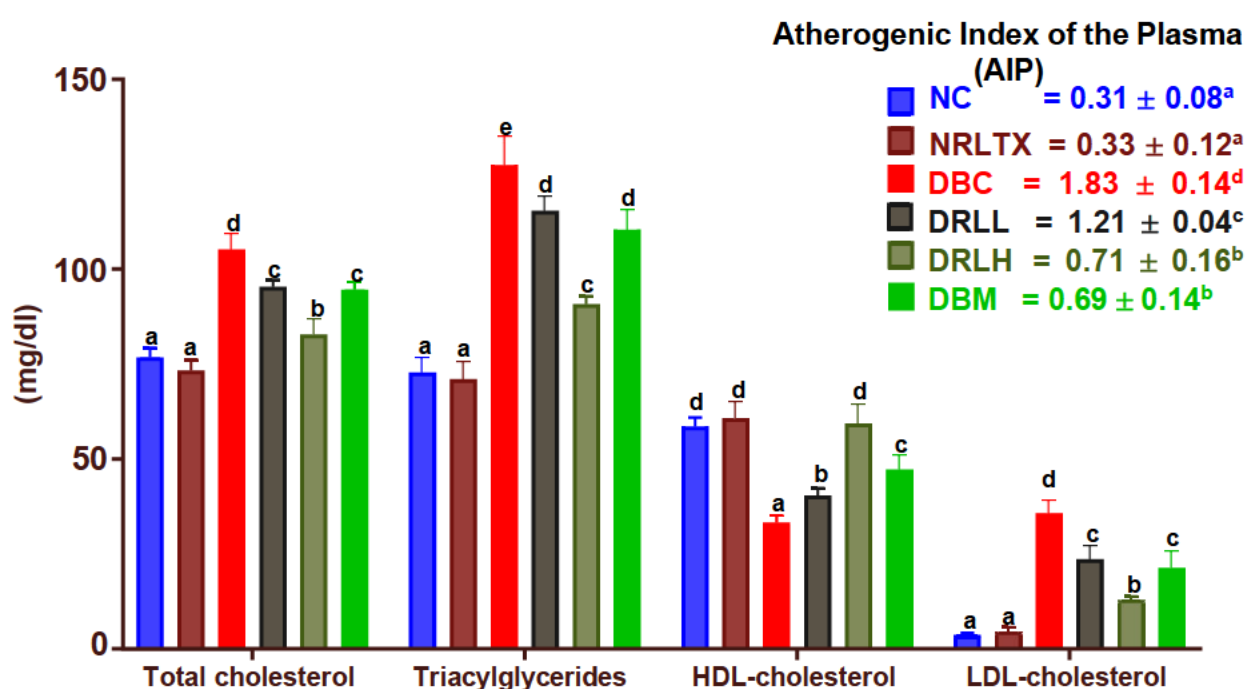


Figure 5.5.7: Effect of oral treatment on lipid profiles. *NC*= Normal Control, *NRLTX* = Normal rats + *R. longipes* high dose, *DBC*= Diabetic Control, *DRLL*= Diabetic rats + *R. longipes* low dose, *DRLH* =Diabetic rats + *R. longipes* high dose, *DBM*: Diabetic rats + Metformin (positive control). Different superscripts a-e represent statistical significance of $p<0.05$. Atherogenic index of plasma (AIP) = $\log \text{triglyceride}/\text{high-density lipoprotein cholesterol (HDLc)}$.

Diabetes induction resulted in significantly ($p<0.05$) elevated MDA and NO levels, while significantly($p<0.05$) decreasing GSH levels and reducing the activities of both SOD and CAT activity in both serum and pancreatic tissue of animals in the DBC group when compared to the NC group as shown in Table 5.5.3 and Table 5.5.4 respectively. Treatment with DRLH significantly ($p<0.05$) reduced NO levels and MDA equivalents while increasing GSH levels and the activities of SOD and CAT concurrently upon treatment with DRLH in both serum and pancreatic tissue.

Table 5.5.3: Effect of oral treatment on various pancreatic tissue oxidative stress linked enzymes.

	NC	NRLTX	DBC	DRLL	DRLH	DBM
MDA (mol/L)	42,63±1,33 ^a	44,28±1,06 ^a	81,76±5,43 ^d	59,13±2,37 ^c	48,26±1,09 ^b	61,33±2,35 ^c
GSH (mol/L)	71,45±2,17 ^d	73,73±3,81 ^d	33,28±1,88 ^a	59,27±1,02 ^b	63,19±1,11 ^c	58,23±3,09 ^b
SOD (U/mg protein)	93,71±3,54 ^d	92,37±5,67 ^d	41,2±2,22 ^a	76,24±3,99 ^b	83,27±3,32 ^c	81,23±1,85 ^c
CAT (U/mg protein)	702,357±9,36 ^e	696,28±11,03 ^e	395,15±6,31 ^a	640,09±11,21 ^b	673,57±10,37 ^d	657,34±7,38 ^c
NO (µml)	0,063±0,003 ^a	0,062±0,003 ^a	0,151±0,007 ^e	0,098±0,002 ^d	0,071±0,005 ^b	0,078±0,002 ^c

Table 5.5.4: Effect of oral treatment on various serum oxidative stress linked enzymes.

	NC	NRLTX	DBC	DRLL	DRLH	DBM
MDA (mol/L)	39,75±2,07 ^a	40,91±2,64 ^a	76,17±6,24 ^e	48,11±1,67 ^c	43,67±1,20 ^b	52,37±4,56 ^d
GSH (mol/L)	121,37±5,78 ^d	123,37±5,12 ^d	62,74±2,37 ^a	98,37±3,89 ^b	112,34±5,37 ^c	100,25±3,31 ^b
SOD (U/mg protein)	583,22±4,37 ^d	578,14±8,03 ^d	176,37±6,13 ^a	386,23±9,21 ^b	493,71±9,11 ^c	497,21±11,17 ^c
CAT (U/mg protein)	801,1±8,63 ^d	807,37±9,37 ^d	709,76±3,77 ^a	743,23±8,92 ^b	778,37±12,38 ^c	741,134±9,08 ^b
NO	0,31±0,006 ^a	0,29±0,004 ^a	0,53±0,008 ^d	0,45±0,003 ^c	0,38±0,001 ^b	0,42±0,002 ^c

MDA= melanoaldehyde equivalence, GSH=Glutathione SOD=Superoxide dismutase, CAT= Catalase, NO=Nitric oxide. **NC**= Normal Control, **NRLTX** = Normal rats + *R. longipes* high dose, **DBC**= Diabetic Control, **DRLL**= Diabetic rats + *R. longipes* low dose, **DRLH** =Diabetic rats + *R. longipes* high dose, **DBM**: Diabetic rats + Metformin (positive control). Different superscripts a-e represent statistical significance of $p<0.05$.

Table 5.5.5. displays the indices of hepatic and renal damage of the different animal groups. Among these indices, LDH, urea, uric acid, ALP, AST, and CK-MB levels were significantly ($P < 0.05$) elevated in the DBC group when compared to NC. Except for ALT levels, treatment with DRLH significantly ($P < 0.05$) reduced these parameters. Except for the ALT, treatment with a high dose significantly reduced these parameters. Interestingly, no statistically significant difference was observed for all the parameters between the NC and NRLTX groups, thus, strongly suggesting the safety of *R. longipes* on both renal and hepatic tissues.

Table 5.5.5: Effect of oral treatment on renal and hepatic function indices.

	NC	NRLTX	DBC	DRLH	DRLL	DBM
Lactate dehydrogenase U/L	387,2 ± 33,05 ^b	391,31±26,14 ^b	911 ± 58,21 ^e	417,67±31,74 ^c	534,12±24,37 ^d	284,4±27,87 ^a
Urea mg/dl	87,6 ± 3,58 ^a	88,07±3,78 ^a	96,6 ± 4,28 ^c	89,87±3,74 ^b	91,74±2,07 ^b	87,0 ± 2,92 ^a
Uric acid mg/dl	0,586 ± 0,32 ^a	0,611±0,34 ^a	2,246 ± 0,57 ^e	0,860±0,31 ^c	1,53±0,11 ^d	0,82 ± 0,19 ^b
Alkaline phosphatase (ALP) U/L	62,2 ± 8,31 ^a	64,37±6,73 ^a	273,2 ± 15,78 ^e	115,34±6,32 ^b	147,36±9,37 ^c	163,4 ± 11,14 ^d
Alanine aminotransferase (ALT) U/L	79,2 ± 4,31 ^a	80,17±5,33 ^a	85,0 ± 2,87 ^b	83,10±3,22 ^b	85,27±4,22 ^b	85,0 ± 3,97 ^b
Aspartate aminotransferase (AST) U/L	85,8 ± 6,41 ^a	83,72±4,12 ^a	120,6 ± 7,33 ^c	109,734±6,34 ^b	111,17±3,97 ^b	114,8 ± 5,89 ^b
Creatine Kinase-Myocardial Band (CK-MB) U/L	367,46 ± 44,25 ^a	348,27±34,30 ^a	790,12 ± 57,12 ^e	438±42,34 ^b	502±22,18 ^c	577,84 ± 33,25 ^d

NC= Normal Control, **NRLTX** = Normal rats + *R. longipes* high dose, **DBC**= Diabetic Control, **DRLL**= Diabetic rats + *R. longipes* low dose, **DRLH** =Diabetic rats + *R. longipes* high dose, **DBM**: Diabetic rats + Metformin (positive control). Different superscripts a-d represent statistical significance of $p < 0.05$ (Tukey's multiple range *post hoc* test at $p < 0.05$).

Histopathological microscopical analysis of the pancreatic tissue revealed a healthy islet of Langerhans morphology characterized by a substantial number of β cells surrounded by intact acinar cells in the NC group as depicted in Fig 5.5.8. In contrast, diabetes induction led to a notable reduction in the size of these islets, a decrease in β cell population, and damage to acinar cell morphology as observed in the DBC group. However, treatment with both doses of the extract resulted in improved pancreatic islet of Langerhans morphology. Treatments with *R. longipes* led to larger islets with an increased density of β cells and improved peripheral acinar cell morphology, indicating their positive therapeutic effect against T2D-induced pancreatic tissue damage.

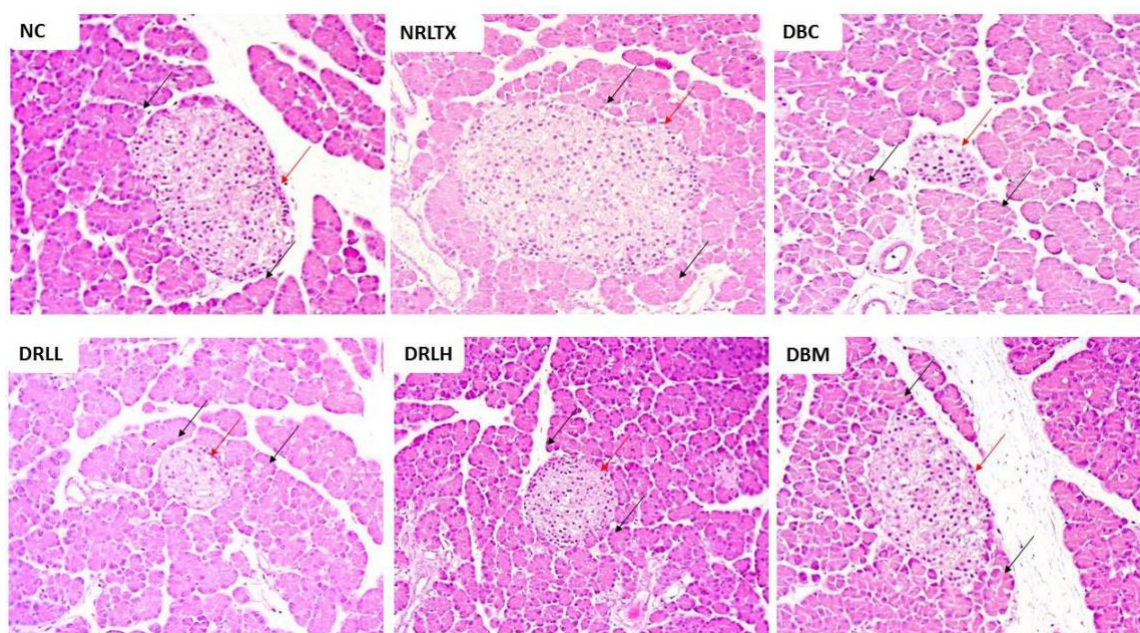


Figure 5.5.8: Pancreatic histopathological examinations illustrating the effects of oral treatments in different animal groups during the experimental period. Magnification=100X. Red arrow shows the pancreatic islets of Langerhans. Black arrows show pancreatic acinar cells **NC**= Normal Control, **NRLTX** = Normal rats + *R. longipes* high dose, **DBC**= Diabetic Control, **DRLL**= Diabetic rats + *R. longipes* low dose, **DRLH** =Diabetic rats + *R. longipes* high dose, **DBM**: Diabetic rats + Metformin (positive control).

5.5.5 Discussion

The rampant rise in the global prevalence of T2D prompts the unceasing development of novel antidiabetic pharmacological agents with high efficacy, bioavailability, and fewer undesirable side effects (Sun *et al.*,2022). Indeed, folkloric medicinal plants have emerged as a promising avenue for the development of novel antidiabetic due to their inherent phytoconstituents which have previously been reported to restore and maintain euglycemic blood glucose levels under T2D conditions (Patel *et al.*,2012). *R. longipes*, a folkloric medicinal plant which was previously shown to have enhanced antioxidant and carbohydrate-digesting enzyme inhibitory activities *in vitro* and *ex vivo* (Beseni *et al.*,2022), was investigated in the current study to establish its antidiabetic capabilities in a T2D rat model.

Acute signs of uncontrolled T2D such as polydipsia and polyphagia observed in the DBC group (Fig. 5.5.1) are attributed to altered blood osmotic potential, glycosuria and increased renal osmotic diuresis (Feldman, *et al.*,2020; Mukhtar *et al.*,2020; Alam *et al.*,2021). Chronic glucosuria in T2D patients coupled with dysfunctional glucose assimilation machinery siphons out glucose, thereby depriving cells of energy consequently leading to polyphagia with concomitant body weight loss as observed in the DBC group in the current study (Fig 5.5.2). The ability of the extract to reduce diabetic-induced polyphagia and polydipsia (Fig. 5.5.1) while retarding excessive body weight loss (Fig 5.5.2), indicates its ability to improve glucose metabolic pathways in T2D. Conceivably, *R. longipes* may function by augmenting insulin secretion in the β pancreatic cells or its sensitivity in peripheral tissues, attenuating the activation of several proteins downstream of the insulin receptor (IR) involved in the insulin transduction pathway to abate several underlying diabetic pathophysiological pathways.

Insulin deficiency, its aberrant action or both are implicated in T2D pathophysiology leading its hallmark signs including chronic hyperglycaemia and glucose intolerance (Ohiagu *et al.*,2021). The depleted insulin levels (Fig.5.5.5a), and reduced HOMA- β scores, with concomitant increase in HOMA-IR scores (Fig.5.5.5b) may have contributed to the elevated weekly fasting blood glucose levels (Fig 5.5.3) and acute glucose intolerance (Fig 5.5.4) observed in the DBC group. Reduced HOMA β scores indicate impeded insulin secretion usually due to pancreatic β cell tissue damage and while increased HOMA IR scores insinuates elevated systemic insulin resistance in peripheral tissues (Petersen *et al.*,2018). Therefore, the ability of the *R. longipes* extracts to improve HOMA- β and reduce HOMA-IR scores insinuate

their potency to improve glucose metabolism by abating pancreatic- β cell dysfunction and increasing the sensitivity of peripheral tissues to circulating insulin.

Exacerbated oxidative stress which elevates chronic bombardment of tissues by free radicals in T2D is among the most glaring factors implicated in pancreatic β cell damage leading to their diminished insulin production capacity (Petersen *et al.*,2018). Evidently, T2D induction elevated lipid peroxidation while significantly diminishing GSH level, SOD and CAT activities in the DBC group as observed (Tables 5.5.3 and Table 5.5.4) leading to increased oxidative tissue damage, which may have consequently led to pancreatic β cell destruction in the group as revealed through histopathological examinations (Figure 5.5.8). Therefore, the ability of the extracts to increase the potency of endogenous antioxidant defence systems and reduce lipid peroxidation, insinuates their protective effects against oxidative stress induced tissue injury (Table 5.5.3& Table 5.5.4) and may be responsible for the improved β pancreatic islets population and tissue morphology as opposed to the DBC group (Figure 5.5.8). This data is consistent with previous results on this plant in our laboratories that showed that the antidiabetic potency *R. longipes* extracts is strongly linked to enhanced antioxidant capacity (*in vitro* and *ex vivo*) which protects against oxidative tissue damage (Beseni *et al.*,2022).

Chronic low-grade inflammation which ensues due to persistent oxidative tissue damage in T2D is mediated through the upregulation of proinflammatory factor production including interleukins, cytokines, and nitric oxide (Van Greevenbroek *et al.*,2013; Tsalamandris *et al.*,2019). Proinflammatory factors mediate immune system responses against foreign bodies, disease, tissue injury, however continued production in T2D aggravates cardiac, pancreatic, renal, and hepatic tissue damage (Tsalamandris *et al.*,2019). The elevated NO in the serum and pancreatic tissue observed in the DBC group as seen in Tables 5.5.3 and Table 5.5.4 is indicative of increased chronic low-grade inflammation. Therefore, the ability of treatments by both doses of the extract (Tables 5.5.3 and Table 5.5.4) to reduce NO levels indicates its potential to reduce chronic low-grade inflammation and thus recede inflammation linked complications in T2D including cardiovascular diseases (CVD), nephrotoxicity and hepatotoxicity (Oguntibeju, 2019).

The liver which detoxifies the blood is among the most metabolically active organs in the body and is therefore predisposed to damage by excessive RS and low-grade inflammation especially in T2D (Harris, 2005). Furthermore, hepatic tissue injury may result due to bruising, cholestatic liver injury, trauma, necrosis, or diseases including cirrhosis and non-alcoholic liver

disease (Lucchesi et al 2015). Diabetic hepatocellular tissue damage leads to the release of several enzymes including alanine transaminase (ALT), Aspartate transaminase (AST), and Alkaline phosphatase (ALP) from their hepatocyte confinements into the blood (Lucchesi et al 2015). Indeed, elevated serum levels of AST ALT and ALP (Table 5.5.5) in the DBC group are indicative of liver damage and impaired liver function. Therefore, the ability of the high dose to reduce serum levels of AST, ALT and ALP implies hepatoprotective activity against hepatocellular tissue damage indicating the extracts' ability to decrease T2D hepatic tissue-linked complications. Furthermore, an insignificant difference between the NRLTX and NC groups indicates the extracts' toxicological safety.

The kidney which maintains homeostatic electrolyte balance in the body is as metabolically active as the liver (Oshima *et al.*,2021). The effectiveness of the kidney to remove toxins and excessive salts which is influenced by several factors including age, sex and body size is measured by the glomerular filtration rate (GFR). The reduced glomerular filtration rates in T2D led to elevated blood serum uric acid and urea levels as observed in Table 5.5.5 in the DBC group are indicative of kidney function impairment due to damage to the basement of glomerular capillaries and disruption of protein crosslinking (Alam *et al.*,2021). However, treatment with the high dose ameliorated elevated serum level of serum uric acid and urea suggesting its nephroprotective effect against T2D renal tissue damage (Table 5.5.5). The extract may thus alleviate T2D linked chronic renal tissue damage which is a major factor leading to end stage diabetic nephropathy, ketoacidosis, and premature mortality in patients (Hovind *et al.*,2011).

Insulin and glucagon regulate blood glucose levels by modulating the activities of several enzymes including glycogen phosphorylase, glucose 6-phosphatase and fructose-1,6-bisphosphatase that works in tandem to mediate hepatic glycogenesis, gluconeogenesis and glycogenolysis (Röder *et al.*,2016). Indeed, depletion of hepatic glycogen stores observed in the DBC group in Figure 5.5.6 taken together with the overactivity of hepatic glucogenic enzyme activity observed in Table 5.5.1 insinuates dysfunctional hepatic glucose metabolism, insulin resistance and which may aggravate chronic hyperglycaemia (Röder *et al.*,2016). Therefore, the restored glycogen stores observed upon treatment with *R. longipes* may stem from their ability to reduce the activities of the glucogenic enzymes (Table 5.5.1) thereby reducing the degradation of hepatic glycogen stores and reducing hepatic glucose output thus implying an antihyperglycemic effect. Furthermore, despite their increased relative liver weights rats in the DBC group had depleted glycogen stores insinuating that the hepatomegaly

these rats was due to fatty deposits rather than increased glycogen stores a candid sign of advanced dyslipidaemia in T2D (Tangvarasittichai, 2015).

T2D dyslipidaemia is linked to dysregulated carbohydrate and fatty acid metabolism resulting from overactivity of hydrolytic enzymes such as lipases and amylases (Tangvarasittichai, 2015). Indeed, T2D induction significantly increased the activities of α amylase and lipase in both the serum and the pancreas as observed in Table 5.5.2 insinuating increased complex carbohydrate and lipid degradation aggravating hyperglycaemia and dyslipidaemia. The ability of *R. longipes* to inhibit these key hydrolytic enzymes as observed in Table 5.5.2 reduces the rate of complex carbohydrate breakdown thereby allows effective circulatory blood glucose clearance thereby abating postprandial blood glucose spikes. This observation is coherent with previous analysis of this plant in our laboratory that reported its potency in inhibiting α amylase, α glucosidase and intestinal glucose absorption while increasing muscular glucose uptake using *in vitro* and *ex vivo* models (Beseni *et al.*,2022).

Diabetic dyslipidaemia due to Insulin deficiency in T2D is strongly implicated in the pathological changes in serum lipid and lipoprotein profiles and is implicated as an aggravating factor in the development of cardiovascular diseases (CVD) associated with T2D (Soran *et al.*,2016). Similarly in the current study, T2D induction notably increased total serum cholesterol, triglycerides and LDL-cholesterols with a concomitant decrease in HDL-cholesterol in the DBC group when compared to the NC group as observed in Figure 5.5.7. Treatment with the extracts of *R. longipes* significantly reversed hyperlipidaemia and reduced the atherogenic index of the plasma suggesting their strong capacity to abate diabetic dyslipidaemia and curb the progression of associated cardiovascular diseases (CVD). Hyperlipidaemia is mainly due to hyperactivity of lipid hydrolysing enzymes and dysregulated lipolytic hormones on the fat depots in the adipose that arise from the impairment of insulin secretions in T2D. Therefore, the ability of the extracts to inhibit lipases observed in Table 5.5.2 and increased insulin secretion (Figure. 5.5.5 A) which counteracts the hydrolytic capacity of the lipolytic hormones in fat reserves may be responsible for improvement of these atherogenic indices.

5.5.6 Conclusion

The current study demonstrates the ability of *R. longipes* to improve glucose metabolism and modulate biological activities linked to T2D and its complications. Evidently, *R. longipes* restored euglycemic levels, improved glucose tolerance, mitigated dyslipidaemia with a concomitant reduction in the atherogenic index, and amelioration of hepatic and renal oxidative stress.

CHAPTER 6

IN VITRO, EX VIVO AND IN VIVO STUDIES FOR INVESTIGATING THE ANTIOXIDANT AND ANTIDIABETIC ACTIVITIES OF *CELTIS AFRICANA* (Burm. F.)

6.1 *Celtis africana* (Burm.f.)

6.1.1 Background and plant description

Celtis africana Burm.f. of the family Cannabaceae is a deciduous medium-to-tall tree with a branched, dense, semi-circular canopy that, depending on the surrounding ecosystem, may grow between 12 and 25 m in height (Bussmann, 2006; Bussmann *et al.*,2020). The genus name *Celtis* is of Latin origin, and in Greek history, the plant is likened to the louts of the ancients. The pale grey to white bark is the most distinguishing feature of *C. africana* (Bussmann, 2006). *C. africana* bears simple light green triangular leaves that alternate in the stalk and have distinct basal veins while they are slightly toothed towards the apex (Ts' ehlanga, 2005). The new leaf sprouts emerge as bright, fresh green and hairy, maturing to a darker green and smoother texture. Inflorescences are small, inconspicuous, greenish, and star-shaped, appearing in the spring season with both sexes on the same tree (Nchabeleng, 2017; Phiri *et al.*,2022). Bee-pollinated flowers yield berry-like fruits that appear yellow, brown, or black when ripe. *C. africana* is commonly found in the wild in dense forests, on rocky outcrops, in bushveld, in open grassland, on mountain slopes, on coastal dunes, along riverbanks, and in kloofs (Siebert, 2018). While *C. africana* is indigenous to southern Africa, it is geographically well distributed across Africa, reaching as far as Ethiopia (Nchabeleng, 2017; Siebert, 2018).



Figure 6.1: *Celtis africana* (Burm. f.): Common names: white stinkwood; witstinkhout (Afr.); umVumvu (Xhosa); uSinga lwesalukazi (Zulu); Modutu (Sotho & Tswana); Mpopano (Venda)
Adapted without permission from www.sanbi.org.

6.1.2 Ethnobotanical uses

C. africana wood is generally hard to work but is used to make planks, shelving, yokes, tent-bows, and furniture. Pegs of *C. africana* are used to ward off evil spirits and the wood is mixed with crocodile fat as a charm against lightning (Adedapo *et al.*,2009). Different parts of *C. africana* are traditionally used to remedy the symptoms resulting from a wide range of ailments, including eye infections, rheumatism, cancer, diabetes, and sexually transmitted diseases (Nchabeleng, 2017). The leaves of *C. africana* are traditionally used to treat indigestion and oedema in both human and veterinary medicine (Krief *et al.*,2005; Al-Taweel *et al.*,2012). The sun-dried powdered bark and roots are traditionally widely used in South Africa for the treatment of cancer (Perveen *et al.*,2015).

6.1.3 Pharmacological and biological activities

Flavone C-glucosides isolated from the butanol subfraction of the ethanolic extract of *C. africana* were shown to possess remarkable antiurelitic properties (Modolo *et al.*,2015). Previous studies by Adedapo and colleagues in 2009 revealed the strong antioxidant potential of the methanolic leaf and stem extracts of *C. africana* through their enhanced capabilities to scavenge the DPPH radical. These results were further corroborated by an independent study conducted in 2012 by Al-Taweel and colleagues that showed *C. africana* extract to exhibit potent anti-inflammatory *in vivo* and *in vitro* antioxidant potential with concomitant acetylcholinesterase enzyme inhibitory activity. Various crude leaf and stem extracts of *C. africana* have been shown to have potential in the treatment of malaria as they exhibit antiparasmodial activity (Lacroix *et al.*,2011; Al-Musayeib *et al.*,2012; Laryea *et al.*,2021). Furthermore, a glucosphingolipid isolated from the ethyl acetate extracts of the aerial parts of *C. africana* was shown to exhibit potent cytotoxicity against L5178Y cells (a mouse lymphoma cell line), further justifying the use of this plant against cancer (Perveen *et al.*,2015).

6.1.4 Phytochemistry

Trans-N-coumaroyltyramine, *trans-N*-feruloyltyramine C-glycosylflavonoids and *trans-N*-caffeoyltyramine have previously been isolated from the *n*-butanol fraction of *C. africana* (Krief *et al.*, 2005; Koduru *et al.*,2007; Perveen *et al.*,2011). Another study identified vitexin, orientin, isoswertiajaponin and isoswertisin as the most active C-glycosylflavonoids in the *n*-butanol fraction of *C. africana* (Modolo *et al.*,2015). Several fatty acids have previously been identified in *C. africana* including lauric acid, palmitic acid, oleic acid, oleanolic acid and β -sitosterol (Perveen *et al.*,2009; Abe *et al.*,2009; Gohari *et al.*,2009).

6.2 *Celtis africana* (N.L.Burm.) leaf extracts regulate glucose homeostasis and mitigate cardiac muscle tissue oxidative injury

Brian K. Beseni¹, Veronica F. Salau^{1,2}, Ochuko L. Erukainure¹, * Md. Shahidul Islam¹

1. Department of Biochemistry, University of KwaZulu-Natal, Westville Campus, Durban 4000, South Africa.

2. Department of Pharmacology, School of Clinical Medicine, Faculty of Health Sciences, University of the Free State, Bloemfontein 9300, South Africa.

***Corresponding author:** islamd@ukzn.ac.za

6.2.1 Abstract

Folkloric traditional medicinal plants, including *Celtis africana*, are endowed with bioactive secondary metabolites that can be developed into novel hypoglycaemic therapeutic agents. Thus, the current study undertook to quantify the total phenolic and flavonoids before identifying the bioactive compounds in *C. africana* through gas chromatography mass spectrometry (GC-MS). Thereafter, *in vitro* models were used to establish the extracts' antioxidant capacity and their inhibitory capabilities on key lipid and carbohydrate-digesting enzymes.. Furthermore the effects of the extracts on muscular glucose uptake, hepatic glucogenic enzymes, intestinal glucose absorption, and cardiac muscle tissue oxidative stress we determined using *ex vivo* models. Finally, *in silico* models (molecular docking) were used to demonstrate the binding affinities of identified compounds against selected target proteins. The *C. africana* leaf extracts contained substantial amounts of both phenolics and flavonoids among an array of bioactive phytochemicals revealed through GC-MS analysis. Noteworthy, such bioactive compounds may have been responsible for the enhanced antioxidant ability, α amylase, and α glucosidase inhibitory capabilities observed for *C. africana*. Furthermore, the extracts inhibited glucogenic hepatic enzymes and retarded intestinal glucose absorption while increasing its uptake by psoas muscle tissues and yeast cells. *C. africana* reduced lipid peroxidation while concomitantly augmenting reduced glutathione (GSH) levels and the activities of superoxide dismutase (SOD) and catalase (CAT) in iron-mediated oxidative cardiac injury. Methoxy-phenyl-oxime and 3-carboxy-1H-indol-5-olate had the best binding affinities against the target proteins among all the docked ligands. The leaf extracts of *C. africana* exhibited the enhanced capacity to scavenge excess reactive species, inhibit carbohydrate hydrolysing enzymes, modulate hepatic glucose output, and

modulate muscular uptake and absorption while concurrently upregulating the level and activities of intrinsic antioxidant defence systems. *In silico* analysis insinuates a strong link between indole-based compounds and observed bioactivities. Further, *in vivo* experiments are necessary to verify these findings and elucidate their probable modes of action.

Keywords: *Celtis africana*, Antioxidant, Antidiabetic, Indole, Enzyme inhibition, Molecular docking.

6.2.2 Introduction

Diabetes mellitus (DM) is a metabolic condition characterised by chronic aberrant glycaemic homeostasis owing to defects in insulin production by β pancreatic cells, its action on its responsive cell surface receptors, or both (Petersmann *et al.*,2018). Type 1, type 2, and gestational are the most prevalent aetiologies of DM, with type II diabetes mellitus (T2D) accounting for over 90% of all reported cases (Kaiser *et al.*,2018). Globally, in 2019, an estimated 463 million people were living with DM, and this accounted for 4.2 million deaths in the same period (IDF 2019).

Oxidative stress is implicated among the multifactorial contributors to the pathogenesis and progression of T2D (Bansal, 2015). Under conditions of oxidative stress, surplus reactive species (RS) disruptively react with functional biomolecules, including DNA, lipids, and various protein cell surface receptors (Rehman and Akash, 2017). Damage to insulin receptors leads to insulin resistance. Insulin resistance reduces glucose uptake and directly results in abnormally elevated blood glucose levels, termed hyperglycaemia (Halim and Halim, 2019). Hyperglycaemia further aggravates the production of RS by upregulating several biochemical pathways, including polyol, hexosamine, protein kinase C, and the production of advanced glycation end-products (AGEs) (Ighodaro, 2018). Uncontrolled chronic hyperglycaemia culminates in the development of complications that are associated with diabetes.

Type 2 diabetes (T2D) patients have a higher risk of developing cardiovascular diseases (CVD), including incident coronary heart disease (Bertoluci and Rocha, 2017). Diabetes-linked cardiovascular diseases are categorised as macrovascular and microvascular complications (Gregg *et al.*,2019). Macrovascular complications include ischemic heart disease, peripheral vascular disease, and cerebrovascular disease (Gregg *et al.*,2019). While retinopathy, nephropathy, and neuropathy are categorised as microvascular complications (Gregg *et al.*,2019).

Several pharmacological management strategies primarily aimed at glycaemic control exist for the management of diabetes (Verspohl, 2012). Depending on their modes of action, they are classified as sulphonyl-ureas, glinides, subtype 2 sodium-glucose transport protein (SGLT2) inhibitors, biguanides, α -glucosidase inhibitors, thiazolidinedione, gliflozins, and dipeptidyl peptidase-4 inhibitors (Brunerova *et al.*,2018). Despite the efficacy of these oral pharmacological agents, their use may be accompanied by several undesirable side effects. These side effects include flatulence, excessive weight loss or gain, hypoglycaemic episodes, among others (Capoccia *et al.*,2016). Furthermore, these medications are beyond the financial reach of people in poverty-stricken regions such as those in Sub-Saharan Africa (Mapa-Tassou *et al.*,2019). This has prompted the search for novel, affordable pharmacological agents with relatively fewer side effects. Medicinal plants have emerged as a source of therapeutic agent templates emanating from their bioactive secondary metabolites.

Celtis africana, of the family Cannabaceae, is a deciduous tree commonly referred to as the white stinkwood. It is a folkloric traditional medicinal plant. It has a characteristic whitish bark and bears small flowers that are pale yellow in color. Sun-dried bark and roots are powdered and infused in water or milk and taken orally to remedy cancer (Krief *et al.*,2005; Koduru *et al.*,2007). The leaves of *C. africana* have been reported to remedy indigestion in South Africa (Krief *et al.*,2005). While Borquaye and colleagues (2020) reported that its leaf decoction is used to alleviate pain and remedy eye infections, Sphingolipid glucoside isolated from the aerial parts of *C. africana* were shown to have strong cytotoxic activity against L5178Y mouse lymphoma cells (Perveen *et al.*,2015). The chloroform-soluble fraction of *C. africana* was shown to contain oleanolic acid, lauric acid, palmitic acid, and several others that exhibited potent antioxidant and anti-inflammatory capabilities (Al-Taweel *et al.*,2012). The current study was aimed at characterizing compounds in the leaf extract of *Celtis africana* and determining their antioxidant, antiobesity, and antidiabetic activities using *in vitro*, *ex vivo*, and *in silico* models.

6.2.3 Materials and methods

Please refer to Chapter 2, sections 2.4.1 to 2.11.5 for detailed materials and methods.

6.2.4 Results

Table 6.2.1 shows the compounds identified in the *C. africana* leaf extracts based on the retention time. Octadecatrienoic acid (30.26%), pentadecanoic acid (23.72%), and phytol acetate (9.66%) were the most abundant compounds in the aqueous extract. Tetramethylheptadecan-4-olide (0.44%), caryophyllene oxide (0.58%), and squalene (0.68%) had the least relative abundance in the aqueous extract. Glycine was eluted first after 5.29 min in the aqueous extract, while squalene was eluted last at the 24.81 min mark. Phytol (15.08%), Ethyl 9,12,15-octadecatrienoate (12.77%), and 1-(+)-Ascorbic acid 2,6-dihexadecanoate (11.24%) were the most abundant compounds in the methanol extract. The ethanolic extract had the most identified compounds among all the leaf extracts of *C. africana*. The ethyl acetate extract had the fewest identified compounds among all the leaf extracts. Phytol and phytol acetate were common in all the extracts.

Table 6.2.1: Phyto-compounds identified by Gas Chromatography Mass Spectrometry (GC-MS) in *C. africana* leaf extracts.

Compounds	Retention time(min)	Molecular Formula	Molecular weight (g/mol)	Relative abundance (%)
Aqueous				
Glycerine	5.29	C ₃ H ₈ O ₃	92.09	0.87
Tetramethyl heptadecane	13.02	C ₂₁ H ₄₄	296.6	0.82
Caryophyllene oxide	13.07	C ₁₅ H ₂₄ O	220.35	0.58
Phytol acetate	15.68	C ₂₂ H ₄₂ O ₂	338.6	9.66
Trimethyl-2-pentadecanone	15.74	C ₁₈ H ₃₆ O	268.5	1.37
Tetramethyl-2-hexadecenol	16.13	C ₂₀ H ₄₀ O	296.5	2.83
Pentadecanoic acid	17.09	C ₁₅ H ₃₀ O ₂	242.4	23.72
Phytol	18.43	C ₂₀ H ₄₀ O	296.5	5.83
Octadecatrienoic acid	18.83	C ₁₈ H ₃₀ O ₂	278.4	30.26
Tetramethylheptadecan-4-olide	20.62	C ₂₁ H ₄₀ O ₂	324.5	0.44
3-pentadecyl-phenol	21.93	C ₂₁ H ₃₆ O	304.5	1.51

5,8-Decadien-2-one, 5,9-dimethyl-, (E)-	23.82	C ₁₂ H ₂₀ O	180.29	1.50
2-Isoamyl-6-methylpyrazine	23.97	C ₁₀ H ₁₆ N ₂	164.25	2.25
Squalene	24.81	C ₃₀ H ₅₀	410.7	0.68
Methanol				
Hexestrol	14.93	C ₁₈ H ₂₂ O ₂	270.4	0.49
Tetradecanoic acid	15.00	C ₁₄ H ₂₈ O ₂	228.37	0.77
Phytol acetate	15.69	C ₂₂ H ₄₂ O ₂	338.6	3.86
Trimethyl-2-pentadecanone	15.76	C ₁₈ H ₃₆ O	268.5	1.07
Tetramethyl-2-hexadecenol	16.14	C ₂₀ H ₄₀ O	296.5	1.01
Trimethylpentadecatrien-2-one	16.50	C ₁₈ H ₃₀ O	262.4	0.55
Methyl ester hexadecanoic acid	16.60	C ₁₇ H ₃₄ O ₂	270.5	1.00
l-(+)-Ascorbic acid 2,6-dihexadecanoate	17.29	C ₃₈ H ₆₈ O ₈	652.9	11.24
Methyl ester octadecatrienoic acid	18.34	C ₂₄ H ₄₀ O ₄	392.6	2.19
Phytol	18.49	C ₂₀ H ₄₀ O	296.5	15.08
Ethyl octadecatrienoate	18.97	C ₂₀ H ₃₄ O ₂	306.5	12.77
Octadecanoic acid	19.18	C ₁₈ H ₃₆ O ₂	284.5	5.34
β-D- Farnesyl-manno-furanoside	20.82	C ₂₁ H ₃₆ O ₆	384.5	0.76
Di-isooctyl phthalate	22.16	C ₂₄ H ₃₈ O ₄	390.6	4.90
Ethanol				
1-amino-cyclobutane carboxylic acid	4.35	C ₅ H ₉ NO ₂	115.13	6.79
6-Methylheptane-1,6-diol	4.42	C ₈ H ₁₈ O ₂	146.23	6.71
Methoxy-phenyl- oxime	4.77	C ₈ H ₉ NO ₂	151.16	13.86
5-hydroxy-1H-Indole-3-carboxylic acid,	4.90	C ₉ H ₇ NO ₃	177.16	1.57

1,2-Cyclopentanedione	5.11	C ₅ H ₆ O ₂	98.1	1.73
Glycerine	5.61	C ₃ H ₈ O ₃	92.09	0.54
α-1-methylethyl cyclo pentanemethanol	7.48	C ₉ H ₁₇ NO ₃	187.24	0.43
Glutarimide	7.57	C ₅ H ₇ NO ₂	113.11	0.89
Isosorbide	8.34	C ₆ H ₁₀ O ₄	146.14	0.63
Caprolactam	8.97	C ₆ H ₁₁ NO	113.16	0.26
1-Tetradecene	10.46	C ₁₄ H ₂₈	196.37	0.59
n-Pentadecanol	12.98	C ₁₅ H ₃₂ O	228.41	1.67
Ethyl-α-D-glucopyranoside	13.67	C ₈ H ₁₆ O ₆	208.21	0.93
3-O-Methyl-D-glucose	13.99	C ₇ H ₁₄ O ₆	194.18	3.60
Octadecanoic acid	15.00	C ₁₈ H ₃₆ O ₂	284.5	0.41
Phytol acetate	15.71	C ₂₂ H ₄₂ O ₂	338.6	9.60
Tetramethyl-2-hexadecenol	16.17	C ₂₀ H ₄₀ O	296.5	2.99
Nonadecanenitrile	16.41	C ₁₉ H ₃₇ N	279.5	0.26
Pentadecanoic acid	17.09	C ₁₅ H ₃₀ O ₂	242.4	3.85
9-Octadecene, 1-(2,3- dimethoxypropoxy)	18.08	C ₂₃ H ₄₆ O ₃	370.6	0.32
n-Heptadecanol-1	18.24	C ₁₇ H ₃₆ O	256.5	2.82
Phytol	18.48	C ₂₀ H ₄₀ O	296.5	0.49
2-(2-hydroxyethoxy)ethyl octadecanoic acid	19.04	C ₂₂ H ₄₄ O ₄	372.6	1.61
Hexadecanamide	19.17	C ₁₆ H ₃₃ NO	255.44	0.81
4-Chlorophenylacetic acid	20.68	C ₈ H ₇ ClO ₂	170.59	0.59
13-Docosenamide	20.88	C ₂₂ H ₄₃ NO	337.6	1.27
Umbellulol	21.89	C ₁₀ H ₁₆ O	152.23	0.40
11-Tetradecynol	22.19	C ₁₄ H ₂₆ O	210.36	0.29
Ethyl acetate				
Ribitol	7.17	C ₅ H ₁₂ O ₅	152.15	1.22
1,2,3-Propanetriol, 1- acetate	8.70	C ₅ H ₁₀ O ₄	134.13	3.75
Acetic acid, chloro-, hexadecyl ester	12.95	C ₁₈ H ₃₅ ClO ₂	318.9	1.26

Phytol acetate	15.69	C ₂₂ H ₄₂ O ₂	338.6	2.98
Pentadecanoic acid	17.43	C ₁₅ H ₃₀ O ₂	242.4	23.27
Hexadecatrienal	18.23	C ₁₆ H ₂₆ O	234.38	1.04
Phytol	18.51	C ₂₀ H ₄₀ O	296.5	10.39
9,12-Octadecadien-1-ol,	19.32	C ₁₈ H ₃₄ O	266.5	38.36
2H-Pyran-2-one,	20.72	C ₁₈ H ₃₄ O ₂	282.5	1.08
tetrahydro-6-tridecyl-				
Eicosanoic acid	20.96	C ₂₀ H ₄₀ O ₂	312.5	2.03
Triphenyl phosphate	21.27	C ₁₈ H ₁₅ O ₄ P	326.3	0.75
Diisooctyl phthalate	22.19	C ₂₄ H ₃₈ O ₄	390.6	3.27
Squalene	24.95	C ₃₀ H ₅₀	410.7	4.37

As depicted in Figure 6.2.1A, the *C. africana* leaf extracts exhibited statistically significant ($p < 0.05$) variations in their polyphenol content, with the ethanol extract displaying significantly ($p < 0.05$) the most abundant polyphenol content of 46.49 mg/g gallic acid equivalents, while the ethyl acetate extract exhibited the least polyphenol content of 7.98 mg/g gallic acid equivalents. A similar trend is discernible in Figure 6.2.1B, where the ethanol extract again showcased significantly ($p < 0.05$) the highest levels of flavonoids, quantified at 31.44 mg/g quercetin equivalents, while the ethyl acetate extract had the least flavonoid content of 11.99 mg/g quercetin equivalents.

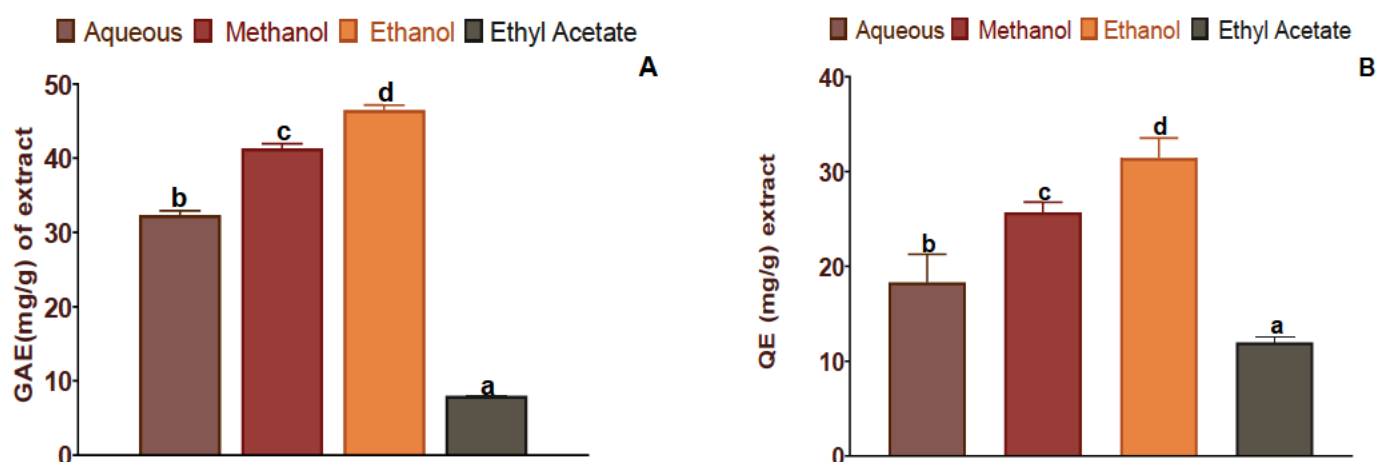


Figure 6.2.1: The total phenolic content of the *C. africana* crude leaf extracts represented as (A) Gallic acid equivalents (GAE mg/g) and the total flavonoid content represented as (B) Quercetin equivalents (QE mg/g). Bars with superscripts a-d for each extract are statistically different at $p < 0.05$ level of significance.

The results of the antioxidant capabilities of *C. africana* leaf extracts determined through DPPH, FRAP, and NO scavenging activity assays are depicted in Figs. 6.2.2A to 6.2.2C. Evidently as shown in Fig. 6.2.2A, the ethanol extract exhibited the best DPPH scavenging activities among all the extracts with an IC_{50} value of $7,35 \pm 0,41 \mu\text{g/ml}$ (Table 6.2.4), which was comparable to that of vitamin C (positive control) of $9,21 \pm 0,12 \mu\text{g/ml}$ (Table 6.2.4). Conversely, the ethyl acetate extract, which had an IC_{50} value $=120,56 \pm 0,51 \mu\text{g/ml}$ as seen in Table 6.2.4, exhibited the least DPPH scavenging activities among all the extracts. In the FRAP assay, depicted in Figure 6.2.2B, the extracts displayed dose-dependent electron donating activity, with most extracts showing similar electron donating capacity across the tested concentrations, except for the ethyl acetate extract. At the highest concentration of $240 \mu\text{g/ml}$, there was no statistically significant difference ($p > 0.05$) in electron-donating activity between the aqueous, methanol, and ethanol extracts when compared to the positive control, vitamin C. Figure 6.2.2C reveals that the aqueous, methanol, and ethanol extracts exhibited comparable nitric oxide scavenging activity to that of quercetin. In contrast, the ethyl acetate extract exhibited the lowest nitric oxide (NO) scavenging activity, with an IC_{50} value of $5.09 \pm 0.06 \mu\text{g/ml}$.

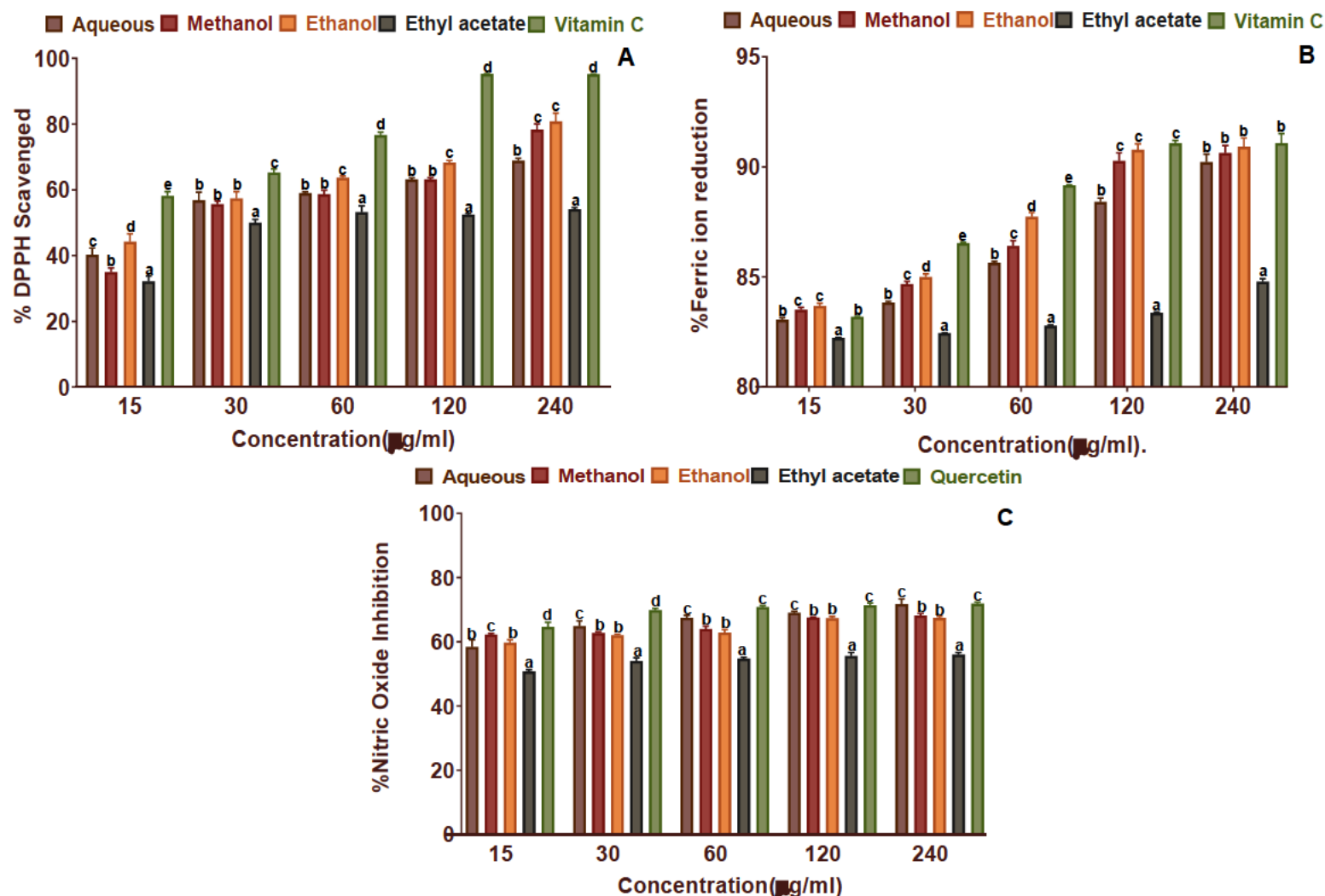


Figure 6.2.2: *C. africana* leaf extracts (A) DPPH scavenging (B) ferric reduction and (C) nitric oxide scavenging activities. Bars with superscripts a-e for each concentration are statistically different at $p < 0.05$ level of significance.

The *C. africana* leaf extracts exhibited dose-dependent inhibitory activities against pancreatic lipase, α -glucosidase, and α -amylase, as shown in Figs. 6.2.3A–6.2.3C. The aqueous and methanol leaf extracts of *C. africana*, especially at the highest concentration of 240 $\mu\text{g/ml}$, exhibited the best pancreatic lipase inhibitory capacity among all the extracts. Evidently, they had comparable lipase inhibitory capabilities, with no significant difference ($p > 0.05$) between them, displaying IC_{50} values of $162,50 \pm 0,39 \mu\text{g/ml}$ and $161,45 \pm 0,99 \mu\text{g/ml}$ (Table 6.2.4), which were comparable to those observed for orlistat (positive control). The ethanol extract had the third-best pancreatic lipase inhibitory capabilities, while the ethyl acetate extract exhibited the least pancreatic lipase inhibitory capacity among all the extracts, with an IC_{50} value of $239,96 \pm 0,46 \mu\text{g/ml}$, as shown in Table 6.2.4. As seen in Fig. 6.2.3 B, the ethanol extract exhibited the best α -glucosidase inhibitory activity among all the extracts, with an IC_{50} value of $103,63 \pm 0,69 \mu\text{g/ml}$ displayed in Table 6.2.4. There was no significant difference in

the ethanol and acarbose α -glucosidase inhibitory activity at all the tested concentrations except at 60 $\mu\text{g/ml}$. However, the ethyl acetate extract had the least α -glucosidase inhibitory activity with an IC_{50} value of $294,32 \pm 0,59 \mu\text{g/ml}$ (Table 6.2.4). Evidently, the aqueous extract exhibited the best α -amylase inhibitory activity, especially at the highest concentration of 240 $\mu\text{g/ml}$, significantly ($p < 0.05$) surpassing all other extracts, including acarbose (the positive control). Evidently, the aqueous extract exhibited comparable α -amylase inhibitory activity to that of acarbose with an IC_{50} value of $147,04 \pm 0,32 \mu\text{g/ml}$, as seen in Table 6.2.4, while the ethyl acetate extract showed the least α -amylase inhibitory activity among all tested extracts.

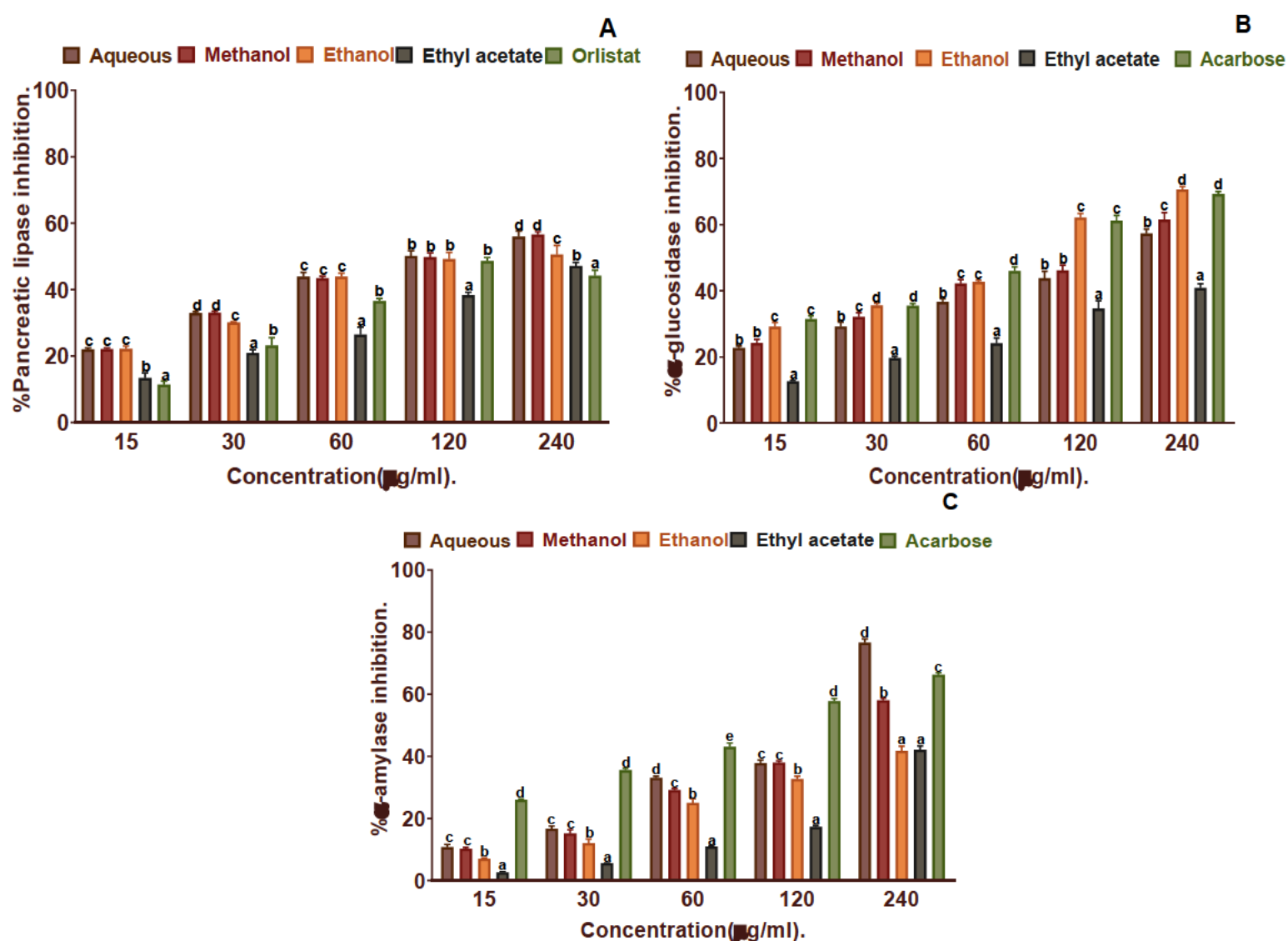


Figure 6.2.3: *C. africana* leaf extracts (A) pancreatic lipase (B) α -glucosidase (C) α -amylase inhibitory activities. Bars with superscripts a-e for each concentration are statistically different at $p < 0.05$ level of significance.

The data on the free binding energy of secondary metabolites from *C. africana* leaf extracts with carbohydrate and lipid digestive enzymes from the *in silico* analysis are presented in Fig. 6.2.4, Table 6.2.2, and Table 6.2.3. Among the phytochemicals from *C. africana* leaf extracts, methoxy-phenyl-oxime exhibited the lowest binding affinities with α amylase (-10.55 kcal/mol) and α glucosidase (-13.1 kcal/mol). The two-dimensional receptor-ligand complex images in Fig. 6.2.4A reveal that methoxy-phenyl-oxime formed a donor hydrogen bond with GLU 271, while other non-covalent interactions such as ionic-ionic and Pi-H, detailed in Table 6.2.3, also stabilized the methoxy-phenyl-oxime- α -glucosidase complex. In Fig. 6.2.4B, the methoxy-phenyl-oxime- α -amylase complex was stabilized by a donor hydrogen bond with GLU 265, while ionic interactions were observed between methoxy-phenyl-oxime and ASP 332, as further indicated in Table 6.2.3. Furthermore, while methoxy-phenyl-oxime exhibited a low binding affinity of -12.91 kcal/mol with pancreatic lipase, the 3-carboxy-1H-indol-5-olate formed a more stable complex with pancreatic lipase, as evidenced by the lower binding affinity of -12.91 kcal/mol observed in Table 6.2.2. As observed in Fig. 6.2.4C, the 3-carboxy-1H-indol-5-olate-pancreatic lipase complex was stabilized by an H-donor bond between the ligand and GLU 253. Additionally, other interactions noted in Table 6.2.3 include the presence of two ionic-ionic interactions between the distal O- of 3-carboxy-1H-indol-5-olate with ARG 265 and LYS 239, further stabilizing this complex and resulting in the low binding affinity reported in Table 6.2.2.

Table 6.2.2: Free binding energy of secondary metabolites from *C. africana* leaf extracts with carbohydrate and lipid digestive enzymes.

Compounds	Glucosidase (kcal/mol)	Amylase (kcal/mol)	Pancreatic lipase (kcal/mol)
Isosorbide	-9.59	-7.1	-8.41
Methoxy-phenyl- oxime	-13.1	-10.55	-12.91
Hexestrol	-11.76	-8.42	-12.6
Glutarimide	-8.53	-6.3	-6.99
Cyclopentanemethanol, α -(1-methylethyl	-9.92	-8.0	-9.5
Caryophyllene oxide	-9.81	-8.2	-10.56
3-carboxy-1H-indol-5-olate	-11.23	-8.4	-13.5
3-pentadecyl-phenol	-10.9	-9.2	-12.2
2-isoamyl-6-methylpyrazine	-7.38	-6.1	-9.28
1,2-cyclopentanedione	-7.5	-6.1	-6.44
Acarbose	-19.6	-14.1	-
Orlistat	-	-	-10.2

Table 6.2.3: Free binding energy and interactions between of secondary metabolites from *C. africana* leaf extracts showing the best inhibitory capabilities against carbohydrate and lipid digestive enzymes.

Compounds	Protein	Type of bonds
Methoxy-phenyl-oxime	A glucosidase	H- donor H-donor Ionic Ionic Pi-H
	Amylase	H-donor Ionic Ionic
3-carboxy-1H-indol-5-olate	Lipase	H-donor H-acceptor H-acceptor H-acceptor Ionic Ionic Ionic Ionic Ionic Pi-H
	Glucosidase	H- donor H- donor H- donor H- donor H- donor H- donor H-acceptor H-acceptor H-acceptor
Acarbose	Amylase	H-donor H-donor H-donor H-acceptor H-acceptor H-acceptor H-pi
Orlistat		H-acceptor

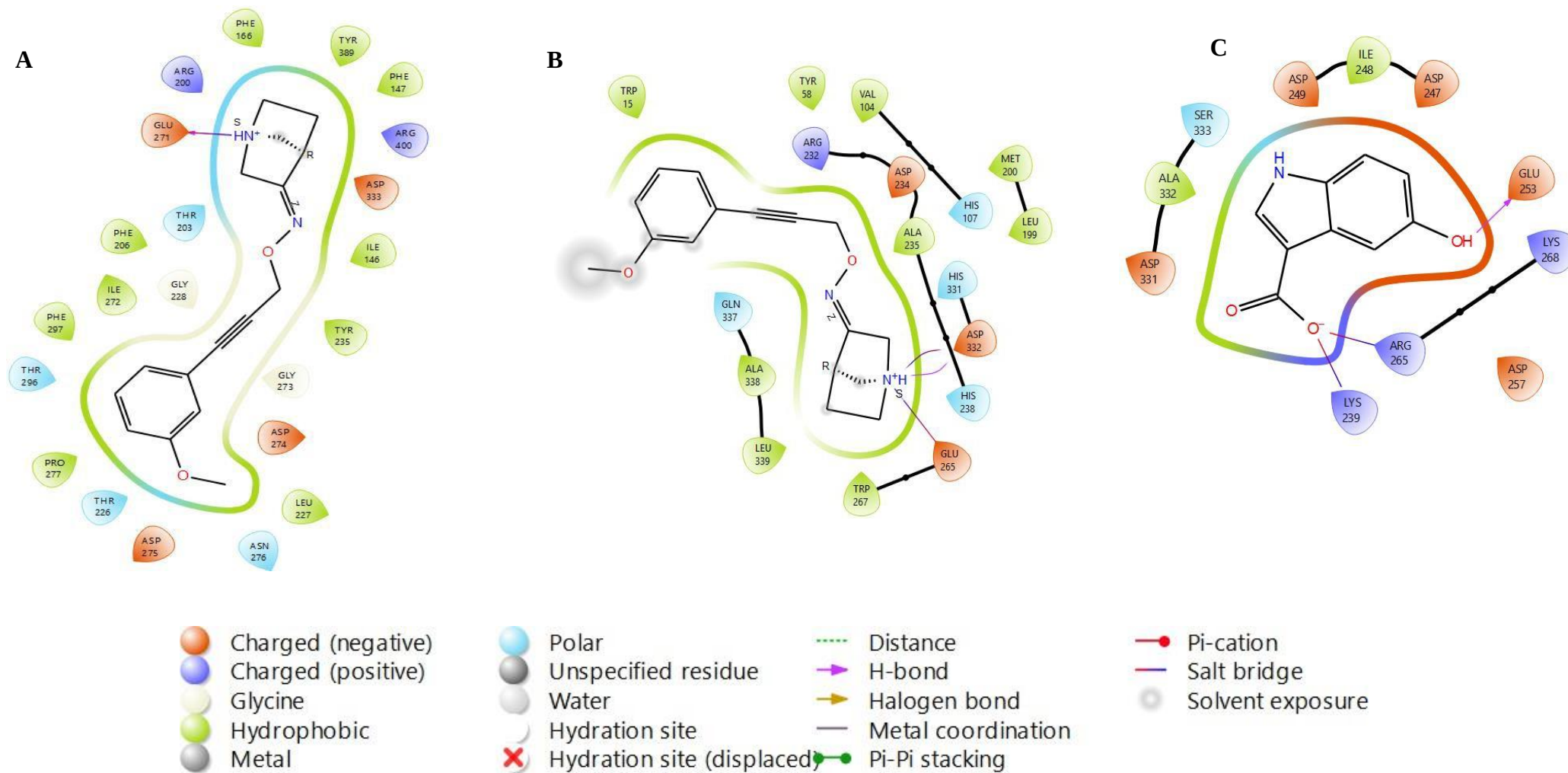


Figure 6.2.4: showing the interactions between the active site of A) α glucosidase and methoxy phenyl oxime, B) α amylase and methoxy-phenyl- oxime and C) pancreatic lipase and 3-carboxy-1H-indol-5-olate.

Table 6.2.4: The IC₅₀ values of different *C. africana* extracts for different assayed parameters.

	Aqueous Extract (µg/ml)	Methanol Extract (µg/ml)	Ethanol Extract(µg/ml)	Ethyl acetate Extract (µg/ml)	Vitamin C (µg/ml)	Quercetin (µg/ml)	Acarbose (µg/ml)	Orlistat(µg/ml)
DPPH	12,00±0,63	38,94±0,62	7,35±0,41	120,56±0,51	9,21±0,12	-	-	-
FRAP	6,06±0,57	4,83±0,142	4,38±0,518	31,98±0,92	6,67±0,88	-	-	-
NO	0,99±0,22	0,54±0,49	0,33±0,86	5,09±0,06	-	1,17±0,58	-	-
Pancreatic lipase	162,50±0,39	161,45±0,99	191,71±0,97	239,96±0,46	-	-	-	114,52±0,83
α-glucosidase	177,26±0,38	151,68±0,34	103,63±0,69	294,32±0,59	-	-	100,68±0,09	-
α-amylase	147,04±0,32	190,43±0,41	272,80±0,44	293,30±0,29	-	-	118,57±0,97	-

Data is presented as mean ± standard deviation. Key: DPPH = 2,2-diphenyl-1-picrylhydrazyl. FRAP = Ferric reducing antioxidant power. NO = Nitric oxide assay.

As illustrated in Figure 6.2.5A, the *C. africana* leaf extracts exhibited a dose-dependent increase in glucose uptake in the yeast suspension. Remarkably, the data highlights that the aqueous extract consistently exhibited the highest glucose uptake across most of the tested concentrations. Notably, at the highest concentrations of 120 and 240 µg/ml, all extracts, except for the ethyl acetate extract, induced significantly ($p<0.05$) greater glucose uptake in the yeast suspension when compared to metformin, the positive control. In Figs. 6.2.5B to 6.2.5D, evidently, the ethanol extract stimulated significantly ($p<0.05$) higher glucose uptake in rat psoas skeletal muscle and liver tissue while simultaneously demonstrating superior capabilities in retarding intestinal glucose absorption compared to all other *C. africana* leaf extracts. At concentrations above 60 µg/ml, the ethanol extracts stimulated rat psoas skeletal muscle (Fig. 6.2.5B) glucose uptake significantly ($p<0.05$) better than insulin (positive control), while a similar effect was only observed at a concentration of 240 µg/ml in the hepatic tissue, as seen in Fig. 6.2.5C. As observed in Fig. 6.2.5D, the ethanol extract had significantly ($p<0.05$) the best intestinal glucose absorption retardation among all the extracts. In contrast, the data indicates that the ethyl acetate extract stimulated significantly ($p<0.05$) the least glucose uptake in rat psoas skeletal muscle and exhibited the lowest intestinal glucose absorption-retarding capacity among all the tested extracts.

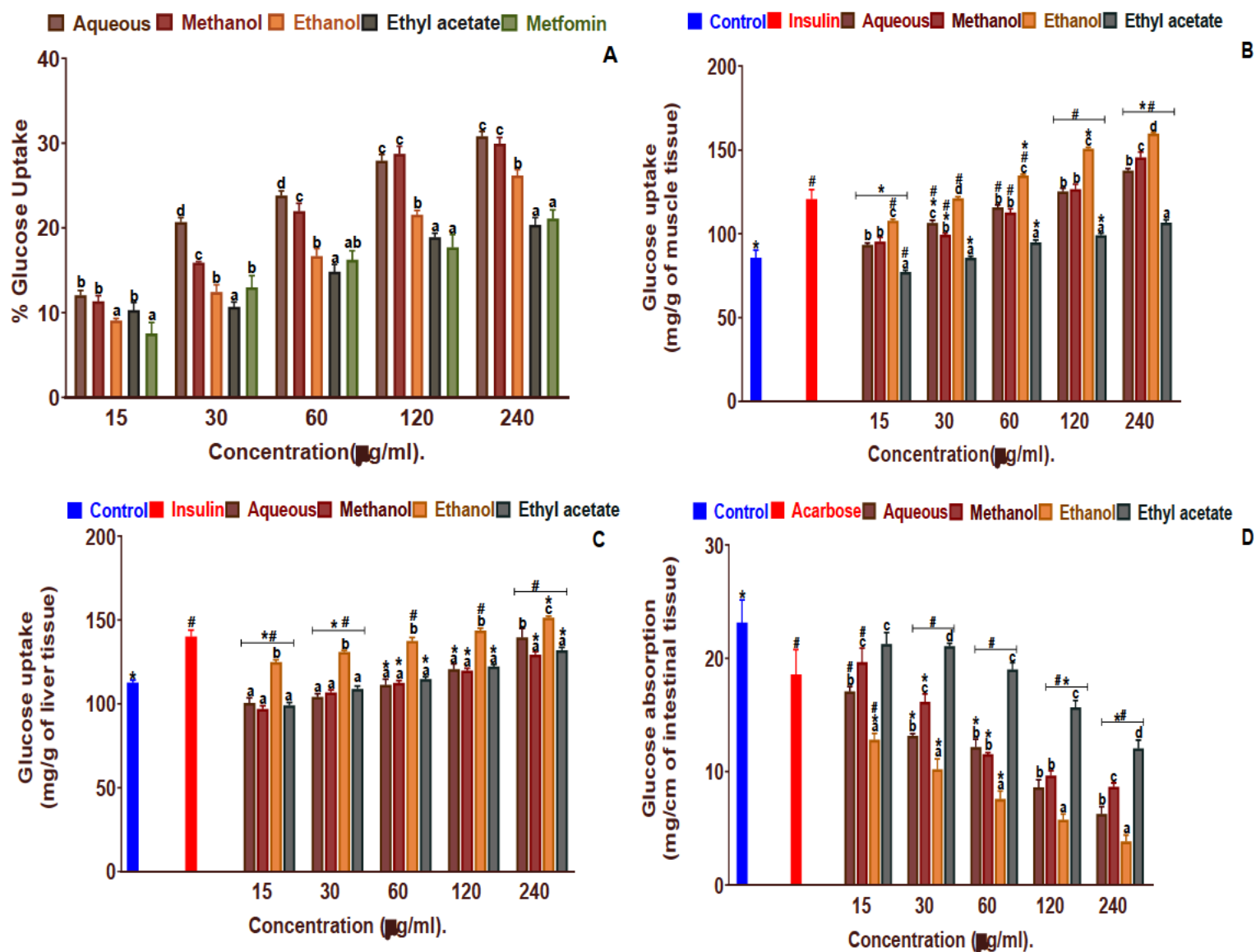


Figure 6.2.5: The effects of *C. africana* leaf extracts on (A) glucose uptake in yeast suspension (B) glucose uptake in excised rat psoas muscle (C) glucose absorption in the intestinal jejunum. Bars with superscripts a-b are statistically different at $p < 0.05$ whereas those with * and # are different from the acarbose/insulin treatment positive and control, respectively.

As depicted in Figs. 6.2.6, A-D oxidative cardiac tissue injury significantly ($p < 0.05$) elevated lipid peroxidation while concomitantly diminishing SOD and CAT enzyme activities and reducing GSH levels. However, as shown in Fig. 6.2.6A, treatment with particularly the ethanol leaf extracts of *C. africana* significantly ($p < 0.05$) reduced MDA equivalents, showing its enhanced lipid peroxidation reduction capabilities. The lipid peroxidation reduction capabilities of the ethanol extract were closely followed by those of the aqueous and methanol extracts, which were comparable at all the tested concentrations, and there were no significant differences ($p > 0.05$) between them at all the tested concentrations. Except for the ethyl acetate

extract at 240 µg/ml, the extracts exhibited significantly better lipid peroxidation activity than vitamin C (positive control) at all the tested concentrations. In Fig. 6.2.5B, treatment with the methanol and ethanol extracts, which were not significantly ($p < 0.05$) different from one another, resulted in the best SOD activity among all the extracts. Furthermore, the methanol and ethanol extracts at concentrations beyond 60 µg/ml resulted in SOD activities significantly ($p < 0.05$) higher than those stimulated by vitamin C (positive control). Only the ethyl acetate extract, which resulted in the least SOD activity, did not surpass SOD activity to greater extents than those resulting from vitamin C treatment. Treatment with the ethanol extract resulted in CAT activities significantly better than those exhibited by the rest of the leaf extracts of *C. africana* at all concentrations except 240 µg/ml, as depicted in Fig. 6.2.6 C. The aqueous and methanol extracts, which had the following best stimulatory activity to that of the ethanol, had comparable activities at most of the tested concentrations, which were not significantly ($p < 0.05$) different from each other. Furthermore, all the extracts except the ethyl acetate extract exhibited significantly higher CAT activity than vitamin C, which was employed as the positive control. Finally, as depicted in Fig. 6.2.6 D, a similar trend as that in Fig. 6.2.6 C was observed, in which the ethanol extract resulted in significantly the highest GSH levels among all the extracts. Furthermore, while the aqueous and methanol extracts showed comparable activity between themselves, their resulting GSH levels were not significantly different from those of the vitamin C (positive control). The ethyl acetate extract resulted in the lowest GSH levels and was the only extract that could not surpass the levels obtained for vitamin C (positive control).

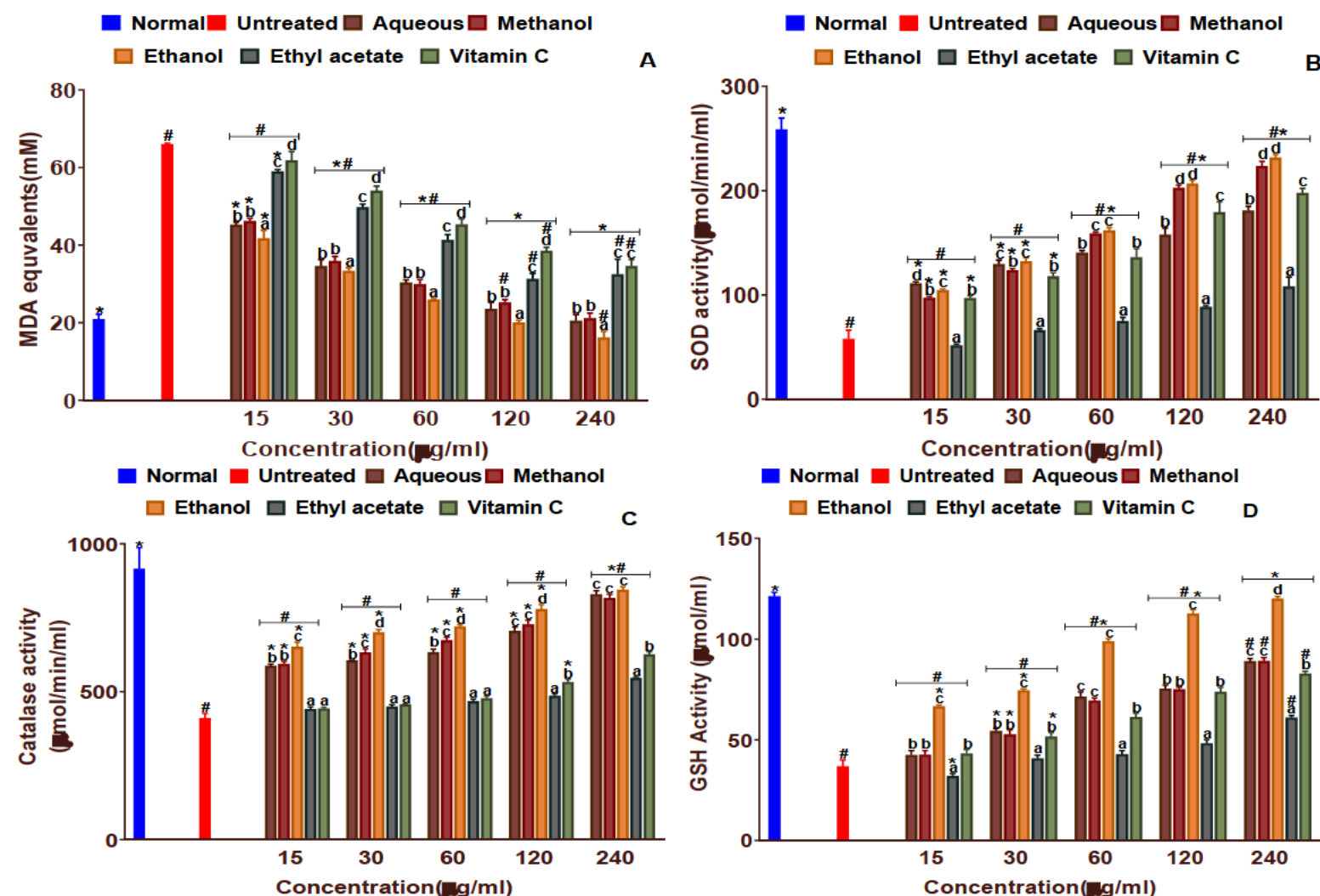


Figure 6.2.6: Effects of *C. africana* leaf extracts on (A) lipid peroxidation, (B) SOD activity, (C) Catalase activity and (D) GSH activity in oxidative cardiac muscle injury. Data are presented as mean $\pm$ standard deviation of triplicate determinations. Different letters (a-e) above the bars for a given concentration are significantly different from each other ($p < 0.05$). *Significantly different from untreated sample and #significantly different from normal sample.

As depicted in Figs. 6.2.7A–6.2.7C, hyperglycaemia induces hyperactivity of hepatic ATPase, glycogen phosphorylase (GP), and fructose-1,6-bisphosphatase (FBP) activity. As observed in Fig. 6.2.7A, treatment with the leaf extracts of *C. africana* significantly reduced hepatic ATPase activities when compared to the untreated group at all the tested concentrations. Except for the ethyl acetate extract, all the extracts exhibited comparable ATPase inhibitory activities to those of quercetin across all tested concentrations. As depicted in Fig. 6.2.7B, the *C. africana* leaf extracts significantly ($p < 0.05$) reduced hepatic glycogen phosphorylase activities at all the tested concentrations when compared to the untreated group and quercetin (positive control

group). The aqueous extract exhibited significant ($p < 0.05$) superior glycogen phosphorylase inhibitory activity among all extracts, while the ethyl acetate extract had the least inhibitory capabilities. Finally, as displayed in Fig. 6.2.7C, at concentrations from 30 $\mu\text{g/ml}$ and beyond, the *C. africana* leaf extracts significantly ($p < 0.05$) reduced fructose 1,6-bisphosphatase activities when compared to the untreated group. Further, no significant ($p > 0.05$) difference was observed between the fructose 1,6-bisphosphatase inhibitory capabilities of the aqueous and ethanol extracts, which showed the best potency among all the extracts. The ethyl acetate extracts displayed significantly ($p < 0.05$) the least fructose 1,6-bisphosphatase inhibitory

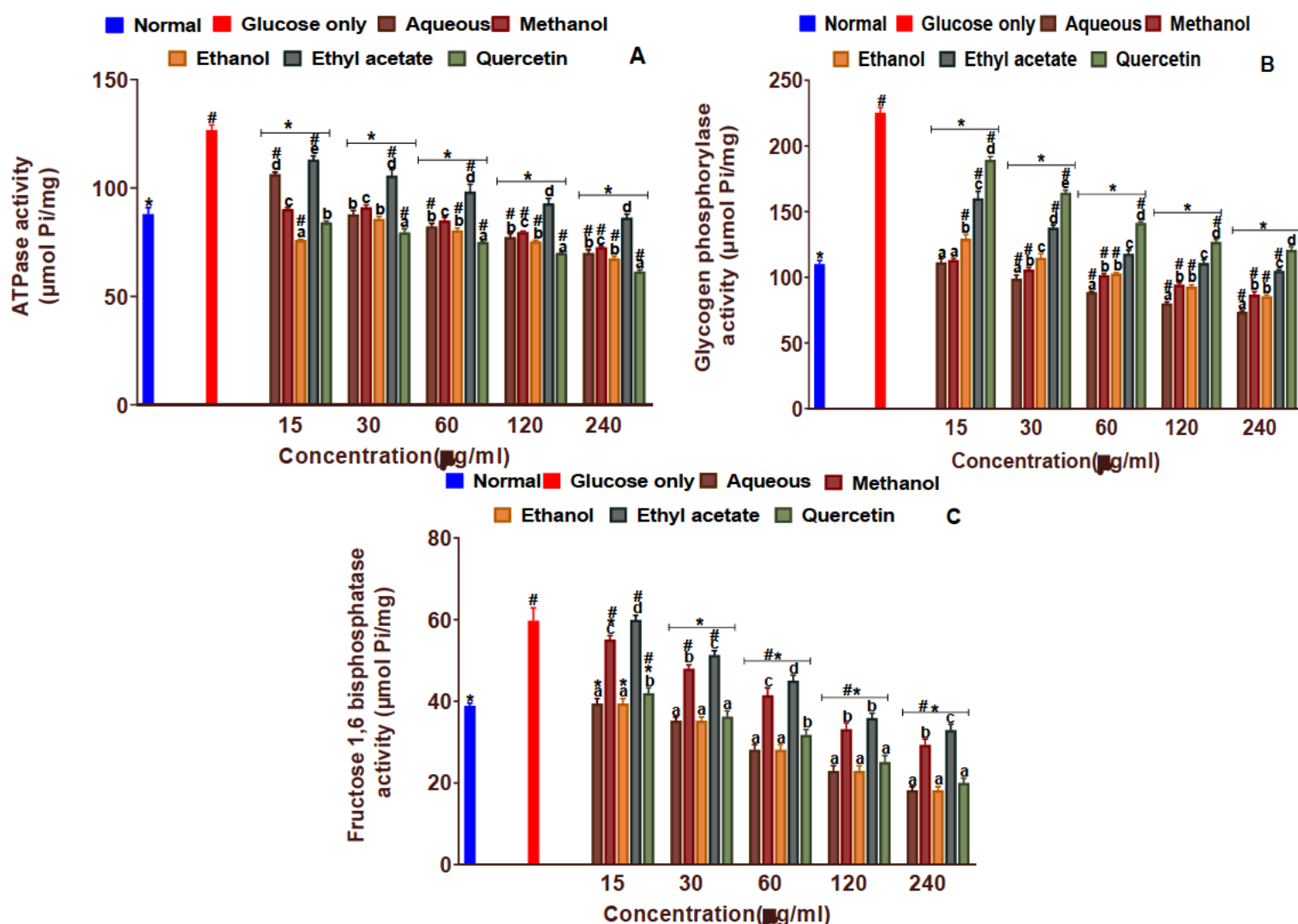


Figure 6.2.7: Effects of *C. africana* leaf extracts on the activities of hepatic (A) ATPase, (B) Glycogen phosphorylase and (C) Fructose 1,6 bisphosphatase enzymes. Data are presented as mean $\pm$ standard deviation of triplicate determinations. Different letters (a-d) above the bars for a given concentration are significantly different from each other ($p < 0.05$). *Significantly different from untreated sample and #significantly different from normal sample ($p < 0.05$).

6.2.5 Discussion

The global prevalence of diabetes has peaked, resulting in an upsurge in morbidity and mortality associated with its chronic complications (Dessie *et al.*,2020). Current pharmacological diabetes management strategies have several drawbacks, including undesirable side effects and unaffordability (Khalidi *et al.*,2014; Chikara *et al.*,2018). Medicinal plants, such as *C. africana*, have thus emerged as a promising source of bioactive therapeutic agent templates (Naveen *et al.*,2021).

Secondary metabolites, including phenols and flavonoids, observed in the leaf extracts of *C. africana* (Table 6.2.1 and Fig. 6.2.1), have multiple therapeutic potentials against many human ailments, including T2D (Das and Gezici, 2018). Previous studies associate enhanced antioxidant activity of plant extracts with high phenolic content (Velderrain-Rodríguez *et al.*,2014), as those in the leaf extracts of *C. africana* in Fig. 6.2.1, with the phenolic compounds' abilities to readily donate their delocalized Pi bond electrons to neighbouring reactive species (RS), enabling them to achieve an octet stable state. Sacrificial antioxidants capable of donating electrons or proton (H⁺) ions (Fig. 6.2.2A and 6.2.2B) to RS are beneficial as they protect against irreversible oxidative damage to functional biomolecules in the body (Velderrain-Rodríguez *et al.*,2014; Huang, 2018). Cumulative oxidative damage is implicated in the development and progression of numerous degenerative conditions, including T2D (Rani *et al.*,2016). Controlled homeostatic levels of RS are necessary for the normal metabolic activity of the body, as they function as immune response factors, cell signalling mediators, and in ATP generation (Lobo *et al.*,2010). Under homeostatic conditions, nitric oxide (NO) mediates the tissue injury inflammatory response (Farah *et al.*,2018); however, in T2D, elevated NO levels are implicated in chronic low-grade hyperinflammation and oxidative stress, leading to exacerbated tissue damage (Lobo *et al.*,2010). Evidently, antioxidant compounds such as those in the leaf extracts of *C. africana* that regulate the release of NO, as observed in Fig. 6.2.2C, may therefore abate excessive oxidative stress-induced tissue damage in T2D (Velagic *et al.*,2020). Previous studies have shown that inhibition of nitric oxide synthase, which mediates the release of NO, improves ischemia and wound healing in T2D (Malone-Povolny *et al.*,2019).

Ingested dietary complex carbohydrates are successively broken down by different hydrolytic enzymes prior to their assimilation into the blood stream, which inevitably leads to hyperglycaemic spikes in T2D patients with dysregulated glucose metabolic machinery. The inhibition of key carbohydrate-digesting enzymes (Fig. 6.2.3A and 6.2.3B) is a cornerstone

mechanism of action of several potent antidiabetic pharmacological agents currently on the market (Mahmood, 2016). Therefore, the inhibitory capabilities of the leaf extracts of *C. africana* on α amylase (Fig. 6.2.3A) and α glucosidase (Fig. 6.2.3B) are coherent with previous studies that show the hypoglycemic effect of such bioactivities (Alam *et al.*,2019). The indole moieties, including 3-[4-Methyl-1-(isopropenyl)-hex-3-en-1-yl]-1H-indole, have a wide range of therapeutic benefits, particularly as α -amylase and α -glucosidase inhibitors (Figure 6.2.4) (El Khatabi *et al.*,2022). Thus, the carbohydrate-digesting enzyme inhibitory abilities and high binding affinities of phytoconstituents in the leaf extracts of *C. africana* insinuate its hypoglycaemic activity.

Absorbed dietary free fatty acids stored in the adipose tissues provide energy reserves utilized under glucose-deficient periods; however, excessive accumulation of excess free fatty acids leads to obesity, which is implicated in the development of T2D and cardiovascular diseases (CDV) (Gregg *et al.*,2019). Compounds that inhibit the digestion of dietary free fatty acids (Fig. 6.2.3C) subsequently aid in the regulation of their absorption. The acquisition of energy from the free fatty acids stored in the adipose tissue through β -oxidation has been implicated in the exacerbation of oxidative stress. Therefore, the results show the antiobesity ability of *C. africana* and its ability to reduce obesogenic-related oxidative stress.

Compounds that retard glucose absorption by the small intestine (Fig. 6.2.5A) delay the rate at which glucose goes into the blood by modulating the action of sodium-glucose co-transporter 2 (Whalen *et al.*,2015). A reduced rate of glucose uptake in diabetic patients facilitates more efficient glucose disposal, thereby counteracting postprandial blood glucose spikes (Whalen *et al.*,2015). Enhanced glucose uptake, as observed in the current study (Fig. 6.2.5B), helps regulate hyperglycaemia in T2D patients. Such compounds may function by abating the mismatch between calorie intake and energy output at the cellular level that is implicated in the development and progression of glucose intolerance and T2D (Olson and Humphries, 2020). Thus, the results insinuate the ability of *C. africana* to potentiate improved glycaemic control through reduced intestinal glucose uptake and increased muscular uptake.

Extrinsic dietary compounds exhibit their antioxidant effect by directly reacting with ROS or by upregulating the activity and levels of intrinsic antioxidant mechanisms (Fig. 6.2.6A–6.2.6D). Iron-induced oxidative stress results in elevated lipid peroxidation (Fig. 6.2.6A), diminished levels of reduced GSH (Fig. 6.2.6B), and reduced activity of SOD (Fig. 6.2.6C) and catalase (Fig. 6.2.6D). Lipids are an integral structural component of cell membranes and

are particularly prone to oxidative damage. Oxidative damage to lipids results in cellular and eventually systemic damage that is implicated in insulin resistance. Reduction of lipid (Fig. 6.2.6A) peroxidation is therefore a key therapeutic avenue in the management of diabetes. The leaf extract of *C. africana* was shown to have an oxidative protective effect by increasing the levels of GSH and increasing the enzyme activities of SOD and CAT. Compounds that bind to the allosteric site of intrinsic antioxidant moieties aid in the maintenance of homeostatic oxidative status in the body by scavenging excess RS. Previous studies have documented the strong potential capacity of medicinal plant-derived indoles to modulate enzyme activity and enhance the therapeutic potential of plants (El Khatabi *et al.*,2022). Thus, these results show the ability of the leaf extracts of *C. africana* to ameliorate oxidative stress.

6.2.6 Conclusion

Results from this study show the great capacity of the ethanolic leaf extract of *C. africana* in its ability to mop up excess RS, inhibit carbohydrate hydrolysing enzymes, modulate glucose uptake and absorption, while concurrently upregulating the level and activities of intrinsic antioxidant defence systems. Identified compounds, especially indole-based ones, may have significantly contributed to these observed activities. Further *in vivo* experiments are necessary to verify these findings and elucidate a probable mode of action.

6.3 *Celtis africana* (N.L.Burm.) bark extracts regulate glucose metabolism and mitigate pancreatic oxidative injury

Brian K. Beseni¹, Ochuko L. Erukainure¹, Kolawole A. Olofinisan¹, * Md. Shahidul Islam¹

1.Department of Biochemistry, University of KwaZulu-Natal, Westville Campus, Durban 4000, South Africa.

***Corresponding author:** islamd@ukzn.ac.za

6.3.1 Abstract

Plants such as *Celtis africana* have been employed in traditional medicine since time immemorial. Studies have revealed that these therapeutic benefits stem from bioactive secondary metabolites, which can be harnessed to develop novel therapeutic agents against a plethora of human ailments, including T2D. Thus, in the current study, the bioactive compounds in *C. africana* stem bark extracts of varying polarity were identified and characterised through gas chromatography mass spectrometry (GC-MS), after which the total phenolic and flavonoids were quantified. Thereafter, the antioxidant and inhibitory capabilities of the extracts on key lipid and carbohydrate-digesting enzymes were determined using *in vitro* models. The modulatory capabilities of the extracts on muscular glucose uptake, hepatic glucogenic enzymes, intestinal glucose absorption, and pancreatic tissue oxidative stress-linked parameters were investigated using *ex vivo* models. Finally, the binding affinities and best poses of identified compounds against selected target proteins were analysed using *in silico* models (molecular docking). *C. africana* stem bark contained several bioactive phyto-compounds, including phytol, glutarimide, l-(+)-Ascorbic acid, 2,6-dihexadecanoate, squalene, and 7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-dien, as revealed through GC-MS, with substantial amounts of both phenolics and flavonoids. Identified compounds may have contributed to the augmented therapeutic bioactivities observed for the *C. africana* stem bark, such as enhanced antioxidant activity, pancreatic lipase, α amylase, and α glucosidase inhibitory capabilities. Ethyl isoxazole-5-carboxylate and 2-Methoxy-4-vinylphenol had the best binding affinities when docked against α glucosidase, α amylase, and pancreatic lipase ligands. Furthermore, *C. africana* stem bark extracts significantly ($p < 0.05$) inhibited glucogenic hepatic enzymes and retarded intestinal glucose absorption while increasing its uptake by psoas muscle tissues and yeast cell suspension. The extracts significantly ($p < 0.05$) reduced lipid peroxidation while concomitantly augmenting reduced glutathione (GSH) levels

and the activities of superoxide dismutase (SOD) and catalase (CAT) in iron sulphate mediated pancreatic tissue oxidative injury. *In silico* analysis insinuates a strong link between isoxazole-based compounds and observed bioactivities. Data from the current study indicates the remarkable potential of *C. africana* stem bark extracts against T2D, which warrants further *in vivo* and molecular experiments to ascertain these findings and deduce their precise modes of action.

Key words: *Celtis africana*, Antioxidant, Antidiabetic, Isoxazole derivatives, Enzyme inhibition, Molecular docking.

6.3.2 Introduction

Diabetes mellitus (DM) continues to be a global health concern, with its prevalence projected to continue soaring. Currently, an estimated 536.6 million adults are living with DM globally, and its incidence is expected to reach 783.2 million by 2045 (Ogurtsova *et al.*,2022; Sun *et al.*,2022). DM is a heterogenous group of chronic metabolic disorders that lead to hyperglycemia (Himanshu *et al.*,2020). Hyperglycemia observed in DM is due to insufficient β pancreatic cell insulin secretion, reduced response to circulatory insulin by target tissues, or both. Type 1, type 2, and gestational DM are the most prevalent etiologies of the condition and are classified according to their pathogenesis. DM is the multifactorial outcome of genetic, epigenetic, and environmental factors that result in aberrant lipid, protein, and carbohydrate metabolism (Himanshu *et al.*,2020). Risk factors for DM include age, ethnicity, physical activity engagement, diet, urbanization, and westernised culture (Al-Goblan *et al.*,2014).

Uncontrolled hyperglycemia in DM patients, which leads to glucose variability, increased blood pressure, elevated serum uric acid, and increased plasma lipids, has been implicated as the major cause of end-stage diabetic complications (Ceriello and Prattichizzo, 2021). DM complications are the major cause of increased morbidity and premature mortality among patients and are broadly classified as either macrovascular or microvascular (Ceriello and Prattichizzo, 2021). Microvascular DM complications include diabetic nephropathy, neuropathy, and retinopathy, while macrovascular complications include atherosclerosis, stroke, and cardiovascular diseases (CVD) (Papatheodorou *et al.*,2016). Excessive formation of advanced glycation end products (AGEs), the development of a pro-inflammatory milieu, and the induction of oxidative stress are only a few of the processes via which chronic hyperglycemia in diabetes (DM) drives the development of these complications (Papatheodorou *et al.*,2016).

Currently, several classes of antidiabetic oral agents exist for the management of DM, including biguanides, meglitinides, and sodium-glucose cotransporter (SGLT2) inhibitors, thiazolidinedione (TZD), dipeptidyl peptidase 4 (DPP-4) inhibitors, α -glucosidase inhibitors, and sulfonylureas (Chaudhury *et al.*,2017). These are selected based on the severity of the DM, age, and whether the patient is taking other medication and are usually prescribed as monotherapy together with intensive lifestyle management. (Mane *et al.*,2012; Chaudhury *et al.*,2017). While most antidiabetic agents function by reducing the circulatory blood glucose levels, few classes of these pharmacological agents have antioxidant activity, therefore they are not able to reduce oxidative stress and its accompanying ill effects (He *et al.*,2015). They are also marred by several ill side effects, including contraindications, weight loss or gain, hypoglycaemic shock, headaches, fluctuance, diarrhoea and nausea or vomiting (Stein *et al.*,2013; Tschöpe *et al.*,2013). Conversely, phytotherapy has long been recognised as a lucrative avenue for the development of novel pharmacological treatments for the treatment of diabetes. Several previous studies have demonstrated the efficacy of traditional medicinal plants as antioxidants and antidiabetic agent reservoirs. They have been documented to stimulate their therapeutic benefit through several mechanisms, including inhibition of α -glucosidase and AGE formation, the increase of GLUT-4 and PPAR expression, and antioxidant activity. Despite the large amount of literature available in support of the efficacy of traditional medicinal plants in the management of DM, their precise clinical modes of action remain controversial. There is therefore a constant need to evaluate and document the mechanisms of action associated with traditional medicinal plants such as *Celtis africana* (Governa *et al.*,2018).

Celtis africana, a deciduous tree of the family Cannabaceae, can be easily identified by its characteristic whitish bark and small, pale-yellow flowers. It is commonly used as an ornamental plant and has a rich history of traditional medicinal applications. This tree, which belongs to the Cannabaceae family, typically grows to heights ranging from 12 to 25 meters, forming a densely branched, semi-circular canopy. Notably, previous studies have reported the potential anticancer properties of powdered bark and root infusions from *C. africana* (Krief *et al.*,2005; Koduru *et al.*,2007). This observation was further supported by research conducted by Perveen and colleagues in 2015, who found that sphingolipid glucosides isolated from the aerial parts of *C. africana* exhibited strong cytotoxic activity against L5178Y mouse lymphoma cells. In addition to its potential in cancer treatment, leaf decoctions of *C. africana* have been traditionally used to remedy indigestion, pain relief, and the treatment of eye infections (Krief

et al.,2005; Borquaye *et al.*,2020). The current study was aimed at characterizing compounds in the stembark extract of *Celtis africana* and determining their antioxidant, antiobesity, and antidiabetic activities using *in vitro*, *ex vivo*, and *in silico* models.

6.3.3 Materials and methods

Please refer to Chapter 2, sections 2.4.1 to 2.11.5 for detailed materials and methods.

6.3.4 Results

As shown in Table 6.3.1, the different *C. africana* bark extracts contained a myriad of compounds including glutarimide, squalene, millitene, 1,4-Dihydro-2-methylbenzoic acid, 4-tert-Octylphenol and 7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-dien as revealed by Gas Chromatography Mass Spectroscopy (GC-MS) analysis. 9,12-Octadecadienoic acid and 9,12,15-Octadecatrienoic acid with 28.57% and 19.70% relative abundance were the most abundant compounds identified in the aqueous stem bark extract of *C. africana*. Octadecadienoic acid with a relative abundance of 16.03% was the most abundant compound in the methanol extract closely followed by Hexadecatrienal with a relative abundance of 12.30%. Octadecadien-1-ol 26.93 %, l-(+)-Ascorbic acid 2,6-dihexadecanoate 18.93%, Phytol (12.54%) were the most abundant compounds in the ethanol stembark extract of *C. africana*. Octadecatrienoic acid (21.23%) Pentadecanoic acid (16.34%) Phytol acetate (14.42%) were the most common compounds in the ethyl acetate stembark *C. africana* extract.

Table 6.3.1: Phyto-compounds identified by Gas Chromatography Mass Spectrometry (GC-MS) in the *C. africana* stembark extracts.

Compounds	Retention time(min)	Molecular Formula	Molecular weight (g/mol)	Relative abundance (%)
Aqueous				
Glutarimide	7.45	C ₅ H ₇ NO ₂	113.11	0.68
α-Methyl manno-furanoside	14.05	C ₇ H ₁₄ O ₆	194.18	0.92
Phytol acetate	15.68	C ₂₂ H ₄₂ O ₂	338.6	9.54
Tetramethyl-2-hexadecene	15.73	C ₂₀ H ₄₀	280.5	1.44
Tetramethyl-2-hexadecen-1-ol	16.14	C ₂₀ H ₄₀ O	296.5	3.02
Pentadecanoic acid, 14-methyl-, methyl ester	16.61	C ₁₇ H ₃₄ O ₂	270.5	0.87
n-Hexadecanoic acid	17.11	C ₁₆ H ₃₂ O ₂	256.42	16.38
Methyl ester octadecadienoic acid	18.27	C ₁₉ H ₃₄ O ₂	294.5	1.28

Eicosatrienoic acid, methyl ester	18.33	C ₂₁ H ₃₆ O ₂	320.5	0.71
Phytol	18.45	C ₂₀ H ₄₀ O	296.5	3.38
Octadecadienoic acid	18.82	C ₁₈ H ₃₂ O ₂	280.4	28.57
Octadecanoic acid	19.03	C ₁₈ H ₃₆ O ₂	284.5	6.68
n-Tetracosanol-1	19.18	C ₂₄ H ₅₀ O	354.7	1.33
Tetramethylheptadecan-4-olide	20.63	C ₂₁ H ₄₀ O ₂	324.5	0.48
Spiro[androst-5-ene-17,1'-cyclobutan]-2'-one	20.81	C ₂₂ H ₃₂ O ₂	328.5	0.52
Dichloroacetic acid, heptadecyl ester	20.92	C ₁₉ H ₃₆ Cl ₂ O ₂	367.4	0.76
Diisooctyl phthalate	22.12	C ₂₄ H ₃₈ O ₄	390.6	0.45
Squalene	24.88	C ₃₀ H ₅₀	410.7	1.23
Methanol				
2-butoxy-ethanol	4.37	C ₆ H ₁₄ O ₂	118.17	0.38
2-Heptenal	4.87	C ₇ H ₁₂ O	112.17	1.66
2,4-Heptadienal	5.30	C ₇ H ₁₀ O	110.15	0.29
2-Cyano-N-(2-p-tolyloxy-ethyl)-acetamide	6.38	C ₁₂ H ₁₄ N ₂ O ₂	218.25	0.18
1,4-Dihydro-2-methylbenzoic acid	6.78	C ₈ H ₁₀ O ₂	138.16	0.34
Glutarimide	7.41	C ₅ H ₇ NO ₂	113.11	0.34
5-Amino-1-benzoyl-1H-pyrazole-3,4-dicarbonitrile	7.51	C ₁₂ H ₇ N ₅ O	237.22	0.18
1-3-methylphenyl-ethanone	7.81	C ₉ H ₁₀ O	134.17	0.39
3-methyl-2-Cyclopenten-1-one	7.94	C ₆ H ₈ O	96.13	0.21
8-methyl-tricyclo-octan-3-one	8.08	C ₉ H ₁₂ O	136.19	0.22
Terpinolene	8.30	C ₁₀ H ₁₆	136.23	0.88
10-Chlorodecyl 2-methylbutanoate	8.44	C ₁₅ H ₂₉ ClO ₂	276.84	0.23
2,6,10,15-tetramethyl-heptadecane	11.33	C ₂₁ H ₄₄	296.6	0.38
Dihydroactinidiolide	12.45	C ₁₁ H ₁₆ O ₂	180.24	0.22
Hexahydro-1H-indene	13.69	C ₁₅ H ₂₆	206.37	0.19
p-octyl-anisole	13.99	C ₁₅ H ₂₄ O	220.35	0.18
2-Butanone, 4-(4-methoxyphenyl)-	14.29	C ₁₁ H ₁₄ O ₂	178.23	0.49
2-Methyl-4-tert-octylphenol	14.49	C ₁₅ H ₂₄ O	220.35	1.34
O-acetyl-hexestrol	14.71	C ₂₀ H ₂₄ O ₃	312.4	0.83
4'-Propoxy-2-methylpropiophenone	14.76	C ₁₃ H ₁₈ O ₂	206.28	0.50
4-tert-Octylphenol	14.93	C ₁₄ H ₂₂ O	206.32	2.05

3-methyl-2-[4-(1-methylethyl pentanoic acid	15.66	C ₁₆ H ₂₃ NO ₃	277.36	0.54
Phytol acetate	15.72	C ₂₂ H ₄₂ O ₂	338.6	1.18
Tetramethyl-2-hexadecen-1-ol	16.17	C ₂₀ H ₄₀ O	296.5	0.31
Millitene	16.35	C ₁₂ H ₁₈	162.27	0.32
7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-dien	16.51	C ₁₇ H ₂₄ O ₃	276.37	0.19
Methyl 10-trans,12-cis- octadecadienoate	18.29	C ₁₉ H ₃₄ O ₂	294.5	0.50
Methyl ester eicosatrienoic acid	18.36	C ₂₁ H ₃₆ O ₂	320.5	0.27
Phytol	18.47	C ₂₀ H ₄₀ O	296.5	2.68
Octadecadienoic acid	18.87	C ₁₈ H ₃₂ O ₂	280.4	16.03
Hexadecatrienal	18.92	C ₁₈ H ₃₀ O ₂	278.4	12.30
Octadecanoic acid	19.09	C ₁₈ H ₃₆ O ₂	284.5	3.67
n-Tetracosanol-1	19.25	C ₂₄ H ₅₀ O	354.7	2.72
Phytol acetate	19.40	C ₂₂ H ₄₂ O ₂	338.6	0.78
Tetramethylheptadecan-4-olide	20.67	C ₂₁ H ₄₀ O ₂	324.5	0.58
Diisooctyl phthalate	22.22	C ₂₄ H ₃₈ O ₄	390.6	3.63
Ethanol				
2-butoxy-ethanol	4.30	C ₆ H ₁₄ O ₂	118.17	3.69
Dimethyl-O-(1-methyl-butyl)- hydroxylam	6.40	C ₇ H ₁₇ NO	131.22	0.81
D-Mannitol	7.11	C ₆ H ₁₄ O ₆	182.17	0.88
2H-Pyrrol-2-one, 1,5-dihydro-1- methyl	7.77	C ₅ H ₇ NO	97.12	0.60
Acetin	8.65	C ₅ H ₁₀ O ₄	134.13	2.56
Ethyl isoxazole-5-carboxylate	8.98	C ₆ H ₇ NO ₃	141.12	0.47
2-Methoxy-4-vinylphenol	9.53	C ₉ H ₁₀ O ₂	150.17	0.54
Trans-9-Hexadecen-1-ol	12.94	C ₁₆ H ₃₂ O	240.42	0.98
Tetradecanoic acid	15.02	C ₁₄ H ₂₈ O ₂	228.37	1.05
Phytol acetate	15.68	C ₂₂ H ₄₂ O ₂	338.6	2.90
Tetramethyl-2-hexadecen-1-ol	16.14	C ₂₀ H ₄₀ O	296.5	0.80
l-(+)-Ascorbic acid 2,6- dihexadecanoate	17.29	C ₃₈ H ₆₈ O ₈	652.9	18.93
Phytol	18.49	C ₂₀ H ₄₀ O	296.5	12.54

Octadecadien-1-ol	19.08	C ₁₈ H ₃₂ O ₂	280.4	26.93
Octadecanoic acid	19.22	C ₁₈ H ₃₆ O ₂	284.5	11.34
Phytol acetate	19.38	C ₂₂ H ₄₂ O ₂	338.6	1.35
2H-Pyran-2-one, tetrahydro-6-tridecyl-	20.67	C ₁₈ H ₃₄ O ₂	282.5	0.56
Ethyl 14-methyl-hexadecanoate	20.94	C ₁₉ H ₃₈ O ₂	298.5	0.59
Diisooctyl phthalate	22.16	C ₂₄ H ₃₈ O ₄	390.6	4.58
Squalene	24.89	C ₃₀ H ₅₀	410.7	1.77
Ethyl acetate				
Dodecanoic acid	12.78	C ₁₂ H ₂₄ O ₂	200.32	0.65
Trans-3(10)-carene-2-ol	13.61	C ₁₀ H ₁₆ O	152.23	0.45
Spirio-10-(2,11-dioxabicyclo[4.4.1]undeca-3,5	14.25	C ₁₄ H ₂₀ O ₃	236.31	0.53
Tetradecanoic acid	15.05	C ₁₄ H ₂₈ O ₂	228.37	0.94
Acetic acid, 2-(2,2,6-trimethyl-7-oxa-bicyclo[4	15.47	C ₁₄ H ₂₂ O ₃	238.32	2.49
Phytol acetate	15.721	C ₂₂ H ₄₂ O ₂	338.6	14.42
Trimethyl-2-pentadecanone	15.78	C ₁₈ H ₃₆ O	268.5	2.77
Pentadecanoic acid	16.08	C ₁₅ H ₃₀ O ₂	242.4	0.55
Tetramethyl-2-hexadecen-1-ol	16.16	C ₂₀ H ₄₀ O	296.5	5.68
7,9-Di-tert-butyl-1-oxaspiro [4.5]deca-6,9-diene-2,8-dione	16.58	C ₁₇ H ₂₄ O ₃	276.4	3.89
Pentadecanoic acid	17.45	C ₁₅ H ₃₀ O ₂	242.4	16.34
1-Heneicosyl formate	18.25	C ₂₂ H ₄₄ O ₂	340.6	0.62
Phytol	18.50	C ₂₀ H ₄₀ O	296.5	8.42
Octadecatrienoic acid	19.23	C ₁₈ H ₃₀ O ₂	278.4	21.23
Trans-9-Hexadecen-1-ol	20.16	C ₁₆ H ₃₂ O	240.42	5.51
Methyl ester eicosatrienoic acid,	20.39	C ₂₁ H ₃₆ O ₂	320.5	0.35
9-Methyl-10-pentadecen-1-ol	20.71	C ₁₆ H ₃₂ O	240.42	0.53
Diisooctyl phthalate	22.17	C ₂₄ H ₃₈ O ₄	390.6	1.18
2-methylhexacosane	24.72	C ₂₇ H ₅₆	380.7	1.08

As depicted in Fig. 6.3.1A, the methanolic extract contained significantly ($p < 0.05$) the highest polyphenolic content of 31.24 mg/g gallic acid equivalent, while the ethyl acetate (10.65 mg/g gallic acid equivalent) extract contained the least polyphenolic content among all the extracts. Furthermore, the data shows that there was no significant ($p > 0.05$) difference in the polyphenolic content in the aqueous and ethanol extracts, which contained 25.73 and 26.93 mg/g gallic acid equivalents, respectively. As displayed in Fig. 6.3.1B, the ethanol extract contained significantly ($p < 0.05$) the highest amounts of flavonoids (19.44 mg/g quercetin equivalents), while the ethyl acetate contained significantly ($p < 0.05$) the least amounts of flavonoids (7.48 mg/g quercetin equivalents). Evidently, no statistically significant ($p > 0.05$) difference in the flavonoid content was observed in the aqueous (14.60 mg/g quercetin equivalents) and methanol (15.71 mg/g quercetin equivalents) extracts.

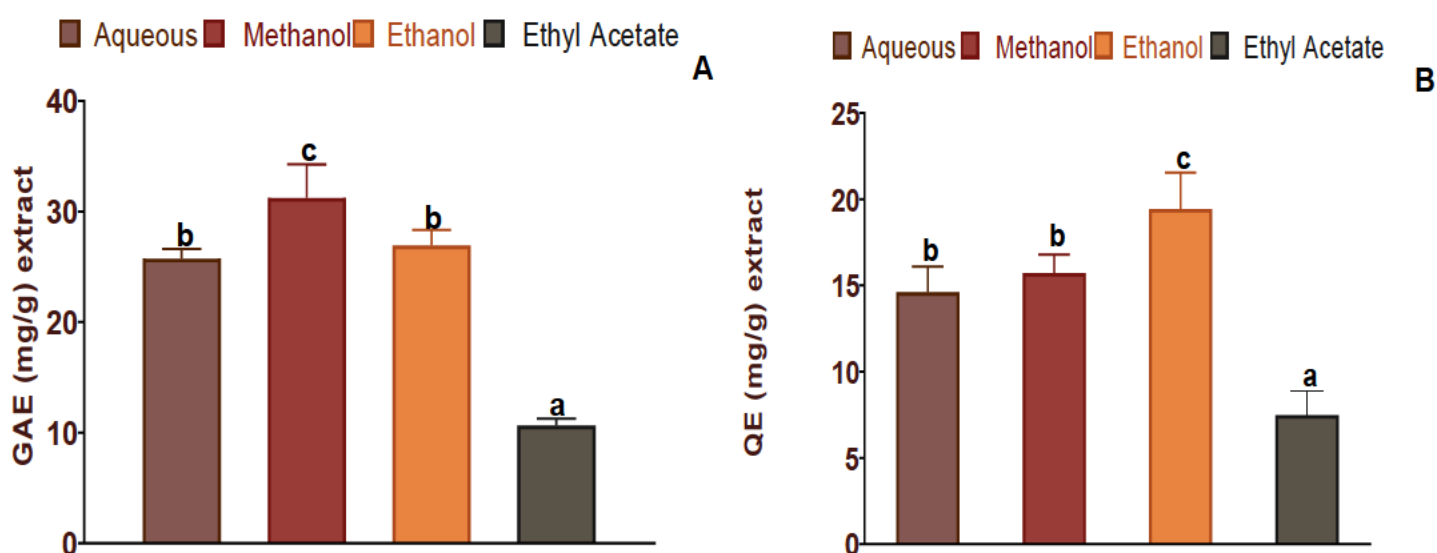


Figure 6.3.1: The total phenolic content of the *C. africana* crude bark extracts represented as (A) Gallic acid equivalents (GAE mg/g) and the total flavonoid content represented as (B) Quercetin equivalents (QE mg/g). Bars with superscripts a-c for each extract are statistically different at $p < 0.05$ level of significance.

The FRAP, DPPH, and NO radical scavenging capabilities of the *C. africana* bark extracts are displayed in Figs. 6.3.2A–6.3.2C. As seen in Figure 6.3.2A, the extracts exhibited dose-dependent DPPH radical scavenging activities, with the ethanol extract showing superior activity as shown by its IC₅₀ value of 7,56±0,37 µg/ml (Table 6.3.2), which was lower than that of Vitamin C 9,21±0,12 (positive control). The aqueous and methanol extracts exhibited comparable DPPH radical scavenging activities with no significant (p>0.05) difference between them at all concentrations except at 30 µg/ml, while the ethyl acetate extract significantly exhibited the least DPPH radical scavenging activities with an IC₅₀ value of 165,71±0,92 µg/ml as shown in Table 6.3.2. As depicted in Figure 6.3.2 B, the methanol *C. africana* bark extract displayed significantly (p<0.05) the best electron-donating activities among all the extracts, with an IC₅₀ value of 0,15±0,68 µg/ml. The aqueous and ethanol extracts displayed comparable electron-donating activities with IC₅₀ values of 0,39±0,08 µg/ml and 0,41±0,34 µg/ml, respectively, as shown in Table 6.3.2, while the ethyl acetate extract exhibited the least capability among all the bark extracts. Evidently, the bark extracts of *C. africana* exhibited dose-dependent nitric oxide scavenging activities, as depicted in Figure 6.3.2C. Except at lower concentrations (15 µg/ml and 30 µg/ml), the aqueous extract exhibited the best nitric oxide scavenging activities, with an IC₅₀ value of 86,96±0,74 µg/ml shown in Table 6.3.2. At all the concentrations, no statistically significant (p>0.05) difference was observed between the nitric oxide (NO) scavenging activities of the methanol and ethanol extracts. Conversely, the ethyl acetate extract exhibited the least nitric oxide scavenging activities at all the assayed concentrations.

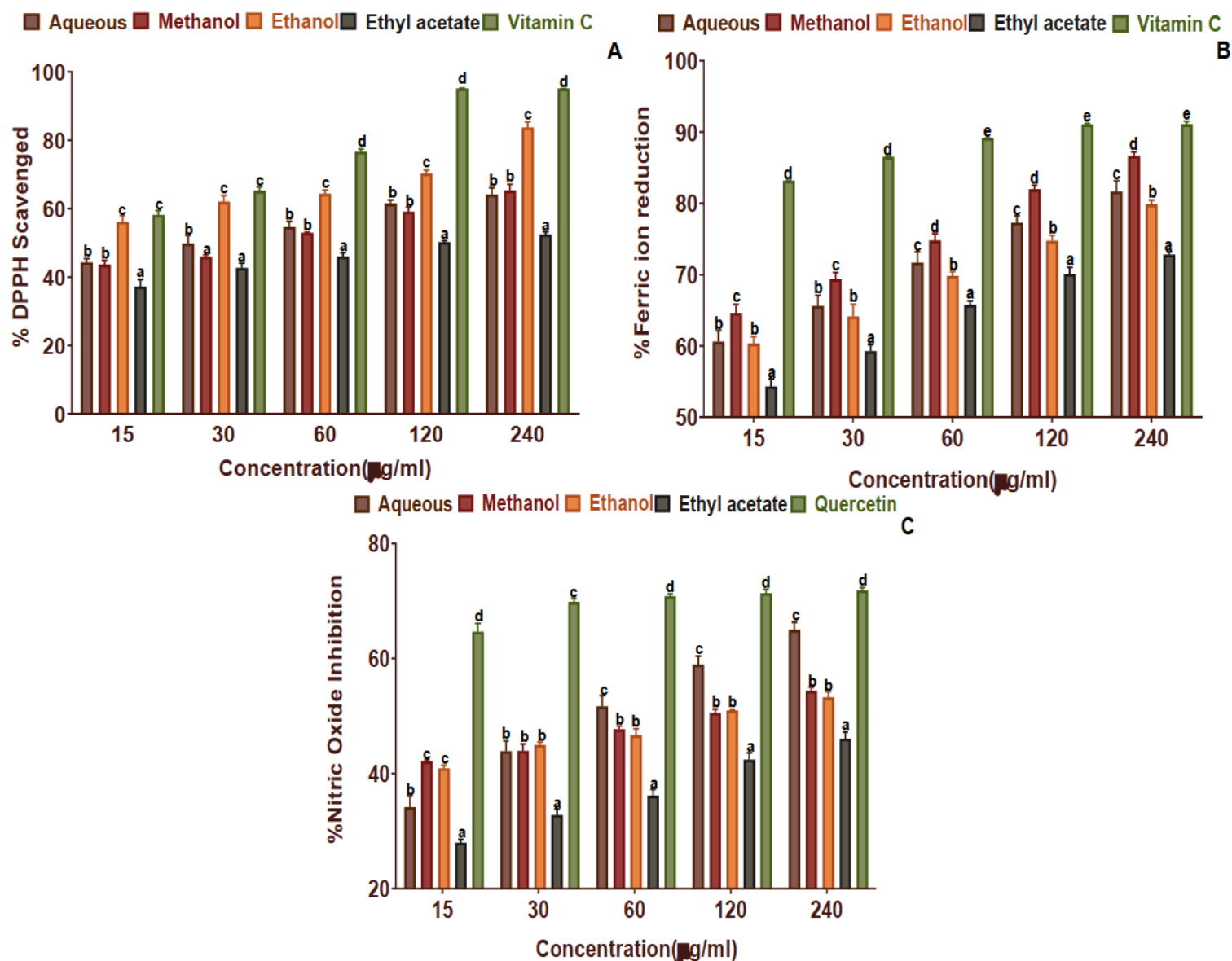


Figure 6.3.2: *C. africana* bark extracts (A) DPPH scavenging (B) ferric reduction and (C) nitric oxide scavenging activities. Bars with superscripts a-e for each concentration are statistically different at $p < 0.05$ level of significance.

The inhibitory capabilities of the *C. africana* bark extracts on key lipid and carbohydrate hydrolytic enzymes are shown in Figs. 6.3.3A–6.3.3C. The ethanolic extract exhibited significantly ($p < 0.05$) the best pancreatic lipase inhibitory capabilities among all other extracts, with an IC_{50} value of $678,77 \pm 0,98 \mu\text{g/ml}$ (Table 6.3.2), seconded by the ethyl acetate extract. Furthermore, no significant differences ($p > 0.05$) in pancreatic lipase inhibitory activities were observed for the aqueous and methanol extracts across all the assayed concentrations. Evidently, the methanol and ethanol extracts showed comparable α -glucosidase inhibitory activities at all the tested concentrations except at 120–240 $\mu\text{g/ml}$, whereby the ethanol extract showed significantly higher α -glucosidase inhibitory capabilities, as depicted in Fig. 6.3.3B.

Furthermore, the ethanol extract, which had an IC_{50} value of $41,87 \pm 0,29 \mu\text{g/ml}$, displayed superior α -glucosidase inhibitory capabilities than acarbose, which had an IC_{50} value of $100,68 \pm 0,09 \mu\text{g/ml}$, as shown in Table 6.3.2. Evidently, no significant ($p > 0.05$) differences in the α -glucosidase inhibitory capabilities of the aqueous and ethyl acetate extracts were observed at all the tested concentrations. Finally, as illustrated in Fig. 6.3.3C, the *C. africana* bark extracts exhibited dose-dependent α -amylase inhibitory activities. The aqueous extract exhibited the overall best α -amylase inhibitory activity with an IC_{50} value of $138,90 \pm 0,98 \mu\text{g/ml}$, comparable to the $118,57 \pm 0,97 \mu\text{g/ml}$ in Table 6.3.2 obtained for acarbose (positive control). Conversely, the ethyl acetate extract exhibited the least α -amylase inhibitory activity with an IC_{50} value of $436,03 \pm 0,57 \mu\text{g/ml}$ (Table 6.3.2).

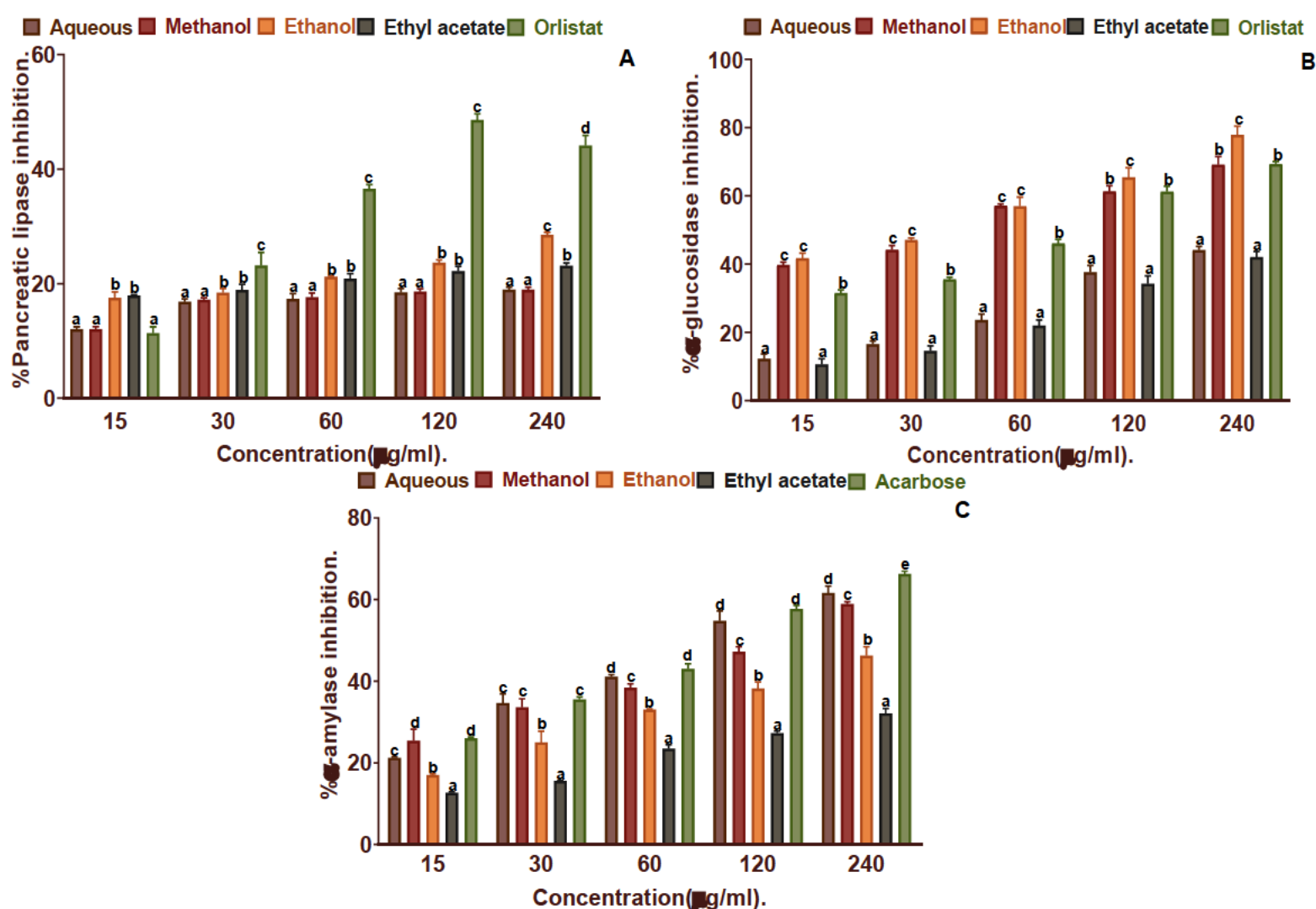


Figure 6.3.3: *C. africana* bark extracts (A) pancreatic lipase (B) α -glucosidase (C) α -amylase inhibitory activities. Bars with superscripts a-e for each concentration are statistically different at $p < 0.05$ level of significance.

Table 6.3.2 summarizes the *in silico* free binding energies (kcal/mol) of various secondary metabolites from *C. africana* stem bark extracts against with three key digestive enzymes: α glucosidase, α amylase, and pancreatic lipase. 5-Amino-1-benzoyl-1H-pyrazole-3,4-dicarbonitrile demonstrated strong binding affinity across all three enzymes, particularly towards α glucosidase (-10.84 kcal/mol). Interestingly, while Ethyl isoxazole-5-carboxylate displays the strongest binding energy with glucosidase (-10.87 kcal/mol), it exhibits lower affinity for amylase (-10.05 kcal/mol). Spiro[androst-5-ene-17,1'-cyclobutan]-2'-one exhibited high binding affinities, against pancreatic lipase (-11.0 kcal/mol) and glucosidase (-10.51 kcal/mol). Furthermore, 2-Methoxy-4-vinylphenol merits attention for exhibiting the strongest binding affinity with pancreatic lipase (-11.82 kcal/mol), closely followed by 2-Methyl-4-tert-octylphenol (-11.71 kcal/mol). These findings support the potential inhibitory effects of *C. africana* stem bark extracts on these respective enzymes as observed experimentally in Fig. 6.3.3.

Table 6.3.2: Free binding energy of secondary metabolites from *C. africana* stembark extracts with carbohydrate and lipid digestive enzymes.

Compound	Glucosidase (kcal/mol)	Amylase (kcal/mol)	Pancreatic lipase (kcal/mol)
α -Methyl manno-furanoside	-8.44	-7.11	-6.83
Spiro[androst-5-ene-17,1'-cyclobutan]-2'-one	-10.51	-10.01	-11.0
5-Amino-1-benzoyl-1H-pyrazole-3,4-dicarbonitrile	-10.84	-8.5	-9.74
1-3-methylphenyl-ethanone	-9.76	-8.72	-6.32
3-methyl-2-Cyclopenten-1-one	-7.32	-5.81	-7.53
8-methyl-tricyclo-octan-3-one	-8.43	-6.92	-7.43
Terpilene	-6.9	-5.72	-7.4
Hexahydro-1H-indene	-10.15	-7.67	-9.79
p-octyl-anisole	-6.33	-8.47	-7.11
4-(4-methoxyphenyl)-2-Butanone	-7.55	-7.94	-8.77
2-Methyl-4-tert-octylphenol	-9.39	-7.6	-11.71
O-acetyl-hexestrol	-10.22	-7.38	-10.36
Millitene	-7.84	-6.0	-9.27
Acetin	-9.77	-6.88	-8.78
Ethyl isoxazole-5-carboxylate	-10.87	-10.05	-8.81
2-Methoxy-4-vinylphenol	-9.42	-6.96	-11.82
Tetrahydro-6-tridecyl-2H-Pyran-2-one	-7.3	-5.31	-6.93
Acarbose	-19.6	-14.1	-
Orlistat	-	-	-10.2

Table 6.3.3: The IC₅₀ values of different *C. africana* stembark extracts for different assayed parameters.

	Aqueous Extract (µg/ml)	Methanol Extract (µg/ml)	Ethanol Extract (µg/ml)	Ethyl acetate Extract (µg/ml)	Vitamin C (µg/ml)	Quercetin (µg/ml)	Acarbose (µg/ml)	Orlistat(µg/ml)
DPPH	32,16±0,14	56,65±0,25	7,56±0,37	165,71±0,92	9,21±0,12	-	-	-
FRAP	0,39±0,08	0,15±0,68	0,41±0,34	0,78±0,74	6,67±0,88	-	-	-
NO	86,96±0,74	136,82±0,79	147,01±0,61	267,82±0,14	-	1,17±0,58	-	-
Pancreatic lipase	1661,88	1724,138	678,77±0,98	1459,256	-	-	-	114,52±0,83
α-glucosidase	257,31±0,91	51,91±0,36	41,87±0,29	275,61±0,73	-	-	100,68±0,09	-
α-amylase	138,90±0,98	161,11±0,44	250,73±0,23	436,03±0,57	-	-	118,57±0,97	-

Data is presented as mean ± standard deviation. Key: DPPH = 2,2-diphenyl-1-picrylhydrazyl. FRAP = Ferric reducing antioxidant power. NO = Nitric oxide assay.

The modulatory effects of the *C. africana* stem-bark extracts on glucose uptake in yeast suspension, rat psoas skeletal muscle, liver tissue, and absorption in rat intestinal jejunum are depicted in Figs. 6.3.4A–6.3.4D. The ethanol extract significantly ($p < 0.05$) increased glucose uptake in the yeast suspension better than metformin (positive control), especially at the highest concentrations of 120 $\mu\text{g/ml}$ to 240 $\mu\text{g/ml}$, as observed in Fig. 6.3.4A. Evidently, apart from the ethyl acetate extract, the *C. africana* bark extracts significantly ($p < 0.05$) increased glucose uptake in the rat psoas skeletal muscle when compared to the normal control, as seen in Fig. 6.3.4 B. At concentrations of 60 $\mu\text{g/ml}$ and beyond the aqueous, methanol and ethanol extract stimulated glucose uptake in the rat psoas skeletal muscle better than insulin (positive control). Furthermore, the data in Fig. 6.3.4B shows that the ethanol extract exhibited the overall best psoas skeletal muscle glucose uptake stimulatory activity among all the extracts. As displayed in Fig. 6.3.4 C, the aqueous and ethanol extracts exhibited the best hepatic tissue glucose uptake stimulatory activities. Furthermore, at the highest concentration (240 $\mu\text{g/ml}$), no significant ($p > 0.05$) difference was observed between the hepatic glucose uptake stimulatory activity of insulin and the aqueous and ethanol extracts. Overall, the aqueous extract stimulated the best hepatic glucose uptake. Finally, Fig. 6.3.4D depicts the glucose-absorption-retarding effects of *C. africana* bark extracts in rat intestinal jejunum. The ethanol extract displayed significantly ($p < 0.05$) the best intestinal glucose absorption retarding capability among all the extracts, superseding that observed for acarbose at concentrations of 30 $\mu\text{g/ml}$ and beyond. At 240 $\mu\text{g/ml}$, all the extracts significantly ($p < 0.05$) surpassed the rat intestinal jejunum glucose absorption inhibitory capacity of acarbose. The ethyl acetate extract had the least rat intestinal jejunum glucose absorption inhibitory capabilities among all tested extracts.

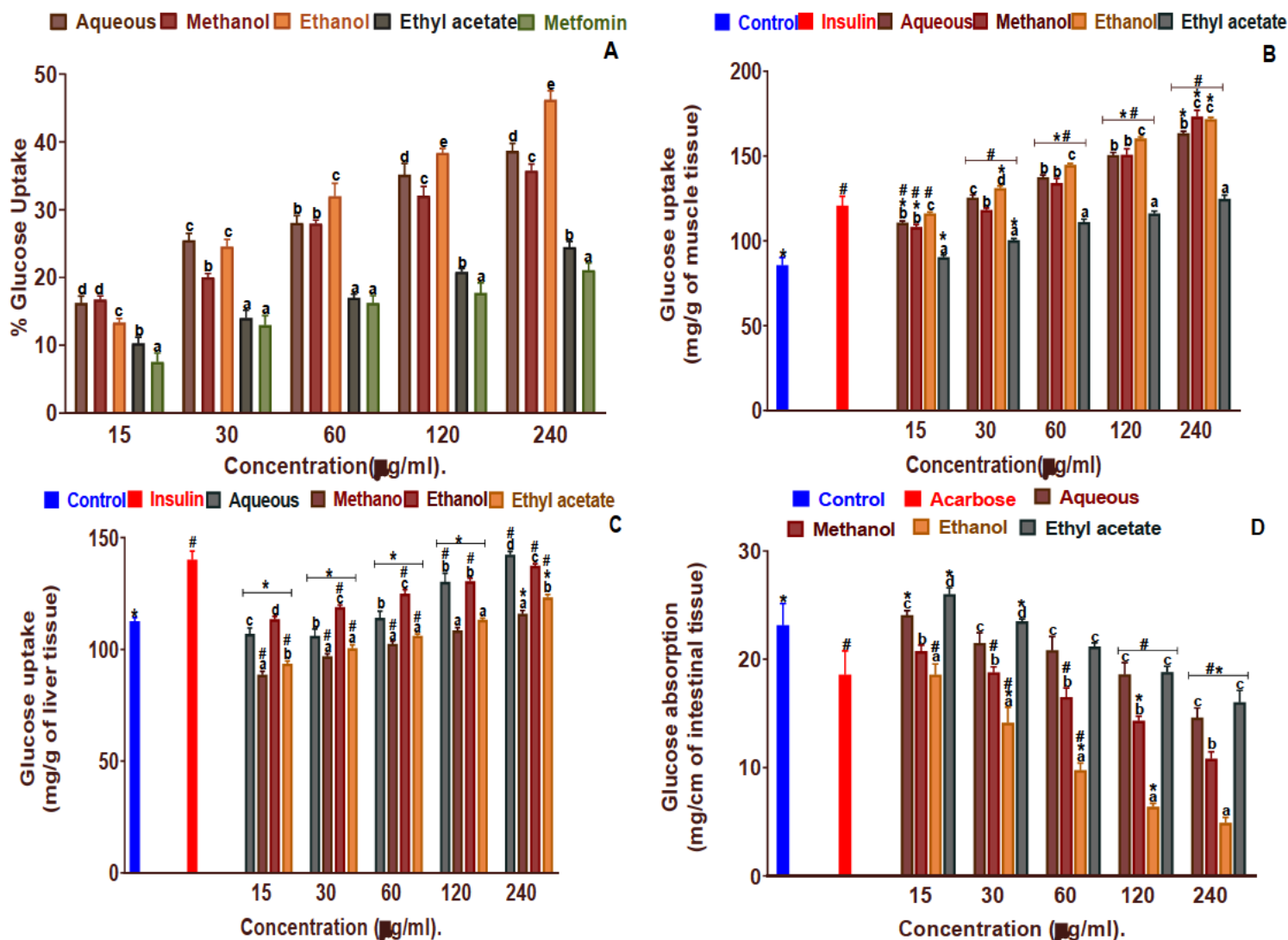


Figure 6.3.4: The effects of *C. africana* bark extracts on (A) glucose uptake in yeast suspension (B) glucose uptake in excised rat psoas muscle (C) liver glucose uptake (D) glucose absorption in the intestinal jejunum. Bars with superscripts a-b are statistically different at $p < 0.05$ whereas those with * and # are different from the acarbose/insulin treatment positive and control, respectively.

As depicted in Fig. 6.3.5, A-D oxidative pancreatic tissue injury significantly ($p < 0.05$) increased lipid peroxidation with a concomitant reduction in SOD and CAT activities and reduced GSH levels. The bark extracts of *C. africana* significantly ($p < 0.05$) reduced lipid peroxidation, particularly at 240 µg/ml, as displayed in Fig. 6.3.5A. The ethanol extract of *C. africana* showed the best lipid peroxidation capability, resulting in near-normal MDA concentrations at 240 µg/ml, which were insignificantly ($p > 0.05$) different from those in the normal tissue. All the extracts had significantly better lipid peroxidation capability when compared to Vitamin C (positive control), suggesting their enhanced effectiveness against lipid peroxidation. A similar trend was observed in Fig. 6.3.5 B-D, in which treatment with the

aqueous extract resulted in the best SOD and CAT activities and stimulated the highest GSH levels. Furthermore, the aqueous, methanol, and ethanol extracts resulted in better SOD, CAT activities, and GSH levels than vitamin C (positive control). As observed in Fig. 6.3.5B, only the ethyl acetate extract failed to augment SOD activity beyond that of vitamin C at all the tested concentrations. However, in Figs. 6.3.5C and 6.3.5D, the ethyl acetate extract showed comparable activity to that of vitamin C at all concentrations except at 240 µg/ml, wherein vitamin C exhibited significantly better CAT activity and GSH levels.

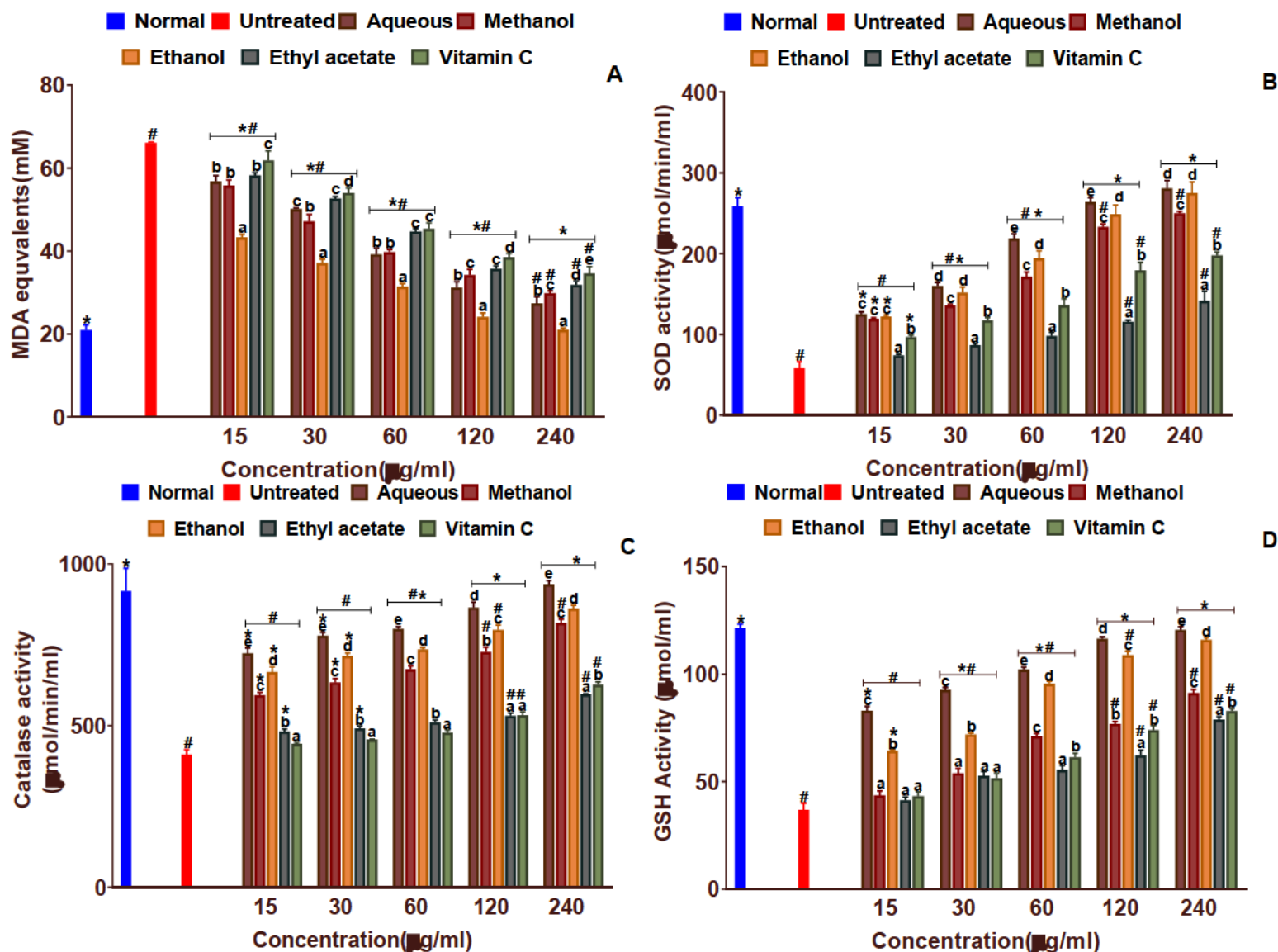


Figure 6.3.5: Effects of *C. africana* bark extracts on (A) lipid peroxidation, (B) SOD activity, (C) Catalase activity and (D) GSH activity in oxidative pancreatic injury. Data are presented as mean $\pm$ standard deviation of triplicate determinations. Different letters (a-e) above the bars for a given concentration are significantly different from each other ($p < 0.05$). *Significantly different from untreated sample and #significantly different from normal sample.

As depicted in Figs. 6.3.6A–6.3.6C, hyperglycaemia induces hyperactivity of hepatic ATPase, glycogen phosphorylase (GP), and fructose-1,6-bisphosphatase (FBP) activity. Treatment with the *C. africana* bark extracts significantly ($p < 0.05$) reduced ATPase activity, as shown in Fig. 6.3.5 A. The ethanol extract had the best ATPase inhibitory activity, which was comparable to that of quercetin (the positive control), particularly at concentrations of 60 $\mu\text{g/mL}$ and beyond. In contrast, the ethyl acetate extract exhibited the least ATPase inhibitory capability among all the extracts at all the tested concentrations. In Fig. 6.3.6B, the aqueous and ethanol extracts, which had the best glycogen phosphorylase (GP) inhibitory capability, showed comparable activity at all the tested concentrations with no significant ($p > 0.05$) difference between them. The aqueous and ethanol extracts effectively inhibited glycogen phosphorylase (GP) significantly ($p < 0.05$) better than quercetin (the positive control). Only the ethyl acetate extract, which was shown to have the least glycogen phosphorylase (GP) inhibitory capability among all the extracts, failed to surpass quercetin's inhibitory capability. In Fig. 6.3.6C Treatment with the *C. africana* bark extracts resulted in dose-dependent fructose 1,6-bisphosphatase (FBP) inhibitory activity. The aqueous and ethanol extracts had the overall best fructose 1,6-bisphosphatase (FBP) inhibitory capabilities among all the extracts, with no significant ($p > 0.05$) difference between them. The aqueous and ethanol extracts inhibited fructose 1,6-bisphosphatase (FBP) activity significantly ($p < 0.05$) better than quercetin (positive control). The ethyl acetate extract had the least fructose 1,6-bisphosphatase (FBP) inhibitory capabilities among all the extracts, which showed comparable activity to quercetin (the positive control) with no significant ($p > 0.05$) difference.

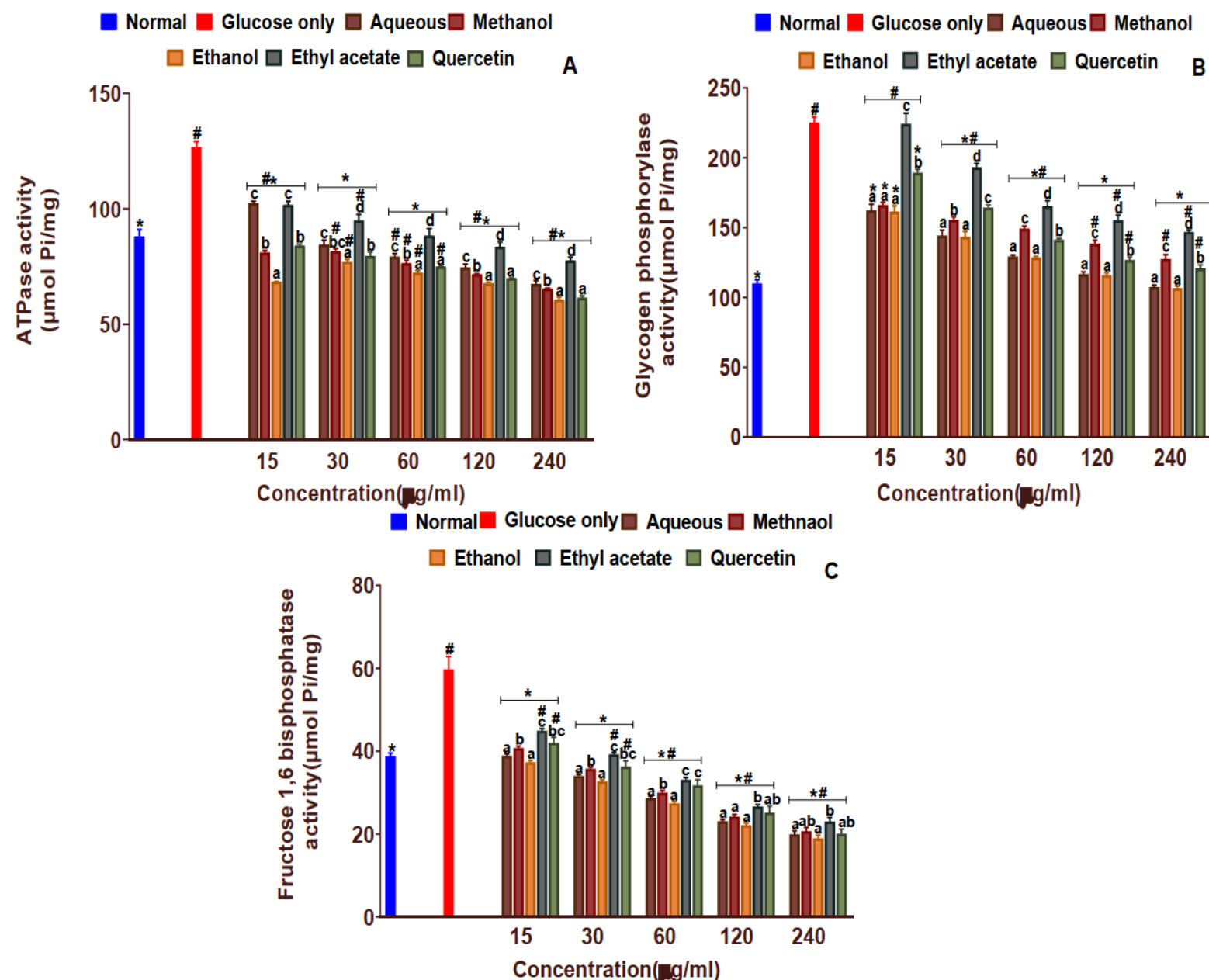


Figure 6.3.6: Effects of *C. africana* bark extracts on the activities of hepatic (A) ATPase, (B) Glycogen phosphorylase and (C) Fructose 1,6 bisphosphatase enzymes. Data are presented as mean $\pm$ standard deviation of triplicate determinations. Different letters (a-d) above the bars for a given concentration are significantly different from each other ($p < 0.05$). *Significantly different from glucose only sample and #significantly different from normal sample ($p < 0.05$).

6.3.5 Discussion

The global incidence of type 2 diabetes (T2D) continues to soar, considerably escalating morbidity and premature mortality among affected individuals (Zheng *et al.*,2018). Traditional medicinal plants rich in therapeutic bioactive compounds provide novel alternative antidiabetic drug development templates that are affordable, readily available, and have high efficacy with increased bioavailability and reduced undesirable side effects (Prasathkumar *et al.*,2021). The current study investigated the antidiabetic and antioxidant activities of the bark extracts of *C. africana*, a plant used in folkloric traditional medicine.

Inherent traditional medicinal plant secondary metabolites, including phenolics, alkaloid tannins, and flavonoids, have previously been shown to alleviate several ailments, including wound healing, tuberculosis (TB), cancer, T2D, cardiovascular diseases (CVD), asthma, and hypertension (Prasathkumar *et al.*,2021). As seen in Figs. 6.3.1A, 6.3.1B, and Table 6.3.1, the presence of high amounts of phenolics, flavonoids, and a plethora of bioactive compounds strongly insinuates the capabilities of the stembark extracts of *C. africana* as potential lucrative therapeutic pharmacological agent development templates. Indeed, the therapeutic benefits of nutraceuticals in the management of T2D are unrivalled, with previous studies showing that their modes of action may include sacrificial electron donation, AMPK and PPAR pathway activation, inhibitory capacity against carbohydrate hydrolytic enzymes, elevation of glucose uptake, and insulin sensing by peripheral tissues (Al-Ishaq *et al.*,2019; Deka *et al.*,2022). Squalene, which was present in the stembark extracts of *C. africana*, was previously shown to possess anticancer and antioxidant capabilities using *in vivo* and *in vitro* models (Kim and Karadeniz, 2012). The study further highlights the potential of squalene in the nutraceutical and pharmaceutical industries as a detoxifier, emollient, skin-hydrating, and drug carrier (Kim and Karadeniz, 2012). On the other hand, 7,9-Ditert-butyl-1-oxaspiro [4.5] -6,9-diene-2,8-dione, which has also previously been identified in *Euphorbia pulcherrima* and *Clathria baltica*, was reported to have therapeutic benefits against intestinal parasites, skin diseases, gonorrhoea, and warts in folkloric medicine (Sharif *et al.*,2015), and this was further corroborated by further studies that showed the antimicrobial and antiviral effects of this compound (Tatipamula *et al.*,2019). Interestingly, the cyclic monoterpene terpinolene, which is a primary terpene found in cannabis, was also identified in the stembark extracts of *C. africana* and is known to have several health benefits, including sedative applications (Ito and Ito, 2013). Terpinolene has been demonstrated to prevent LDL oxidation and therefore may play a pivotal role in the management of T2D-linked atherogenesis and coronary artery disease (Maestri *et al.*,2006; Kendall, and Alexander, 2017).

Undeniably, this brief account highlights the immense potential of plant-derived nutraceuticals, and furthermore, previous studies allude to their enhanced potency and efficacy, which may be due to their ability to work harmoniously and synergistically on several targets to stimulate a wholistic therapeutic effect as opposed to their synthetic counterparts (Mohammed and Tajuddeen, 2022).

Oxidative stress supervenes when the inherent antioxidant defense systems fail to mop up excess reactive species (RS), including reactive oxygen species (ROS), chloride species (RCS), hydroxy species (RHS), and nitrogen species (RNS) (Zhang *et al.*, 2020). The irreversible deleterious effects of highly reactive RS with unpaired valency shell electrons on functional electron-rich biomolecules, including proteins, lipids, and DNA, are implicated as the driving force behind several degenerative conditions, including cancer, Alzheimer's, and T2D (Asmat *et al.*, 2016). Indeed, the ability of the bark extracts of *C. africana* to donate free electrons (FRAP) (Fig. 6.3.2B) and scavenge free radicals (DPPH and NO) in Figs. 6.3.2A and 6.3.2C, coupled with their capacity to reduce lipid peroxidation (Fig. 6.3.5A), in pancreatic tissues oxidatively injured with FeSO₄ (pro-oxidant), is indicative of their enhanced antioxidant capabilities. Additionally, the ability of the bark extracts of *C. africana* to augment the activities of antioxidant enzymes SOD (Fig. 6.3.5B) and CAT (Fig. 6.3.5C) while increasing GSH levels (Fig. 6.3.5D) in oxidatively injured pancreatic tissues corroborates their antioxidant potential and protective capabilities against oxidative tissue damage. Furthermore, enhanced extrinsic and intrinsic antioxidants may mitigate the deleterious effects of chronic oxidative stress, including immune system suppression, cardiovascular diseases, reduced wound healing, chronic inflammation, and systemic peripheral tissue insulin resistance in T2D, thereby improving glycaemic control in patients (Deng *et al.*, 2021; Iacobini *et al.*, 2021).

Dietary carbohydrates are vital biomolecules that serve as a primary source of energy, blood glucose regulation, and as part of biological recognition as glycoconjugate molecules (Wei and Prasad, 2015). Typically, these carbohydrates are consumed in the form of polysaccharides and are subsequently broken down into their monosaccharide, glucose, through a series of hydrolytic enzymes, such as α -amylase and α -glucosidase. Ultimately, it is in the monosaccharide form that they are absorbed into the bloodstream through the lumen of the small intestine by an active, facilitated diffusion process mediated by sodium-glucose-linked transporter 1 (SGLT1). Consequently, excessive intake of dietary carbohydrates by T2D patients with dysregulated blood glucose metabolic machinery results in sustained post-prandial blood glucose spikes, leading to chronic hyperglycaemia (O'Neill, 2020). Therefore,

the ability of the stembark *C. africana* extracts to inhibit α glucosidase and α amylase, as observed in Figs. 6.3.3B and 6.3.3C, taken together with their rat intestinal jejunum glucose absorption retarding effects as seen in Fig. 6.3.4D, insinuates strong antihyperglycemic potential under T2D conditions. Indeed, this data suggests that the extracts may contently function by delaying the conversion of complex sugars to glucose and retarding its assimilation rate into the blood through the small intestines, thereby tweaking the mismatch between glucose assimilation and clearance in T2D patients, allowing more effective glucose clearance and thereby abating chronic hyperglycaemia. Furthermore, the computational studies suggest that ethyl isoxazole-5-carboxylate, which formed strong binding affinity in the active sites of both α glucosidase and α amylase, as seen in Table 6.3.2, is among the prominent compounds leading to enhanced key carbohydrate digesting inhibitory capabilities of the stembark *C. africana* extracts. This data is corroborated by Ullah and colleagues in 2021, who showed that isoxazole derivatives exhibited binding interactions with critical amino acid residues of the α -glucosidase enzyme. Furthermore, a previous study by Nie and colleagues in 2020 reported the ability of isoxazole-based flavonoid derivatives to improve glucose uptake in insulin-resistant (IR) HepG2 cells. Data from previous studies allude to the ability of plant extracts to retard glucose absorption in the small intestine due to their SGLT1 inhibitory capacity (Song *et al.*,2016).

Ingested dietary fatty acids undergo emulsification by bile and hydrolysis by lipases before being either stored in adipose tissue or used as an energy source through the β -oxidation pathway (Sridhar *et al.*,2019). An imbalance between calorie intake and expenditure, often caused by dysregulated lipid metabolism, results in altered secretion of adipokines and an excessive accumulation of fatty acids in the adipose, liver, skeletal muscle, and pancreas tissues (Liu *et al.*,2020). This imbalance is a contributing factor to the development of obesity, which is both a risk factor and a comorbidity of T2D (Stinkens *et al.*,2015; Liu *et al.*,2020). Furthermore, obesity aggravates systemic inflammation, oxidative stress, lipotoxicity, and insulin resistance in T2D (Stinkens *et al.*,2015; Chen *et al.*,2022). Consequently, the inhibitory capabilities of the bark extracts of *C. africana* against pancreatic lipase, as demonstrated in the current study (see Fig. 6.3.3B), may have the potential to slow down the hydrolysis of dietary fatty acids, thus potentially mitigating obesity, and the exacerbation of oxidative stress in T2D patients (Sridhar *et al.*,2019). Molecular docking further revealed that 2-methyl-4-vinylphenol, which had the highest binding affinity among the compounds found in the stembark extract of *C. africana*, formed several strong interactions in the binding site of pancreatic lipase,

suggesting its inhibitory role. Indeed, the phenolic compound 2-Methoxy-4-vinylphenol, which is present in red cabbage (Rubab *et al.*,2020), has been documented to have several health benefits, including anti-inflammatory, antioxidant (Jeonget *et al.*,2011), antimicrobial, and antigermination activities (Hameed *et al.*,2015). Furthermore, previous *in silico* studies have alluded to the enhanced inhibitory capacity of 2-methyl-4-vinylphenol on several carbohydrate and lipid hydrolytic enzymes and thus its potential antidiabetic capacity (Ferdosi *et al.*,2021).

Peripheral tissues, including the liver, adipose tissue, and skeletal muscles, constitute most of the glucose disposal mechanisms, which are thereby pivotal in maintaining glucose homeostasis in the body (Chadt and Al-Hasani, 2020). Undoubtedly, pharmacological agents that stimulate increased glucose uptake by peripheral tissues aid in abating hyperglycaemia in T2D through their ability to increase blood glucose clearance (Zhao *et al.*,2019). Indeed, the bark extracts of *C. africana* increased glucose uptake in yeast suspension (Fig 6.3.4A), excised rat psoas muscle (Fig 6.3.4B), and liver glucose (Fig 6.3.C), indicating their capabilities to restore glucose homeostasis under insulin resistance conditions in T2D. Previous studies show that bioactive phytoconstituents that increase glucose uptake in peripheral tissues may function by enhancing their insulin sensitivity, upregulating translocation of GLUT4 to the cell membrane, and modulating gene expression (Naresh *et al.*,2012; Zhao *et al.*,2019; Johannesen *et al.*,2020).

Intrinsic blood glucose level sensing mechanisms, together with several hormones, including glucagon and insulin, work together to maintain homeostatic glucose levels during periods of fasting by regulating hepatic glucose output (Sharabi *et al.*,2015; Rines *et al.*,2016). Hepatic glucose output is mediated through essential pathways, including glycogenolysis and gluconeogenesis, which are in turn regulated by a network of enzymes such as glycogen phosphorylase and fructose 1,6-bisphosphatase (Sharabi *et al.*,2015; Hatting *et al.*,2018). Glycogen phosphorylase is instrumental in breaking down glycogen in glycogenolysis, while fructose 1,6-bisphosphatase is pivotal in the regulation of gluconeogenesis, in which glucose is synthesized from non-carbohydrate sources, including amino acids and lipids, when there is an unmet high demand (Hatting *et al.*,2018). Indeed, the hyperactivity of hepatic glucogenic enzymes, including glycogen phosphorylase and fructose 1,6-bisphosphatase, displayed in Fig. 6.3.1 insinuates hyperglycaemia under T2D conditions through increased glycogenolysis and gluconeogenesis. Therefore, the inhibitory capabilities of the bark extracts of *C. africana* on hepatic glucogenic enzymes are indicative of their abilities to counteract hyperglycaemia arising from internal sources such as glycogenolysis and gluconeogenesis. The findings from

the current study are coherent with previous studies that show that glucogenic enzyme inhibition is a lucrative antidiabetic mechanism that reduces hepatic glucose output, thereby promoting the build-up of glycogen stores in both the liver and muscle tissues (Rines *et al.*,2016; Li *et al.*,2022).

6.3.6 Conclusion

The stem bark extracts of *C. africana* exhibited the enhanced capacity to scavenge excess reactive species, inhibit carbohydrate hydrolysing enzymes, modulate hepatic glucose output, and modulate muscular uptake and absorption while concurrently upregulating the level and activities of intrinsic antioxidant defence systems. Identified compounds, especially phenolic and isoxazole derivatives, may have significantly contributed to these observed activities. Further, *in vivo* experiments are necessary to verify these findings and elucidate a probable mode of action.

6.4 *Celtis africana* (N.L.Burm.) improves glucose metabolism, reduces atherogenic indices, and ameliorates oxidative stress in streptozotocin induced type 2 diabetic rat model

Brian K. Beseni¹, Kolawole A. Olofinisan¹, Ochuko L. Erukainure¹ *Islam Shahidul Md¹

1.Department of Biochemistry, University of KwaZulu-Natal, Westville Campus, Durban 4000, South Africa

***Corresponding author:** islamd@ukzn.ac.za

6.4.1 Abstract

The rampant upsurge in the global incidence of Type 2 diabetes mellitus (T2D) prevalence has reached an unsurpassed high, prompting scientists to investigate the development of novel antidiabetic pharmacological agents. Plants such as *Celtis africana*, which are employed in folkloric medicine, have emerged as a lucrative source of bioactive drug development templates. Therefore, the antioxidant, antihyperglycemic, and antihyperlipidemic capabilities of *C. africana* were investigated in the current study *in vivo* using a T2D rat model. T2D induction was achieved by an intraperitoneal injection of streptozotocin (40 mg/kg b.w.) into rats fed with 10% fructose solution *ad libitum* for two weeks. Prior to T2D confirmation, intervention was carried out by daily administration of a low dose (150 mg/kg) and a high dose (300 mg/kg) of the *C. africana* extract during the trial period. Mean food and fluid intake were recorded daily; body weight and blood glucose levels were measured weekly; and the oral glucose tolerance test (OGTT) was performed in the last week of treatment. In particular, the high dose of *C. africana* (DCAH) abated T2D-induced polydipsia and polyphagia, leading to restored euglycemic levels and improved glucose tolerance. Furthermore, the extracts mitigated diabetic dyslipidaemia by decreasing total cholesterol and triacyl glycerides while concomitantly increasing HDL cholesterol levels, thereby reducing the atherogenic index of plasma. The extracts abated fatty acid deposits linked to hepatic enlargement and restored hepatic glycogen stores through the modulation of hepatic glucogenic enzymes. *C. africana* reversed oxidative injury on pancreatic tissue as revealed by histopathological examinations, potentially by augmenting the antioxidant defence system and ameliorating redox imbalance. Furthermore, the extracts improved indices of hepatic and renal T2D-linked damage. Data from the current study suggests that *C. africana* has enhanced ameliorative capabilities against several T2D-linked pathologies that may occur through several mechanisms, including

improved oxidative status, reduced tissue damage, and upregulation of insulin sensitivity and secretion through the regeneration of pancreatic β -cells. These findings warrant further clinical trials to elucidate the precise mode of antidiabetic action shown by *C. africana* in the current study.

Keywords: *Celtis africana*, Antioxidant, Antidiabetic, Dyslipidaemia, *in vivo*, T2D rat model

6.4.2 Introduction

The prevalence of diabetes mellitus (DM) has reached an unsurpassed high, with an estimated 10.5% of the adult population globally currently living with the condition, posing an unsustainable burden on healthcare systems (Forouhi and Wareham, 2019; Sun *et al.*,2022). Several DM etiologies exist, but the most prevalent types are type 1, type 2, and gestational diabetes. Type I diabetes (T1D) stems from an autoimmune distraction of insulin-producing β pancreatic cells, while type II diabetes (T2D) has a strong link to individuals' lifestyles and accounts for over 80% of all DM cases (Sun *et al.*,2022). Furthermore, gestational diabetes manifests during the 3rd trimester in pregnant women who were not diabetic prior to the pregnancy. DM is a group of chronic metabolic disorders characterized by chronic hyperglycemia whose pathogenesis is attributed to several predisposing risk factors that result in relative insulin deficiency, impaired action, or both (Petersmann *et al.*,2018).

In T2D, dysregulated insulin metabolism manifests as insulin resistance, in which homeostatic blood insulin levels fail to facilitate glucose transportation into insulin-responsive peripheral tissues, leading to chronic hyperglycemia (Sun *et al.*,2022). Furthermore, the precise mechanism of insulin resistance pathogenesis remains poorly elucidated but is attributed to chronic oxidative stress (Yaribeygi *et al.*,2020). Oxidative stress is a redox imbalance between the body's endogenous antioxidant defense system and excessive reactive species (RS). Excessive reactive species (RS), including reactive oxygen species (ROS), reactive nitrogen species (RNS), and reactive chloride species (RCS), have deleterious effects on various functional and structural moieties, including lipids, DNA, and proteins, whose change in their precise chemical structure impedes their function (Zhang *et al.*,2020). Oxidative tissue damage is implicated in the development and progression of associated chronic complications of T2D that are broadly categorized as macrovascular and microvascular complications (Yaribeygi *et al.*,2020). T2D linked complications severely reduce the quality of life among sufferers and are the leading cause of blindness and amputation of extremities, reported to account for about 5%

of mortality in the year 2021 (Ogurtsova *et al.*,2022). Therefore, as the incidence of T2D soars globally, there is a constant need for the development of new antidiabetic therapeutic agents that are potent and cost-effective.

T2D management strategies are broadly classified as non-pharmacological or pharmacological therapies (Khursheed *et al.*,2019). Non-pharmacological T2D management strategies, which include medical nutrition therapy, behavioral change, and increased physical activity, are first-line strategies effective against mild forms of the condition (Khursheed *et al.*,2019). Despite being cost-effective, they have the major drawback of patients relapsing (Lambrinou *et al.*,2019). Pharmacological strategies, which include oral agents largely classified as biguanides, sulfonylureas, meglitinides, thiazolidinediones, and α -glucosidase inhibitors, are widely used for the management of T2D (Dowarah and Singh, 2020). Despite their efficacy, pharmacological T2D management strategies are not cost-effective and have a host of undesirable side effects, including weight gain or loss, nausea, diarrhea, and flatulence, with some being associated with severe liver and kidney damage (Stein *et al.*,2013). These observations have thus prompted an investigation into the development of novel antidiabetic pharmacological agents with high efficacy, mild side effects, and increased bioavailability (Yatoo *et al.*,2017). Plants that are used in traditional and complementary medicine have thus emerged as a reservoir of novel natural antidiabetic agent templates (Yagasaki and Muller, 2022). Ongoing scientific research on traditional medicinal plants is aimed at elucidating their modes of action, establishing their safety, and validating their health benefits (Yatoo *et al.*,2017; Dowarah and Singh, 2020). The therapeutic potential of folkloric traditional medicinal plants such as *C. africana* stems from their inherent secondary metabolites, which include phenolics, flavonoids, terpenes, coumarins, and tannins.

Celtis africana Burm.f. is a small to medium deciduous tree belonging to the family Cannabaceae whose different parts have traditionally been used to alleviate eye infections, rheumatism, cancer, diabetes, and sexually transmitted diseases (Bussmann, 2006; Nchabeleng, 2017). While the leaves of *C. africana* are traditionally used to treat indigestion and oedema (Krief *et al.*,2005; Al-Taweel *et al.*,2012), the bark and roots are used to treat cancer in different parts of South Africa (Perveen *et al.*,2015). Indeed, previous studies have characterized several bioactive compounds from the chloroform-soluble fraction of *C. africana*, including oleanolic acid, lauric acid, and palmitic acid, which were further shown to be potent antioxidant and anti-inflammatory agents with acetylcholinesterase enzyme inhibitory activity (Al-Taweel *et al.*,2012). Therefore, the current study was aimed at

investigating the antidiabetic and antioxidant capabilities of *C. africana* *in vivo* using a T2D rat model.

6.4.3 Materials and methods

The materials and methods for this study are detailed in Chapter 2 section 2.12

6.4.4 Results

Diabetes induction led to marked polyphagia and polydipsia, as evidenced by the significantly ($p < 0.05$) increased food and fluid intake in the DBC group when compared to the NC group, as displayed in Figure 6.4.1. Indeed, treatment with a high dose of *C. africana* significantly ($p < 0.05$) lowered the fluid intake. However, despite DCAH reducing the food intake, the resulting difference was not statistically significant ($p > 0.05$) to that measured for animals in the DBC group. Furthermore, treatment with a low dose of *C. africana* did not have a significant ($p > 0.05$) on both food and fluid intake when compared to the DBC group. Similarly, there was no significant ($p > 0.05$) difference between the food and fluid intake of the NC and NCATX groups.

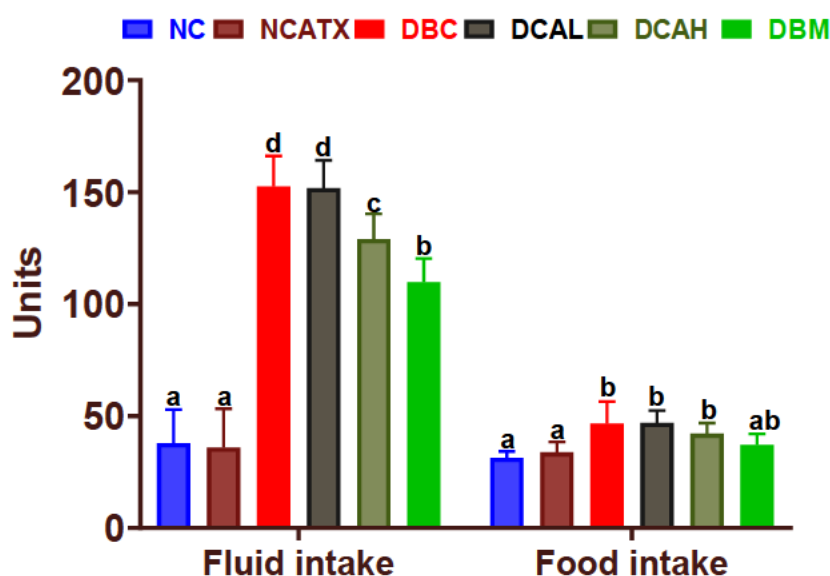


Figure 6.4.1: Food and Fluid intake in different animal groups during the intervention period. NC= Normal Control, NCATX = Normal rats + *C. africana* high dose, DBC= Diabetic Control, DCAL= Diabetic rats + *C. africana* low dose, DCAH =Diabetic rats + *C. africana* high dose, DBM: Diabetic rats + Metformin (positive control). Different superscripts a-d represent statistical significance of $p < 0.05$ (Tukey's multiple range *post hoc* test at $p < 0.05$).

Consistent mean body weight gain was observed in the NC group during successive weeks of the trial period, with rats in this group displaying significantly ($p < 0.05$) higher mean body weight gain with respect to the DBC group as determined through the AUC (Area under the curve) calculation depicted in Fig 6.4.2. Remarkably, there was no significant ($p > 0.05$) difference in mean body weight observed between the animals in the NC and NCATX groups. Evidently, diabetic-linked glucose metabolism dysfunction impeded body weight gain in the DBC group, resulting in animals in that group exhibiting significantly ($p < 0.05$) the least overall body weight among all groups, as shown by the AUC calculation. However, treatment with both high and low doses of *C. africana* resulted in a significant ($p < 0.05$) increase in mean body weight gain compared to the DBC group. While the high dose of *C. africana* resulted in slightly more body weight gain than the low dose, there was no significant ($p > 0.05$) difference between them. Indeed, both high and low doses of *C. africana* led to a higher body weight gain that was significantly ($p < 0.05$) greater than that observed in the DBM group, as denoted by the AUC value.

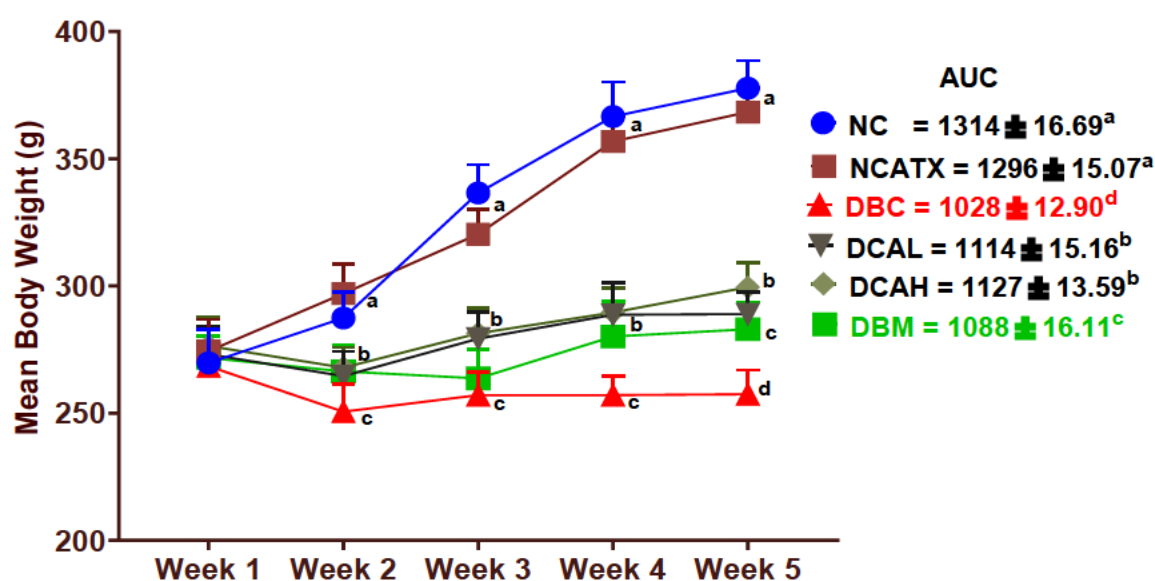


Figure 6.4.2: Effect of oral treatment on mean body weight in different animal groups during the 5-week intervention period. NC= Normal Control, NCATX = Normal rats + *C. africana* high dose, DBC= Diabetic Control, DCAL= Diabetic rats + *C. africana* low dose, DCAH =Diabetic rats + *C. africana* high dose, DBM: Diabetic rats + Metformin (positive control). Different superscripts a-d represent statistical significance of $p < 0.05$ (Tukey's multiple range *post hoc* test at $p < 0.05$).

As denoted in Fig. 6.4.3, a gradual increase in the weekly blood glucose levels was observed in the DBC group, resulting in significantly ($p < 0.05$) the highest weekly blood glucose levels throughout the experimental period. Conversely, the NC group, which had significantly ($p < 0.05$) the lowest weekly blood glucose levels, maintained constant weekly blood glucose levels throughout the experimental period. Treatment with a low dose of *C. africana* resulted in lower blood glucose levels from the third week onwards, while a significant ($p < 0.05$) decrease was observed following treatment with a high dose of *C. africana* after the first week of treatment. There was no significant difference ($p > 0.05$) in the weekly blood glucose levels between a high dose of *C. africana* and DBM (positive control) on week 5. Despite the inability of the low dose of *C. africana* to reduce the weekly blood glucose levels to a proportion comparable to the high dose of *C. africana* and DBM, which were almost normal, it significantly ($p < 0.05$) reduced the blood glucose levels lower than the DBC group, especially at week 5.

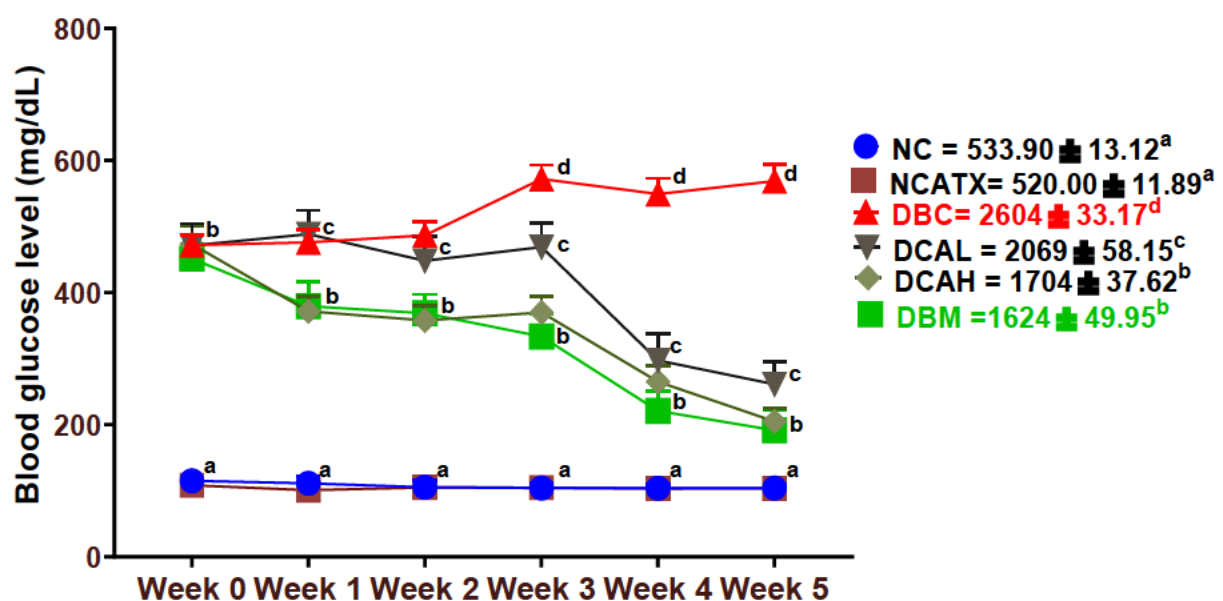


Figure 6.4.3: Effect of oral treatment on blood glucose in different animal groups during the intervention period. NC= Normal Control, NCATX = Normal rats + *C. africana* high dose, DBC= Diabetic Control, DCAL= Diabetic rats + *C. africana* low dose, DCAH =Diabetic rats + *C. africana* high dose, DBM: Diabetic rats + Metformin (positive control). Different superscripts a-d represent statistical significance of $p < 0.05$ (Tukey's multiple range *post hoc* test at $p < 0.05$).

Following treatment, the oral glucose tolerance test (OGTT) was conducted and depicted in

Fig. 6.4.4. Oral administration of a glucose load resulted in a significant ($p<0.05$) spike in the blood glucose levels in the DBC group after 30 minutes. In contrast, only a slight, insignificant ($p>0.05$) increase in the blood glucose levels was observed for the NC. There was no significant difference in the blood glucose levels between the NC and the NCATX groups, which remained constant at all the sampled time points. The blood glucose levels in the diabetic groups (DBC, DCAL, DCAH, and DBM) peaked 60 minutes after glucose administration and gradually decreased nearing the 120-minute time point. The high and low doses of *C. africana* treatment groups had significantly lower blood glucose levels with respect to the DBC group after 120 minutes. However, notably after 120 minutes, the blood glucose levels had not returned to their initial levels in the diabetic groups but had returned to their initial levels in the normal NC and NCATX groups.

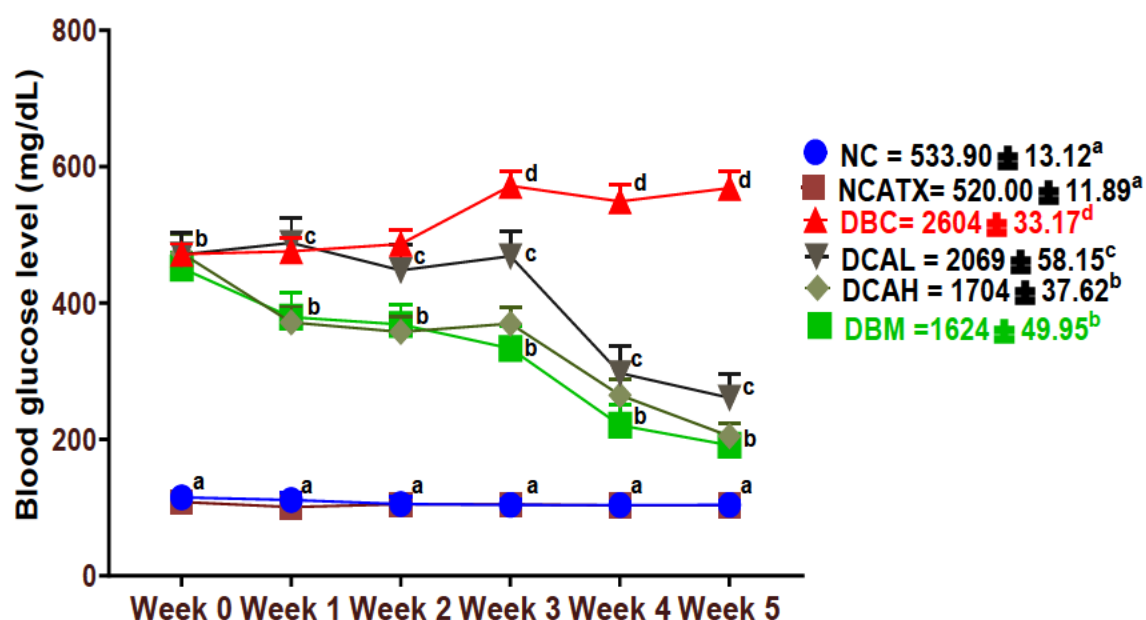


Figure 6.4.4: Oral glucose tolerance test (OGTT) of different animal groups in the last week of experimental period. **NC**= Normal Control, **NCATX** = Normal rats + *C. africana* high dose, **DBC**= Diabetic Control, **DCAL**= Diabetic rats + *C. africana* low dose, **DCAH** =Diabetic rats + *C. africana* high dose, **DBM**: Diabetic rats + Metformin (positive control). Different superscripts a-d represent statistical significance of $p<0.05$ (Tukey's multiple range *post hoc* test at $p < 0.05$).

Evidently, T2D induction significantly ($p < 0.05$) diminished insulin levels (Fig. 6.4.5A) and significantly ($p < 0.05$) reduced the HOMA β score while concomitantly significantly ($p < 0.05$) increasing the HOMA IR scores in the DBC group, as displayed in Fig. 6.4.5B. While treatment with both the low dose and high dose of *C. africana* significantly ($p < 0.05$) increased insulin levels with respect to the DBC group, only the high dose of *C. africana* significantly ($p < 0.05$) surpassed the insulin levels of DBM (the positive control) to proportions comparable to those observed for the NC group, with no significant ($p > 0.05$) difference as depicted in Fig. 6.4.5A. Furthermore, there was no significant ($p > 0.05$) difference in insulin levels between the NC group and the NCATX group. Indeed, both the high and low doses of *C. africana* significantly ($p < 0.05$) increased HOMA β scores and concomitantly reduced HOMA IR scores. However, the data shows that the high dose of *C. africana* exhibited significantly ($p < 0.05$) superior activities than the low dose of *C. africana*, resulting in significantly ($p < 0.05$) lower HOMA IR scores and higher HOMA β levels statistically insignificant from the DBM group.

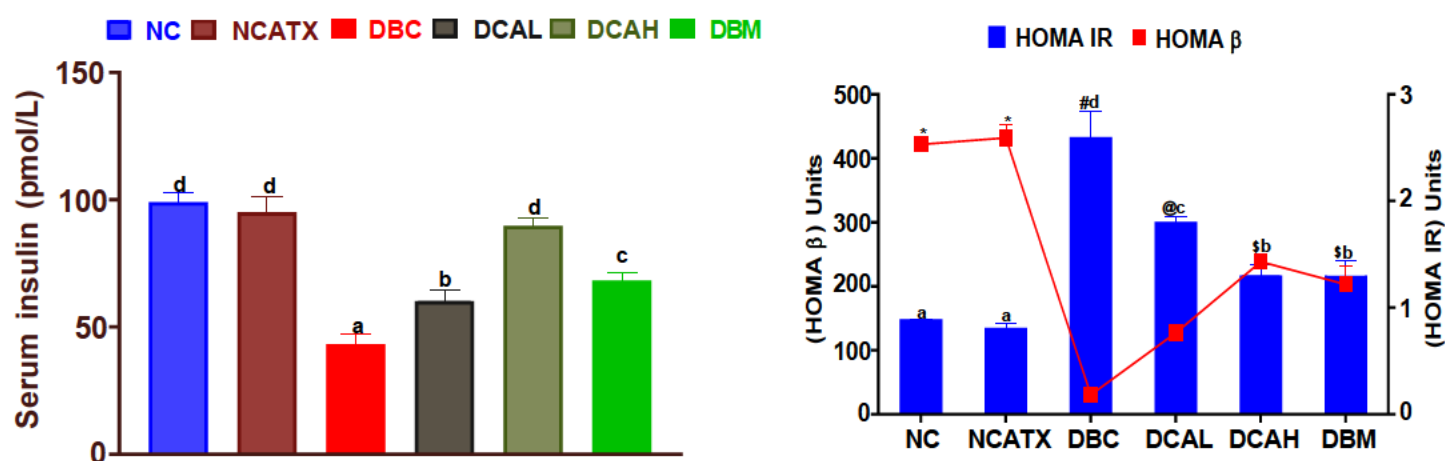


Figure 6.4.5: Effect of oral treatment on (A) serum insulin levels (B) homeostatic assessment model (HOMA IR and HOMA β) in different animal groups during the experimental period. NC= Normal Control, NCATX = Normal rats + *C. africana* high dose, DBC= Diabetic Control, DCAL= Diabetic rats + *C. africana* low dose, DCAH =Diabetic rats + *C. africana* high dose, DBM: Diabetic rats + Metformin (positive control). Different superscripts a-d represent statistical significance of $p < 0.05$ (Tukey's multiple range *post hoc* test at $p < 0.05$).

As depicted in Fig. 6, 4.6 rats in the diabetic control (DBC) group exhibited significantly ($p<0.05$) increased relative liver weight with significantly ($p<0.05$) diminished glycogen content when compared to the NC group. Indeed, both the low dose and high dose of *C. africana* significantly ($p<0.05$) reduced the relative liver weight while concomitantly increasing their respective glycogen content with respect to the DBC group. Furthermore, there was no significant ($p>0.05$) difference in both the relative liver weights and glycogen content of the rats treated with the high dose of *C. africana* and those administered with metformin (DBM group).

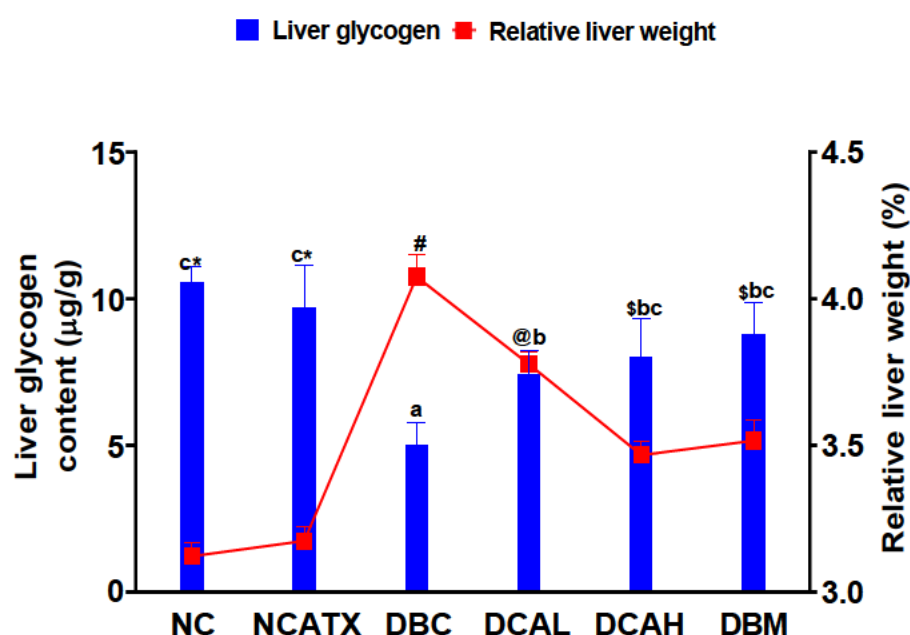


Figure 6.4.6: Effect of oral treatment on relative liver weight and glycogen content in different animal groups during the experimental period. NC= Normal Control, NCATX = Normal rats + *C. africana* high dose, DBC= Diabetic Control, DCAL= Diabetic rats + *C. africana* low dose, DCAH =Diabetic rats + *C. africana* high dose, DBM: Diabetic rats + Metformin (positive control). Different superscripts a-d represent statistical significance of $p<0.05$ (Tukey's multiple range *post hoc* test at $p < 0.05$).

As depicted in Table 6.4.1, diabetes induced significant ($p<0.05$) hyperactivity of hepatic glycogen phosphorylase, glucose-6 phosphatase, and fructose-1,6 bisphosphatase while concomitantly significantly ($p<0.05$) reducing ATPase activity. Indeed, treatment with both high and low doses of *C. africana* significantly ($p<0.05$) reduced hepatic glycogen phosphorylase, glucose-6 phosphatase, and fructose-1,6 bisphosphatase activities to proportions insignificantly ($p>0.05$) different from the NC group. Furthermore, a high dose of *C. africana* significantly ($p<0.05$) increased ATPase activity to proportions insignificantly ($p>0.05$) different from the NC group. There was no significant ($p>0.05$) difference between the ATPase stimulatory activities of the high dose of *C. africana* and those administered with metformin (DBM).

Table 6.4.1: The effects of oral treatment of hepatic glucogenic and nucleotide hydrolysing enzymes.

	NC	NCATX	DBC	DCAL	DCAH	DBM
Glycogen phosphorylase*	153,673±9,65 ^a	149,27±9,72 ^a	218,367±11,21 ^c	155,37±9,37 ^a	150,11±9,37 ^a	176,37±8,11 ^b
Glucose 6 phosphatase*	148,76±5,81 ^a	146,76±11,27 ^a	228,71±10,37 ^c	152,46±10,24 ^a	149,73±10,20 ^a	183,97±5,67 ^b
Fructose 1,6 bisphosphatase*	180,21±7,31 ^a	177,28±12,32 ^a	253,67±12,35 ^c	189,16±6,97 ^{ab}	179,68±6,71 ^a	200,07±7,92 ^b
ATPase*	301,68±10,36 ^c	306,16±924 ^c	210,32±10,37 ^a	269,73±10,77 ^b	297,73±7,33 ^c	268,13±8,36 ^b

*Units ($\mu\text{mol Pi/mg}$), **NC**= Normal Control, **NCATX** = Normal rats + *C. africana* high dose, **DBC**= Diabetic Control, **DCAL**= Diabetic rats + *C. africana* low dose, **DCAH** =Diabetic rats + *C. africana* high dose, **DBM**: Diabetic rats + Metformin (positive control). Different superscripts a-d represent statistical significance of $p<0.05$ (Tukey's multiple range *post hoc* test at $p < 0.05$).

Diabetes induction significantly ($p<0.05$) elevated the activities of both α amylase and lipase in the serum and pancreas, as observed in the diabetic control group (DBC), as displayed in Table 6.4.2. Treatment with the high dose of *C. africana* significantly ($p<0.05$) reduced both α amylase and lipase in the serum and pancreas when compared to the DBC group. Indeed, the high dose of *C. africana* exhibited superior carbohydrate and lipid hydrolysing enzyme inhibitory capabilities than the low dose of *C. africana* and metformin (DBM).

Table 6.4.2: Effect of oral treatment on carbohydrate and lipid hydrolysing enzymes.

	NC	NCATX	DBC	DCAL	DCAH	DBM
Serum α amylase U/L	22,37 $\pm$ 1,08 ^a	20,06 $\pm$ 0,98 ^a	76,49 $\pm$ 3,71 ^d	32,01 $\pm$ 2,46 ^b	29,23 $\pm$ 1,24 ^b	46,37 $\pm$ 2,48 ^c
Pancreatic α amylase U/L	30,21 $\pm$ 0,37 ^a	33,07 $\pm$ 0,11 ^a	83,57 $\pm$ 2,55 ^d	51,38 $\pm$ 1,05 ^c	40,21 $\pm$ 1,23 ^b	43,16 $\pm$ 1,54 ^b
Serum lipase U/L	21,45 $\pm$ 1,11 ^a	22,71 $\pm$ 0,84 ^a	66,97 $\pm$ 2,82 ^d	43,20 $\pm$ 0,53 ^c	34,78 $\pm$ 0,41 ^b	33,89 $\pm$ 1,23 ^b
Pancreatic lipase U/L	37,367 $\pm$ 2,33 ^a	39,21 $\pm$ 1,23 ^a	92,74 $\pm$ 5,34 ^d	46,36 $\pm$ 3,03 ^b	40,18 $\pm$ 3,88 ^a	53,67 $\pm$ 3,53 ^c

NC= Normal Control, **NCATX** = Normal rats + *C. africana* high dose, **DBC**= Diabetic Control, **DCAL**= Diabetic rats + *C. africana* low dose, **DCAH** =Diabetic rats + *C. africana* high dose, **DBM**: Diabetic rats + Metformin (positive control). Different superscripts a-d represent statistical significance of $p<0.05$ (Tukey's multiple range *post hoc* test at $p < 0.05$).

Dysregulated lipid metabolism in T2D resulted in significantly ($p<0.05$) elevated total cholesterol, triacylglycerols, and LDL cholesterol, with concomitant diminished HDL cholesterol levels in the rats in the diabetic control group (DBC) when compared to the NC group, as depicted in Fig. 6.4.7. Consequently, this data indicates that rats in the DBC group had an increased predisposition to develop cardiovascular diseases (CVD), as evidenced by a significantly ($p<0.05$) increased atherogenic index of plasma (AIP). Treatment with a high dose of *C. africana* significantly ($p<0.05$) decreased total cholesterol and triacyl glycerides and increased HDL cholesterol levels to near-normal levels, as seen in Fig. 6.4.7. Furthermore, DCAH significantly ($p<0.05$) reduced LDL levels, significantly reducing the atherogenic index of plasma when compared to the DBC group, suggesting its potency against T2D dyslipidaemia, and associated cardiovascular diseases (CVD).

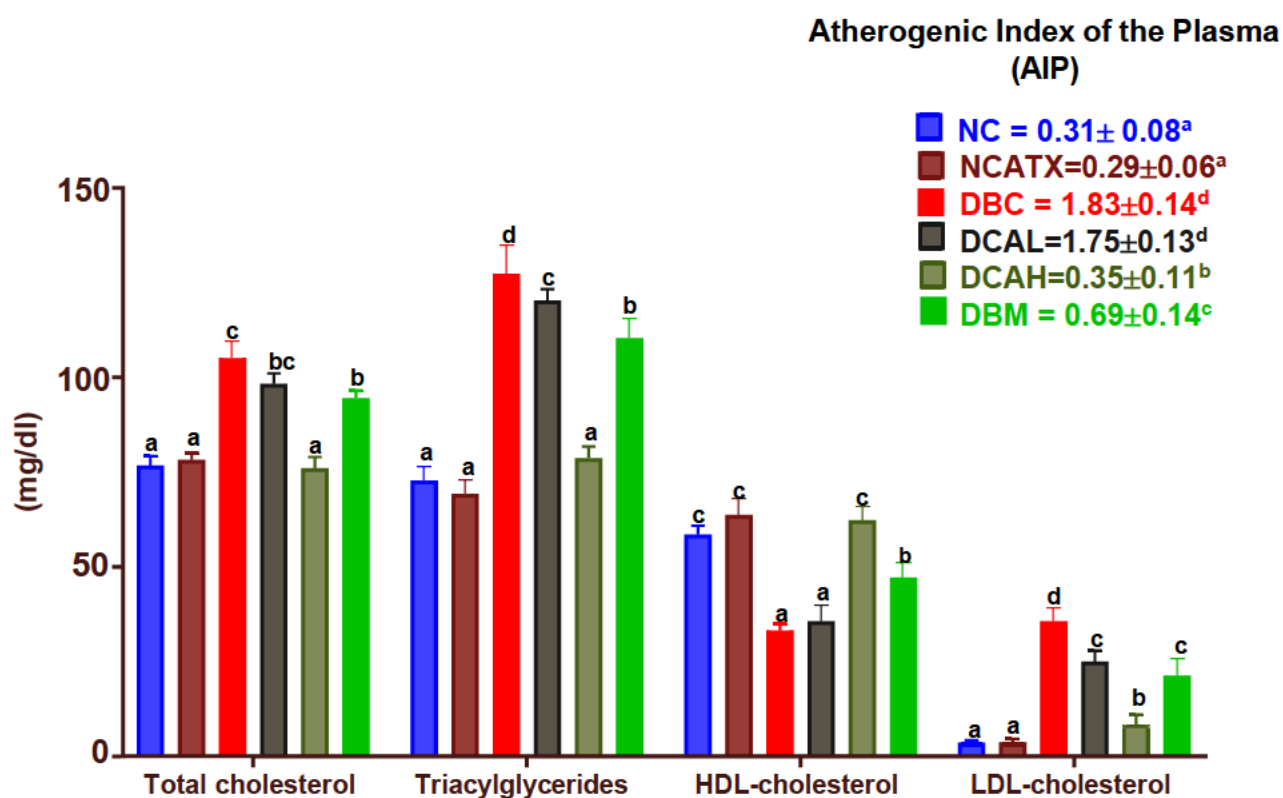


Figure 6.4.7: Effect of oral treatment on lipid profiles. NC= Normal Control, NCATX = Normal rats + *C. africana* high dose, **DBC**= Diabetic Control, **DCAL**= Diabetic rats + *C. africana* low dose, **DCAH** =Diabetic rats + *C. africana* high dose, **DBM**: Diabetic rats + Metformin (positive control). Different superscripts a-d represent statistical significance of $p<0.05$ (Tukey's multiple range *post hoc* test at $p < 0.05$). Atherogenic index of plasma (AIP) = log triglyceride/high-density lipoprotein cholesterol (HDLc).

Diabetes induction resulted in elevated MDA and NO levels, as well as a significant ($p<0.05$) decrease in GSH levels, SOD, and CAT activity in both serum and pancreatic tissue, as shown in Tables 6.4.3 and 6.4.4, respectively. There was a significant ($p<0.05$) reduction in MDA equivalents upon treatment with the high dose of *C. africana* in both serum and pancreatic tissue. Treatment with the high dose of *C. africana* decreased lipid peroxidation in both the serum and pancreatic tissue to near-normal levels, insignificantly ($p>0.05$) different from the NC group. Furthermore, DCAH increased SOD and CAT activities and GSH levels while decreasing NO concentrations significantly ($p<0.05$) better than the low dose of *C. africana*.

Table 6.4.3: Effect of oral treatment on various serum oxidative stress linked enzymes.

	NC	NCATX	DBC	DCAL	DCAH	DBM
MDA (mol/L)	39,75±2,07 ^a	41,21±2,14 ^a	76,17±6,24 ^c	53,09±1,41 ^b	44,27±3,41 ^a	52,37±4,56 ^b
GSH (mol/L)	121,37±5,78 ^d	125,74±9,73 ^d	62,74±2,37 ^a	102,37±6,69 ^b	115,37±7,32 ^c	100,25±3,31 ^b
SOD (U/mg protein)	583,22±4,37 ^c	591,74±14,83 ^c	176,37±6,13 ^a	501,23±10,38 ^b	513,76±11,94 ^c	497,21±11,17 ^b
CAT (U/mg protein)	801,1±8,63 ^e	811,37±13,04 ^e	709,76±3,77 ^a	754,64±11,97 ^c	781,37±13,14 ^d	741,134±9,08 ^b
NO(μml)	0,31±0,006 ^a	0,28±0,004 ^a	0,53±0,008 ^d	0,39±0,002 ^b	0,35±0,003 ^b	0,42±0,002 ^c

Table 6.4.4: Effect of oral treatment on various pancreatic oxidative stress linked enzymes.

	NC	NCATX	DBC	DCAL	DCAH	DBM
MDA (mol/L)	42,63±1,33 ^a	40,19±2,07 ^a	81,76±5,43 ^d	48,71±3,13 ^b	43,11±2,67 ^a	61,33±2,35 ^c
GSH (mol/L)	71,45±2,17 ^c	71,09±3,79 ^c	33,28±1,88 ^a	60,37±7,24 ^b	66,13±6,11 ^c	58,23±3,09 ^b
SOD (U/mg protein)	93,71±3,54 ^c	90,08±8,82 ^c	41,2±2,22 ^a	77,12±2,36 ^b	88,36±7,38 ^c	81,23±1,85 ^b
CAT (U/mg protein)	702,357±9,36 ^e	713,23±14,37 ^e	395,15±6,31 ^a	671,02±11,37 ^c	687,37±14,19 ^d	657,34±7,38 ^b
NO(μml)	0,063±0,003 ^a	0,061±0,002 ^a	0,151±0,007 ^c	0,079±0,001 ^b	0,067±0,004 ^a	0,078±0,002 ^b

MDA= melanoaldehyde equivalence, GSH=Glutathione SOD=Superoxide dismutase, CAT= Catalase, NO=Nitric oxide. **NC**= Normal Control, **NCATX** = Normal rats + *C. africana* high dose, **DBC**= Diabetic Control, **DCAL**= Diabetic rats + *C. africana* low dose, **DCAH** =Diabetic rats + *C. africana* high dose, **DBM**: Diabetic rats + Metformin (positive control). Different superscripts a-d represent statistical significance of $p<0.05$ (Tukey's multiple range *post hoc* test at $p < 0.05$).

Table 6.4.5 displays the indices of hepatic and renal damage. Lactate dehydrogenase (LDH), urea, uric acid, alkaline phosphate, aspartate aminotransferase AST, and Creatine Kinase-Myocardial Band (CK- MB) levels were significantly higher in the DBC compared to the control group (NC). Indeed, treatment with a high dose of *C. africana* significantly ($p<0.05$) reduced lactate dehydrogenase, aspartate aminotransferase, and Creatine Kinase-Myocardial Band levels. Treatment with both high and low doses of *C. africana* significantly ($p<0.05$) reduced urea levels to proportions insignificantly ($p>0.05$) different from the NC group. Furthermore, treatment with a high dose of *C. africana* significantly ($p<0.05$) reduced uric acid levels to near-normal levels, insignificantly ($p>0.05$) different from the NC group. Treatment with both high and low doses of *C. africana* significantly ($p<0.05$) reduced alkaline phosphatase levels when compared to the DBC group. However, no significant ($p>0.05$) difference in the level of Alanine aminotransferase was observed among the normal and diabetic groups.

Table 6.4.5: Effect of oral treatment on renal and hepatic function indices.

	NC	NCATX	DBC	DCAL	DCAH	DBM
Lactate dehydrogenase U/L	387,2 ± 33,05 ^b	387,2±28,41 ^b	911 ± 58,21 ^d	665±57,38 ^c	457,4±21,56 ^b	284,4±27,87 ^a
Urea mg/dl	87,6 ± 3,58 ^a	87,4 ± 2,70 ^a	96,6 ± 4,28 ^b	84,8 ± 3,96 ^a	84,0 ± 2,55 ^a	87,0 ± 2,92 ^a
Uric acid mg/dl	0,586 ± 0,32 ^a	0,69 ± 0,24 ^a	2,246 ± 0,57 ^c	1,266 ± 0,16 ^b	0,676 ± 0,17 ^a	0,82 ± 0,19 ^a
Alkaline phosphatase (ALP) U/L	62,2 ± 8,31 ^a	66,2 ± 7,91 ^a	273,2 ± 15,78 ^d	222,8 ± 16,74 ^c	220,8 ± 12,57 ^c	163,4 ± 11,14 ^b
Alanine aminotransferase (ALT) U/L	82,2 ± 4,31	83,8 ± 4,01	85,0 ± 2,87	82,8 ± 3,12	85,6 ± 2,53	85,0 ± 3,97
Aspartate aminotransferase (AST) U/L	85,8 ± 6,41 ^a	83,0 ± 4,57 ^a	120,6 ± 7,33 ^c	109,0 ± 4,35 ^{ab}	98,8 ± 5,12 ^a	114,8 ± 5,89 ^b
Creatine Kinase-Myocardial Band (CK-MB) U/L	367,46 ± 44,25 ^a	366,36 ± 23,25 ^a	790,12 ± 57,12 ^c	562,06 ± 53,30 ^b	553,1 ± 30,74 ^b	577,84 ± 33,25 ^b

NC = Normal Control, NCATX = Normal rats + *C. africana* high dose, DBC = Diabetic Control, DCAL = Diabetic rats + *C. africana* low dose, DCAH = Diabetic rats + *C. africana* high dose, DBM: Diabetic rats + Metformin (positive control). Different superscripts a-d represent statistical significance of $p < 0.05$ (Tukey's multiple range *post hoc* test at $p < 0.05$).

Diabetes induction led to the destruction of pancreatic islet tissue morphology and a significant decrease in the number of β pancreatic cells in the DBC group when compared to the NC group, as revealed by histopathological analysis of the pancreatic tissue displayed in Fig. 6.4.8. Indeed, treatment with the high dose of *C. africana* reversed pancreatic islet destruction, increasing the number of insulin-secreting cells while increasing the overall size and improving islet tissue morphology. However, the extract did not reduce cytoplasmic vacuolation, as seen in Fig. 6.4.8.

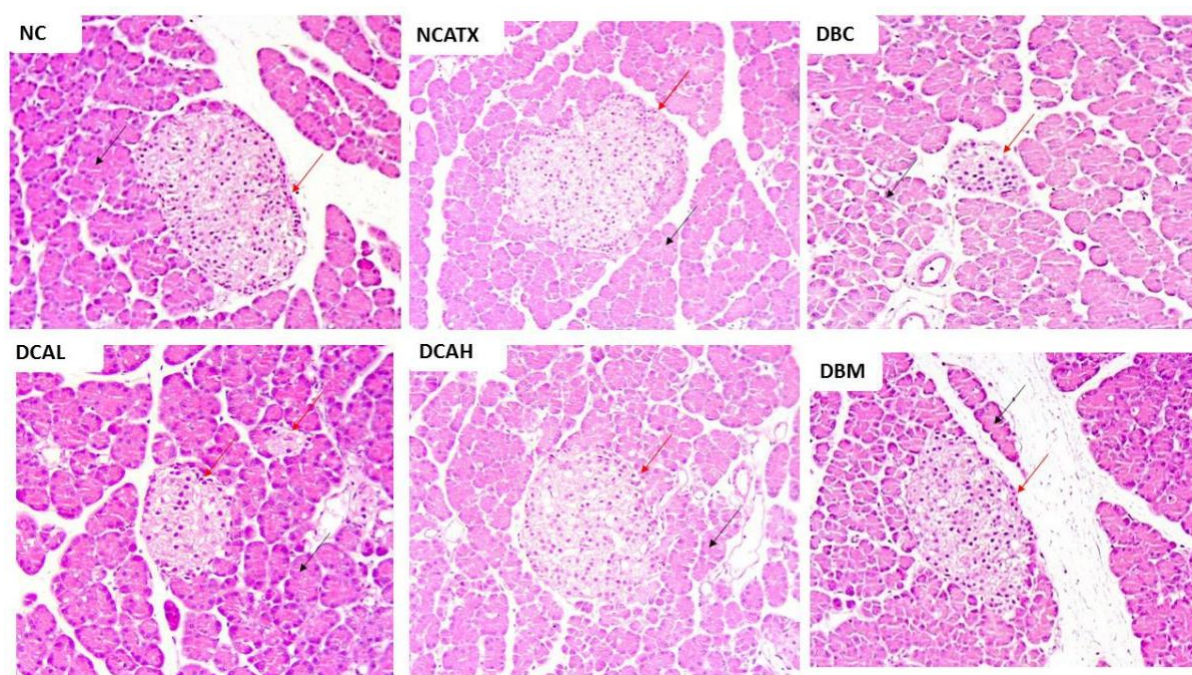


Figure: 6.4.8. Pancreatic histopathological examinations illustrating the effects of oral treatments in different animal groups during the experimental period. Magnification=100X. Red arrow shows the pancreatic islets of Langerhans. Black arrows show pancreatic acinar cells. **NC**= Normal Control, **NCATX** = Normal rats + *C. africana* high dose, **DBC**= Diabetic Control, **DCAL**= Diabetic rats + *C. africana* low dose, **DCAH** =Diabetic rats + *C. africana* high dose, **DBM**: Diabetic rats + Metformin (positive control).

6.4.5 Discussion

The global upsurge in the prevalence of T2D has prompted rigorous research into the development of novel, cost-effective anti-diabetic pharmacological agents with high efficacy, increased bioavailability, and reduced undesirable side effects when compared to current management strategies (Jacob and Narendhirakannan 2019). Indeed, plants have been used since time immemorial in folkloric medicine and have been shown to be endowed with countless therapeutic bioactive phytoconstituents, which pose them as a promising avenue for the development of novel antidiabetic agents (Jacob and Narendhirakannan 2019). Considering this, the current study investigated the therapeutic potential of *C. africana* against several diabetes-linked pathophysiologies in a type 2 diabetic model.

The onset of acute signs and symptoms associated with T2D, often linked to disrupted metabolic pathways, may present as increased thirst (polydipsia) and excessive hunger (polyphagia), as illustrated in Fig. 6.4.1. While the development, progression, and severity of acute T2D symptoms may vary among individuals, they are influenced by hyperglycaemia-induced alterations in glucose assimilation, blood osmotic potential, and energy redox balance (Pippitt *et al.*, 2016; Petersmann *et al.*, 2019). Furthermore, these symptoms are accompanied by a significant decrease in body weight, as observed for the diabetic control group (DBC) shown in Fig. 6.4.2. Data from the current study indicates that *C. africana* reduced food and fluid intake, as depicted in Fig. 6.4.1, suggesting that the extract may alleviate polydipsia and polyphagia under T2D conditions, possibly by regulating hunger and thirst centers in the hypothalamus and modulating levels of the satiety hormones ghrelin and leptin (Schalla and Stengel, 2019). Furthermore, the data demonstrates the extracts' potential to increase mean body weight gain by potentially mitigating peripheral tissue insulin resistance, allowing for more efficient assimilation and storage of glucose, a high-energy molecule. Indeed, this observation aligns with previous studies indicating that weight gain results from cell growth, division, and increased storage of polymerized glucose in the form of glycogen in muscles and/or as fatty acids in adipose tissue (Ginzberg *et al.*, 2015).

Impaired glucose uptake and disposal by peripheral tissues in T2D elevates circulatory blood glucose levels, directly leading to chronic hyperglycaemia as displayed by rats in the diabetic control group (DBC) in Fig. 6.4.3. Indeed, chronic hyperglycaemia (Fig. 6.4.3) and acute glucose intolerance, as seen in Fig. 6.4.4, are hallmark characteristics of T2D, indicating aberrant glucose metabolic pathways, particularly peripheral tissue insulin resistance (Tangvarasittichai, 2015). Therefore, the ability of the *C. africana* extracts to reduce the weekly

fasting blood glucose levels (Fig. 6.4.4) and improve the animal's glucose tolerance response (Fig. 6.4.4) is indicative of their ability to achieve and maintain homeostatic glucose levels, insinuating their potential potency as an antihyperglycemic agent in T2D. Furthermore, the ability of *C. africana* extracts may have reduced the weekly fasting blood glucose levels (Fig. 6.4.4) and improved glucose tolerance by inhibiting α amylase in both the serum and pancreas, as displayed in Table 6.4.2. Inhibition of carbohydrate hydrolytic enzymes retards dietary carbohydrate breakdown, thereby abating the mismatch between circulatory blood glucose assimilation and its disposal by peripheral tissues and allowing for more effective glucose metabolism (Agarwal and Gupta, 2016). This pharmacological activity forms a cornerstone of T2D management, with some drugs such as acarbose and miglitol currently on the market being classified as α amylase inhibitors (Dastjerdi *et al.*,2015). Indeed, such compounds abate postprandial blood glucose spikes, enabling the achievement of euglycemic levels, which is pivotal to T2D management, circumventing the development and progression of its associated complications (Bashary *et al.*,2020). Alternatively, *C. africana* extracts may reduce weekly fasting blood glucose levels and improve glucose tolerance in T2D by increasing insulin secretion by β pancreatic cells or improving its sensitivity in insulin- responsive peripheral tissues, including the skeletal muscle and the liver (Kazeem and Davies, 2016).

Insulin is an anabolic endocrine hormone secreted by the β pancreatic cells that mediates cell growth and/or differentiation, glucose uptake in major peripheral glycogen and protein synthesis, and inhibition of gluconeogenesis (Lankatillake *et al.*,2019). Indeed, relative insulin deficiency, as illustrated in Fig. 6.4.5A, together with reduced HOMA β scores, as indicated in Fig. 6.4.5B, insinuate impeded pancreatic β cell function and destruction in the diabetic control group (DBC). Additionally, elevated HOMA IR scores observed in Fig. 6.4.5B are indicative of systemic insulin resistance in the diabetic control group (DBC). The data therefore shows an additive effect of insulin deficiency and insulin resistance, thereby predisposing animals in the DBC group to aggravated chronic hyperglycaemia leading to the rapid development of end-stage complications associated with T2D. Therefore, the ability of *C. africana* to upregulate insulin secretion (Fig. 6.4.5A) and improve pancreatic β -cell function with a concomitant reduction in systemic insulin resistance in peripheral tissues (Fig. 6.4.5B) is indicative of the extracts' enhanced potential to achieve and maintain homeostatic blood glucose levels and abate T2D-linked complications (Shahwan *et al.*,2022). These pharmacological capabilities are further corroborated by the histopathological examinations of the pancreatic tissue in Fig. 6.4.8, which revealed that *C. africana* reversed β pancreatic cell damage, increased their number, and

improved overall pancreatic tissue health and morphology when compared to the diabetic control group (DBC group). Indeed, improved β pancreatic tissue health is associated with improved insulin secretion, which is pivotal for the regulation of glucose uptake in peripheral tissues, metabolism, and homeostasis (Lankatillake *et al.*,2019). Additionally, the extracts may increase insulin sensitivity by modulating the activities of several downstream proteins other than the IR (insulin receptor), thus resulting in increased GLUT4 translocation to the membranes and subsequently increasing glucose clearance from the blood (Oh, 2015; Tran *et al.*,2020).

During the fed state, increased glucose influx triggers hepatic glycogen synthesis through glycogenesis to store the excess glucose for later use during intermittent fasting periods (Petersen *et al.*,2017). Blood glucose regulation through hepatic glucose output is a delicate, multistep process tightly regulated by several key hormones and enzymes, including insulin, glucose-6 phosphatase, and glycogen phosphorylase (Petersen *et al.*,2017; Rajas *et al.*,2019). Consequently, diminished hepatic glycogen reserves observed in the diabetic group DBC displayed in Fig. 6.4.6 are indicative of dysregulated hepatic glucose metabolism, presumably through T2D-induced hyperactivity of glycogenic enzymes including glucose-6 phosphatase and glycogen phosphorylase, as depicted in Table 6.4.1. Indeed, diminished hepatic glycogen stores (Fig. 6.4.6) insinuate increased hepatic glucose output, which, when taken together with elevated fructose 1,6-bisphosphatase activity (Table 6.4.1), mediates gluconeogenesis and culminates in T2D-linked chronic hyperglycaemia as observed in the DBC group in Fig. 6.4.3. Therefore, the ability of *C. africana* to restore the depleted glycogen reserves in diabetic animals through the regulation of glucogenic enzymes indicates the extracts' ability to abate dysregulated glycogen degradation, therefore suggesting antihyperglycemic potential against T2D (Rajas *et al.*,2019). Furthermore, the fortified glycogen stores in the liver may stem from the insulin secretagogue activity of *C. africana* extract observed in Fig. 6.4.5A, as increased circulatory insulin availability inhibits glycogenolysis and its associated regulatory enzymes (Rines *et al.*,2016).

Diabetic dyslipidaemia stemming from dysregulated lipid metabolism is characterized by hyperlipidaemia and altered lipoprotein ratios aggravate cardiovascular diseases (CVD) in T2D (Vergès, 2015; Filippatos *et al.*,2017). Several additive factors, including polyphagia (Fig. 6.4.1) and relative insulin deficiency (Fig. 6.4.5A), observed in the DBC group lead to the development of diabetic dyslipidaemia (Filippatos *et al.*,2017). Significantly increased lipase activities in both the serum and pancreases of the DBC group depicted in Table 6.4.2 insinuate increased dietary lipid hydrolysis and assimilation, thereby predisposing the animals to hyperlipidemia. Furthermore, T2D induction significantly elevated total cholesterol,

triacylglycerols, and LDL cholesterol while concomitantly diminishing HDL cholesterol levels, as depicted in Fig. 6.4.7. This data is indeed consistent with the increased risk of cardiovascular disease (CVD) observed among T2D patients, as further corroborated by the elevated atherogenic index of the plasma (AIP) displayed in Fig. 6.4.7. Therefore, the lipase inhibitory capabilities of *C. africana*, taken together with the extracts' capacity to reduce cholesterol, triacylglycerols, and LDL cholesterol while elevating HDL cholesterol levels, strongly suggest its potential to abate diabetic dyslipidaemia, thereby retarding the onset and severity of associated cardiovascular diseases (CVD) (Katsiki *et al.*,2017). Additionally, excessive hyperlipidaemia is implicated in the development and progression of non-alcoholic fatty liver disease (NAFLD) (Kosmalski *et al.*,2022). The enlargement of the liver despite exhausted glycogen stores observed in Figure 6.4.6 suggests fatty acid infiltration in the liver. The abilities of *C. africana* to restore a normal lipid profile and reduce liver enlargement strongly suggest that the extracts may help abate NAFLD in T2D, which in severe cases may present with cirrhosis or hepatic steatosis, signifying extensive damage to the organ (Kosmalski *et al.*,2022).

The hepatic and renal tissues are among the most metabolically active tissues in the body, performing critical processes ranging from blood detoxification, osmotic and electrolyte balance, immune response, and blood pressure regulation (Ozougwu, 2017; Velho and Velho, 2013; Rivadeneyra-Domínguez *et al.*,2018). These processes are mediated by several enzymes, including lactate dehydrogenase (LDH), urea, uric acid, alkaline phosphate (ALP), aspartate aminotransferase (AST), and Creatine Kinase-Myocardial Band (CK-MB). Indeed, damage to hepatic tissue compromises the membrane barrier that confines these enzymes in the hepatocytes, resulting in their spillage into the blood, thereby making the assessment of the blood concentrations of these enzymes (biomarkers) a routine procedure when liver or kidney damage is suspected. Evidently, the significant increase of these biomarkers, as seen in Table 6.4.5, is indicative of T2D-induced hepatic damage. Consequently, as the renal tissue performs blood filtration functions, an increase in urea and uric acid in the rats in the diabetic control group in Table 6.4.5 is indicative of impeded kidney function under T2D conditions. Furthermore, the current data study suggests that hyperglycaemic-induced oxidative stress and chronic low-grade inflammation may have contributed to renal and hepatic tissue damage through elevated lipid peroxidation and nitric oxide production with concomitant diminished GSH levels and reduced activities of superoxide dismutase (SOD) and catalase (CAT), as observed for the DBC group in Table 6.4.3. Under oxidative stress conditions in T2D, excessive electron-deficient reactive species with lone pairs damage several tissue moieties, thereby

aggravating associated complications. Therefore, the ability of *C. africana* to reduce hepatic damage biomarker indices and reduce urea and uric acid serum levels, as seen in Table 6.4.5, is indicative of their capabilities against hepatic and renal tissue damage. This could possibly be due to the ability of the extracts to reduce T2D oxidative tissue damage by augmenting the activities of key antioxidants such as SOD and CAT, increasing the levels of GSH, and concomitantly reducing lipid peroxidation and NO production. Taken together, these results indicate the protective capacity of *C. africana* against oxidative tissue damage, which may aid in retarding the onset of T2D complications that result from impeded metabolic tissue function.

6.4.6 Conclusion

The ability of the *C. africana* extracts to reduce the weekly fasting blood glucose levels and improve glucose tolerance may be attributed to their ability to reverse β pancreatic cell damage, thereby abating insulin deficiency and systemic resistance. Furthermore, the extracts restored the depleted glycogen reserves in diabetic animals, possibly through the regulation of glucogenic enzymes. *C. africana* abated dyslipidaemia through reduced cholesterol, triacylglycerols, and LDL cholesterol while elevating HDL cholesterol levels. Indeed, *C. africana* restored homeostatic redox balance and reduced T2D-linked hepatic and renal damage, suggesting its protective capacity against oxidative stress-linked tissue damage. These results show the enhanced potential of *C. africana* as a source of novel antidiabetic drug templates that may ameliorate T2D-linked complications. These findings warrant further clinical trials to elucidate the precise mode of antidiabetic action shown by *C. africana* in the current study.

CHAPTER 7

IN VITRO, EX VIVO AND IN VIVO STUDIES FOR INVESTIGATING THE ANTIOXIDANT AND ANTIDIABETIC ACTIVITIES OF *SCHOTIA BRACHYPETALA* (Sond.)

7.1 *Schotia brachypetala* (Sond.)

7.1.1 Background and plant description

Schotia brachypetala of the family Fabaceae is a medium-to-large tree with a rough brown to brownish gray bark characterized by a wide, spreading round crown (Watt and Breyer-Brandwijk, 1962). The branches hold the deep red flowers produced during the spring season and are a distinct feature of this plant. The bright, rich, deep red flowers produce copious nectar around September and October, which attracts birds and several insects (Du, 2011). The flowers give rise to hard, flattened woody pods with pale brown seeds about 2 cm in diameter, characterised by conspicuous yellow arils (Du, 2011). Mature pods split open to release the seeds, usually during the late summer season. *S. brachypetala* is found growing in different types of habitats, ranging from bushvelds to deciduous woodland and on the banks of rivers, usually associated with termite mounds. In South Africa, it is widespread in the Eastern Cape, through KwaZulu-Natal, Mpumalanga, and Limpopo Province, and extends into Mozambique and Zimbabwe. Several reports have detailed the use of *S. brachypetala* in ornamental gardens as wood for furniture and the bark for tanning leather (Venter and Venter, 1996). Similarly, the plant is an invaluable part of several traditional medicinal decoctions (Du, 2011).

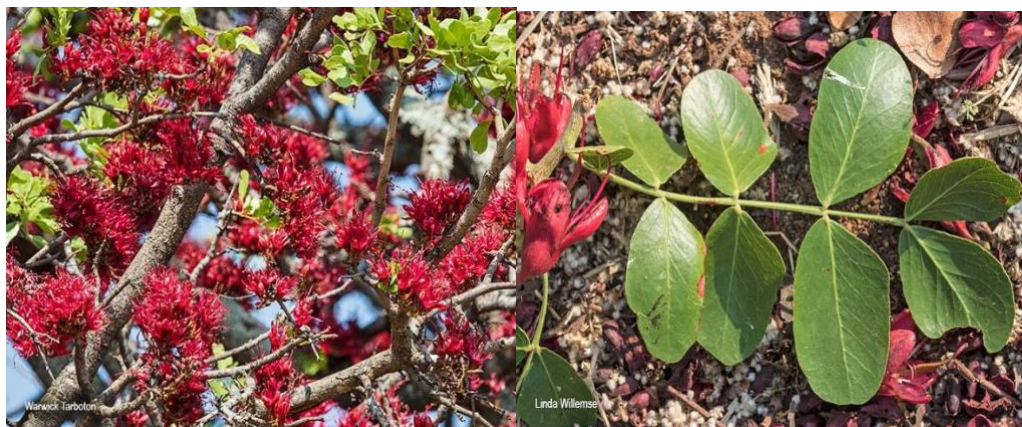


Figure 7.1: *Schotia brachypetala* (Sond). Common names: weeping boer-bean (English); huilboerboon (Afr.); *Umfofofo* (Xhosa), *Uvovovo* (Zulu), *Molope* (Northern Sotho), *Mulibi* (Venda), *Nwavilombe* (Tsonga); *Umutwa* (Tswana). Adapted without permission from www.zimbabweflora.co.zw.

7.1.2 Ethnobotanical uses

Schotia brachypetala is used against diabetes mellitus by Bapedi and Vhavenda ethnic groups in the Limpopo province, South Africa (Silas and Alfred, 2019). In South Africa, the stem bark decoction of *S. brachypetala* is traditionally used to remedy diarrhoea by the northern-Sotho-speaking people of the Limpopo province (Grace *et al.*,2002; Mathabe *et al.*,2006), while in the KwaZulu Natal Province it is used to remedy heartburn and pimples (Hutchings, 1996; Coopoosamy and Naidoo, 2012). The plant is traditionally used to wash the body to reduce body swelling, which may be a result of several chronic conditions, including diabetes (Van Wyk, and Gericke, 2000). The bark decoction of *S. brachypetala* is taken to treat heartburn and hangover from excessive alcoholic beverage consumption (Watt and Breyer-Brandwijk, 1962).

7.1.3 Pharmacological and biological activities

S. brachypetala exhibited potential for development into a therapeutic agent against Alzheimer's disease due to its capacity to enhance non-amyloidogenic processing of β -amyloid precursor protein and thus decrease A β 42 levels (Thakur *et al.*,2019). This was further reiterated by independent workers who established the antioxidant and acetylcholinesterase inhibiting capacity of secondary compounds in the *S. brachypetala* extracts for the treatment of memory loss due to Alzheimer's disease (Adewusi *et al.*,2011). McGaw and colleagues, in 2002, documented the anti-microbial activity of leaf and root ethanolic extracts of *S. brachypetala*. *S. brachypetala* leaf extracts exhibited enhanced antioxidant activity *in vitro* against DPPH, ABTS, and FRAP, with the concomitant ability to decrease reactive oxygen species (ROS) production and attenuate *hsp16.2* expression in *C. elegans* under oxidative stress conditions (Sobeh *et al.*,2016).

7.1.4 Phytochemistry

Trans-3,3,4,5,5-pentahydroxystilbene and trans-3,3,4,5-tetrahydroxystilbene are among the major polyhydroxystilbenes that have been identified and isolated from water: methanol extract of heartwood of *S. brachypetala* (Drewes and Fletcher, 1974). Furthermore, more recent *in silico* studies have predicted the potential benefits of 3,3,4,5,5-pentahydroxystilbene against diabetes (Pereira *et al.*,2019). Linolenic acid and methyl-5,11,14,17-eicosatetraenoic acid have been isolated from the leaf ethanolic extracts of *S. brachypetala* (McGaw *et al.*,2022). In a previous study, Sobeh and colleagues (2016) identified several secondary metabolites in *S. brachypetala*, including polyphenols, flavonoid derivatives, dihydrochalcones, procyanidins, anthocyanins, hydroxybenzoic acid derivatives, and hydrolysable tannins. The study further isolated and identified three novel compounds, namely gallic acid, myricetin-3-O- α -L-1C4-rhamnoside, and quercetin-3-O-L-1C4-rhamnoside, which had not previously been identified in the *Scotchia* species (Sobeh *et al.*,2016).

7.2 *Schotia brachypetala* (Sond) crude leaf extract exhibit potent antioxidant, antihyperglycemic, and enhanced protective activities against FeSO₄ induced oxidative pancreatic cell injury

Brian K Beseni¹, Kolawole A Olofinisan¹, Erukainure L Ochuko¹ * Shahidul Md Islam¹

1.Department of Biochemistry, University of KwaZulu-Natal, Westville Campus, Durban 4000, South Africa.

***Corresponding author:** islamd@ukzn.ac.za

7.2.1 Abstract

The total flavonoids and phenolics of *S. brachypetala* leaf crude extracts were quantified prior to the determination of their *in vitro* free radical scavenging, electron-donating capacity, and nitric oxide production inhibiting capabilities. Thereafter, the carbohydrate and lipid-digesting enzyme inhibitory activities of the extracts were also determined *in vitro*. The modulation of hepatic glycogenic enzymes and endogenous antioxidant moieties, intestinal glucose absorption, and muscular uptake were investigated *ex vivo*. The extracts contained significant amounts of both total flavonoid and phenolic constituents, which may be responsible for the different pharmacological activities observed for *S. brachypetala*. The ethanolic extract exhibited the best DPPH scavenging activity among all extracts, with an IC₅₀ value of 26,44±0,57 µg/ml. The aqueous extract exhibited the most potent pancreatic lipase and α-amylase inhibitory capabilities. Furthermore, the ethanolic extract significantly stimulated glucose uptake in the yeast suspension and psoas muscle tissue, while the aqueous extract significantly retarded intestinal glucose absorption among all extracts. The *S. brachypetala* leaf crude extracts lowered lipid peroxidation, increased cellular reduced glutathione (GSH) concentration, and increased the activities of superoxide dismutase (SOD) and catalase (CAT) in pancreatic homogenate treated with pro-oxidant FeSO₄. The aqueous and ethanol leaf extracts of *S. brachypetala* significantly (p<0.05) lowered hepatic ATPase, glycogen phosphorylase (GP), and fructose 1,6-bisphosphatase (FBP) activities to normal ranges. The findings in this study warrant further molecular and *in vivo* analysis to ascertain the precise mechanism of action for the bioactivities observed, particularly by the aqueous and ethanolic extracts.

Keywords: *S. brachypetala*, Antidiabetic, Antioxidant, Enzyme inhibition.

7.2.2 Introduction

The prevalence of diabetes mellitus (DM) has reached epidemic levels, with its prevalence soaring at an alarming rate globally (Hostalek, 2019; Forouhi and Wareham, 2019). The International Diabetes Federation (IDF) estimated in 2021 that about 536.6 million people globally were living with DM. Furthermore, the DM incidence is projected to reach about 783.2 million people by the year 2045 (IDF 2021). DM is a metabolic disorder characterized by chronic hyperglycemia stemming from aberrant glucose metabolism (Kerner and Brückel, 2014; Punthakee *et al.*, 2018). While several DM etiologies are recognized, the most prevalent include type 1 (T1D), type 2 (T2D), and gestational diabetes (ADA, 2014; Punthakee *et al.*, 2018). T2D, which accounts for over 90% of all DM cases, is associated with several comorbidities, including hypertension, obesity, and cardiovascular diseases (CVD) (Iglay *et al.*, 2016; Rubino *et al.*, 2020). Globally, it is estimated that the annual mortality due to T2D has surpassed the 1 million mark, qualifying it as one of the leading causes of death (Khan *et al.*, 2020).

The pathogenesis and progression of T2D are multifactorial and scantily understood but can be attributed to several risk factors, including genetic predisposition, diet, sedentary lifestyle, age, and ethnicity (Bellou *et al.*, 2018). Recent reports implicate oxidative stress as a major aggravating factor in the development and progression of T2D. (Asmat *et al.*, 2016). Oxidative stress occurs when the body's endogenous antioxidant defense systems are overwhelmed by the production of reactive species (RS) (Oguntibeju, 2019). Reactive species (RS), including reactive oxygen species (ROS), reactive nitrogen species (RNS), and reactive chlorine species (RCS), function under homeostatic conditions as secondary messengers, mediators of cell signaling pathways, and several other processes (Asmat *et al.*, 2016; Forrester *et al.*, 2018; Jakubczyk *et al.*, 2020). However, excessive reactive species with unstable unpaired electrons under oxidative stress conditions disrupt the structure of several biomolecules, including lipids, proteins, and DNA, thereby impeding their function (Forrester *et al.*, 2018; Halim and Halim, 2019). Widespread cell surface receptor and tissue damage at molecular levels is responsible for the development of several degenerative conditions and is implicated as the major driving force behind insulin resistance in T2D (Asmat *et al.*, 2016; Kurdekar & Sushma, 2020). Therefore, antioxidant molecules that can counter the harmful effects of excessive reactive species under oxidative stress conditions are widely sought after as beneficial therapeutic agents in the management of T2D and its related complications (Matough *et al.*, 2012; Asmat *et al.*, 2016).

Current management strategies for diabetes, although effective, have several drawbacks, including undesirable multiple side effects (Capoccia *et al.*, 2016). Side effects of current DM medication include weight loss or gain, diarrhea, water retention, vomiting, and nausea, with some having been reported to cause cancer and kidney and liver damage (Sterrett *et al.*, 2016; Stahnke *et al.*, 2020). Furthermore,

some may be out of reach, particularly for people living in impoverished regions of the world, especially in sub-Saharan Africa. It is estimated that about 80% of people in the African region rely on the use of traditional medicinal plants for either their primary health care needs or to supplement western medicine regimens (Thomford *et al.*,2015; Buwa-Komoren *et al.*,2019). There has been a rise in interest by scientists throughout the world to determine the modes of operation of these medicinal plants in terms of their efficacy and safety (Thomford *et al.*,2015). The eventual goal is to develop pharmacological agents that have reduced side effects and are affordable to people in impoverished regions of the world. *S. brachypetala* is one such plant that is used in Africa for medicinal purposes.

S. brachypetala is a handsome, medium-to-large tree with a wide spreading, densely branched, rounded crown whose bark and root mixtures are widely used as blood purifiers and in the management of nervous heart conditions and diarrhea (Khumalo *et al.*,2021). The Bapedi and Vhavenda ethnic groups in the Limpopo province of South Africa have been reported to use *Schotia brachypetala* in the management of diabetes mellitus and related complications (Silas and Alfred, 2019). While the Northern-Sotho ethnic group in the same region reportedly uses the stem bark decoction of *S. brachypetala* to remedy diarrhea (Grace *et al.*,2002; Mathabe *et al.*,2006). Considering these observed traditional uses, the present study explored the antioxidant and antidiabetic properties of various crude leaf extracts of *S. brachypetala* using *in vitro* and *ex vivo* experimental models.

7.2.3 Materials and methods

Please refer to Chapter 2, sections 2.4.1 to 2.11.5 for detailed materials and methods.

7.2.4 Results

As shown in Fig. 7.2.1A and Fig. 7.2.1B, the ethanol extract contained significantly ($p < 0.05$) the highest amounts of both phenolics (34.34 mg/g gallic acid equivalent) and flavonoids (26.16 mg/g gallic acid equivalent). The methanol extract contained the second most abundant phenolic content 30.10 mg/g gallic acid equivalent followed by the aqueous extracts 20.92 mg/g gallic acid equivalent while the ethyl acetate extract contained the least total polyphenols 7.03 mg/g gallic acid equivalent. In Fig. 7.2.1B similarly the methanol extract contained the second most abundant flavonoid content 21.09 however there was no significant ($p > 0.05$) difference in the flavonoid content in the aqueous 7.86 mg/g gallic acid equivalent and ethyl acetate 8.41 mg/g gallic acid equivalent extracts.

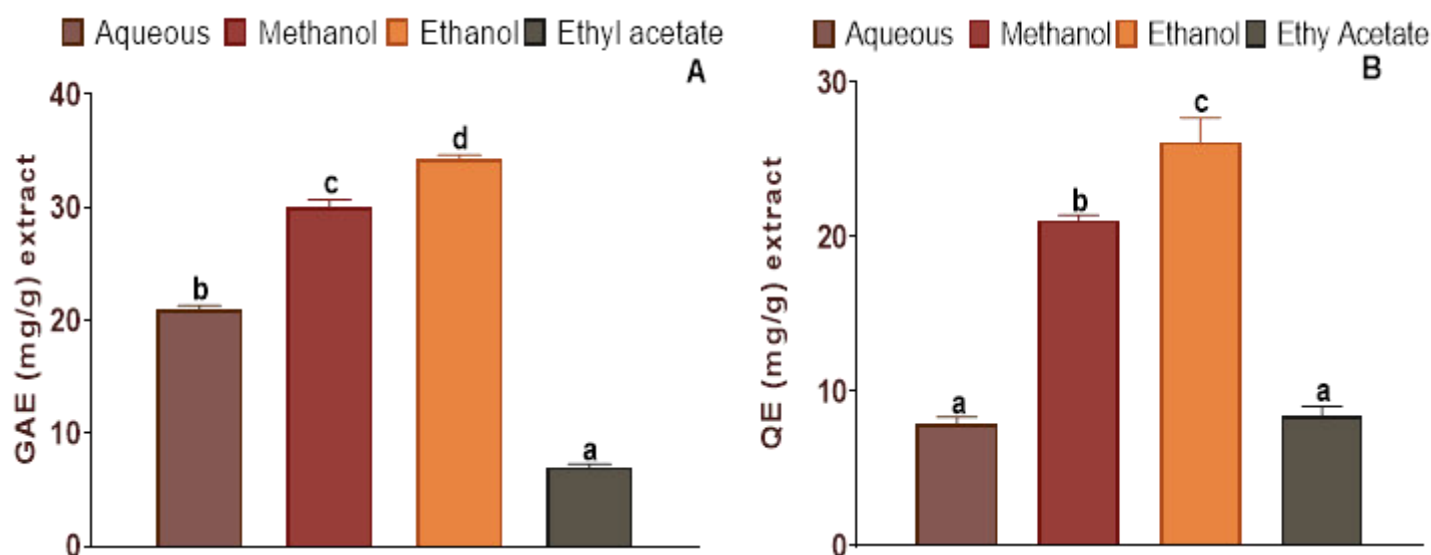


Figure 7.2.1: The total phenolic content of the *S. brachypetala* crude leaf extracts represented as A) Gallic acid equivalents (GAE mg/g) and the total flavonoid content represented as B) Quercetin equivalents (QE mg/g). Bars with superscripts a-d for each extract are statistically different at $p < 0.05$ level of significance.

As shown in Fig. 7.2.2A, the ethanolic extract exhibited the best DPPH scavenging activity among all extracts with an IC_{50} value of $26,44 \pm 0,57$ μ g/ml, closely followed by the aqueous extract with an IC_{50} value of $76,77 \pm 0,23$ μ g/ml. Furthermore, the methanol and ethyl acetate extracts had comparable DPPH radical activities at most of the assayed concentrations. The methanolic extract had the best electron-donating capacity among all the extracts, as shown in Fig. 7.2.2 B, with an IC_{50} value of $7,86 \pm 0,34$ μ g/ml. Evidently, the methanol and ethyl acetate extracts, which had IC_{50} values of $178,33 \pm 0,61$ μ g/ml and $178,18 \pm 0,53$ μ g/ml, had the best nitric oxide scavenging capabilities among all *S. brachypetala* leaf extracts, as depicted in Fig. 7.2.2 C.

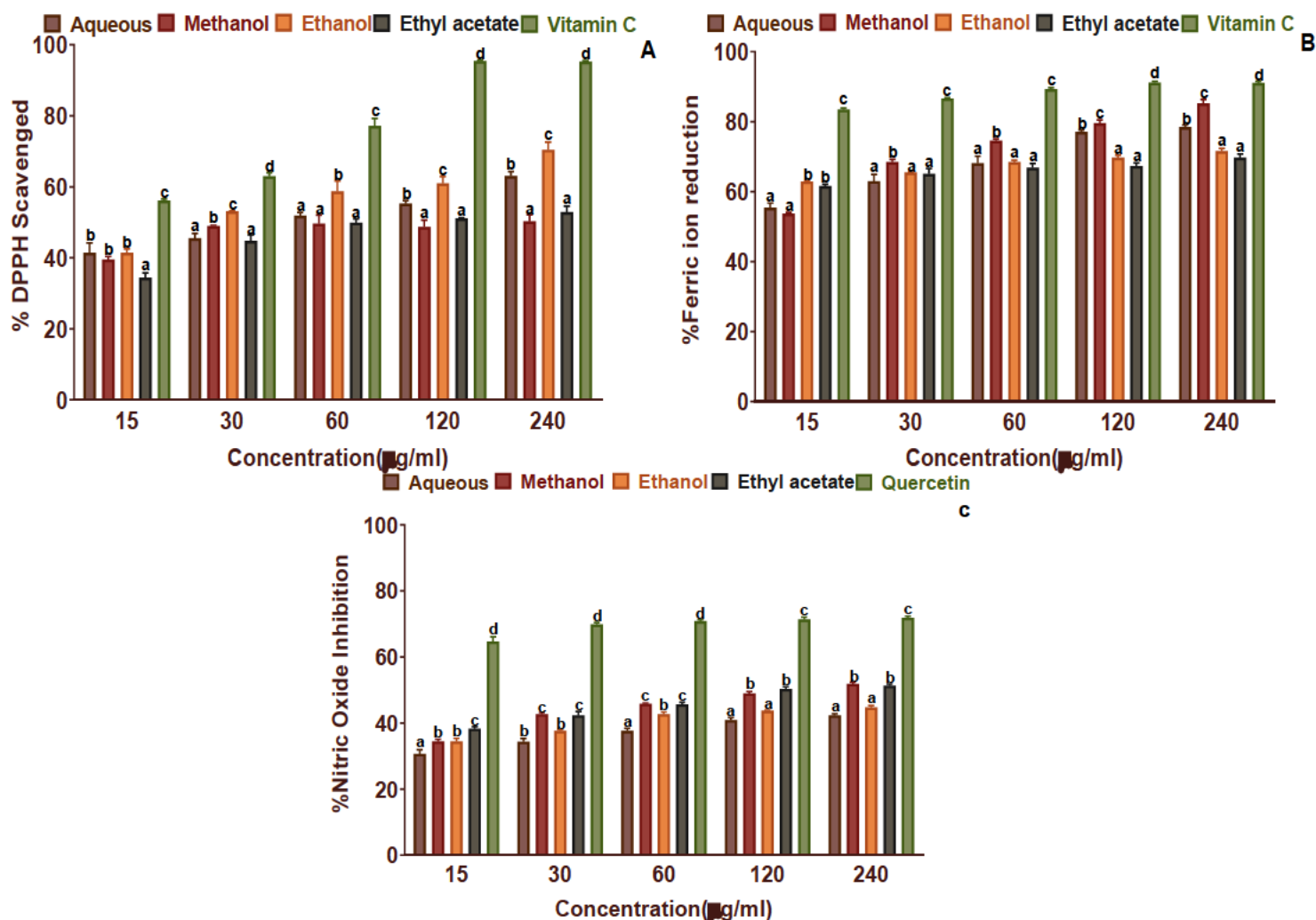


Figure 7.2.2: *S. brachypetala* leaf extracts (A) DPPH scavenging (B) ferric reduction and (C) nitric oxide scavenging activities. Bars with superscripts a-e for each concentration are statistically different at $p < 0.05$ level of significance.

As depicted in Fig. 7.2.3A, the aqueous extract exhibited the most potent pancreatic lipase inhibitory capability among all other extracts, with an IC_{50} value of $133,25 \pm 0,25$ µg/ml. Evidently, at the highest concentration of 240 µg/ml, all the extracts exhibited significantly ($p < 0.05$) better pancreatic lipase inhibitory capabilities than orlistat (the positive control). In Fig. 7.2.3 B, the methanolic extract exhibited the best α -glucosidase activity with an IC_{50} value of $60,14 \pm 0,347$ µg/ml. The methanolic extract's α -glucosidase inhibitory capacity was closely followed by that of the ethanol extract, which had an IC_{50} value of $108,88 \pm 0,82$ µg/ml. The aqueous and ethyl acetate extracts showed comparable α -glucosidase inhibitory activities at the tested concentrations. Finally, as observed in Fig. 7.2.3 C, the aqueous extract exhibited the most potent α -amylase inhibitory capabilities among all the extracts, with an IC_{50} value of $124,01 \pm 0,25$ µg/ml. Furthermore, no significant difference ($p > 0.05$) was observed in the α -amylase inhibitory capabilities of the aqueous and methanol extracts with those of acarbose at

the highest concentration of 240 µg/ml.

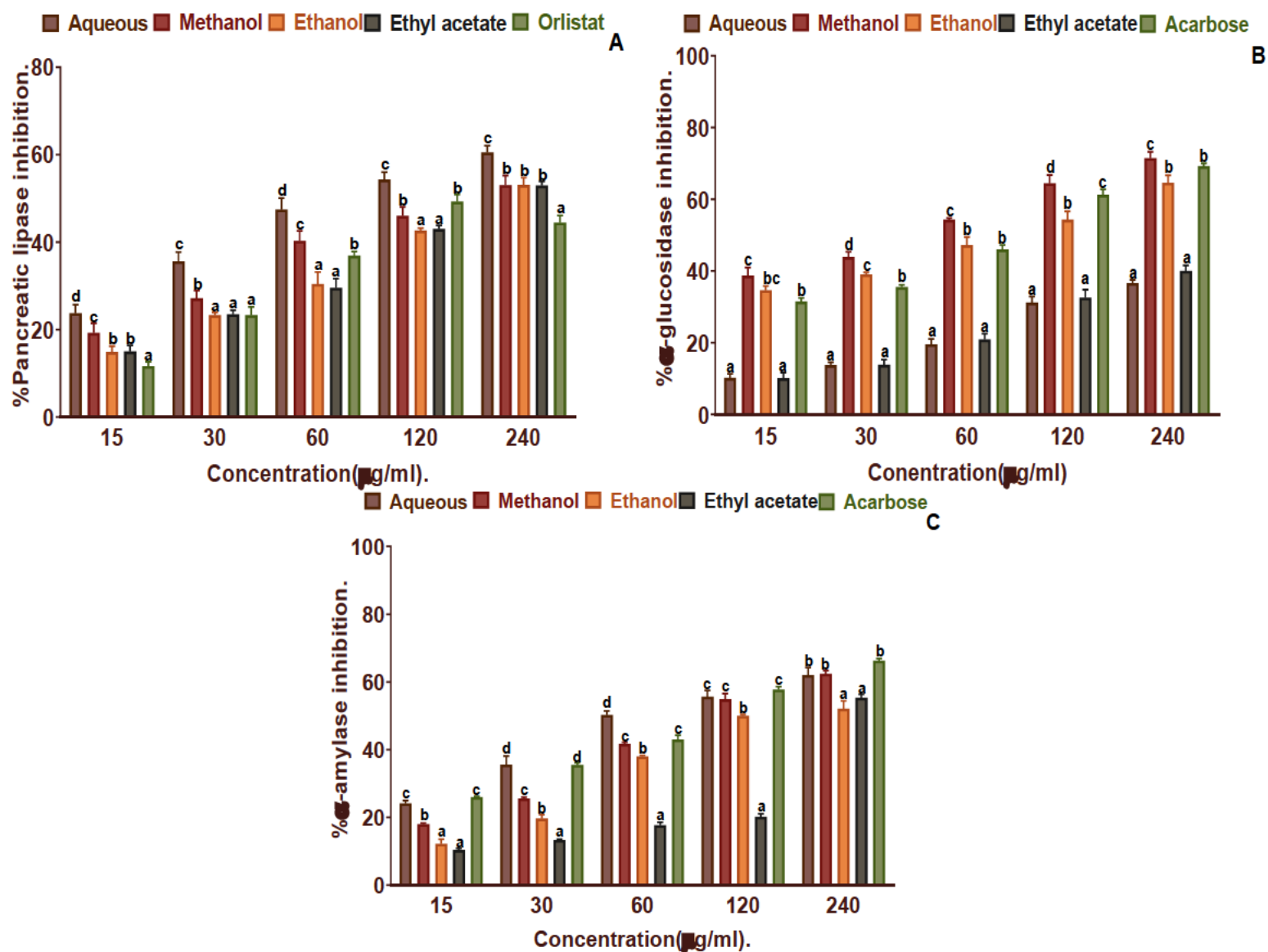


Figure 7.2.3: *S. brachypetala* leaf extracts (A) pancreatic lipase (B) α -glucosidase (C) α -amylase inhibitory activities. Bars with superscripts a-e for each concentration are statistically different at $p < 0.05$ level of significance.

Table 7.2.1: The IC₅₀ values of different leaf extracts of *S. brachypetala* under different assayed parameters.

	Aqueous	Methanol	Ethanol	Ethyl acetate	Vitamin C	Quercetin	Acarbose	Orlistat
DPPH (µg/ml)	76,77±0,23	188,85±0,19	26,44±0,57	149,58±0,19	9,21±0,12	-	-	-
FRAP (µg/ml)	8,91±0,22	7,86±0,34	10,67±0,56	12,97±0,98	6,67±0,88	-	-	-
NO (µg/ml)	374,83±0,52	178,33±0,61	336,43±0,23	178,18±0,53	-	1,17±0,58	-	-
Pancreatic lipase(µg/ml)	133,25±0,25	141,38±0,98	200,73±0,45	201,22±0,83	-	-	-	114,52±0,83
α-glucosidase(µg/ml)	330,03±0,42	60,14±0,347	108,88±0,82	293,99±0,85	-	-	100,68±0,09	-
α-amylase (µg/ml)	124,01±0,25	143,57±0,37	186,97±0,95	231,48±0,75	-	-	118,57±0,97	-

Data is presented as mean ± standard deviation. Key: DPPH = 2,2-diphenyl-1-picrylhydrazyl. FRAP = Ferric reducing antioxidant power, NO = Nitric oxide assay.

The glucose uptake stimulatory capabilities of *S. brachypetala* leaf extracts in yeast suspension depicted in figure 7.2.4 A indicate that the ethanol extract, closely followed by the aqueous extract, exhibited the best activity among all the extracts. Furthermore, except at the lowest concentration, all the extracts surpassed the yeast suspension glucose uptake stimulatory activities of metformin, which was used as the positive control. Similarly, the ethanol extracts stimulated the highest glucose uptake in skeletal muscles, as shown in Fig. 7.2.4 B. Furthermore, at concentrations of 60 µg/ml and beyond, the ethanol extract stimulated significantly better skeletal muscle glucose uptake than insulin (positive control). While the extracts significantly ($p<0.05$) increased glucose uptake in the muscle tissues higher than the normal control, the ethyl acetate extract had the least stimulatory activity among all extracts. However, as shown in Fig 7.2.4 C, the methanolic extracts followed by the aqueous extract had significantly the best hepatic tissue glucose uptake stimulatory activities among all extracts at most of the tested concentrations. Finally, the glucose absorption-retarding effects of the *S. brachypetala* leaf extracts on rat jejunum are depicted in Fig. 7.2.4 D. As observed, the aqueous and ethanol extracts had the overall best capabilities to retard glucose absorption in rat jejunum. Furthermore, the extracts outperformed acarbose at all the tested concentrations.

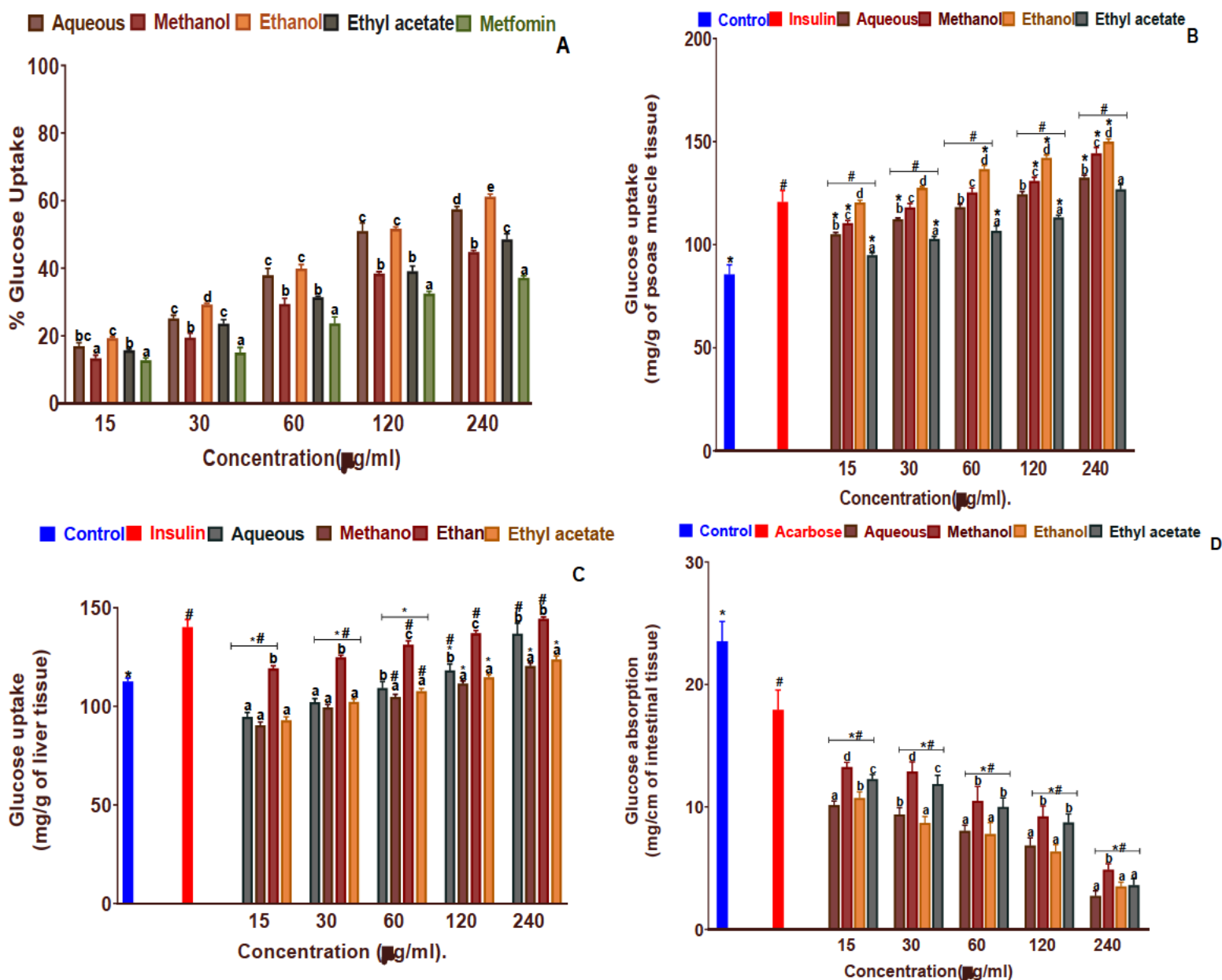


Figure 7.2.4: The effects of *S. brachypetala* leaf extracts on (A) glucose uptake in yeast suspension (B) glucose uptake in excised rat psoas muscle (C) glucose absorption in the intestinal jejunum. Bars with superscripts a-b are statistically different at $p < 0.05$ whereas those with * and # are different from the acarbose/insulin treatment positive and control, respectively.

Oxidative pancreatic tissue injury significantly elevated MDA equivalents while concomitantly diminishing SOD and CAT activities and reducing GSH levels. As depicted in Fig. 7.2.5A, the aqueous, methanol, and ethanol extracts exhibited comparable capabilities to reduce MDA equivalents, especially at the highest concentrations of 120 µg/ml to 240 µg/ml. While these three extracts significantly outperformed vitamin C, the ethyl acetate extract was the least effective in reducing LPO but had comparable activity to that of vitamin C, with which there

was no significant difference at all the tested concentrations, as observed in Fig. 7.2.5A. As depicted in Fig. 7.2.5 B, the ethanol extract exhibited the best SOD activity, especially at concentrations of 60 µg/mL and beyond, where it also significantly outperformed vitamin C. The ethyl acetate extract exhibited the least SOD-stimulating activity among all the extracts at all the tested concentrations. The aqueous and methanol extracts showed comparable SOD-stimulating activities at all the tested concentrations except at 240 µg/ml, where the methanol significantly outperformed the aqueous extract. As observed in Fig. 7.2.5 C, the ethanol extract showed the best CAT-stimulating capabilities among all the extracts. The CAT- stimulating capabilities of the ethanol extract were closely followed by those of the aqueous and methanol extracts. The aqueous methanol and ethanol extracts in Fig. 7.2.6 C were shown to increase CAT activities to levels significantly higher than those observed for vitamin C (positive control) at all the tested concentrations. The ethyl acetate extract had the least CAT-stimulating capabilities, which were comparable to those of vitamin C. Lastly, as observed in Fig. 7.2.5 D, the ethanol extracts had the overall best GSH level, significantly outperforming all the extracts and vitamin C (positive control) at all the tested concentrations. Similarly, the ethanol extracts and vitamin C did not show a significant difference at all the tested concentrations amongst themselves. Only the ethyl acetate extracts failed to reach and surpass the GSH-stimulatory activities of vitamin C (positive control).

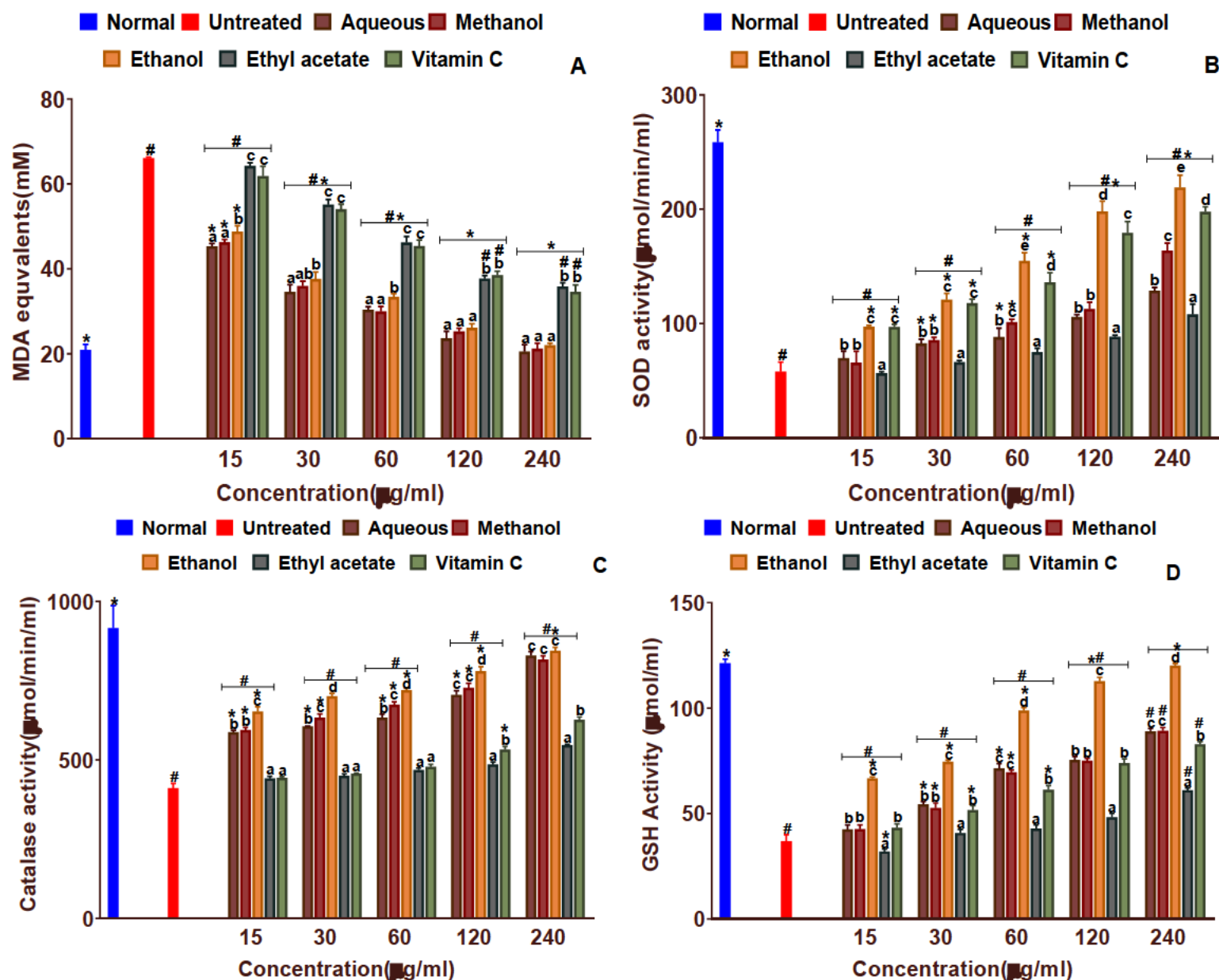


Figure 7.2.5: Effects of *S. brachypetala* leaf extracts on (A) lipid peroxidation, (B) SOD activity, (C) Catalase activity and (D) GSH activity in oxidative pancreatic injury. Data are presented as mean $\pm$ standard deviation of triplicate determinations. Different letters (a-e) above the bars for a given concentration are significantly different from each other ($p < 0.05$). *Significantly different from untreated sample and #significantly different from normal sample.

As observed in Fig. 7.2.6, A-C hyperglycemia induced hyperactivity of hepatic ATPase, glycogen phosphorylase (GP), and fructose-1,6-bisphosphatase (FBP) activity. As shown in Fig 7.2.6 A, treatment with particularly the aqueous and ethanol leaf extracts of *S. brachypetala* significantly ($p<0.05$) lowered ATPase activity to levels comparable to those observed for quercetin. The ethyl extract had significantly ($p<0.05$) the least ATPase inhibitory capability among all the extracts at the tested concentrations. Similarly, as shown in Fig. 7.2.6 B, the aqueous and ethanol extracts showed the best glycogen phosphorylase inhibitory capabilities among all other extracts, especially at concentrations of 60 $\mu\text{g/mL}$ and beyond. The aqueous, methanol, and ethanol extracts restored glycogen phosphorylase to near-normal levels at treatments from 30 $\mu\text{g/ml}$ and beyond and exhibited better inhibitory capabilities than quercetin (positive control). Finally, as observed in Fig. 7.2.6 C, treatment with the aqueous extract resulted in the best fructose 1,6-bisphosphatase (FBP) inhibitory capabilities among all the extracts at all the tested concentrations. This was closely followed by the ethanol extract, which had the second-best fructose 1,6-bisphosphatase (FBP) inhibitory capabilities. Furthermore, the aqueous extract resulted in significantly better fructose 1,6-bisphosphatase (FBP) inhibitory capabilities than quercetin at all the tested concentrations.

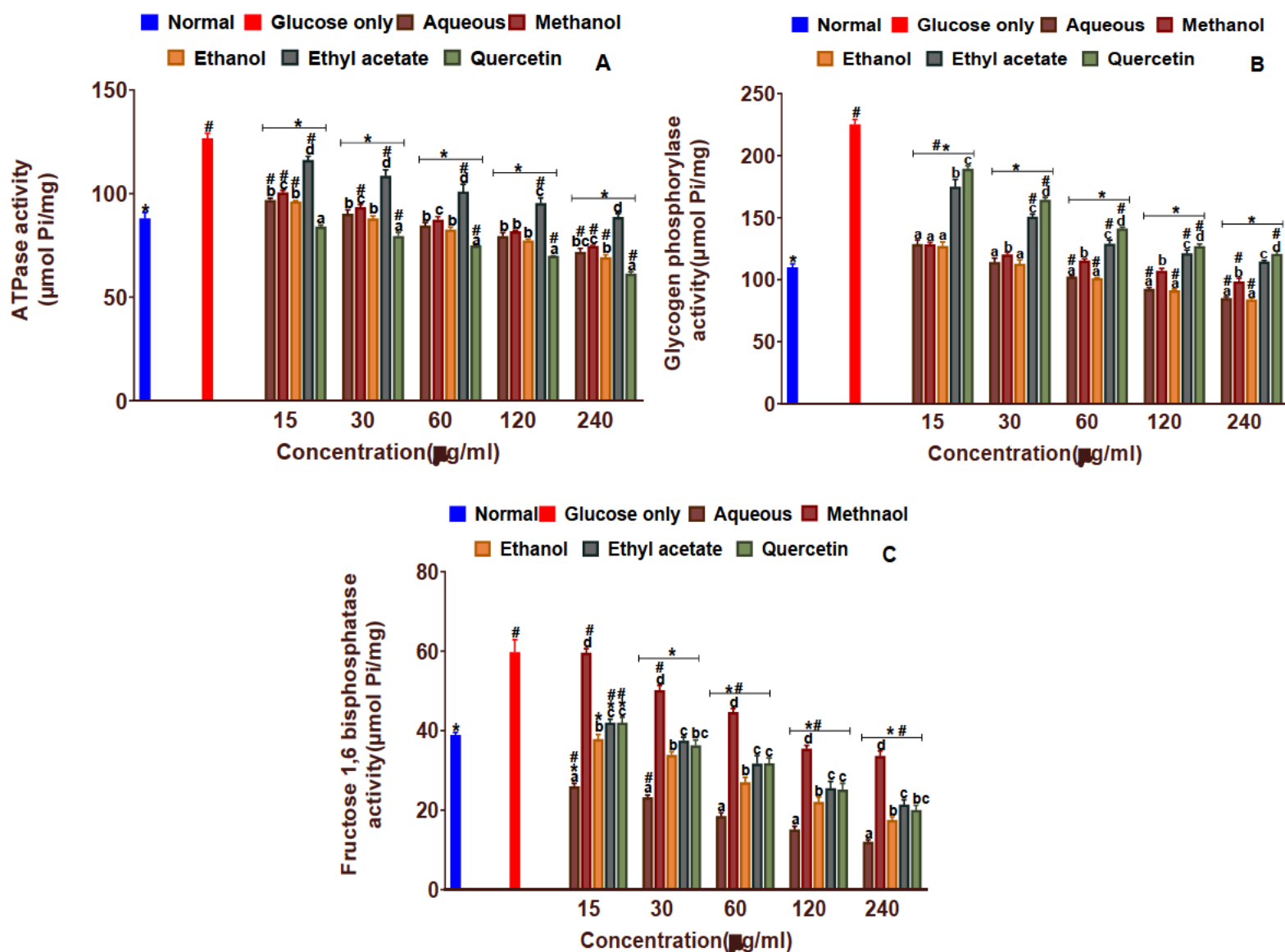


Figure 7.2.6: Effects of *S. brachypetala* leaf extracts on the activities of liver (A) ATPase, (B) Glycogen phosphorylase and (C) Fructose 1,6 biphosphatase enzymes. Data are presented as mean $\pm$ standard deviation of triplicate determinations. Different letters (a-d) above the bars for a given concentration are significantly different from each other ($p < 0.05$). *Significantly different from untreated sample and #significantly different from normal sample ($p < 0.05$).

7.2.5 Discussion

As the incidence of diabetes mellitus T2D continues to rise, there is an urgent need to develop novel antidiabetic therapeutic agents with increased efficacy and bioavailability (Forouhi and Wareham, 2019). Traditional medicinal plants have thus emerged as an alternative source of therapeutic pharmacological agent development templates against an assortment of ailments, including cancer, tuberculosis (TB), and T2D (Nazarian-Samani *et al.*, 2018). The therapeutic capabilities of folkloric traditional medicinal plants stem from specialized secondary metabolites, including phenols, flavonoids, terpenes, stilbenoids, alkaloids, coumarins, bibenzyls, phenanthrenes, anthocyanins, and polysaccharide derivatives (Teoh, 2016). Considering this, the high amounts of phenolic and flavonoid content observed for the *S. brachypetala* leaf extracts, as depicted in Fig. 7.2.1, insinuate the extracts potential as novel therapeutic antidiabetic pharmacological templates (Marella, 2017). Several studies corroborate the antidiabetic capabilities of flavonoid and phenolic compounds through their ability to inhibit α -glucosidase and α -amylase and mitigate oxidative stress via potent antioxidant activity (Vinayagam *et al.*, 2016).

Chronic hyperglycaemia promotes the production of deleterious reactive species, leading to exacerbated oxidative stress and the onset of chronic low-grade inflammation (Asmat *et al.*, 2016). Oxidative stress leads to insulin resistance and is linked to tissue damage through lipid peroxidation, consequently leading to the end-stage complications associated with T2D (Asmat *et al.*, 2016). Therefore, the ability of the *S. brachypetala* leaf extracts to reduce lipid peroxidation in the pancreatic tissue shown in Fig. 7.2.5A and scavenge free radicals (DPPH and NO) in Figs. 7.2.2A and 7.2.2B is indicative of their capabilities to abate the deleterious effects of excessive free radicals. Previous reports suggest that the extracts function by donating hydrogen protons, thereby quenching free radicals before they can react with electron-rich lipid membranes (Gulcin, 2020). Furthermore, while nitric oxide (NO) is an essential pro-inflammatory secondary messenger, when in excess, it is an exacerbating factor of chronic low-grade inflammation that is responsible for the development of diabetic complications (Khazan and Hedayati, 2015; Ghimire *et al.*, 2017). Therefore, the ability of the *S. brachypetala* leaf extracts to inhibit NO production observed in Fig. 7.2.2C insinuates their anti-hyperinflammatory capabilities. Additionally, the extracts had enhanced electron-donating capabilities, as indicated by their ability to donate electrons, and reduce the ferric ion in the FRAP assay, as observed in Fig. 7.2.2B, suggesting they may participate in sacrificial antioxidant reactions with free radicals, thereby protecting functional biomolecules (Gulcin, 2020; Zeb, 2020). Furthermore, the ability of the extracts to attenuate the activities of endogenous

defence systems implies their beneficial capacity to mop up excess free radicals that are present under conditions of oxidative stress. The extracts' capabilities to augment glutathione levels and bolster the activities of superoxide dismutase and catalase, as shown in Fig. 7.2.5B-D, further corroborate their antioxidant capacity and protective effects against pancreatic tissue injury (Zeb, 2020).

Obesity, which is an imbalance between calorie intake and expenditure, is both a risk factor and a consequence of T2D (Verma and Hussain, 2017; Malone and Hansen, 2019). Excessive ingestion of dietary fats has been implicated in the development of dyslipidaemia and obesity (Verma and Hussain, 2017). Obese individuals are predisposed to exacerbate oxidative stress and chronic low-grade inflammation (Gadde *et al.*, 2018). Therefore, the ability of the *S. brachypetala* leaf extracts in Fig.

7.2.3A to inhibit the lipid hydrolytic capacity of pancreatic lipase may be beneficial in reducing the ill effects of fatty acid metabolism. The *S. brachypetala* leaf extracts may function by modulating the β oxidation pathway, which, when elevated, exacerbates oxidative stress, leading to aggravated insulin resistance in T2D patients (Chandel *et al.*, 2018).

Carbohydrates are essential energy providers and macromolecules in the body. Complex dietary carbohydrates are successively broken down to glucose by a series of hydrolytic enzymes, including α amylase and α glucosidase (Chandel, 2021). In T2D patients, glucose absorbed through the small intestine into the blood leads to post-prandial glucose spikes and chronic hyperglycaemia (Chandel, 2021). The ability of the *S. brachypetala* leaf extracts to inhibit α glucosidase and α amylase as observed in Fig 7.2.2B-C and retard glucose absorption by the small intestine as shown in Fig. 7.2.4D is indicative of the extracts' antihyperglycemic capabilities. Indeed, several previous studies show that the inhibition of carbohydrate hydrolysing enzymes reduces the rate of carbohydrate digestion (Joshi *et al.*, 2015; Usman *et al.*, 2019). Therefore, when taken together with retarded intestinal absorption, it enables effective circulatory blood glucose clearance and circumvents postprandial hyperglycaemia spikes. The *S. brachypetala* leaf extracts may function by inhibiting SGLT1, which facilitates the transportation of glucose from the small intestine lumen into the bloodstream (Rieg and Vallon, 2018).

Blood glucose clearance by peripheral tissues is pivotal in maintaining blood glucose homeostasis (Rines *et al.*, 2016). The GLUT4 molecule is the most potent glucose transporter in insulin-responsive tissue (Rines *et al.*, 2016). Therefore, the ability of the *S. brachypetala* leaf extracts to augment glucose uptake in yeast suspension, muscle, and liver tissue observed in Fig 7.2.4 A-C may be due to their upregulating effects on different molecules involved in the GLUT4

translocation pathway, further corroborating their antihyperglycemic capabilities. Under conditions of low blood glucose, GLUT4 is found sequestered in intracellular vacuoles. An increase in blood glucose levels triggers a series of biochemical reactions that culminate in the translocation and fusion of the GLUT4 molecules, resulting in an exponential increase in glucose flux.

Blood glucose levels are tightly regulated by several interconnected organs and hormones. During feeding conditions, excess glucose is converted to glycogen and stored in the liver for use during extended fasting periods. Glycogen phosphorylase and fructose 1,6-bisphosphatase are key mediators of glycogenolysis and gluconeogenesis, both of which raise blood glucose levels (Agius, 2015; Kaur *et al.*, 2017). Therefore, hyperactivity of hepatic glycogen phosphorylase in Fig. 7.2.6B and fructose 1,6- bisphosphatase in Fig. 7.2.6C suggests heightened glycogenolysis and gluconeogenesis, insinuating aggravated hyperglycaemia as observed in T2D. Consequently, the inhibitory effects of the *S. brachypetala* leaf extracts on both hepatic glycogen phosphorylase and fructose 1,6-bisphosphatase may contribute to mitigating chronic hyperglycaemia in T2D patients. These findings corroborate previous data that highlights the therapeutic potential of agents exhibiting inhibitory activities on glucogenic hepatic enzymes as promising antidiabetic agents (Agius, 2015; Kaur *et al.*, 2017).

ATPase plays a pivotal role in energy production by converting ATP to ADP, and an inorganic phosphate molecule is essential for maintaining the body's energy redox balance and ensuring a homeostatic ATP:ADP ratio. Hyperactivity of hepatic ATPase, as observed in Fig. 7.2.6A, implies ATP depletion, indicating a shift in the homeostatic ATP:ADP ratio. Depletion of ATP in Type 2 diabetes (T2D) is associated with persistent fatigue, as tissues lack the energy needed for crucial reactions such as GLUT4 translocation and muscle contraction (Radosinska and Vrbjar, N., 2016). Therefore, the capacity of the *S. brachypetala* leaf extracts in Fig. 7.2.6A to inhibit hepatic ATPase activity suggests their potential to regulate energy redox in T2D patients with imbalanced ATP:ADP ratios.

7.2.6 Conclusion

The current study highlights the capabilities of phenolic and flavonoid bioactive phytoconstituents of the leaf extract of *S. brachypetala* to augment antioxidant defence systems and bolster radical scavenging capacity, inhibit carbohydrate hydrolysing enzymes, modulate glucose uptake and retard its absorption. Furthermore, the extracts mitigated hyperactivity of hepatic glucogenic enzymes and nucleotide metabolising enzymes. Further, *in vivo* experiments are necessary to verify these findings and elucidate a probable mode of action.

7.3 *Schotia brachypetala* (Sond) crude bark extract exhibit potent antioxidant, antihyperglycemic, and enhanced protective activities against FeSO₄ induced oxidative cardiac tissue injury

Brian K Beseni¹, Kolawole A Olofinisan¹, Erukainure L Ochuko¹ * Shahidul Md Islam¹

1.Department of Biochemistry, University of KwaZulu-Natal, Westville Campus, Durban 4000, South Africa.

***Corresponding author:** islamd@ukzn.ac.za

7.3.1 Abstract

Therefore, the current study investigated the antioxidant and antidiabetic capabilities of sequentially extracted *S. brachypetala* crude bark extracts. The total flavonoids and phenolics were quantified prior to gas-chromatography mass spectroscopy (GC-MS) analysis for the identification of the bioactive constituents in extracts. Thereafter, their antioxidant capabilities and inhibitory effects on carbohydrate and lipid-digesting enzymes were investigated *in vitro*. Thereafter, their glucose uptake-enhancing capabilities in liver and muscle tissues, together with their glucose absorption-retarding effects in small intestinal tissues, were determined using *ex vivo* experimental models. Similarly, *ex vivo* experimental models were employed to evaluate the protective effects of the extracts on oxidative cardiac tissue injury and the modulatory effects of the plant extracts on key hepatic glucogenic and nucleotide metabolizing enzymes. The binding affinities of identified compounds on carbohydrate and lipid digestive enzymes were also screened via *in silico* computational analysis. The extracts contained abundant phenolic and flavonoid content, including a myriad of compounds such as pyrogallol, O-Menthan-3-one, 2,3- diphenyl-quinoxaline, and l-(+)-Ascorbic acid 2,6-dihexadecanoate. The methanolic extract had the best electron-donating capabilities, DPPH, and NO scavenging activities. While the ethanol extract showed the best lipid and carbohydrate-inhibiting capabilities, Furthermore, 2,3-diphenyl-quinoxaline had the best binding affinities with selected lipid and carbohydrate-digesting enzymes. The ethanolic extract stimulated the best glucose uptake in the yeast suspension, muscle, and liver tissue, while it concomitantly had the best glucose absorption-retarding capability in the small intestinal tissue. The *S. brachypetala* crude bark extracts significantly reduced lipid peroxidation and augmented glutathione levels while bolstering the activities of superoxide dismutase and catalase, suggesting enhanced protective capabilities

against cardiac tissue oxidative damage. Finally, the ability of the extracts to reduce the activities of hepatic ATPase, glycogen phosphorylase, and fructose 1,6-bisphosphatase is indicative of their potential to regulate energy redox balance and reduce hepatic glucose output. These results warrant further studies using molecular and animal models to ascertain the mode of action, particularly for the ethanolic *S. brachypetala* crude bark extract.

Keywords: *S. brachypetala*, Extract, Antioxidant, Antidiabetic

7.3.2 Introduction

The prevalence of diabetes mellitus (DM) is escalating globally, with an estimated 536 million individuals currently affected (Sun *et al.*,2022). Projections indicate a further increase to 642 million cases among individuals aged 20–79 years by 2040 (Ogurtsova *et al.*,2017). DM is characterized by chronic hyperglycaemia and elevated glycosylated haemoglobin (HbA1c) levels, resulting from dysfunction in lipid, protein, and carbohydrate metabolism (Glovaci *et al.*,2019). The most common aetiologies of DM include Type 1, Type 2 (T2D), and gestational diabetes (Glovaci *et al.*,2019). Modifiable risk factors for T2D encompass aspects such as diet, smoking, alcohol consumption, and engagement in physical activity, while non-modifiable factors include genetic predisposition, age, and ethnicity (Alam *et al.*,2014). The underlying cause of T2D involves relative insulin deficiency due to impaired β pancreatic cell function and insulin resistance in insulin-responsive peripheral tissues or a combination of both (Kharroubi and Darwish, 2015). Improper insulin secretion and action result in retained glucose in the circulation, leading to the chronic hyperglycaemia observed in T2D (Kharroubi and Darwish, 2015).

Several mechanisms are implicated in the development of insulin metabolic dysfunction, including oxidative stress (Yang *et al.*,2011). Oxidative stress is an imbalance between endogenous antioxidants and reactive species (RS). Reactive species (RS) are generated under homeostatic conditions to mediate several reactions, including mitochondrial energy production, or as by-products of environmental factors such as chemical carcinogens (Yang *et al.*,2011). Under homeostatic conditions, these RS are neutralized by several antioxidant defense systems, including superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase, glutathione levels, and vitamins (Matough *et al.*,2012). When endogenous antioxidants fail to mop up excess RS, the homeostatic redox balance is negatively affected, leading to oxidative stress (Matough *et al.*,2012). The excess reactive species (RS) with unpaired electrons are known to descriptively react with functional and structural tissue moieties, including lipids, proteins,

and DNA, rendering them non-functional (Oguntibeju, 2019). The severity and appearance of DM-linked complications differ greatly among individuals depending on the type and extent of tissues. Acute DM signs include blurred vision, polyuria, weight loss or gain, polydipsia, and polyphagia (Dwivedi and Pandey, 2020). Conversely, chronic T2D-linked complications may appear over a given period of uncontrolled hyperglycaemia, including macrovascular and microvascular complications (Lotfy *et al.*, 2017). Chronic macrovascular diabetic complications include coronary artery disease (CAD), cardiovascular diseases (CVD), and atherosclerosis, while microvascular complications include neuropathy, nephropathy, and retinopathy (Papatheodorou *et al.*, 2017).

Currently, several non-pharmacological and pharmacological management strategies exist for T2D. Non-pharmacological strategies include behavioural change, physical activity engagement, and nutritional therapy (Evert *et al.*, 2019). Non-pharmacological management strategies for T2D are effective against mild forms of the condition and are additionally met with high resistance, with most patients reverting to their unhealthy behaviour (Evert *et al.*, 2019). Pharmacological therapies are prescribed for the management of more severe forms of T2D, usually in combination with non-pharmacological strategies. Major classes of oral antidiabetic pharmacological agents subdivided based on their modes of action include biguanides, sulfonylureas, meglitinides, thiazolidinedione (TZD), and α -glucosidase inhibitors (Chaudhury *et al.*, 2017). Despite their effectiveness, oral pharmacological antidiabetic agents are marred with several undesirable side effects, including flatulence, diarrhoea, hypoglycaemia, excessive weight loss or gain, and contraindications with other chronic medications.

Traditional medicinal plants are currently being investigated for the development of antidiabetic pharmacological agents that are readily available with reduced side effects. *Schotia brachypetala* of the family Fabaceae is a medium- to large tree with a myriad of therapeutic benefits against several human ailments. In the KwaZulu Natal Province, *S. brachypetala* is used to remedy heartburn and pimples (Hutchings, 1996; Coopooosamy and Naidoo, 2012). The plant is traditionally used to wash the body to reduce body swelling, which may be a result of several chronic conditions, including T2D (Van Wyk, and Gericke, 2000). The bark decoction of *S. brachypetala* is taken to treat heartburn and hangover from excessive alcoholic beverage consumption (Watt and Breyer-Brandwijk, 1962). In 2002, McGaw and collaborators recorded the antimicrobial properties of ethanolic extracts from the leaves and roots of *S. brachypetala*. The leaf extracts from *S. brachypetala* demonstrated enhanced antioxidant activity *in vitro* against

DPPH, ABTS, and FRAP. Additionally, they displayed the capability to reduce reactive oxygen species (ROS) production and mitigate hsp16.2 expression in *C. elegans* under oxidative stress conditions (Sobeh *et al.*,2016). Considering these observed traditional uses and pharmacological uses, the present study explored the antioxidant and antidiabetic properties of various crude bark extracts of *S. brachypetala* using *in vitro*, *ex vivo*, and *in silico* experimental models.

7.3.3 Materials and methods

Please refer to Chapter 2, sections 2.4.1 to 2.11.5 for detailed materials and methods.

7.3.4 Results

As shown in Table 7.3.1, the different *S. brachypetala* bark extracts contained a myriad of compounds, including pyrogallol, O-Menthan-3-one, 2,3-diphenyl-quinoxaline, and l-(+)-Ascorbic acid 2,6- dihexadecanoate, as revealed by gas chromatography mass spectrometry (GC-MS) analysis. Phytol acetate and phytol were the most common compounds among the extracts. Phytol acetate and palmitic acid, with relative abundances of 26.00% and 12.83%, respectively, were the most abundant compounds in the aqueous extract. Conversely, benzenestrol and tetradecanoic acid, which were eluted after 14.32 min and 14.99 min, respectively, had the least relative abundances among all other identified compounds in the same extract. Tetratetracontane, which had a relative abundance of 8.38%, was eluted last in the aqueous extract. Phytol acetate, 9,12,15-Octadecatrienoic acid, and phytol, with relative abundances of 34.22%, 12.54%, and 10.28%, respectively, were the most abundant constituents in the methanol *S. brachypetala* leaf extract. Conversely, 3,7,11-trimethyl-1-dodecanol was the least abundant constituent in the methanol, while eicosane was the last compound to be eluted after 24.04 min. Phytol and phytol acetate, with relative abundances of 40.52% and 7.88%, respectively, were the most abundant constituents in the ethanol extract. While ethyl behenate and pyrogallol, with relative abundances of 1.12% and 1.42%, respectively, were the least abundant constituents in the ethanol extract, Lastly, phytol and l-(+)-Ascorbic acid 2,6-dihexadecanoate, with relative abundances of 23.86% and 13.41%, respectively, were the most abundant constituents in the ethyl acetate extract. Ribitol, which was eluted first at 6.99 min, had the least relative abundance of 0.58% in the ethyl acetate extract.

Table 7.3.1: Phyto-compounds identified by Gas Chromatography Mass Spectrometry (GC-MS) in *S. brachypetala* bark extracts.

Compounds	Retention time (min)	Molecular Formula	Molecular weight (g/mol)	Relative abundance (%)
Aqueous				
Benzestrol	14.32	C ₂₀ H ₂₆ O ₂	298.40	0.26
Tetradecanoic acid	14.99	C ₁₄ H ₂₈ O ₂	228.37	0.71
Phytol acetate	15.69	C ₂₂ H ₄₂ O ₂	338.60	26.00
Phytene 2	15.74	C ₂₀ H ₄₀	280.50	2.42
Palmitic acid	17.10	C ₁₆ H ₃₂ O ₂	256.42	12.83
Ethyl behenate	17.28	C ₂₄ H ₄₈ O ₂	368.60	1.67
Phytol	18.45	C ₂₀ H ₄₀ O	296.50	2.57
7,10,13-Hexadecatrienal	18.82	C ₁₆ H ₂₆ O	234.38	7.39
9-Octadecenoic acid, ethyl ester	18.94	C ₂₀ H ₃₈ O ₂	310.50	3.03
O-Menthan-3-one	19.31	C ₁₀ H ₁₈ O	154.25	1.13
10-Methyl-E-11-tridece-1-ol acetate	19.33	C ₁₆ H ₃₀ O ₂	254.41	1.23
9-Octadecenal	20.38	C ₁₈ H ₃₄ O	266.50	0.74
1-Heptacosanol	20.92	C ₂₇ H ₅₆ O	396.70	1.81
2,3-diphenyl-quinoxaline	22.38	C ₂₀ H ₁₄ N ₂	282.30	1.06
Tetratetracontane	24.33	C ₄₄ H ₉₀	619.20	8.38
Methanol				
Butoxyethanol	4.46	C ₆ H ₁₄ O ₂	118.17	4.61
Tetradecane	10.53	C ₁₄ H ₃₀	198.39	1.46
Phytol acetate	15.68	C ₂₂ H ₄₂ O ₂	338.60	34.22
Phytene 2	15.74	C ₂₀ H ₄₀	280.50	3.87
3,7,11-trimethyl-1-dodecanol	16.57	C ₁₅ H ₃₂ O	228.41	0.74
Benzenepropanoic acid, 3-(1,1-dimethylethyl)-4-hydroxy-, methyl ester	16.71	C ₁₄ H ₂₀ O ₃	236.31	1.50

Pentadecanoic acid	17.07	$C_{15}H_{30}O_2$	242.40	7.06
Hexadecanoic acid, ethyl ester	17.28	$C_{18}H_{36}O_2$	284.50	1.28
Phytol	18.44	$C_{20}H_{40}O$	296.50	10.28
9,12,15-Octadecatrienoic acid	18.82	$C_{18}H_{30}O_2$	278.40	12.54
Eicosane	24.04	$C_{20}H_{42}$	282.50	2.09
Ethanol				
Ethanol, 2-butoxy-	4.32	$C_6H_{14}O_2$	118.17	7.66
1,2,3-Propanetriol, 1-acetate	6.63	$C_5H_{10}O_4$	134.13	4.50
Pyrogallol	11.30	$C_6H_6O_3$	126.11	1.42
Phytol acetate	15.68	$C_{22}H_{42}O_2$	338.60	7.88
Phytene 2	15.74	$C_{20}H_{40}$	280.50	1.58
n-Hexadecanoic acid	17.10	$C_{16}H_{32}O_2$	256.42	2.04
Hexadecanoic acid, ethyl ester	17.28	$C_{18}H_{36}O_2$	284.50	4.52
Phytol	18.47	$C_{20}H_{40}O$	296.50	40.52
9,12,15-Octadecatrienoic acid	18.83	$C_{18}H_{30}O_2$	278.40	2.32
Octadecanoic acid	18.99	$C_{18}H_{36}O_2$	284.50	2.43
Ethyl behenate	19.17	$C_{24}H_{48}O_2$	368.60	1.12
Ethyl acetate				
Ribitol	6.99	$C_5H_{12}O_5$	152.15	0.58
2-Oxazolidinethione	8.65	C_3H_5NOS	103.15	3.07
1,2,3-Propanetriol, 1-acetate	8.69	$C_5H_{10}O_4$	134.13	3.47
Heptadecane	13.03	$C_{17}H_{36}$	240.50	1.15
Hexestrol	14.94	$C_{18}H_{22}O_2$	270.40	0.86
Acetic acid, 2-(2,2,6-trimethyl-7-oxa-bicyclo[4	15.39	$C_{14}H_{22}O_3$	238.32	0.81
Phytol acetate	15.69	$C_{22}H_{42}O_2$	338.60	8.25
6,10,14-trimethylpentadecan-2-one	15.77	$C_{18}H_{36}O$	268.50	1.07

l-(+)-Ascorbic acid 2,6-dihexadecanoate	17.36	$C_{38}H_{68}O_8$	652.90	13.41
Phytol	18.54	$C_{20}H_{40}O$	296.50	23.86
Dichloroacetic acid, tridec-2-ynyl ester	18.98	$C_{15}H_{24}Cl_2O_2$	307.30	9.98
Octadecanoic acid	19.13	$C_{18}H_{36}O_2$	284.50	4.27

As shown in Fig. 7.3.1A, the aqueous extract contained significantly ($p<0.05$) the highest amounts of phenolics, 34.15 mg/g gallic acid equivalent. This was followed by the methanol and ethyl acetate extracts, which contained 26.81 and 26.99 mg/g gallic acid equivalent, respectively, with no significant ($p>0.05$) difference. The ethanol extract contained significantly ($p<0.05$) the least amounts of phenolics (23.24 mg/g gallic acid equivalent). While in Fig. 7.3.1B, the ethanol extract contained significantly ($p<0.05$) the highest amounts of flavonoids (29.19 mg/g gallic acid equivalent), it was not significantly different from the aqueous 26.17 mg/g gallic acid equivalent extract that closely followed. The ethyl acetate 23.34 mg/g gallic acid equivalent extract contained the third most flavonoids, which was significantly different from the ethanol 21.09 mg/g gallic acid equivalent extract, which had the least flavonoid content among all the extracts.

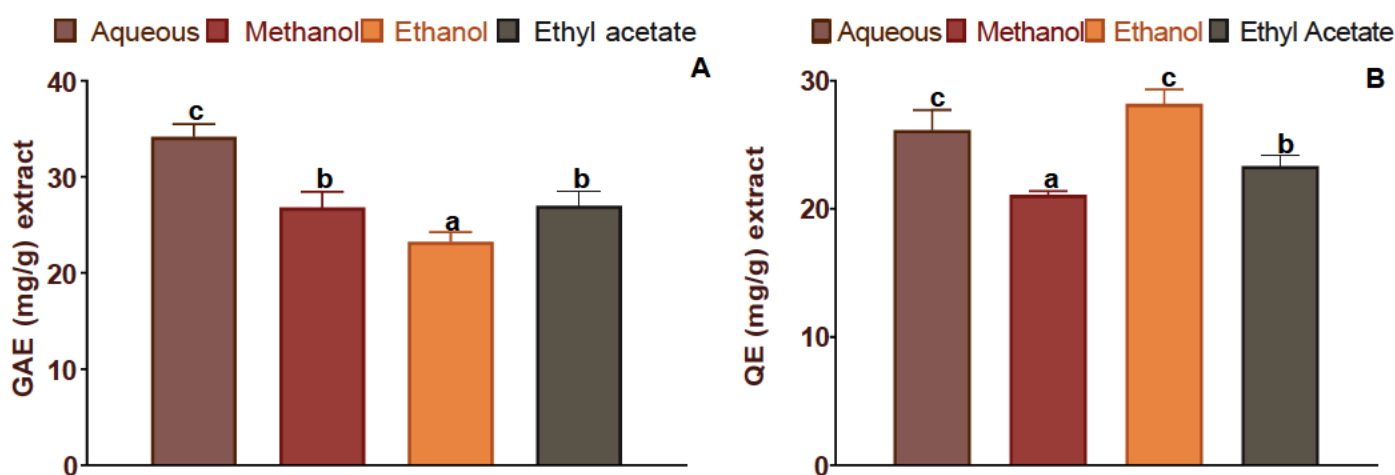


Figure 7.3.1: The total phenolic content of the *S. brachypetala* crude bark extracts represented as **A)** Gallic acid equivalents (GAE mg/g) and the total flavonoid content represented as **B)** Quercetin equivalents (QE mg/g). Bars with superscripts a-d for each extract are statistically different at $p<0.05$ level of significance.

As shown in Fig. 7.3.2A, the methanol bark extract of *S. brachypetala* exhibited the best DPPH scavenging activity among all extracts at all concentrations except 15 $\mu\text{g/ml}$. The methanol had the lowest IC_{50} value of $42,29\pm0,27$ $\mu\text{g/ml}$, which was comparable to that of vitamin C, which was the positive control ($9,21\pm0,12$ $\mu\text{g/ml}$). The aqueous extract had the second-best DPPH scavenging activity, especially at the highest concentrations (120–240 $\mu\text{g/ml}$). As shown in Fig. 7.3.2 B, the methanol bark extract exhibited the best electron-donating capability, followed closely by the aqueous extract with IC_{50} values of $12,60\pm0,96$ $\mu\text{g/ml}$ and $13,84\pm0,58$, respectively. The ethyl acetate extract had the least capacity to donate electrons among all the extracts. As depicted in Fig. 7.3.2 C, the methanol extract exhibited the best nitric oxide scavenging activity, followed by the aqueous extract with IC_{50} values of

239,72±0,49 µg/ml and 459,16±0,67 µg/ml, respectively. The ethyl acetate extract had the least capacity to scavenge nitric oxide among all the extracts.

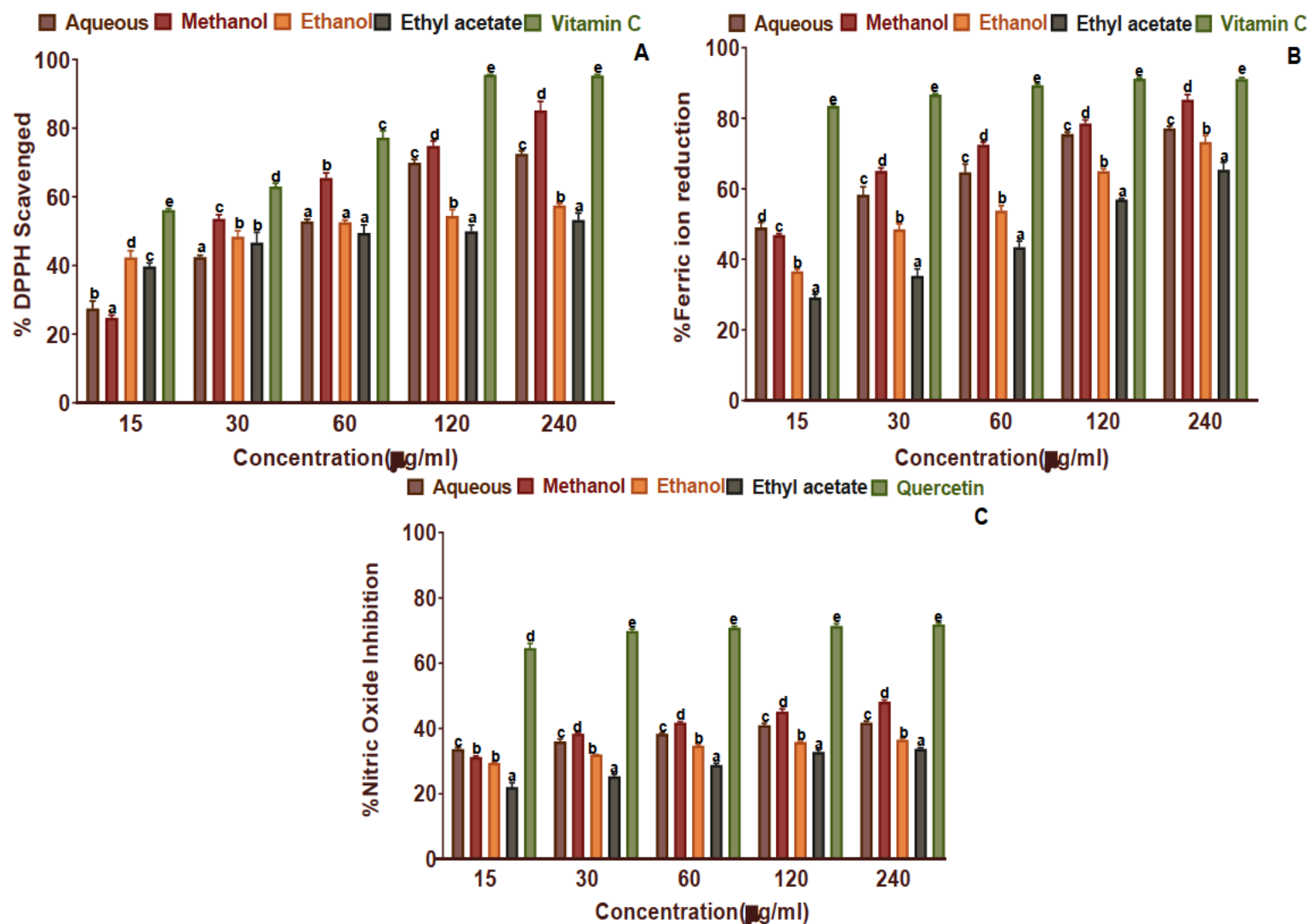


Figure 7.3.2: *S. brachypetala* bark extracts (A) DPPH scavenging (B) ferric reduction and (C) nitric oxide scavenging activities. Bars with superscripts a-e for each concentration are statistically different at $p<0.05$ level of significance.

As depicted in Fig. 7.3.3 A, the aqueous extract exhibited significantly ($p<0.05$) better pancreatic lipase inhibitory capacity at all the tested concentrations except at 240 µg/ml, where there was no significant ($p>0.05$) difference with the ethanolic extract. Despite the aqueous and ethanol extras exhibiting comparably the best lipase inhibitory activity with IC_{50} values of 329,09±0,25 µg/ml and 339,11±0,09 µg/ml, they did not, however, surpass that of orlistat (the positive control), which had an IC_{50} value of 114,52±0,83 µg/ml. The ethyl acetate extract had the highest IC_{50} value of 446,31±0,75 µg/ml, showing that it had the least lipase inhibitory capacity among all the tested extracts. As depicted in Fig 7.3.3 B, the ethanolic extract showed the best α glucosidase inhibitory activity among all the extracts, closely followed by the aqueous extract

with IC_{50} values of $160,32 \pm 0,72 \mu\text{g/ml}$ and $175,97 \pm 0,23 \mu\text{g/ml}$, respectively. The ethanol extract showed comparable α glucosidase inhibition to that of acarbose (positive control), which had an IC_{50} value of $100,68 \pm 0,09 \mu\text{g/ml}$. Similarly, there was no significant ($p > 0.05$) difference in the α glucosidase activities of the methanol and ethyl acetate extracts at all the tested concentrations. In Fig. 7.3.3 C, the ethanol extract had significantly higher α -amylase inhibitory activity than all the extracts at all the tested concentrations. Furthermore, the ethanolic extract had the overall best α -amylase inhibitory capacity among all the extracts, with an IC_{50} value of $187,63 \pm 0,95 \mu\text{g/ml}$ that was comparable to that of acarbose (positive control). Except at the lowest (15 $\mu\text{g/ml}$) and the highest concentration (240 $\mu\text{g/ml}$), there was no significant difference in the α -amylase inhibitory activities of the aqueous and methanol extracts. However, the methanol extract had a slightly lower IC_{50} value ($199,60 \pm 0,56 \mu\text{g/ml}$) than the aqueous extract ($216,57 \pm 0,79 \mu\text{g/ml}$). The ethyl acetate extract showed the least α -amylase inhibitory activity among all tested extracts.

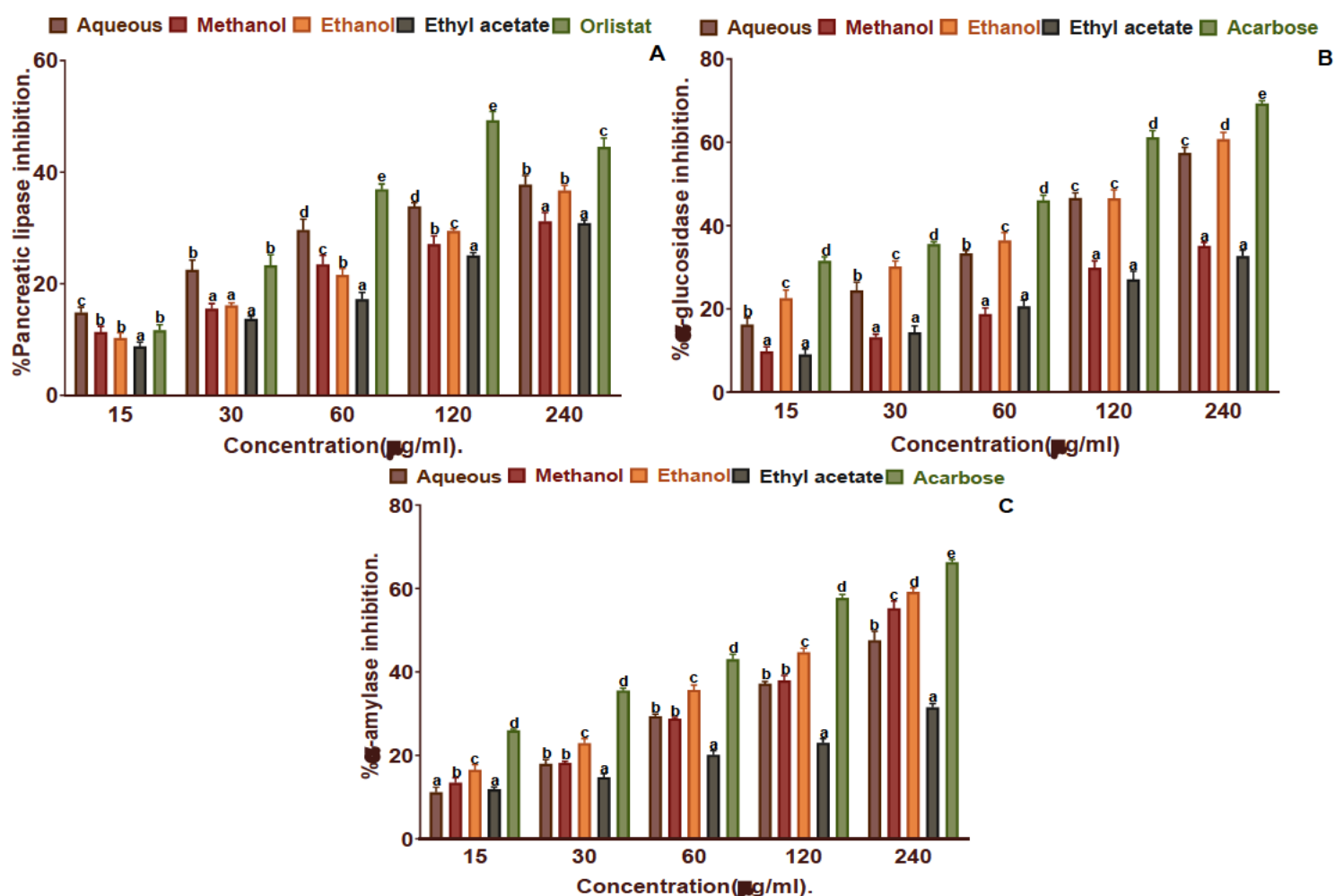


Figure 7.3.3: *S. brachypetala* bark extracts (A) pancreatic lipase (B) α -glucosidase (C) α -amylase inhibitory activities. Bars with superscripts a-e for each concentration are statistically different at $p < 0.05$ level of significance.

Table 7.3.2: IC₅₀ values of *in vitro* biological activities of *S. brachypetala* bark extracts.

Activities	Aqueous (µg/ml)	Methanol (µg/ml)	Ethanol (µg/ml)	Ethyl acetate (µg/ml)	Vitamin C (µg/ml)	Quercetin (µg/ml)	Acarbose (µg/ml)	Orlistat (µg/ml)
DPPH	56,29±0,25	42,29±0,27	74,82±0,56	142,76±0,17	9,21±0,12	-	-	-
FRAP	13,84±0,58	12,60±0,96	44,14±0,19	91,08±0,27	6,67±0,88	-	-	-
NO	459,16±0,67	239,72±0,49	710,30±0,34	547,12±0,47	-	1,17±0,58	-	-
Pancreatic lipase	329,09±0,25	435,89±0,26	339,11±0,09	446,31±0,75	-	-	-	114,52±0,83
α-glucosidase	175,97±0,23	348,30±0,66	160,32±0,72	353,40±0,89	-	-	100,68±0,09	-
α-amylase	216,57±0,79	199,60±0,56	187,63±0,95	456,55±0,26	-	-	118,57±0,97	-

Data is presented as mean ± standard deviation. Key: DPPH = 2,2-diphenyl-1-picrylhydrazyl. FRAP = Ferric reducing antioxidant power , NO = Nitric oxide assay.

As depicted in Fig. 7.3.4 A, the extract showed a dose-dependent glucose uptake stimulatory effect in the yeast suspension. The ethanol extract exhibited the best yeast suspension glucose uptake among all the extracts. This was followed by the aqueous extract. At the highest concentrations of 120 µg/ml to 240 µg/ml, all the back extracts of *S. brachypetala* significantly outperformed the yeast suspension glucose uptake stimulatory effect of metformin (positive control). As shown in Fig. 7.3.4 B, the ethanolic extract exhibited the best muscular glucose uptake stimulatory activity, only closely followed by the aqueous extract at the highest concentration of 240 µg/ml. There was no significant difference between the muscular glucose uptake stimulatory capabilities of both the aqueous and ethanol extracts and those of the insulin (positive control). A similar trend was observed in Fig. 7.3.4 C, in which the ethanol extract outperformed all other extracts in its ability to stimulate hepatic glucose uptake. The hepatic glucose uptake stimulatory activities of the ethanol were closely followed by those of the aqueous extract. At the highest concentration of 240 µg/ml, there was no significant difference between the insulin and ethanol hepatic tissue glucose uptake stimulatory activities. Finally, as observed in Fig. 7.3.4 D, the *S. brachypetala* extracts showed a dose-dependent glucose absorption-retarding effect on rat intestinal jejunum. The methanol and aqueous extracts had the best glucose absorption-retarding capabilities among all extracts, including acarbose, at all the tested concentrations, especially at 240 µg/ml. The ethanol extract exhibited the least intestinal glucose absorption-retarding capability among all the extracts at all the tested concentrations.

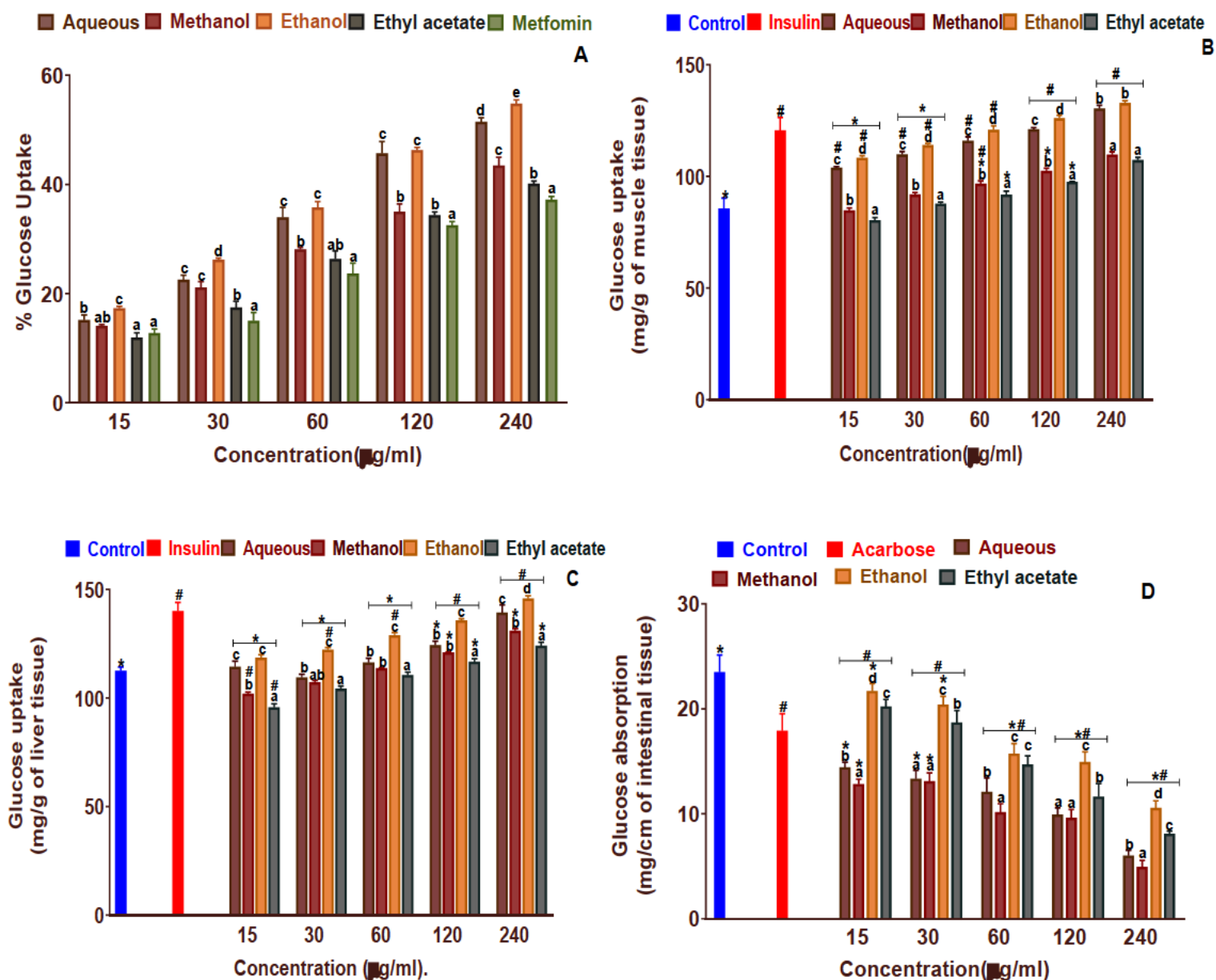


Figure 7.3.4: The effects of *S. brachypetala* bark extracts on (A) glucose uptake in yeast suspension (B) glucose uptake in excised rat psoas muscle (B) glucose uptake in excised rat liver (D) glucose absorption in the intestinal jejunum. Bars with superscripts a-b are statistically different at $p < 0.05$ whereas those with * and # are different from the acarbose/insulin treatment positive and control respectively.

FeSO-4-induced oxidative cardiac tissue injury significantly ($p<0.05$) elevated lipid peroxidation while concomitantly suppressing superoxide dismutase (SOD) and catalase (CAT) activities, as well as glutathione (GSH) levels in the untreated control group, as shown in Fig. 7.3.5 A-D. Treatment with the aqueous, methanol, and ethanol extracts resulted in reduced MDA equivalents, implying comparable lipid peroxidation inhibitory capabilities across all the tested concentrations. Indeed, the aqueous and methanol extracts did not show a significant difference in MDA equivalents with the normal control, highlighting their effective lipid peroxidation capabilities. Furthermore, these extracts had significantly ($p<0.05$) better lipid peroxidation inhibitory capabilities when compared to vitamin C (positive control). The ethyl acetate had the least lipid peroxidation inhibitory activity among all the extracts but was comparable to that of vitamin C at the highest concentration of 240 $\mu\text{g/ml}$. As shown in Fig. 7.3.5 B, treatment with the aqueous and ethanol extracts restored normal SOD activity, particularly at the highest concentrations of 120 $\mu\text{g/ml}$ to 240 $\mu\text{g/ml}$. Furthermore, these extracts showed comparable activity at all tested concentrations and resulted in significantly higher SOD activity than vitamin C. Evidently, the ethyl acetate extracts had the least SOD-stimulating activity among all extracts. However, in Fig. 7.3.5 C, the aqueous extract resulted in significantly the highest CAT activity among all the extracts. For all the tested concentrations, only the ethyl acetate extract failed to surpass the CAT stimulatory capabilities of vitamin C at concentrations of 60 $\mu\text{g/mL}$ and below. Finally, a similar trend was observed in Fig. 7.3.5 D, whereby treatment with the aqueous extract significantly increased GSH levels. The GSH level augmentation capabilities of the aqueous extract were closely followed by treatment with the ethanol extract, especially at the highest concentration of 240 $\mu\text{g/ml}$. Treatment with the aqueous and ethanol extracts resulted in near-normal GSH levels with no significant ($p>0.05$) difference from those of the normal tissue. Furthermore, these two extracts further surpassed the GSH-stimulating activity of vitamin C, which was the positive control.

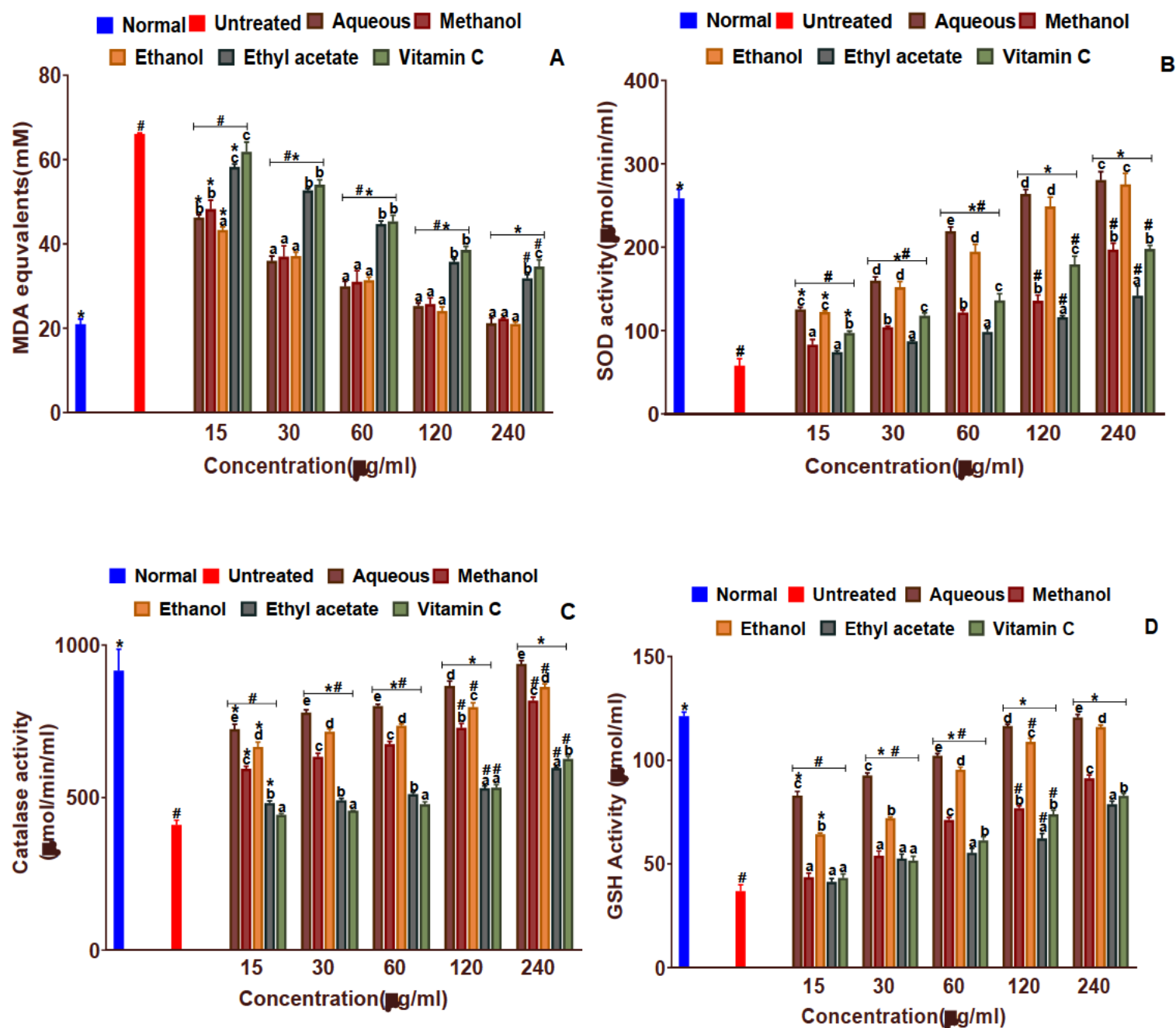


Figure 7.3.5: Effects of *S. brachypetala* bark extracts on (A) lipid peroxidation, (B) SOD activity, (C) Catalase activity and (D) GSH activity in oxidative cardiac muscle injury. Data are presented as mean $\pm$ standard deviation of triplicate determinations. Different letters (a-e) above the bars for a given concentration are significantly different from each other ($p < 0.05$). *Significantly different from untreated sample and #significantly different from normal sample.

As shown in Figs. 7.3.6 A-C, hyperglycaemic toxicity significantly ($p < 0.05$) elevated hepatic ATPase, glycogen phosphorylase, and fructose 1,6-bisphosphatase activities in the untreated group when compared to the normal group. However, as shown in Fig. 7.3.6 A, treatment with the aqueous and methanol bark extracts of *S. brachypetala* significantly ($p < 0.05$) reduced hepatic ATPase activity in a dose-dependent manner. While the aqueous and methanol extracts exhibited comparable hepatic ATPase inhibitory activities across all the tested concentrations, they also showed comparable activity to quercetin. Polarity-dependent inhibitory capabilities on hepatic glycogen phosphorylase were observed in Fig. 7.3.6 B, whereby the aqueous extract (mostly polar) showed the best activity, followed by the methanol ethanol and the ethyl acetate extract, respectively. Evidently, the bark extracts of *S. brachypetala* significantly ($p < 0.05$) reduced hepatic glycogen phosphorylase activity to levels lower than the untreated tissue at all tested concentrations. Finally, as observed in Fig. 7.3.6 C, the aqueous and ethanol extracts showed comparable fructose-1,6-bisphosphatase inhibitory activity at all the assayed concentrations. Similarly, the methanol extract had comparable activity to the quercetin (positive control). The aqueous and ethanol extracts showed the best fructose-1,6-bisphosphatase inhibitory activity among all the extracts. Only the ethyl acetate extracts, which had the least fructose 1,6-bisphosphatase inhibitory activity, failed to surpass that of quercetin. However, the ethyl acetate extract had lower fructose 1,6-bisphosphatase activity capability than the untreated tissue.

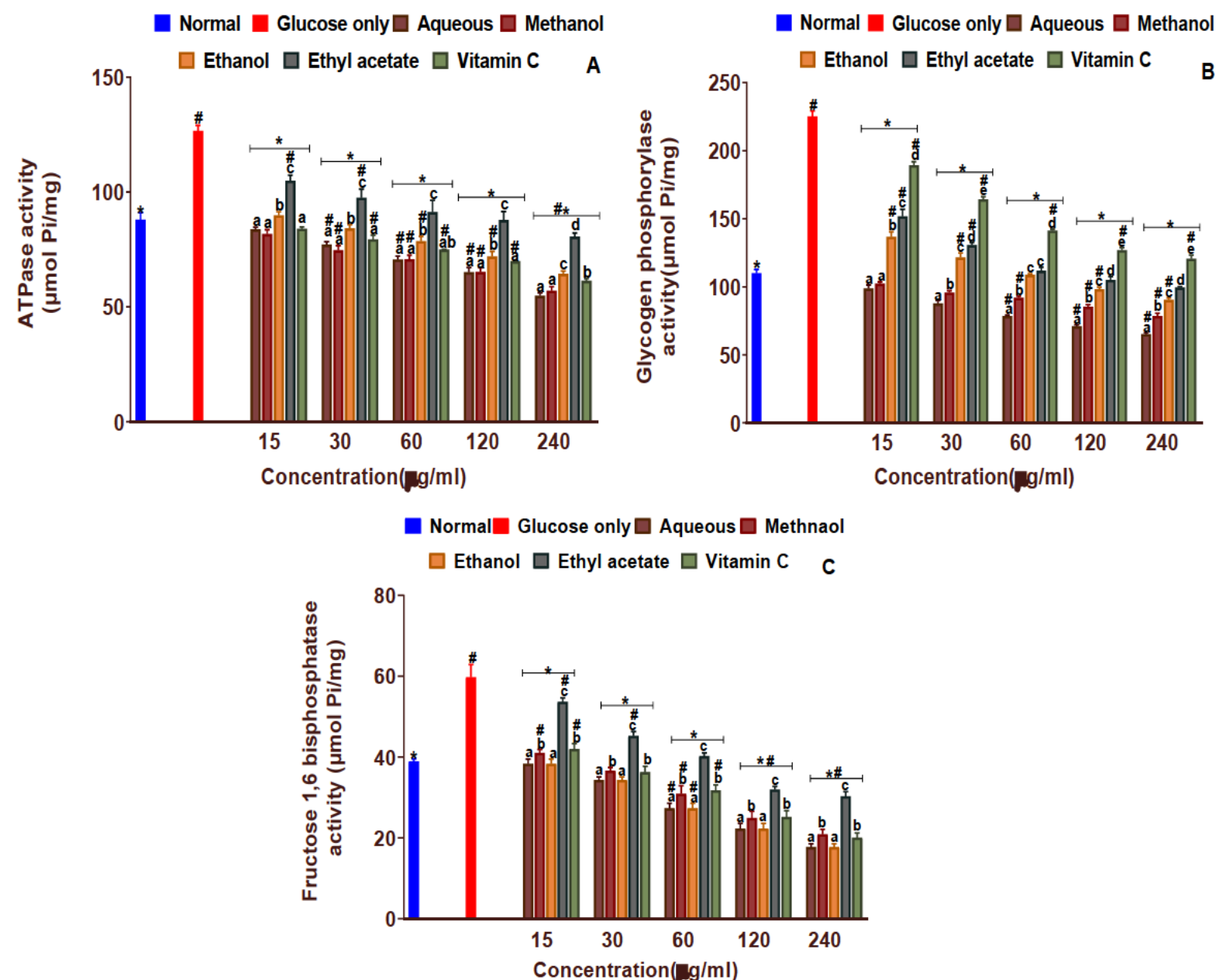


Figure 7.3.6: Effects of *S. brachypetala* bark extracts on the activities of hepatic (A) ATPase, (B) Glycogen phosphorylase and (B) Fructose 1,6 bisphosphatase enzymes. Data are presented as mean $\pm$ standard deviation of triplicate determinations. Different letters (a-d) above the bars for a given concentration are significantly different from each other ($p < 0.05$). *Significantly different from untreated sample and #significantly different from normal sample ($p < 0.05$).

The data of the free binding energy of secondary metabolites from *S. brachypetala* bark extracts with carbohydrate and lipid digestive enzymes from the *in silico* analysis is presented in Fig. 7.3.7 and Table 7.3.3. Among the phytochemicals from *S. brachypetala* bark

extracts, 2,3-diphenyl-quinoxaline had the lowest binding affinities with α -amylase (-8.4 kcal/mol) and α -glucosidase (-9.3 kcal/mol). The 2,3-diphenyl-quinoxaline pancreatic-lipase complex which had a binding affinity score of -9.5 kcal/mol was stabilized by a hydrophobic Pi-Pi stacking interaction between one of the benzene rings and HIS 263. The complex was further stabilized by Pi-cation and Pi-Pi stacking interactions with another the benzene ring of 2,3-diphenyl-quinoxaline as seen in Fig. 7.3.7A. as seen in Fig. 7.3.7B the 2,3-diphenyl-quinoxaline to α -glucosidase was stabilized by Pi-Pi stacking interaction between the first benzene ring and HIS 201. Furthermore, the second benzene ring on 2,3-diphenyl-quinoxaline formed a Pi-Pi stacking with HIS 201 and an H donor interaction with GLU 233. The third benzene ring formed Pi-Pi stacking interaction with TRY 62 further stabilizing the complex. Finally, as seen in Fig. 7.3.7C the 2,3-diphenyl-quinoxaline- α -amylase complex was stabilized by several interactions. The first 2,3-diphenyl-quinoxaline benzene ring formed a Pi-Pi stacking interaction with PHE 1559 on α -amylase. The second benzene ring formed 3 Pi-Pi stacking bonds with TYR 1251, PHE 1559 and TRP 1355. Finally, the last ring formed a Pi-cation bond with ARG1510.

Table 7.3.3: Free binding energy of *S. brachypetala* bark extracts secondary metabolites with carbohydrate and lipid digestive enzymes.

Ligand	Free energy of binding (kcal/mol)		
	α -Glucosidase	α -Amylase	Pancreatic lipase
Acarbose	-7.5	-7.8	-
Orlistat	-	-	-6.7
2-methoxy-4-vinyphenol	-6.4	-5.5	-6.1
Glycerin	-4	-3.9	-3.9
Palmitic acid	-5.2	-6.1	-6.2
Dibutyl phthalate	-6.7	-5.7	-6.9
Octadecanoic acid	-5.2	-5.3	-6.2
Benzestrol	-7.7	-7.9	-7.7
Ribitol	-5.3	-5.2	-4.9
2-methybutanal	-4.3	-3.6	-3.7
Butoxyethanol	-4.6	-3.9	-4.1
1,2,3-Propanetriol, 1-acetate	-4.9	-4.6	-5.2

Diisooctyl phthalate	-7.5	-6.5	-7.1
2,3-diphenyl-quinoxaline	-9.3	-8.4	-9.5
1-Heptacosanol	-5.8	-5.2	-5.8
Phytene 2	-5.1	-6.2	-6.5
7,10,13-Hexadecatrienal	-5.9	-5.3	-6.4
Benzenepropanoic acid, 3-(1,1-dimethylethyl)-4-hydroxy-, methyl ester	-7.2	-6.8	-7.2
Phytol	-6.4	-6	-6.6
9,12,15-Octadecatrienoic acid	-5.8	-5.6	-7
9-Octadecenal	-5.6	-5	-5.9
Acetic acid, 2-(2,2,6-trimethyl-7-oxa-bicyclo [4])	-6.2	-7.2	-6.6
9-Octadecenoic acid, ethyl ester	-5.4	-5.3	-6.3
Phytol acetate	-5.9	-5.8	-6.9
l-(+)-Ascorbic acid 2,6-dihexadecanoate	-6.5	-6.0	-5.9

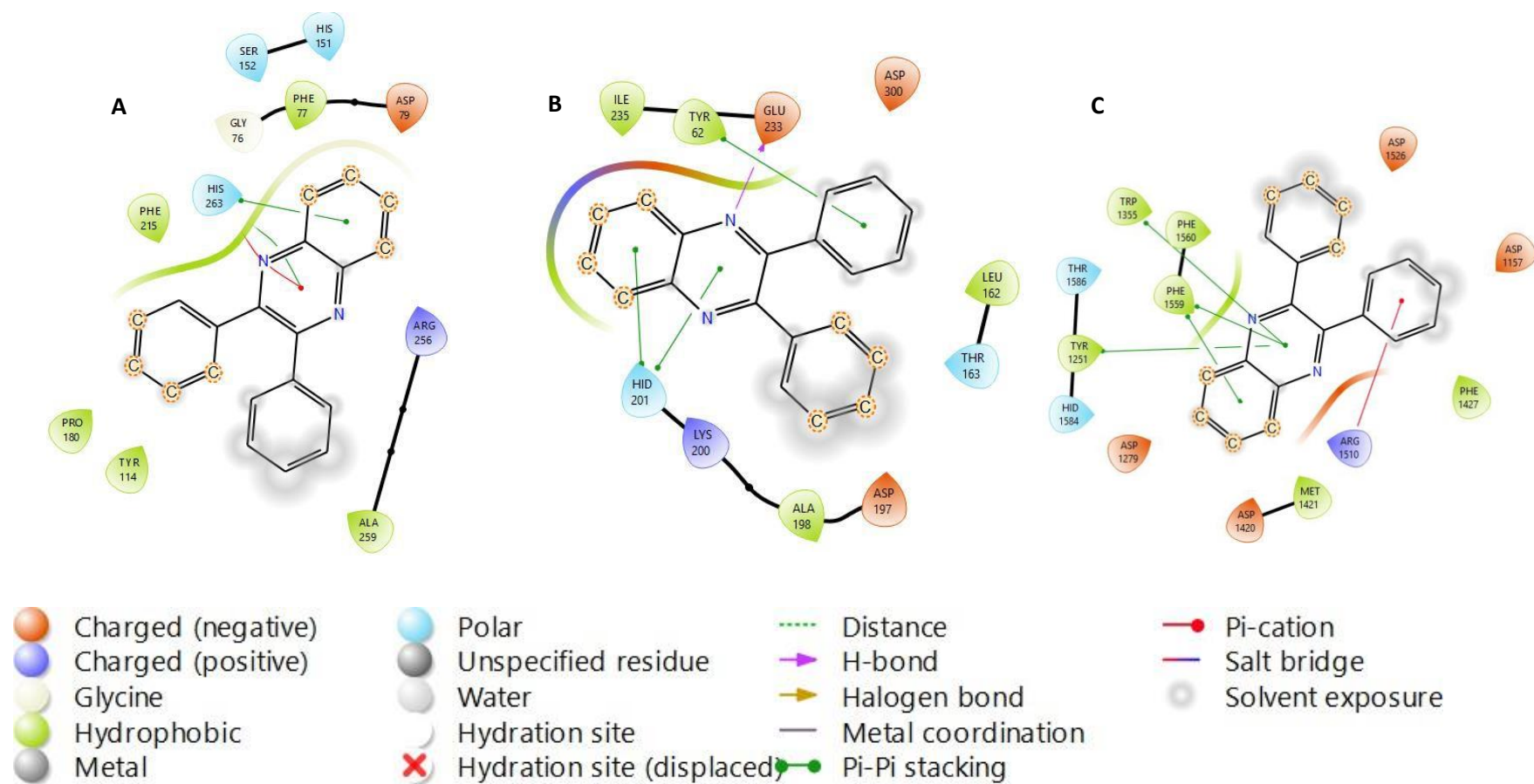


Figure 7.3.7: 2D images of the molecular interactions between 2,3-diphenyl-quinoxaline and active site amino acid residues of (A) pancreatic-lipase (B) α -glucosidase (C) α -amylase.

7.3.5 Discussion

The current global and regional epidemiology data regarding type 2 diabetes (T2D) projects a concerning upward trajectory in its incidence if current preventative and management strategies are not revised (Sun *et al.*,2022). In response, scientists across the globe have intensified their efforts toward the development of cost-effective novel antidiabetic drug templates with increased bioavailability, high efficacy, and reduced side effects (Dowarah and Singh, 2020). Folkloric traditional medical plans have emerged as lucrative reservoirs of bioactive compounds with immense therapeutic capabilities against numerous human ailments, including tuberculosis, cancer, and T2D (Jacob and Narendhirakannan, 2019).

Numerous studies have previously reported the diverse health benefits associated with plant-derived secondary metabolites, including their antioxidant, anti-inflammatory, antimicrobial, anthelmintic, anticoagulant, antidiabetic, and lipid-lowering properties (Teoh, 2016). Phenolics, alkaloids, tannins, and flavonoids are among the most prominent groups of secondary metabolites from which bioactive compounds with therapeutic benefits against several ailments are derived (Tiwari and Rana, 2015). Therefore, the abundant total phenolic and flavonoid content in Fig. 7.3.1, coupled with the array of phytochemicals revealed by GCMS analysis in Table 7.3.1 in the *S. brachypetala* bark extracts, insinuates their potential to be developed into novel antidiabetic agents. This observation is coherent with previous studies that indicate several bioactive phyto-constituents, such as fisetin, a dietary flavonoid, which can reduce hyperglycemia through improved plasma insulin levels and increased antioxidant enzyme capacity (Prasath *et al.*,2013).

Oxidative stress ensues due to an imbalance between intrinsic antioxidant defense systems and excessive reactive oxygen species (ROS) such as superoxide (O₂⁻), hydroxyl (OH), and peroxy (RO₂) (Yaribeygi *et al.*,2020). Aggravated oxidative stress under chronic hyperglycaemic conditions in T2D is implicated in the structural disruption of lipids, DNA, and proteins, impeding their function (Yaribeygi *et al.*,2020). Impaired function of these essential biomolecules leads to tissue degeneration and the manifestation of chronic complications associated with T2D. Cardiac tissue damage is implicated in the development of several diabetic-linked complications, such as coronary artery disease (CAD), arrhythmias, myocardial infarction (heart attack), and diabetic cardiomyopathy, which can eventually lead to heart failure (Miki *et al.*,2013). Therefore, the ability of the *S. brachypetala* bark extracts to reduce lipid peroxidation and augment glutathione levels while increasing the activities of

superoxide dismutase and catalase displayed in Figure 7.3.5 is indicative of the extract's potential to abate oxidative cardiac tissue injury and reduce systolic or diastolic dysfunction that leads to diabetic cardiomyopathy in T2D patients (Shah and Brownlee, 2016). Furthermore, the current data suggests that the extracts can reduce oxidative stress through increased radical scavenging (DPPH and NO). Fig. 7.3.2 and electron-donating capabilities (FRAP) Fig. 7.3.2. Phenolic and flavonoid compounds, as observed in Fig. 7.3.1, are endowed with an abundant valency shell. π electrons can easily donate this electron cloud and therefore participate in sacrificial reactions with reactive species, thereby protecting functional biomolecules (Gutiérrez-Grijalva *et al.*,2016). Previous reports have documented the capabilities of flavanones such as hesperidin to reverse hyperglycemia and hyperlipidemia through downregulation of free radical generation in a diabetic animal model (Visnagri *et al.*,2014). Maintenance of homeostatic redox balance has indeed been shown to improve glycemic control through improved regulation of several processes involved in glucose metabolism, including improved insulin sensitivity in peripheral tissues (Lankatillake *et al.*,2019).

Various enzymes participate in the successive hydrolysis of complex dietary carbohydrates through a multistep process along the digestive tract (Brewer *et al.*,2016). Key among these carbohydrate-digesting enzymes are α -amylases, present in the mouth and secreted by pancreatic acini cells. Additionally, α -glucosidases, primarily located at the intestinal brush border, play a crucial role in catalyzing the final step of carbohydrate digestion before assimilation into the blood as glucose (Ćorković *et al.*,2022). Moreover, the absorption of glucose by intestinal tissues occurs through facilitated diffusion, mediated by SGLT1 found on the enterocytes of the small intestines. Elevated hydrolysis of complex carbohydrates coupled with an increased absorption rate in intestinal tissue contributes to postprandial blood glucose spikes, particularly in T2D patients with impaired glucose metabolic machinery. Therefore, the ability of the *S. brachypetala* bark extracts to inhibit α glucosidase and α amylase as shown in Fig. 7.3.3 B and Fig. 7.3.3 C, together with their enhanced capacity to retard the glucose absorption rate in the small intestines as illustrated in Fig. 7.3.4 D, insinuates their antihyperglycemic capabilities in abating postprandial blood glucose spikes in T2D patients. Furthermore, quinoxaline derivatives were shown to be the most potent enzyme inhibitors, as revealed by *in silico* analysis, as seen in Fig. 7.3.7. Previous reports show the diverse pharmacological capabilities of several quinoxaline derivatives, including their potent α amylase and α glucosidase capabilities (Hameed *et al.*,2022). Previous reports show several

synthetic oral pharmacological agents currently on the market, such as acarbose and miglitol, that are based on their inhibitory activities against carbohydrate hydrolytic enzymes (Dirir *et al.*,2022). Furthermore, the capability of the extracts to retard glucose absorption in intestinal tissue may stem from their capacity to slow down gastric emptying through amylin-like activity, the release of glucagon-like peptide-1 (GLP-1), or inhibiting SGLT1 activity (Alam *et al.*,2019; Rtibi *et al.*,2019). Indeed, reduced intestinal glucose absorption rates allow for effective blood glucose clearance through disposal in peripheral tissues.

Glucose disposal through several peripheral tissues, including skeletal muscle tissue, adipose tissues, the brain, and the liver, constitutes an integral glucose homeostasis mechanism (Ho *et al.*,2017; Chadt and Al-Hasani, 2020). Indeed, glucose is termed the energy currency of the body as it is the main energy-provider molecule being converted to ATP, which drives energy-requiring reactions in the body (Chadt and Al-Hasani, 2020). The brain selectively uses glucose as its major energy molecule, while any excess glucose is polymerised and stored in the liver and muscle tissues as glycogen through the process of glycogenesis for later use (Hajiaghaalipour *et al.*,2015; Ho *et al.*,2017). Therefore, the ability of *S. brachypetala* bark extracts to increase glucose uptake in yeast suspension, liver, and psoas muscle shown in Figure 7.3.4 A-C insinuates their antihyperglycemic capacity through increased glucose disposal. Indeed, dysfunctional glucose disposal mechanisms linked to insulin resistance are implicated in chronic post-prandial glucose spikes in T2D.

Under fed conditions, any excess glucose is polymerized and stored as glycogen in the muscle and liver tissues or as fatty acids in the adipose tissue. During intermittent fasting, hepatic glycogen stores are utilized to maintain homeostatic blood glucose levels through glycogenolysis mediated by several enzymes, including glycogen phosphorylase (Bergman *et al.*,2019). However, in T2D, hyperactivity of glycogen phosphorylase and fructose 1,6-bisphosphatase, as observed in Figs. 7.3.6B and 7.3.6C, exacerbates hyperglycemia by upregulating glycogenolysis and gluconeogenesis. Therefore, the ability of the *S. brachypetala* bark extracts to reduce the activities of hepatic glycogen phosphorylase and fructose 1,6-bisphosphatase insinuates their potential to reduce exacerbated hepatic glucose output, therefore mitigating hyperglycemia in T2D patients. Previous studies have reported on the potential antihyperglycemic capabilities of pharmacological agents that can inhibit the activities of hepatic glycogen phosphorylase and fructose 1,6-bisphosphatase (Donnier-Maréchal and Vidal 2016). Notably, pharmacological agents inhibiting fructose 1,6-bisphosphatase provide an additional benefit by inhibiting gluconeogenesis from all

gluconeogenic substrates while avoiding direct effects on glycogenolysis, glycolysis, and the tricarboxylic acid cycle (Kaur *et al.*, 2017). ATPase, an essential nucleotide-metabolizing enzyme, plays a critical role in energy production by converting ATP to ADP and an inorganic phosphate molecule (Radosinska and Vrbjar, N., 2016). Therefore, hyperactivity of ATPase as observed in Fig. 7.3.6A suggests ATP depletion. The ability of *S. brachypetala* to reduce the activity of ATPase is indicative of the extracts' capacity to preserve the body's homeostatic ATP:ADP ratio, thereby maintaining energy redox balance. Homeostatic energy redox balance may help mitigate the persistent fatigue linked to ATP depletion in T2D needed for muscle contraction (Radosinska and Vrbjar, N., 2016).

7.3.6 Conclusion

The *S. brachypetala* crude bark extracts a myriad of bioactive compounds including phenolics and flavonoids. These compounds may have been responsible for the observed pharmacological activities exhibited by the extracts including potent antioxidant, carbohydrate and lipid-digesting enzyme inhibitory capabilities. Furthermore, the extracts stimulated glucose uptake in the yeast suspension, muscle, and liver tissue while concomitantly retarding glucose absorption in the small intestinal tissue. The extracts mitigated hepatic glucogenic hyperactivity signifying their potential to reduce hepatic glucose output. These results warrant further studies using molecular and animal models to ascertain the mode of action particularly for the ethanolic *S. brachypetala* crude bark extract.

7.4 *Schotia brachypetala* (Sond) ameliorates glucose metabolic dysfunction, abates dyslipidaemia, and mitigates oxidative renal and hepatic tissue damage in streptozotocin induced type 2 diabetic rat model.

Brian K Beseni¹, Kolawole A Olofinisan¹, Erukainure L Ochuko¹ * Shahidul Md Islam¹

1.Department of Biochemistry, University of KwaZulu-Natal, Westville Campus, Durban 4000, South Africa

***Corresponding author:** islamd@ukzn.ac.za

7.4.1 Abstract

The prevalence of type 2 diabetes mellitus (T2D) continues to rise and has reached an unsurpassed high. This has prompted the investigation of traditional medicinal plants as alternative therapeutic drug development templates. *Schotia brachypetala* is a folkloric medicinal plant whose leaf, bark, and root mixtures have been traditionally reported to strengthen the body, purify the blood, and treat nervous heart conditions, diabetes, and diarrhoea. The current study investigated the antioxidant, antilipidemic, and antidiabetic activities of the ethanolic crude extract of *S. brachypetala* using an *in vivo* T2D rat model. T2D was induced by feeding 10% fructose solution ad libitum for two weeks, followed by an intraperitoneal injection of streptozotocin (40 mg/kg body weight). Animals in the diabetic treatment groups were orally administered a low dose (150 mg/kg) and a high dose (300 mg/kg) of the extract daily, five times a week, during the trial period. Food and fluid intake were recorded daily, while body weight and blood glucose levels were determined weekly. An oral glucose tolerance test was performed in the last week of treatment. The extract restored redox balance and euglycemic levels, mitigated dysregulated lipid metabolisms, and reversed tissue damage in treated animals as compared to the diabetic, untreated group. The results of this study revealed that the T2D ameliorative potential of *S. brachypetala* may occur through several mechanisms, including upregulation of insulin sensitivity and secretion through the regeneration of pancreatic β -cells. Further molecular analyses and clinical trials are necessary to completely elucidate the precise mode of antidiabetic action shown in the current study.

Key words: T2D animal model, Antioxidant, Antihyperlipidemic, Antihyperglycemic.

7.4.2 Introduction

Diabetes mellitus (DM) is a group of physiological dysfunctions that result in chronic hyperglycemia (Blair M., 2016; Kaul *et al.*,2013). The global prevalence of diabetes has reached a record high, resulting in serious negative implications for the social, health, and economic status of countries. It is currently estimated that about 536.6 million adults are living with the condition globally (Sun *et al.*,2022). The pathogenesis of DM is complex and multifaceted but is mainly attributed to defective insulin secretion by pancreatic β -cells or the inability of insulin-sensitive tissues to respond to homeostatic circulatory insulin levels or both (Galicia-Garcia *et al.*,2020). Type 1, type 2, and gestational diabetes are the most prevalent forms of the condition. Type 1 diabetes stems from an autoimmune disorder that leads to pancreatic β cell destruction, resulting in poor insulin secretion (Blair, 2016). Type 2, which accounts for over 80% of the cases, results from a combination of poor insulin secretion and insulin resistance.

Hyperglycaemic-induced oxidative stress and low-grade chronic inflammation are implicated in the development and progression of DM-related complications (Oguntibeju, 2019). Hyperglycemia facilitates the change of the environment around tissues to enhance oxidative stress, which in turn stimulates the generation of inflammatory mediators and inflammation, thus resulting in further exacerbation of the production of reactive species (RS) (Oguntibeju, 2019). Reactive species with unpaired electrons react and damage functional and structural moieties, including lipids, proteins, and DNA, leading to their progressive dysfunction. Uncontrolled hyperglycemia, together with other metabolic aberrations in DM patients, is implicated in the development of chronic diabetes-related complications (Goyal and Jialal, 2018). DM-related complications are sub-classified as either macrovascular or microvascular complications (Papatheodorou *et al.*,2017). Microvascular complications of DM include neuropathy, nephropathy, and retinopathy. On the other hand, macrovascular complications consist of cardiovascular disease (CVD), stroke, and peripheral artery disease (Papatheodorou *et al.*,2017).

DM incidence, morbidity, and mortality are projected to rise further in the absence of a cure or improved management strategies (Kaul *et al.*,2013; Sun *et al.*,2022). Current management strategies for DM include non-pharmacological and pharmacological therapies. Non-pharmacological therapies include behavioral change, increased physical activity engagement, and controlled diets. Currently, while type 1 DM is generally treated through insulin replacement therapy, type 2 DM is treated with oral hypoglycemia. The major drug

therapy for type 2 DM comprises insulin secretagogues, biguanides, insulin sensitizers, α glucosidase inhibitors, incretin mimetics, amylin antagonists, and sodium-glucose co-transporter-2 (SGLT2) inhibitors. Dual drug therapies are often recommended in patients who are unable to achieve therapeutic goals with first-line oral hypoglycaemic agents as monotherapy. Despite their appreciable therapeutic benefits, current synthetic pharmacological agents are marred with undesirable side effects, leading to therapy ineffectiveness and patient non-compliance. This has prompted scientists across the globe to look for alternative therapeutic agents with high efficacy and reduced side effects. Thus, traditional medicinal plants such as *Schotia brachypetala* have emerged as probable sources of potent, lucrative antidiabetic agents.

S. brachypetala, of the Fabaceae family, is a sizable tree characterized by rough brown to brownish-grey bark and a wide-spreading, round crown (Watt and Breyer-Brandwijk, 1962). During the spring season, the branches bear distinctive deep red flowers, a prominent feature of this plant. These vibrant flowers, producing abundant nectar around September and October, attract various birds and insects (Du, 2011). Widely distributed in South Africa, from the Eastern Cape through KwaZulu-Natal, Mpumalanga, and Limpopo Province, extending into Mozambique and Zimbabwe, it holds cultural significance. In the KwaZulu-Natal Province, it is traditionally employed to remedy heartburn and pimples (Hutchings, 1996; Cooposamy and Naidoo, 2012). The plant's traditional uses include body washing to alleviate swelling associated with various chronic conditions, including T2D (Van Wyk and Gericke, 2000). Additionally, the bark decoction of *S. brachypetala* is taken to address heartburn and alleviate hangovers resulting from excessive alcoholic beverage consumption (Watt and Breyer-Brandwijk, 1962). The current study investigated the antioxidant, antilipidemic, and antidiabetic activities of the ethanolic crude extract of *S. brachypetala* using an *in vivo* T2D rat model.

7.4.3 Material and methods

The materials and methods for this study are detailed in Chapter 2 section 2.12

7.4.4 Results

As observed in Fig. 7.4.1, the DBC group had significantly ($p < 0.05$) higher food and fluid intake than the NC group during the experimental period. No significant ($p > 0.05$) difference in food and fluid intake was observed between the NC and NSBTX groups. Treatment with *S. brachypetala* at a high dose significantly ($p < 0.05$) reduced the fluid intake with respect to the

DBC group. Furthermore, there was no significant ($p>0.05$) difference in the fluid intake between the high-dose *S. brachypetala* and DBM groups. Similarly, no significant difference in food intake was observed between both the high and low doses of *S. brachypetala* and the DBC group.

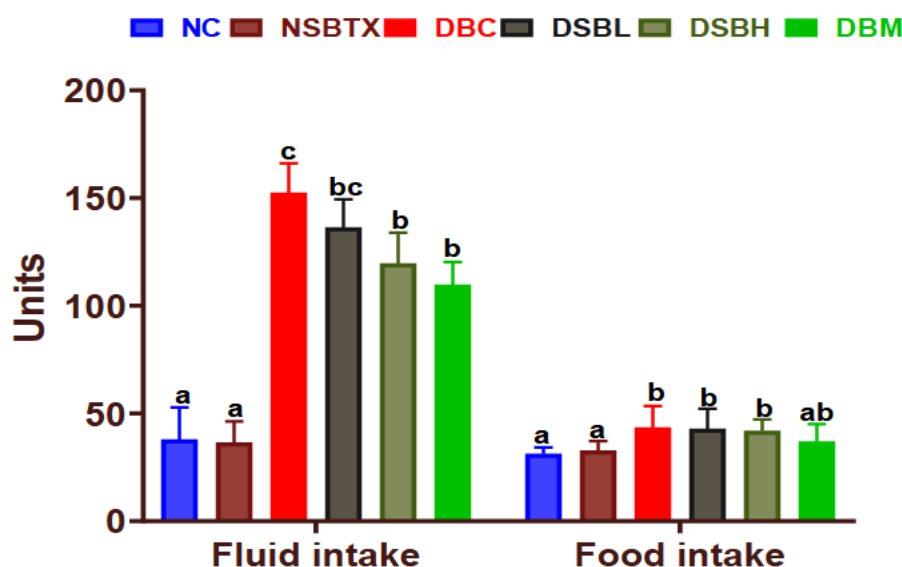


Figure 7.4.1: Food and Fluid intake in different animal groups during the intervention period. *NC*= Normal Control, *NSBTX* = Normal rats + *S. brachypetala* high dose, *DBC*= Diabetic Control, *DSBL*= Diabetic rats + *S. brachypetala* low dose, *DSBH* =Diabetic rats + *S. brachypetala* high dose, *DBM*: Diabetic rats + Metformin (positive control). Different superscripts a-d represent statistical significance of $p<0.05$ (Tukey's multiple range *post hoc* test at $p < 0.05$).

Fig. 7.4.2 shows a gradual increase in the mean body weight of the animals in the NC group during successive weeks of the experimental periods. Evidently, there was no significant($p>0.05$) difference in mean rat body weights between the NC and NSBTX groups throughout the experimental period. The DBC group had significantly ($p<0.05$) the least mean body weight during the experimental period amongst all groups. Treatment with *S. brachypetala* at a high dose resulted in significantly ($p<0.05$) higher body weight gain than the DBC, low dose of *S. brachypetala*, and DBM (positive control).

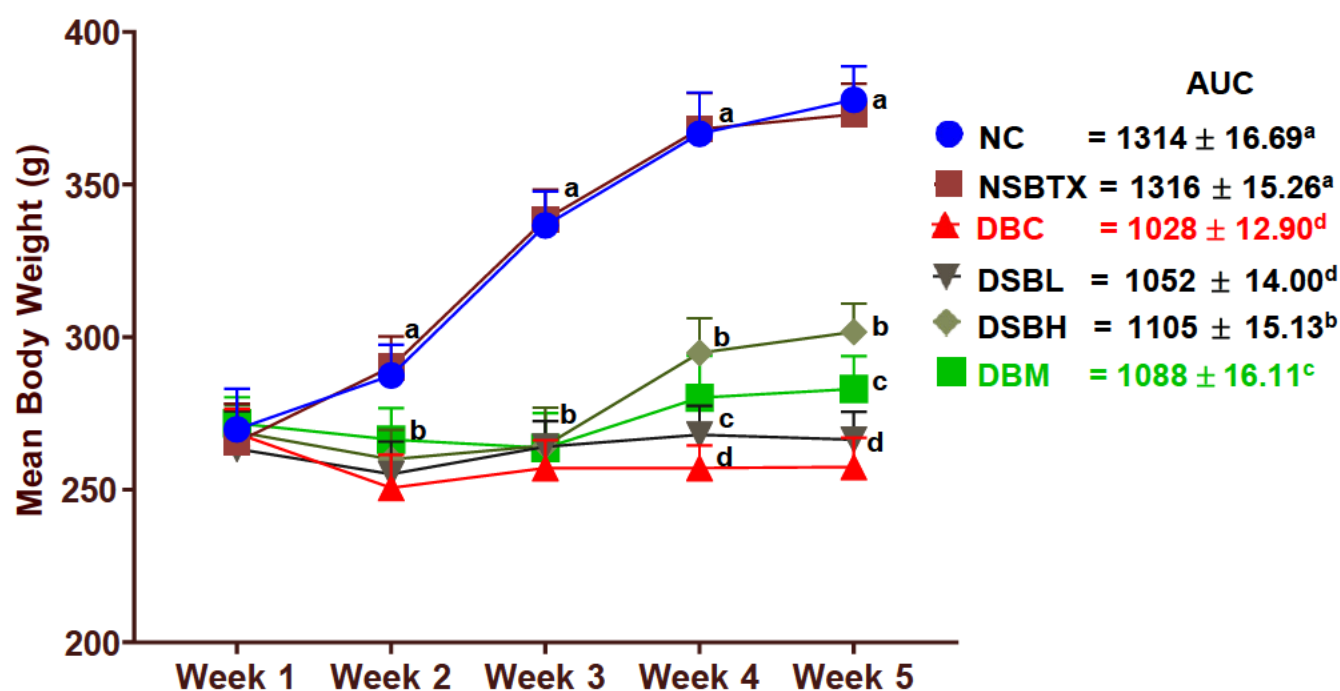


Figure 7.4.2: Effect of oral treatment on mean body weight in different animal groups during the 5-week intervention period. NC= Normal Control, NSBTX = Normal rats + *S. brachypetala* high dose, DBC= Diabetic Control, DSBL= Diabetic rats + *S. brachypetala* low dose, DSBH =Diabetic rats + *S. brachypetala* high dose, DBM: Diabetic rats + Metformin (positive control). Different superscripts a-d represent statistical significance of $p<0.05$ (Tukey's multiple range *post hoc* test at $p < 0.05$).

There was no significant ($p > 0.05$) difference between the NC and the toxicology group, which had the lowest weekly blood glucose levels, as shown in Fig. 7.4.3. Evidently, from the second week onwards, there was a gradual increase in weekly blood glucose levels in the DBC group. Furthermore, the DBC group had significantly ($p < 0.05$) the highest weekly blood glucose levels among all the groups. Treatment with both low and high doses of *S. brachypetala* significantly ($p < 0.05$) reduced the mean weekly blood glucose levels after the second week, with the high dose showing better capabilities than the low dose. No significant difference ($p > 0.05$) in the weekly blood glucose levels was observed for both low and high doses of *S. brachypetala* and DBM at week 5.

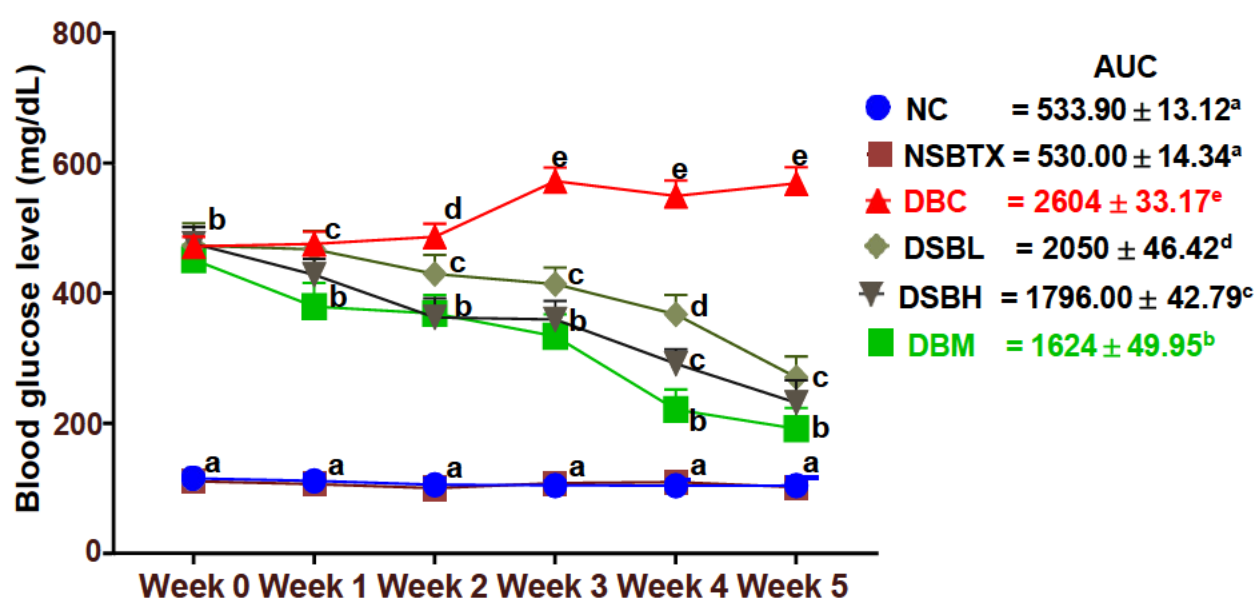


Figure 7.4.3: Effect of oral treatment on blood glucose in different animal groups during the intervention period. NC= Normal Control, NSBTX = Normal rats + *S. brachypetala* high dose, DBC= Diabetic Control, DSBL= Diabetic rats + *S. brachypetala* low dose, DSBH =Diabetic rats + *S. brachypetala* high dose, DBM: Diabetic rats + Metformin (positive control). Different superscripts a-d represent statistical significance of $p < 0.05$ (Tukey's multiple range *post hoc* test at $p < 0.05$).

Figure 7.4.4 shows the oral glucose tolerance test (OGTT) of the different treatment groups. Oral administration of a glucose load resulted in a significant ($p < 0.05$) spike in the blood glucose levels in the DBC group after 30 minutes, while no insignificant ($p > 0.05$) increase was observed for the NC at all the sampled time points. Furthermore, there was no significant ($p > 0.05$) difference in the blood glucose levels between the NC and NSBTX groups. There was no significant ($p > 0.05$) difference between animals in the high dose group and DBM (positive control), which had a significantly ($p < 0.05$) better OGTT response than both the low dose and DBC groups.

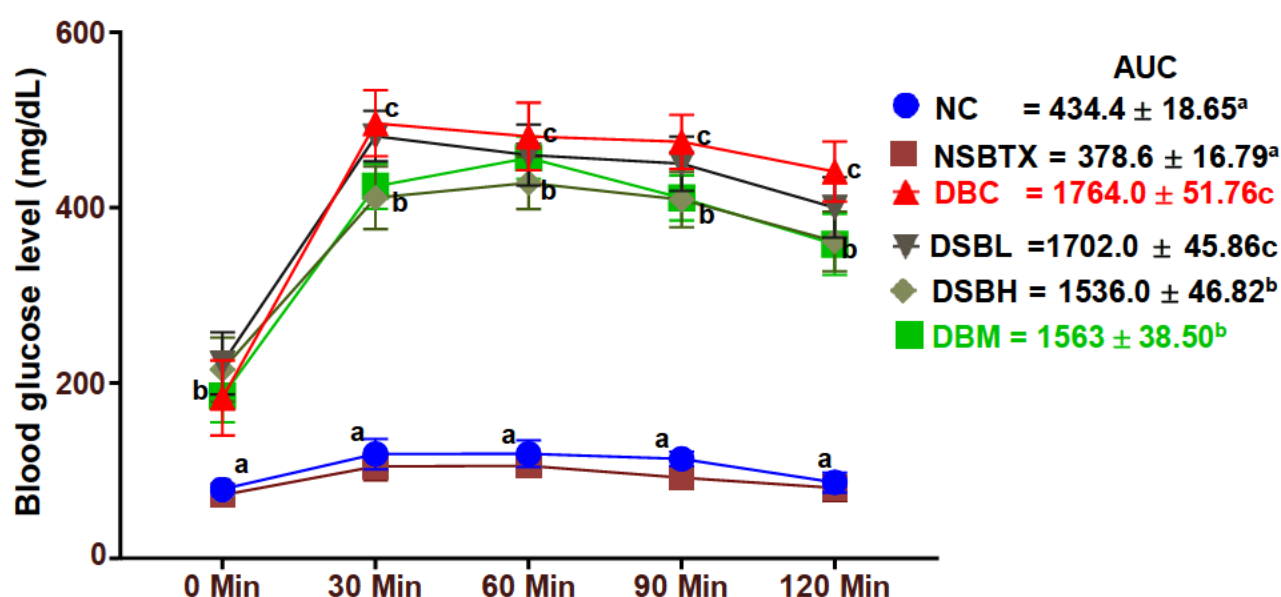


Figure 7.4.4: Oral glucose tolerance test (OGTT) of different animal groups in the last week of experimental period. NC= Normal Control, NSBTX = Normal rats + *S. brachypetala* high dose, DBC= Diabetic Control, DSBL= Diabetic rats + *S. brachypetala* low dose, DSBH =Diabetic rats + *S. brachypetala* high dose, DBM: Diabetic rats + Metformin (positive control). Different superscripts a-d represent statistical significance of $p < 0.05$ (Tukey's multiple range *post hoc* test at $p < 0.05$).

As observed in Fig 7.4.5 A, the DBC group exhibited significantly ($p < 0.05$) diminished insulin levels with respect to the NC group. However, no significant ($p > 0.05$) difference between the NC and NSBTX was observed in Fig. 7.4.5 A. Treatment with the high dose of *S. brachypetala* significantly ($p < 0.05$) increased insulin levels beyond those for both DBC and the low dose. Furthermore, treatment with the high dose significantly ($p < 0.05$) surpassed the insulin levels observed for DBM (positive control). As observed in Fig 7.4.5 B, there was a reduction in pancreatic β cell function with a concomitant increase in peripheral tissue insulin resistance, as indicated by the significantly ($p < 0.05$) reduced HOMA β and significantly ($p < 0.05$) increased HOMA IR scores in the DBC group when compared to the NC group. Consequently, treatment with the high dose significantly ($p < 0.05$) improved the HOMA β scores, surpassing the DBM group. While the low dose of *S. brachypetala* significantly ($p < 0.05$) improved the HOMA β scores when compared to the DBC group, there was no significant difference between this group and the DBM group. Similarly, the high dose lowered HOMA IR scores significantly ($p < 0.05$) better than both DBC and the low dose to proportions comparable to DBM with no significant difference ($p > 0.05$) as observed in Fig. 7.4.5 B.

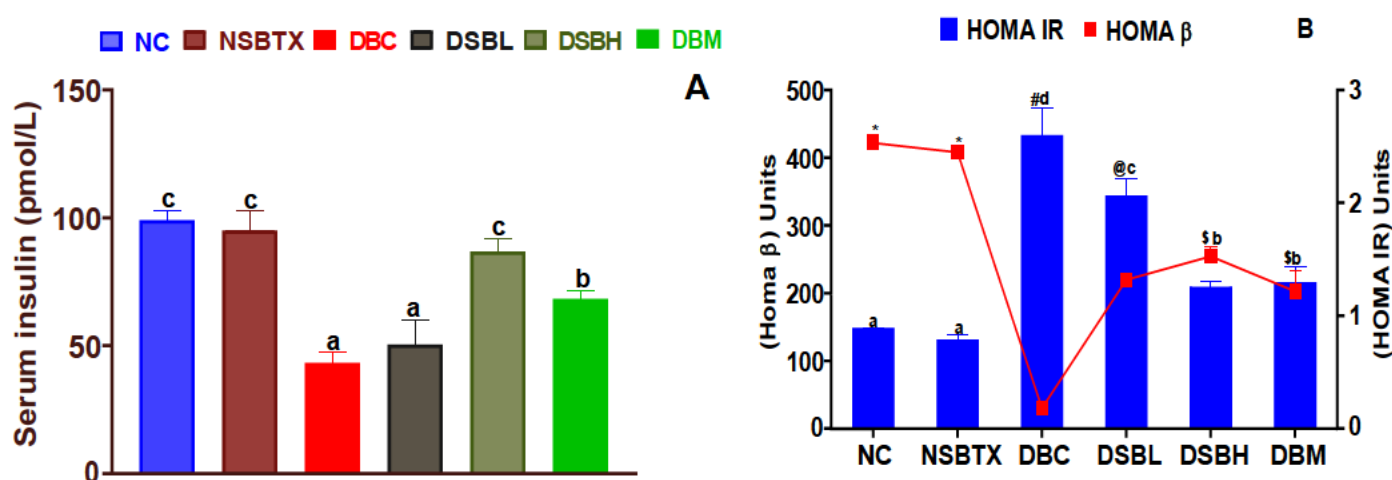


Figure 7.4.5: Effect of oral treatment on A) serum insulin levels B) homeostatic assessment model (HOMA IR and HOMA β) in different animal groups during the experimental period. NC= Normal Control, NSBTX = Normal rats + *S. brachypetala* high dose, DBC= Diabetic Control, DSBL= Diabetic rats + *S. brachypetala* low dose, DSBH =Diabetic rats + *S. brachypetala* high dose, DBM: Diabetic rats + Metformin (positive control). Different superscripts a-d represent statistical significance of $p < 0.05$ (Tukey's multiple range *post hoc* test at $p < 0.05$).

Fig. 7.4.6 shows significantly ($p < 0.05$) depleted hepatic glycogen content with a concomitant increase in relative liver weight in the DBC when compared to the NC. Treatment with both the high and low doses of *S. brachypetala* significantly ($p < 0.05$) reduced the relative liver weights with respect to the DBC group. Notably, there was no significant ($p > 0.05$) difference between the liver weights of the high-dose and DBM groups. Treatment with both high and low doses of *S. brachypetala* ($p < 0.05$) increased hepatic glycogen content to near-normal proportions, insignificantly different from the NC group. Furthermore, both high and low doses of *S. brachypetala* resulted in liver glycogen content significantly ($p < 0.05$) higher than the DBM group (positive control).

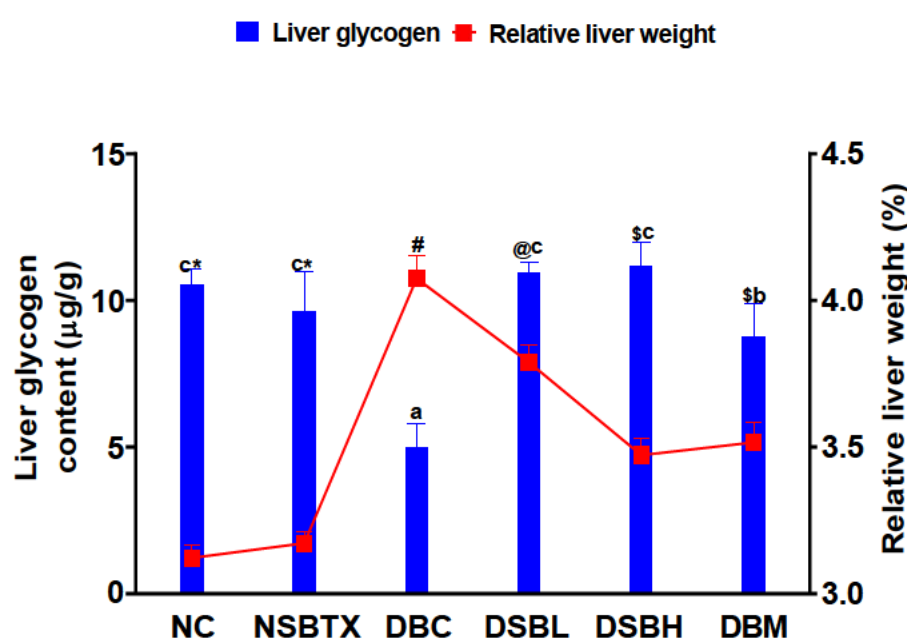


Figure 7.4.6: Effect of oral treatment on relative liver weight and glycogen content in different animal groups during the experimental period. NC= Normal Control, NSBTX = Normal rats + *S. brachypetala* high dose, DBC= Diabetic Control, DSBL= Diabetic rats + *S. brachypetala* low dose, DSBH =Diabetic rats + *S. brachypetala* high dose, DBM: Diabetic rats + Metformin (positive control). Different superscripts a-d represent statistical significance of $p < 0.05$ (Tukey's multiple range *post hoc* test at $p < 0.05$).

As seen in Table 7.4.1, a significant ($p < 0.05$) increase in the activities of hepatic glycogen phosphorylase, glucose 6 phosphatase, and fructose 1,6 bisphosphatase with concomitantly reduced ATPase activity was observed in the DBC group when compared to the NC group. Treatment with the high dose significantly ($p < 0.05$) decreased glycogen phosphorylase activity, which was lower than the low dose of *S. brachypetala* and DBM. Despite the low dose of *S. brachypetala* reducing glycogen phosphorylase activity lower than DBM, the difference between these treatment groups was not significant ($p > 0.05$). Treatment with both high and low doses of *S. brachypetala* resulted in glucose-6 phosphatase activity significantly ($p < 0.05$) lower than both the DBC and DBM groups. Furthermore, no significant difference in the glucose-6 phosphatase activity resulting from the treatment with both high and low doses of *S. brachypetala* was observed. There was no significant ($p > 0.05$) difference between the ATPase activity resulting from treatment with a low dose and that of DBM, both of which were significantly ($p < 0.05$) lower than that of the high dose.

Table 7.4.1: The effects of oral treatment of hepatic glucogenic and nucleotide hydrolysing enzymes.

	NC	NSBTX	DBC	DSBL	DSBH	DBM
Glycogen phosphorylase*	153,673±9,65 ^a	150,37±10,34 ^a	218,367±11,21 ^d	172,53±8,05 ^c	161,37±5,87 ^b	176,37±8,11 ^c
Glucose 6 phosphatase*	148,76±5,81 ^a	151,73±9,76 ^a	228,71±10,37 ^d	170,37±9,12 ^b	163,47±9,23 ^b	183,97±5,67 ^c
Fructose 1,6 Bisphosphatase*	180,21±7,31 ^a	179,99±11,86 ^a	253,67±12,35 ^d	187,67±12,74 ^b	177,37±11,34 ^a	200,07±7,92 ^c
ATPase*	301,68±10,36 ^c	303,73±7,10 ^c	210,32±10,37 ^a	269,31±12,36 ^b	297,37±14,09 ^c	268,13±8,36 ^b

*Units = $\mu\text{mol Pi/mg}$. **NC** = Normal Control, **NSBTX** = Normal rats + *S. brachypetala* high dose, **DBC** = Diabetic Control, **DSBL** = Diabetic rats + *S. brachypetala* low dose, **DSBH** = Diabetic rats + *S. brachypetala* high dose, **DBM**: Diabetic rats + Metformin (positive control). Different superscripts a-d represent statistical significance of $p < 0.05$ (Tukey's multiple range *post hoc* test at $p < 0.05$).

As shown in Table 7.4.2, α amylase and lipase in both the serum and pancreas were significantly ($p<0.05$) elevated in the diabetic animal control group (DBC). However, treatment with the high dose significantly ($p<0.05$) reduced α amylase activity in serum and pancreatic tissue. Furthermore, the high dose of *S. brachypetala* significantly ($p<0.05$) reduced lipase activity in the pancreatic tissue to near-normal levels, insignificantly different from the NC group. Finally, the high dose had better lipase inhibitory activities in the serum than the low dose, significantly ($p<0.05$) reducing lipase activity to levels comparable to those of DBM with no significant ($p>0.05$) difference.

Table 7.4.2: Effect of oral treatment on carbohydrate and lipid hydrolysing enzymes.

	NC	NSBTX	DBC	DSBL	DSBH	DBM
Serum amylase U/L	22,37±1,08 ^a	21,87±1,01 ^a	76,49±3,71 ^d	41,76±2,31 ^c	32,97±1,83 ^b	46,37±2,48 ^c
Pancreatic amylase U/L	30,21±0,37 ^a	32,83±0,41 ^a	83,57±2,55 ^d	63,53±1,03 ^c	39,11±0,96 ^b	43,16±1,54 ^b
Pancreatic lipase U/L	37,367±2,33 ^a	39,11±2,11 ^a	92,74±5,34 ^d	45,24±3,14 ^b	39,73±1,37 ^a	53,67±3,53 ^c
Serum lipase U/L	21,45±1,11 ^a	20,93±0,89 ^a	66,97±2,82 ^d	38,23±1,44 ^c	31,71±1,32 ^b	33,89±1,23 ^b

NC= Normal Control, NSBTX = Normal rats + *S. brachypetala* high dose, DBC= Diabetic Control, DSBL= Diabetic rats + *S. brachypetala* low dose, DSBH =Diabetic rats + *S. brachypetala* high dose, DBM: Diabetic rats + Metformin (positive control). Different superscripts a-d represent statistical significance of $p<0.05$ (Tukey's multiple range *post hoc* test at $p < 0.05$).

As shown by the lipid profile analysis in Fig 7.4.7, diabetes induction significantly ($p < 0.05$) elevated total cholesterol, triacylglycerols and LDL cholesterol while concomitantly diminishing HDL cholesterol in the DBC group when compared to the NC group. Treatment with the high dose of *S. brachypetala* significantly ($p < 0.05$) reduced the total cholesterol level to proportions insignificantly ($p > 0.05$) different from the NC group. Furthermore, the high dose of *S. brachypetala* significantly increased HDL cholesterol levels to proportions insignificantly ($p > 0.05$) different from those in the NC group. Both the high and low doses *S. brachypetala* significantly ($p < 0.05$) reduced triacylglycerol and LDL cholesterol levels when compared to the DBC. Evidently, as observed in Fig 7.4.7, the DBC had significantly ($p < 0.05$) the highest atherogenic index amongst all animals including the NC group. Evidently, treatment with both the high and low doses *S. brachypetala* significantly ($p < 0.05$) reduced the atherogenic index. As shown by the lipid profile analysis in Fig. 7.4.7, diabetes induction significantly ($p < 0.05$) elevated total cholesterol, triacylglycerols, and LDL cholesterol while concomitantly diminishing HDL cholesterol in the DBC group when compared to the NC group. Treatment with the high dose of *S. brachypetala* significantly ($p < 0.05$) reduced the total cholesterol level to proportions insignificantly ($p > 0.05$) different from the NC group. Furthermore, the high dose of *S. brachypetala* significantly increased HDL cholesterol levels to proportions insignificantly ($p > 0.05$) different from those in the NC group. Both the high and low doses of *S. brachypetala* significantly ($p < 0.05$) reduced triacylglycerol and LDL cholesterol levels when compared to the DBC. As observed in Fig. 7.4.7, the DBC had significantly ($p < 0.05$) the highest atherogenic index amongst all animals, including the NC group. Evidently, treatment with both the high and low doses of *S. brachypetala* significantly ($p < 0.05$) reduced the atherogenic index of the plasma (AIP) when compared to the DBC group.

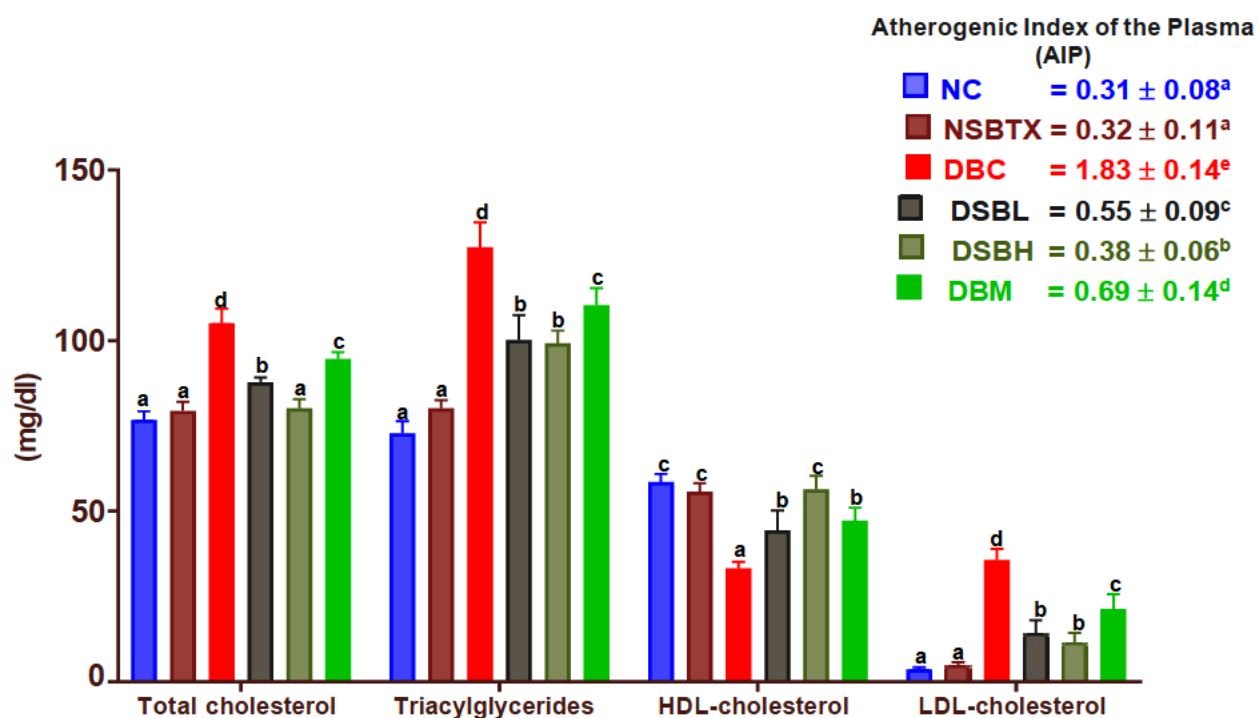


Figure 7.4.7: Effect of oral treatment on lipid profiles. *NC*= Normal Control, *NSBTX* = Normal rats + *S. brachypetala* high dose, *DBC*= Diabetic Control, *DSBL*= Diabetic rats + *S. brachypetala* low dose, *DSBH*=Diabetic rats + *S. brachypetala* high dose, *DBM*: Diabetic rats + Metformin (positive control). Different superscripts a-d represent statistical significance of $p < 0.05$ (Tukey's multiple range *post hoc* test at $p < 0.05$).

Diabetes induction resulted in significantly ($p<0.05$) elevated MDA and NO levels, as well as a significant ($p<0.05$) decrease in GSH levels, SOD, and CAT activity in both serum and pancreatic tissue, as shown in Tables 7.4.3 and 7.4.4, respectively. Treatment with the high dose of *S. brachypetala* significantly ($p<0.05$) reduced lipid peroxidation and NO concentrations in both serum and pancreatic tissue. Furthermore, the high dose concomitantly increased GSH levels and SOD and CAT activities significantly ($p<0.05$) higher than the DBC group.

Table 7.4.3: Effect of oral treatment on various pancreatic tissue oxidative stress linked enzymes.

	NC	NSBTX	DBC	DSBL	DSBH	DBM
MDA (mol/L)	42,63±1,33 ^a	41,98±1,09 ^a	81,76±5,43 ^d	55,76±2,95 ^c	47,17±1,11 ^b	61,33±2,35 ^c
GSH (mol/L)	71,45±2,17 ^c	72,09±4,36 ^c	33,28±1,88 ^a	61,23±3,11 ^b	67,73±6,20 ^b	58,23±3,09 ^b
SOD (U/mg protein)	93,71±3,54 ^d	89,73±5,33 ^d	41,2±2,22 ^a	74,26±6,09 ^b	88,13±3,89 ^d	81,23±1,85 ^c
CAT (U/mg protein)	702,357±9,36 ^e	705,37±12,14 ^e	395,15±6,31 ^a	621,74±6,78 ^b	683,32±12,37 ^d	657,34±7,38 ^c
NO (µml)	0,063±0,003 ^a	0,059±0,003 ^a	0,151±0,007 ^d	0,087±0,004 ^c	0,072±0,002 ^b	0,078±0,002 ^b

NC= Normal Control, NSBTX = Normal rats + *S. brachypetala* high dose, DBC= Diabetic Control, DSBL= Diabetic rats + *S. brachypetala* low dose, DSBH =Diabetic rats + *S. brachypetala* high dose, DBM: Diabetic rats + Metformin (positive control). Different superscripts a-d represent statistical significance of $p<0.05$ (Tukey's multiple range *post hoc* test at $p < 0.05$).

Table 7.4.4: Effect of oral treatment on various serum oxidative stress linked enzymes.

	NC	NSBTX	DBC	DSBL	DSBH	DBM
MDA (mol/L)	39,75±2,07 ^a	38,09±2,78 ^a	76,17±6,24 ^d	52,74±3,24 ^c	45,61±1,73 ^b	52,37±4,56 ^c
GSH (mol/L)	121,37±5,78 ^d	119,32±5,89 ^d	62,74±2,37 ^a	89,75±3,93 ^b	110,73±2,34 ^c	100,25±3,31 ^c
SOD (U/mg protein)	583,22±4,37 ^d	591,73±9,37 ^d	176,37±6,13 ^a	489,73±10,51 ^b	507,37±7,91 ^c	497,21±11,17 ^b
CAT (U/mg protein)	801,1±8,63 ^e	813,17±13,22 ^e	709,76±3,77 ^a	766,21±11,67 ^c	791,37±9,36 ^d	741,134±9,08 ^b
NO (µml)	0,31±0,006 ^a	0,33±0,005 ^a	0,53±0,008 ^c	0,41±0,001 ^b	0,39±0,001 ^b	0,42±0,002 ^b

NC= Normal Control, **NSBTX** = Normal rats + *S. brachypetala* high dose, **DBC**= Diabetic Control, **DSBL**= Diabetic rats + *S. brachypetala* low dose, **DSBH** =Diabetic rats + *S. brachypetala* high dose, **DBM**: Diabetic rats + Metformin (positive control). Different superscripts a-e represent statistical significance of $p<0.05$ (Tukey's multiple range *post hoc* test at $p < 0.05$).

The indices of hepatic and renal function in the different animal treatment groups are shown in Table 7.4.5. Significantly ($p<0.05$) elevated LDH, urea, uric acid, alkaline phosphate, ALT, AST, and CK-MB levels in the DBC as compared to the normal control group (NC) are indicative of hepatic and renal damage. Upon treatment with both the low and high doses, which significantly ($p<0.05$) reduced urea levels, no significant ($p>0.05$) difference was observed between the urea levels in these groups when compared to the NC group. Treatment with the high dose of *S. brachypetala* significantly ($p<0.05$) reduced lactate dehydrogenase, uric acid, alkaline phosphatase, and aspartate aminotransferase with respect to the DBC group. Evidently, no significant ($p>0.05$) difference in ALT levels was observed between the normal (NC and NSBTX) and diabetic groups (DBC, DSBL, DSBH, and DBM).

Table 7.4.5: Effect of oral treatment on renal and hepatic function indices.

	NC	NSBTX	DBC	DSBL	DSBH	DBM
Lactate dehydrogenase U/L	387,2 ± 33,05 ^b	402,56±28,34 ^b	911 ± 58,21 ^e	508,76±15,73 ^d	467,38±36,74 ^c	284,4±27,87 ^a
Urea mg/dl	87,6 ± 3,58 ^a	86,32±4,04 ^a	96,6 ± 4,28 ^b	89,11±4,23 ^a	84,76±3,76 ^a	87,0 ± 2,92 ^a
Uric acid mg/dl	0,586 ± 0,32 ^a	0,617±0,28 ^a	2,246 ± 0,57 ^e	1,063±0,37 ^d	0,953±0,35 ^c	0,82 ± 0,19 ^b
Alkaline phosphatase U/L	62,2 ± 8,31 ^a	67,88±6,34 ^a	273,2 ± 15,78 ^e	198,73±11,37 ^d	157,37±10,74 ^b	163,4 ± 11,14 ^c
Alanine aminotransferase (ALT) U/L	82,2 ± 4,31 ^a	83,24±3,87 ^a	85,0 ± 2,87 ^a	85,01±2,46 ^a	84,14±37 ^a	85,0 ± 3,97 ^a
Aspartate aminotransferase (AST) U/L	85,8 ± 6,41 ^a	86,37±3,21 ^a	120,6 ± 7,33 ^d	118,37±7,37 ^c	103,75±6,71 ^b	114,8 ± 5,89 ^c
Creatine Kinase-Myocardial Band (CK-MB) U/L	367,46 ± 44,25 ^a	358,24±22,41 ^a	790,12 ± 57,12 ^e	517,16±28,37 ^c	477,37±29,67 ^b	577,84 ± 33,25 ^d

NC= Normal Control, **NSBTX** = Normal rats + *S. brachypetala* high dose, **DBC**= Diabetic Control, **DSBL**= Diabetic rats + *S. brachypetala* low dose, **DSBH** =Diabetic rats + *S. brachypetala* high dose, **DBM**: Diabetic rats + Metformin (positive control). Different superscripts a-e represent statistical significance of $p < 0.05$ (Tukey's multiple range *post hoc* test at $p < 0.05$).

Histopathological analysis

The histological analysis of the NC group revealed numerous β pancreatic cells in the islets of Langerhans with distinct morphology, surrounded by numerous acinar cells, as observed in Fig. 7.4.8. However, a significant decrease in the number of β cells with concomitant morphological destruction of pancreatic islets in the DBC group was observed when compared to the NC group. Treatment with the high dose, on the other hand, reversed pancreatic islet destruction, resulting in larger islets with a higher number of β cells.

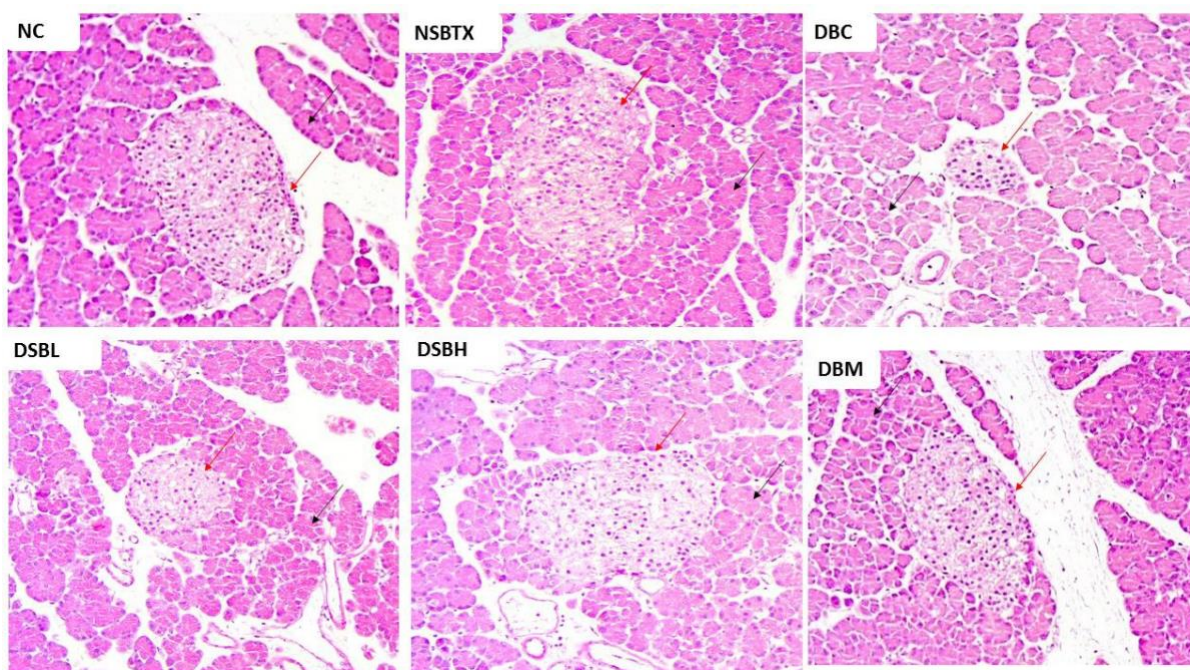


Figure:7.4.8: Pancreatic histopathological examinations illustrating the effects of oral treatments in different animal groups during the experimental period. Magnification=100X. Red arrow shows the pancreatic islets of Langerhans. Black arrows show pancreatic acinar cells. NC= Normal Control, NSBTX = Normal rats + *S. brachypetala* high dose, DBC= Diabetic Control, DSBL= Diabetic rats + *S. brachypetala* low dose, DSBH =Diabetic rats + *S. brachypetala* high dose, DBM: Diabetic rats + Metformin (positive control).

7.4.5 Discussion

The steep rise in the global prevalence of T2D has reduced the quality of life among sufferers and placed an immense burden on health care systems (Goyal *et al.*,2020). Traditional medicinal plants produce secondary metabolites with an assortment of therapeutic benefits against several ailments, including T2D, which have emerged as a lucrative avenue for the development of novel antidiabetic drug templates. Therefore, scientists across the globe continue their search for novel phyto-constituent-based antidiabetic agents with increased efficacy, bioactivity, and reduced side effects (Tayyab *et al.*,2012). The current study investigated the antioxidant, antihyperglycemic, and antihyperglycemic capabilities of *S. brachypetala*, a traditional medicinal plant, in a type 2 diabetic rat model.

The manifestation of typical signs and symptoms of T2D varies among individuals and is influenced by the severity of the condition, with some individuals remaining asymptomatic for as long as 10 years (Pippitt *et al.*,2016). Chronic hyperglycaemia alters the blood milieu, leading to the prominent acute signs associated with T2D, including polyphagia and polydipsia (Grotzke and Jones, 2013), observed in the DBC in Fig. 7.4. 1. Despite abundant circulatory blood glucose, peripheral tissue insulin resistance and increased renal tissue-mediated glucosuria lead to compromised glucose assimilation and weight gain in T2D, as observed in Fig. 7.4. 2. The inability of T2D patients' tissues to assimilate circulating glucose leads to unmet energy demands, prompting proteolysis and body weight loss (Yang *et al.*,2016). Therefore, the ability of the high dose of *S. brachypetala* to reduce fluid intake and stimulate body weight gain insinuates the plant extracts' ability to abate insulin resistance in peripheral tissues and stimulate glucose assimilation.

Dysregulated glucose assimilation pathways in T2D impede the movement of glucose from the blood into peripheral tissues, leading to chronic hyperglycaemia (Alsahli and Gerich, 2017). Indeed, the data in our current study, which showed hyperactivity of carbohydrate digestive enzymes as seen in Table 7.4.1 coupled with increased peripheral tissue insulin resistance as indicated by significantly increased HOMA IR scores in Fig. 7.4.5B, may have contributed to the chronic hyperglycaemia observed in Fig. 7.4.3. Furthermore, the glucose intolerance observed for the DBC group through the OGTT analysis in Fig 7.4.4 may have resulted from reduced insulin levels, as seen in Fig 7.4.5A, due to reduced β pancreatic tissue function, as indicated by the reduced HOMA β scores seen in Fig. 7.4.5B. Therefore, the ability of the high dose of *S. brachypetala* to reduce the weekly blood glucose levels and improve glucose tolerance may stem from their ability to reduce the activities of carbohydrate-digesting

enzymes, ameliorate peripheral tissue insulin resistance, and concomitantly increase insulin secretion. Indeed, the insulin secretagogue capabilities of the high dose of *S. brachypetala* stem from their ability to improve β pancreatic tissue health, morphology, and population, as revealed by the histopathological analysis displayed in Fig. 7.4.8. Under T2D conditions, pancreatic tissue damage is mediated through oxidative stress, as indicated by the significantly increased lipid peroxidation and NO radical production, with a concomitant reduction in intrinsic antioxidant defenses such as superoxide dismutase and catalase activities and diminished glutathione levels, as observed in the DBC group in Tables 7.4.3 and 7.4.4 in both the serum and pancreatic tissues. Hyperglycaemic-induced oxidative stress is implicated as a major contributor to tissue damage, and the pancreatic tissues are particularly prone to oxidative tissue injury, which leads to β cell damage (Gerber *et al.*,2017; Eguchi *et al.*,2021). Therefore, the ability of the extracts to reduce lipid peroxidation and NO radicals while bolstering antioxidant defenses further supports their protective capacity against hyperglycemic-induced oxidative β pancreatic tissue damage under T2D conditions (Wang *et al.*,2021). This insinuates the protective and regenerative capacity of the extracts (Wang *et al.*,2021).

The hepatic and renal tissues are among the most metabolically active tissues in the body and are thus prone to damage by reactive species (Mohamed *et al.*,2016; Forbes and Thorburn, 2018). Hepatic tissue damage results in compromised integrity of the membrane barrier, thereby leading to spillage of intercellular contents into the blood (Mohamed *et al.*,2016). Therefore, the elevation of serum levels of AST, ALP, and LDH as shown in Table 7.4.5, in the DBC group is indicative of hepatic tissue damage. Furthermore, renal tissue damage impedes its glomerular filtering capacity, leading to an elevation of blood area and uric acid (Forbes and Thorburn, 2018), as observed in the DBC group displayed in Table 7.4.5. Consequently, the ability of the *S. brachypetala* extracts to restore hepatic and renal tissue damage indices in the serum is indicative of their capacity to protect against T2D-induced liver and kidney damage, as shown in Table 7.4.5.

The liver regulates blood glucose levels through the action of insulin and glucagon, which mediate several processes, including glycogenesis and glycogenolysis (Han *et al.*,2016). While glycogenesis promotes the polymerization and storage of glucose as glycogen, glycogenolysis occurs during periods of intermittent fasting to break down hepatic glycogen stores to increase blood glucose levels (Han *et al.*,2016). Dysregulated hepatic glucose output in T2D is mediated by the hyperactivity of glucogenic enzymes observed in Table 7.4.1, which diminished glycogen stores in the hepatic tissue of the DBC group shown in Figure 7.4.6, insinuating a

state of chronic hyperglycaemia. Insulin inhibits glycogenolysis by inhibiting the activities of glycogen phosphorylase and fructose 1,6-bisphosphatase, which corroborates the increased activity of these enzymes seen in Table 7.4.1 due to decreased insulin levels observed in Fig. 7.4.5A. The ability of the extracts to increase insulin levels with a concomitant reduction of the activities of the glucogenic enzymes may have resulted in the restoration of hepatic glycogen stores, further insinuating their antihyperglycemic capabilities through regulated hepatic glucose output. Furthermore, the increased relative liver weight observed in Fig. 7.4.6 may have been due to fat deposits in the glycogen-poor hepatic tissue. Fat deposits in the liver are associated with T2D-induced dyslipidaemia.

Unhealthy diets comprised of excessive intakes of dietary fats predispose T2D patients to diabetic dyslipidaemia (Katsiki *et al.*,2016). Diabetic dyslipidaemia is the pathological change in lipid profile resulting from dysregulated lipid metabolism and is a major risk factor for T2D-linked cardiovascular diseases (CVD) (Soran *et al.*,2016). Increased activities of lipid digestion enzymes observed in Table 7.4.2 together with dysregulated lipoprotein metabolizing enzymes may have significantly contributed to elevated total triglycerides, total cholesterol, and LDL cholesterol, with concomitantly significantly reduced HDL cholesterol in the DBC group, as displayed in Fig. 7.4.7. This lipid profile is an aggravating factor in diabetic cardiovascular diseases, as evidenced by the elevated AIP displayed in Fig. 7.4.7. LDL cholesterol transports and deposits cholesterol in peripheral tissues, and its increase is associated with increased atherogenesis, which precedes atherosclerosis in cardiovascular diseases (CVD) (Filippatos *et al.*,2017). Conversely, HDL cholesterol mediates the transportation of cholesterol from peripheral tissues to the liver for metabolism and eventual excretion. Increased HDL levels are therefore associated with a reduced risk of cardiovascular disease (CVD) (Filippatos *et al.*,2017). Consequently, the ability of the high dose of *S. brachypetala* to reduce the activity of lipid-digesting enzymes and regulate other lipolytic hormones improved the lipid profile and may be beneficial in mitigating the deleterious effects of T2D-induced hyperlipidemia, including cardiovascular diseases (CVD) (Filippatos *et al.*,2017).

7.4.6 Conclusion

The present study highlights *S. brachypetalas*' capacity to enhance glucose metabolism and regulate biological activities associated with type 2 diabetes (T2D) and its complications. Notably, *S. brachypetala* restored euglycemic levels, improved glucose tolerance, alleviated dyslipidemia, and improved the atherogenic index while concomitantly ameliorating oxidative pancreatic, hepatic, and renal tissue damage.

CHAPTER 8

GENERAL DISCUSSION AND CONCLUSION

8.1 General discussion

The incidence of diabetes mellitus has reached epidemic levels, with an estimated 536.6 million adults between the ages of 20 and 79 reported to be living with the condition in 2021, and its prevalence is further projected to rise to 783.2 million by 2045 (IDF, 2021; Sun *et al.*,2022). Although reports show that the African region accounts for about 5.3% of the global diabetes prevalence, this figure may be an underestimate as over 50% of the cases in this region go unreported and undiagnosed (Sun *et al.*,2022). In 2021, approximately 12.2% (6.7 million adults) of global deaths were attributed to diabetes and its complications (IDF, 2021). Furthermore, in South Africa, diabetes-related mortality was estimated to be 5.1% and predicted to rise to 5.5% by 2017 (Thomas *et al.*,2019). Furthermore, diabetes is a leading cause of morbidity, including limb amputation, severely reducing the quality of life among afflicted individuals. The high prevalence of diabetes poses a serious financial burden on healthcare systems, with direct costs rising from USD 232 billion in 2007 to USD 966 billion in 2022. Further projections indicate this amount will rise to USD 1.03 trillion by 2030 and USD 1.05 trillion by 2045 (IDF, 2021). This is particularly devastating within the African region, where most of the countries are in the low- to middle-income bracket with small GDPs.

The high cost of diabetes health care facilities and medicine in the African region is a worrisome cause for concern. Current oral pharmacological management agents for diabetes are broadly classified as sulfonylureas, thiazolidinediones, meglitinides, sodium glucose co-transporter (SGLT2), α -glucosidase inhibitors, dipeptidyl peptidase IV inhibitors, and biguanides, depending on their structure and mechanism of action (Hu and Jia, 2019). While these drugs are effective in the management of diabetes and its complications, they are costly for people living in the low- to middle-income countries of the sub-Saharan African region. Furthermore, patient adherence to these medications is a challenge as they are marred with undesirable side effects and adverse events (Sterrett *et al.*,2016; Fernandes *et al.*,2018). Notable side effects of oral synthetic antidiabetic medications include weight loss or gain, nausea, abdominal irritations, diarrhoea, flatulence, and several adverse events such as severe hypoglycaemic shock, congestive heart failure, pancreatitis, and cancer (Sterrett *et al.*,2016). Therefore, people, particularly in the African region, resort to the use of traditional medicine for the management of diabetes and to circumvent the high cost and undesirable side effects

associated with available commercial antidiabetic pharmacological agents. In South Africa, up to 80% of people rely on or have consulted traditional medicine practitioners for their primary health care needs (Van Wyk and Prinsloo, 2018). The pharmacological benefits of traditional medicinal plants stem from their inherent bioactive secondary metabolites. Plant-derived pharmacological agents have relatively fewer undesirable side effects as they have been shown to work harmoniously with the body's biological systems (Raj *et al.*, 2016). Folkloric medicine has been practiced since time immemorial, but despite its widespread use, the modes of action, efficacy, and toxicological profiles of many of the plants used remain poorly elucidated and documented. Therefore, this study evaluated the antioxidative and antidiabetic potentials of selected traditional medicinal plants (*Rhus longipes* Engl.; *Celtis africana* Burm.f.; *Trema orientalis* (L.) Blume; *Albizia adianthifolia* (Schumach.) W.F. Wight; and *Schotia brachypetala* Sond.) using *in vitro*, *ex vivo*, *in silico*, and *in vivo* experimental models.

The leaf and stem bark of *Trema orientalis* (L.), which were the first plant material to be studied, were sequentially macerated in solvents of varying polarity (water, methanol, ethanol, and ethyl acetate) to yield crude extracts. The antioxidant and antidiabetic effects of *T. orientalis* crude leaf and extracts were then evaluated using *in vitro* and *ex vivo* experimental models. *In vitro* analysis revealed a high concentration of both total polyphenol and flavonoid content in *T. orientalis*, suggesting strong therapeutic potential. 2-Methyl-4-tert-octylphenol, methyl hexofuranoside, 3-O-Methyl-d-glucose, 4-Tert-octylphenol, l-(+)-Ascorbic acid 2,6-dihexadecanoate, and squalene were among the most significant bioactive compounds identified via GC-MS analysis in the stem bark extract of *T. orientalis*. Several compounds, including tremetol, sweroside, scopoletin, and (-)-epicatechin, have previously been identified in the stem bark of *T. orientalis*. (Noungoué Tchamo *et al.*, 2001; Kuo *et al.*, 2007). The high total phenolic and flavonoid content and presence of several bioactive compounds corroborate the extracts' strong antioxidant capabilities, shown by their ability to scavenge DPPH and NO radicals and donate electrons (FRAP). Furthermore, the extracts exhibited enhanced α -glucosidase and α -amylase inhibitory activity, with concomitant glucose uptake augmenting capabilities, suggesting their strong antihyperglycemic capabilities. A comparative analysis of IC₅₀ values across various antioxidant and antidiabetic properties, including carbohydrate and lipid digesting enzyme inhibitory activities, revealed distinct patterns in leaf versus stem bark extracts of *T. orientalis*. While the leaf extracts demonstrated superior activity in only the DPPH and pancreatic lipase inhibition assay, the bark extracts exhibited greater activity in the FRAP, and α -glucosidase and α -amylase inhibition assays. These findings suggest that the

stem bark of *T. orientalis* possesses better bioactivities compared to the leaf extracts, warranting further investigation for potential therapeutic applications." The antioxidant and antidiabetic activities observed for *T. orientalis* warrants further studies using *in vivo* models to ascertain its precise modes of action.

Albizia adianthifolia was the next plant whose leaf and stem bark extracts were studied. *A. adianthifolia* has previously been reported as a remedy for sore eyes, cuts, ulcers, sore throat, tonsillitis, tuberculosis, gonorrhoea, diabetes, and heart diseases by the Murehwa and Mutoko people in Zimbabwe (Maroyi, 2018). These observed folkloric medicinal uses suggest that the plant possesses several potent bioactive compounds, as corroborated in the current study, whereby the leaf and stem bark of *A. adianthifolia* was shown to contain significant total phenolics and flavonoids. The remarkable antioxidant activity of *A. adianthifolia* shown in the current study is corroborated by a previous study that documented the antioxidant activities of various extracts of *A. adianthifolia* from different parts against 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging (Sonibare *et al.*, 2017). Furthermore, the extracts increased glucose uptake in muscle and liver tissues while reducing its absorption in the intestinal jejunum, suggesting potent antihyperglycemic capabilities. The extracts improved pancreatic and cardiac oxidative stress biomarkers, indicating both protective capabilities against oxidative tissue damage, which is implicated in the development of several degenerative diseases, including T2D. The extracts inhibited prominent carbohydrate and lipid-digesting enzymes while concomitantly reducing the hyperactivity of hepatic glucogenic enzymes, suggesting strong antihyperglycemic capabilities. While the current study did not identify compounds in the extracts of *A. adianthifolia*, previous reports suggest that these activities may be attributed to compounds including β -caryophyllene, e-geranyl acetone, acorenone, viridiflorol, α -zingiberene, ar-curcumene, selin-11-en-4- α -ol, caryophyllene oxide, and α -humulene (Akande *et al.*, 2018). Comparative analysis of various parameters revealed that while the bark extracts *A. adianthifolia* displayed superior antioxidant capabilities especially in the and NO inhibition assay, the leaf extracts demonstrated significantly greater antidiabetic potential, exhibiting superior inhibitory activity against α -amylase, α -glucosidase and pancreatic lipase. Interestingly, the leaf extract exhibited the strongest FRAP activity. Collectively, these findings suggest that the leaf extracts of *A. adianthifolia* possess a superior antioxidant and antidiabetic bioactivities compared to the stem bark extracts. *A. adianthifolia* extracts exhibited several pharmacological activities, which warrant further study using an experimental animal model to ascertain the results of this study.

The third plant that was studied was *R. longipes*. Firstly, dried leaf and stem bark of *R. longipes* were infused with hot distilled water after overnight defatting in n-hexane. The aqueous leaf and stem bark of *R. longipes* did not show cytotoxicity on normal Vero cells, suggesting the safety of this plant and supporting its use in folkloric medicine. *In vitro* and *ex vivo* studies revealed the antioxidant potency of the extracts, which effectively mopped up free radicals, including DPPH and NO. Furthermore, aqueous leaf and stem bark lowered lipid peroxidation, increased cellular reduced glutathione (GSH) concentration, and increased the activities of superoxide dismutase (SOD) and catalase (CAT) in renal homogenate treated with pro-oxidant (FeSO₄). Renal damage in T2D leads to end-stage nephropathy; therefore, the protective capacity of the aqueous leaf and stem bark against renal oxidative damage may aid in abating such deleterious pathologies in T2D. The antihyperglycemic capabilities of the aqueous leaf and stem bark were further highlighted by *in silico* studies that revealed the low negative free binding energy values of 24,25-dihydroxyvitamin D with digestive enzymes including α -glucosidase, α -amylase, and pancreatic lipase. The GC-MS result revealed the presence of several bioactive phytochemicals, including palmitoleic acid, octadecanamide, 24,25-dihydroxyvitamin D, and L-ascorbic acid, to which the observed pharmacological activities may be attributed.

Secondly, the study employed *in vitro*, *in silico*, and *ex vivo* methods to investigate the antioxidant and antidiabetic capabilities of leaf and stem bark crude extracts prepared by sequential extraction in water, methanol, ethanol, and ethyl acetate. The high phenolics and flavonoids in these extracts may be responsible for their enhanced electron-donating (FRAP), DPPH, and NO radical scavenging capabilities. Furthermore, the extracts demonstrated heightened α glucosidase and α amylase inhibitory activities coupled with increased capabilities to delay glucose absorption in rat jejunum while enhancing its uptake in the yeast suspension, liver, and muscle tissue. The extracts reduced hepatic ATPase and fructose-1,6-bisphosphatase to near-normal proportions. *R. longipes* leaf extracts significantly ($p < 0.05$) reduced lipid peroxidation while concomitantly augmenting superoxide dismutase (SOD) and catalase (CAT) activities, as well as elevating glutathione (GSH) levels in the pancreatic and cardiac muscle tissue. (+)-Ascorbic acid, 2,6-dihexadecanoate, 9-methyl-nonadecane, tetradecanoic acid, tributyl acetyl citrate, and squalene were among some of the bioactive compounds identified in *R. longipes*, which may be responsible for the observed biological activities. Analysis of IC₅₀ values revealed significantly superior bioactivity in the bark extract compared to the leaf extracts of *R. longipes*. The bark extracts of *R. longipes* exhibited

lower IC₅₀ values across most of the assays conducted, indicating their superior activity with respect to the leaf extracts. Specifically, the bark extract demonstrated the strongest inhibitory effects in the DPPH, pancreatic lipase, and α -glucosidase assay. While *R. longipes* shows potential as a source of antioxidant and antihyperglycemic pharmacological agent templates; further molecular and *in vivo* studies are necessary to elucidate the precise mechanisms of action of its phytoconstituents.

Lastly, the study evaluated the antioxidant, antilipidemic, and antidiabetic activities of the ethanolic bark crude extract of *R. longipes* using an *in vivo* T2D rat model. The ethanolic bark crude extract of *R. longipes* was selected based on its potent pharmacological activities observed using *in vitro*, *ex vivo*, and *in silico* studies. The high dose of *R. longipes* (DRLH) reduced polyphagia and polydipsia in the rats, suggesting its capabilities to abate T2D dysregulated satiety pathways. Furthermore, the extract restored euglycemic levels and improved glucose tolerance, indicating its antihyperglycemic capability. The ability of the high dose of *R. Longipes* to mitigate dyslipidaemia with a concomitant reduction in atherogenic index is pivotal in reducing the development of cardiovascular diseases (CVD) in people living with T2D. T2D patients are at a 2-fold risk of developing cardiovascular diseases (CVD), which are among the highest causes of mortality among sufferers. The ability of *R. longipes* to ameliorate redox imbalance by augmenting the antioxidant defense system played a pivotal role in reversing oxidative injury in pancreatic tissue, as revealed by histopathological examinations. As T2D ensues due to dysregulated pancreatic tissue function, the ability of *R. longipes* to upregulate insulin sensitivity and secretion through the regeneration of pancreatic β -cells signifies the extracts' potential to mitigate several T2D-linked pathologies. The ability of *R. longipes* to modulate hepatic glucogenic enzymes and restore hepatic glycogen stores insinuate reduced hepatic glucose output, therefore being a cornerstone pathway in maintaining euglycemia. Current data suggests that *R. longipes* may have several pharmacological activities beneficial against T2D; however, further molecular analysis and clinical trials are necessary to elucidate the precise mode of antidiabetic action shown in the current study.

The fourth plant studied was *Celtis africana*, which is well distributed across Africa, from Ethiopia to South Africa, where it is traditionally used to remedy eye infections, rheumatism, cancer, diabetes, and sexually transmitted diseases (Nchabeleng, 2017). The current study examined the antioxidant and antidiabetic capabilities of the leaf and stem bark extracts of *C. africana* prepared by sequential maceration in solvents of varying polarities. The pharmacological activities of the resulting extracts were evaluated using *in vitro*, *in silico*, and

ex vivo models. An array of bioactive phytochemicals was revealed by GC-MS analysis in both the leaf and stem bark extracts of *C. africana*, including 2H-Pyran-2-one, Squalene-4-Chlorophenylacetic acid, Ethyl- α -D-glucopyranoside, 3-O-Methyl-D-glucose, Isosorbide, Caprolactam, 5-hydroxy-1H-Indole-3-carboxylic acid, and 1-(+)-Ascorbic acid 2,6-dihexadecanoate. These bioactive phytochemicals may have directly contributed, either singularly or synergistically, to the pharmacological activities observed for *C. africana* in the current study. *C. africana* inhibited α amylase and α glucosidase, retarded intestinal glucose absorption, and increased its uptake by psoas muscle tissues and yeast cells, strongly suggesting its antihyperglycemic capabilities, which may aid in maintaining euglycemia in T2D patients. Furthermore, it showed enhanced antioxidant capabilities by its capacity to scavenge free radicals (DPPH and NO), donate electrons, and reduce lipid peroxidation while concomitantly augmenting reduced glutathione (GSH) levels and bolstering the activities of superoxide dismutase (SOD) and catalase (CAT) in FeSO₄-mediated oxidative pancreatic and cardiac injury. *C. africana* exhibited potent antioxidant capacity, carbohydrate/lipid hydrolysing enzyme inhibitory capabilities, modulatory abilities on hepatic glucogenic enzymes, glucose uptake, and absorption, which were linked to the presence of indole-based compounds as revealed by *in silico* analysis. IC₅₀ values revealed superior antioxidant activity in the leaf extract of *C. africana*, particularly for the ethanolic extract, when compared to the stem bark extracts. The leaf extract of *C. africana* exhibited lower IC₅₀ values in the DPPH and NO inhibition assays. The methanolic and aqueous leaf extracts exhibited superior pancreatic lipase and α -amylase inhibitory activities, respectively. Interestingly, the bark ethanolic extract demonstrated the strongest α -glucosidase inhibitory amongst all extracts. Conclusively, the leaf extracts of *C. africana* displayed superior antidiabetic activities compared to the stem bark extracts. Further *in vivo* experiments are necessary to verify these findings and elucidate probable modes of action.

Following *in vitro*, *ex vivo*, and *in silico* analysis, the best-performing *C. africana* extract (ethanolic leaf) extracts' antidiabetic, antioxidant, and antihyperlipidemic pharmacological capabilities were determined using a type 2 diabetic rat model. Evidently, the high dose of *C. africana* (DCAH) showed better capabilities when compared to the low dose in abating the diabetic pathologies analyzed in the current study. *C. africana* restored euglycemic levels and improved glucose tolerance in T2D rats, strongly indicating its antihyperglycemic capabilities. The extracts abated fatty acid deposits linked to hepatic enlargement by decreasing total cholesterol and triacyl glycerides while concomitantly increasing HDL cholesterol levels while concomitantly restoring hepatic glycogen reserves. These pharmacological activities show the extracts' capacity to reduce the atherogenic index of plasma and thus may play a crucial role

in mitigating T2D-linked CVD. *C. africana* improved indices of hepatic and renal T2D-linked damage while concomitantly reversing oxidative injury on pancreatic tissue. This observation may be attributed to their ability to augment the antioxidant defense system and ameliorate redox imbalance, thereby increasing the body's defense against oxidative tissue damage. Evidence from the data in this study suggests that *C. africana* has enhanced ameliorative capabilities against several T2D-linked pathologies that may occur through several mechanisms, including improved oxidative status, reduced tissue damage, and upregulation of insulin sensitivity and secretion through the regeneration of pancreatic β -cells. These findings warrant further clinical trials to elucidate the precise mode of antidiabetic action shown by *C. africana* in the current study.

Schotia brachypetala, of the family Fabaceae, was the last plant studied. *Schotia brachypetala* is widely used against T2D in South Africa (Silas and Alfred, 2019) due to its abundant secondary metabolites in *S. brachypetala*, including polyphenols, flavonoid derivatives, dihydrochalcones, procyanidins, anthocyanins, hydroxybenzoic acid derivatives, and hydrolysable tannins (Sobeh *et al.*, 2016). *S. brachypetala* had a high polyphenolic and flavonoid content with several compounds, including pyrogallol, O-Menthan-3-one, 2,3-diphenyl-quinoxaline, and l-(+)-Ascorbic acid 2,6-dihexadecanoate. The methanolic extract had the best electron-donating capabilities, DPPH, and NO scavenging activities. While the ethanol extract showed the best lipid and carbohydrate-inhibiting capabilities, Furthermore, 2,3-diphenyl-quinoxaline had the best binding affinities with selected lipid and carbohydrate-digesting enzymes. The ethanolic extract stimulated the best glucose uptake in the yeast suspension, muscle, and liver tissue, while it concomitantly had the best glucose absorption-retarding capability in the small intestinal tissue. The *S. brachypetala* crude bark extracts significantly reduced lipid peroxidation and augmented glutathione levels while bolstering the activities of increased cellular reduced glutathione (GSH) concentration and the activities of superoxide dismutase (SOD) and catalase (CAT) in homogenates treated with pro-oxidant (FeSO_4), suggesting enhanced protective capabilities against pancreatic and cardiac tissue oxidative damage. *S. brachypetala* may potentially regulate energy redox balance and reduce hepatic glucose output through their capabilities to reduce the activities of hepatic ATPase, glycogen phosphorylase, and fructose 1,6-bisphosphatase. Comparative analysis of antioxidant and carbohydrate/lipid digesting enzyme inhibitory activities revealed that leaf extracts of *S. brachypetala* exhibited superior bioactivity compared to the stem bark extracts. These results warrant further studies using molecular and *in vivo* models to ascertain the mode of action, particularly for the leaf *S. brachypetala* extracts.

Finally, the current study investigated the antioxidant, antilipidemic, and antidiabetic activities of the ethanolic crude extract of *S. brachypetala* using an *in vivo* T2D rat model. T2D was induced using the fructose-streptozotocin model, after which animals with non-fasting blood glucose (NFBG) levels greater than 200 mg/dl were considered diabetic and used for the experimental trial. Treatment, particularly with the high dose (300 mg/kg bw) of *S. brachypetala* improved glucose tolerance and restored euglycemic levels, possibly through the inhibition of carbohydrate-digesting enzymes and reduced polyphagia, suggesting strong antihyperglycemic capabilities. Furthermore, the ability of *S. brachypetala* to restore redox balance through reducing lipid peroxidation and bolstering antioxidant defence systems may be attributed to the plant's protective capabilities against T2D-induced hepatic, renal, and pancreatic tissue damage. Results of the study showed the capabilities of the extract to improve HOMA β while ameliorating HOMA IR through improved insulin secretion, which resulted from improved pancreatic β -cell health attributed to treatment with *S. brachypetala*. *S. brachypetala* inhibited the action of lipid-digesting enzymes and mitigated dysregulated lipid metabolism, thereby reducing the atherogenic index of the plasma, which is indicative of their capacity to abate end-stage T2D-induced cardiovascular diseases. The results of this study revealed that the T2D ameliorative potential of *S. brachypetala* may occur through several mechanisms, including upregulation of insulin sensitivity and secretion through the regeneration of pancreatic β -cells. Further molecular analyses and clinical trials are necessary to completely elucidate the precise mode of antidiabetic action shown in the current study.

8.2 General conclusion

The current study, as indicated in **Fig. 8.1**, demonstrates the ability of (*Rhus longipes* Engl.; *Celtis africana* Burm.f.; *Trema orientalis* (L.) Blume; *Albizia adianthifolia* (Schumach.) W.F.Wight; *Schotia brachypetala* Sond.) to improve glucose metabolism and modulate biological activities linked to T2D and its complications.

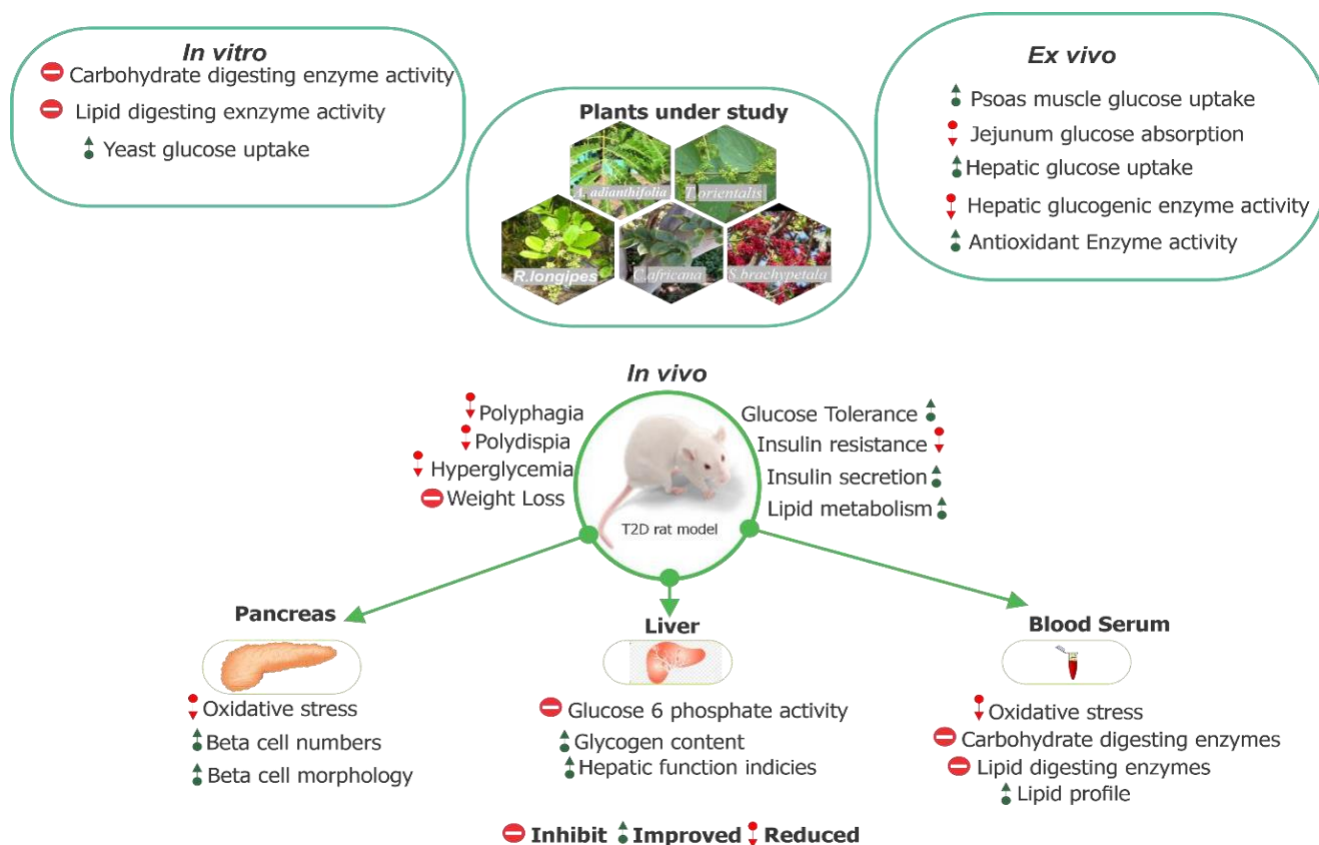


Figure 8.1: Schematic representation of the investigated plants' pharmacological activities and potential mode of action.

The selected plants were shown to possess a high content of both phenolics and flavonoids, coupled with an array of bioactive compounds identified in their different extracts whose presence insinuates their therapeutic capabilities. The extracts showed potent antioxidant capabilities through their enhanced electron-donating (FRAP), DPPH, and NO radical scavenging capabilities while concomitantly reducing lipid peroxidation and augmenting antioxidant defence systems in several examined tissues. Furthermore, the extracts demonstrated heightened pancreatic lipase, α glucosidase, and α amylase inhibitory activities coupled with increased capabilities to delay glucose absorption in rat jejunum while enhancing its uptake in the yeast suspension, liver, and muscle tissue. The plants were shown to potentially regulate energy redox balance and reduce hepatic glucose output through their abilities to

reduce the activities of hepatic glucogenic and nucleotide metabolism. *C. africana*, at both low and high doses, demonstrated the most potent weight loss mitigatory effect among all tested extracts. This effect was closely followed by the high dose of *S. brachypatela*, while *R. longipes* exhibited the least impact on diabetes-induced weight loss. Similarly, the high dose of *C. africana* resulted in the most substantial reduction in blood glucose levels across different diabetic treatment animal groups. The reduced blood glucose levels observed following treatment with the high dose of *C. africana* were closely matched by those observed in animals treated with the high dose of *S. brachypatela*. In the oral glucose tolerance test (OGTT), the high dose of *C. africana* again showed the best performance among different animal groups during the final week of the experimental period. This was closely followed by the results observed for *R. longipes*. The high dose of *C. africana* resulted in the best atherogenic index of the plasma (AIP) index among all tested extracts, closely followed by the high dose of *S. brachypatela*. *R. longipes* had the least effect on the atherogenic index of the plasma (AIP) among all tested extracts.

Overall, the comparative analysis of the anti-diabetic effects of the extracts, including parameters such as blood glucose levels, OGTT, and AIP indices, showed that the high dose of *C. africana* exhibited the most potent activity among all the treatments in this study. This was followed by the high dose of *S. brachypatela*, while the high dose of *R. longipes* had the third best activity. As shown in the *in vivo* studies, *Rhus longipes*, *Celtis africana*, and *Schotia brachypetala* restored euglycemic levels, improved glucose tolerance, mitigated dyslipidaemia with a concomitant reduction in atherogenic index, and ameliorated hepatic and renal oxidative stress. The isolation and characterization of bioactive compounds from these plant extracts may further enhance their anti-diabetic properties. Additionally, understanding the synergistic actions of the bioactive compounds in the different plant extracts could provide deeper insights into the observed antidiabetic activities in this study.

8.3 Recommendations

According to the current study, the folkloric use of the plants used for the management of T2D has been well documented and justified using various models, including *in vitro*, *ex vivo*, *in silico*, and *in vivo*. To the best of our knowledge, this work revealed for the first time the *in vivo* antidiabetic potentials of *Rhus longipes*, *Celtis africana*, and *Schotia brachypetala* in animal studies. Further studies are, however, required to isolate the exact bioactive phytoconstituents of the studied plants and elucidate their precise mode of action using molecular techniques. Successful elucidation of the modus operandi of the compounds could lead to the development of novel, cost-effective antidiabetic agents with high efficacy, increased bioavailability, and reduced undesirable side effects.

REFERENCES

- Aba, P.E. and Asuzu, I.U., 2018. Mechanisms of actions of some bioactive anti-diabetic principles from phytochemicals of medicinal plants: A review. *Indian Journal of Natural Products and Resources*, 9(2), p.85-96.
- Abbas, G., Al-Harrasi, A.S. and Hussain, H., 2016. α -glucosidase enzyme inhibitors from natural products. *Discovery and Development of Antidiabetic Agents from Natural Products*. UK: Elsevier.
- Abe, M., Ito, Y., Suzuki, A., Onoue, S., Noguchi, H. and Yamada, S., 2009. Isolation and pharmacological characterization of fatty acids from saw palmetto extract. *Analytical Sciences*, 25(4), p.553-557.
- Abiodun, O., Gbotosho, G., Ajaiyeoba, E., Happi, T., Falade, M., Wittlin, S., Sowunmi, A., Brun, R. and Oduola, A., 2011. *In vitro* antiplasmodial activity and toxicity assessment of some plants from Nigerian ethnomedicine. *Pharmaceutical biology*, 49(1), p.9-14.
- Adedapo, A.A., Jimoh, F.O., Afolayan, A.J. and Masika, P.J., 2009. Antioxidant Properties of the Methanol Extracts of the Leaves and Stems of *Celtis africana*. *Records of Natural Products*, 3(1), p.23-31.
- Aderibigbe, A.O., 2018. Antidepressant activity of ethanol extract of *Albizia adianthifolia* (Schumacher) W.F. Wight leaf in mice. *African Journal of Medicine and Medical Sciences*, 47(2), p.133-140.
- Adewusi, E.A., Moodley, N. and Steenkamp, V., 2011. Antioxidant and acetylcholinesterase inhibitory activity of selected southern African medicinal plants. *South African Journal of Botany*, 77(3), p.638-644.
- Adinortey, M.B., Galyuon, I.K. and Asamoah, N.O., 2013. *Trema orientalis* Linn. Blume: A potential for prospecting for drugs for various uses. *Pharmacognosy reviews*, 7(13), p.67.
- Adler, A., Bennett, P., Chair, S.C., Gregg, E., Narayan, K.V., Schmidt, M.I., Sobngwi, E., Tajima, N., Tandon, N., Unwin, N. and Wild, S., 2021. Reprint of: classification of diabetes mellitus. *Diabetes Research and Clinical Practice*. UK: Elsevier.
- Adwas, A.A., Elsayed, A., Azab, A.E. and Quwaydir, F.A., 2019. Oxidative stress and antioxidant mechanisms in human body. *J. Appl. Biotechnol. Bioeng*, 6(1), p.43-47.
- Agarwal, P. and Gupta, R., 2016. Alpha-amylase inhibition can treat diabetes mellitus. *Res. Rev. J. Med. Health Sci*, 5(4), p.1-8.

- Agius, L., 2015. Role of glycogen phosphorylase in liver glycogen metabolism. *Molecular aspects of medicine*, 46, pp.34-45.
- Aguilar, T.A.F., Navarro, B.C.H. and Pérez, J.A.M., 2016. Endogenous antioxidants: a review of their role in oxidative stress. *A master regulator of oxidative stress-the transcription factor nrf2*. UK: InTech
- Ahmadovna, R.M. and Sharipovna, Y.H., 2021. Disorders of Carbohydrate Metabolism in Overweight and Obesity. *Ijtimoiy fanlarda innovasiya onlayn ilmiy jurnali*, 1(5), p.90-96.
- Ahmed, A.M., 2002. History of diabetes mellitus. *Saudi medical journal*, 23(4), p.373-378.
- Ahola, A.J. and Groop, P.H., 2013. Barriers to self-management of diabetes. *Diabetic medicine*, 30(4), p.413-420.
- Ajebli, M., Khan, H., and Eddouks, M. 2021. Natural alkaloids and diabetes mellitus: A review. *Endocrine, Metabolic and Immune Disorders-Drug Targets*, 21(1), p.111–130.
- Akande, A., Aboaba, S. and Flamini, G., 2018. Constituents and Anthelmintic Activity Evaluation of Albizia Adiantifolia (Schumach) WF Wright Essential Oils From Nigeria. *International Journal of Chemistry*, 10(2), p.10-15.
- Alam, F., Shafique, Z., Amjad, S.T. and Bin Asad, M.H.H., 2019. Enzymes inhibitors from natural sources with antidiabetic activity: A review. *Phytotherapy Research*, 33(1), p.41-54.
- Alam, S., Hasan, M.K., Neaz, S., Hussain, N., Hossain, M.F. and Rahman, T., 2021. Diabetes Mellitus: insights from epidemiology, biochemistry, risk factors, diagnosis, complications, and comprehensive management. *Diabetology*, 2(2), p.36-50.
- Alam, Y.H., Kim, R. and Jang, C., 2022. Metabolism and health impacts of dietary sugars. *Journal of lipid and atherosclerosis*, 11(1), p.20.
- Al-Goblan, A.S., Al-Alfi, M.A. and Khan, M.Z., 2014. Mechanism linking diabetes mellitus and obesity. *Diabetes, metabolic syndrome and obesity: targets and therapy*, 7, p.587.
- Ali Asgar, M.D., 2013. Antidiabetic potential of phenolic compounds: A review. *International Journal of Food Properties*, 16(1), p.91-103.
- Ali, H., Houghton, P.J. and Soumyanath, A., 2006. α -Amylase inhibitory activity of some Malaysian plants used to treat diabetes; with particular reference to *Phyllanthus amarus*. *Journal of ethnopharmacology*, 107(3), p.449-455.
- Ali, O., 2013. Genetics of type 2 diabetes. *World journal of diabetes*, 4(4), p.114.
- Al-Ishaq, R.K., Abotaleb, M., Kubatka, P., Kajo, K. and Büsselberg, D., 2019. Flavonoids and

- their anti-diabetic effects: Cellular mechanisms and effects to improve blood sugar levels. *Biomolecules*, 9(9), p.430.
- Alkhalidy, H., Moore, W., Wang, Y., Luo, J., McMillan, R.P., Zhen, W., Zhou, K. and Liu, D., 2018. The flavonoid kaempferol ameliorates streptozotocin-induced diabetes by suppressing hepatic glucose production. *Molecules*, 23(9), p.2338.
- Almourani, R., Chinnakotla, B., Patel, R., Kurukulasuriya, L.R. and Sowers, J., 2019. Diabetes and cardiovascular disease: an update. *Current diabetes reports*, 19(12), p.1-13.
- Al-Musayeib, N.M., Mothana, R.A., Al-Massarani, S., Matheeussen, A., Cos, P. and Maes, L., 2012. Study of the *in vitro* antiplasmodial, antileishmanial and antitrypanosomal activities of medicinal plants from Saudi Arabia. *Molecules*, 17(10), p.11379-11390.
- Alsahli, M. and Gerich, J.E., 2017. Renal glucose metabolism in normal physiological conditions and in diabetes. *Diabetes research and clinical practice*, 133, p.1-9.
- Al-Taweel, A.M., Perveen, S., El-Shafae, A.M., Fawzy, G.A., Malik, A., Afza, N., Iqbal, L. and Latif, M., 2012. Bioactive phenolic amides from *Celtis africana*. *Molecules*, 17(3), p.2675-2682.
- American Diabetes Association, 2011. Standards of medical care in diabetes—2011. *Diabetes care*, 34(S1), p. S11-S61.
- American Diabetes Association, 2013. Standards of medical care in diabetes—2013. *Diabetes care*, 36(S1), p.S11-S66.
- American Diabetes Association, 2014. Diagnosis and classification of diabetes mellitus. *Diabetes care*, 37(S1), p.S81-S90.
- American Diabetes Association, 2014. Standards of medical care in diabetes--2014. *Diabetes care*, 37, p.S14.
- American Diabetes Association, 2015. Classification and diagnosis of diabetes. *Diabetes care*, 38(S1), p. S8-S16.
- American Diabetes Association, 2016. 2. Classification and diagnosis of diabetes. *Diabetes care*, 39(S1), p.S13-S22.
- Amuda, A. T., and Berkowitz, S. A. (2019). Diabetes and the built environment: evidence and policies. *Current Diabetes Reports*, 19, p.1–8.
- Amuri, B., Maseho, M., Simbi, L., Okusa, P., Duez, P. and Byanga, K., 2017. Hypoglycemic and antihyperglycemic activities of nine medicinal herbs used as antidiabetic in the region of Lubumbashi (DR Congo). *Phytotherapy research*, 31(7), p.1029-1033.

- Andolfi, C. and Fisichella, P.M., 2018. Epidemiology of obesity and associated comorbidities. *Journal of Laparoendoscopic and Advanced Surgical Techniques*, 28(8), p.919- 924.
- Anhê, F.F., Desjardins, Y., Pilon, G., Dudonné, S., Genovese, M.I., Lajolo, F.M. and Marette, A., 2013. Polyphenols and type 2 diabetes: A prospective review. *PharmaNutrition*, 1(4), p.105-114.
- Anık, A., Çatlı, G., Abacı, A. and Böber, E., 2015. Maturity-onset diabetes of the young (MODY): an update. *Journal of pediatric endocrinology and metabolism*, 28(3-4), p.251-263.
- Artasensi, A., Pedretti, A., Vistoli, G. and Fumagalli, L., 2020. Type 2 diabetes mellitus: a review of multi-target drugs. *Molecules*, 25(8), p.1987.
- Asif, M., 2015. Chemistry and antioxidant activity of plants containing some phenolic compounds. *Chemistry international*, 1(1), p.35-52.
- Asmat, U., Abad, K. and Ismail, K., 2016. Diabetes mellitus and oxidative stress—A concise review. *Saudi pharmaceutical journal*, 24(5), p.547-553.
- Assefa, S.T., Yang, E.Y., Chae, S.Y., Song, M., Lee, J., Cho, M.C. and Jang, S., 2019. Alpha glucosidase inhibitory activities of plants with focus on common vegetables. *Plants*, 9(1), p.2.
- Ayoade, G.W., Olusi, T.A., Amoo, I.A. and Eka-ete, G.A.E., 2014. Composition of some traditional malaria remedies and their antiplasmodial effects on (*Plasmodium berghei*). *International Journal of Scientific and Research Publications*, 4(3), p.1-7.
- Bailey, C.J. and Day, C., 2019. The future of new drugs for diabetes management. *Diabetes research and clinical practice*, 155, p.107785.
- Balahbib, A., El Omari, N., Ghchime, R., Hakkour, M., Sadak, A. and Bouyahya, A., 2020. Anti-Schistosomal Activity of Medicinal Plants: Mini. *Letters in Applied NanoBioScience*, 10(3), p. 2352-2360.
- Balogun, F.O. and Ashafa, A.O.T., 2017. Aqueous root extracts of *Dicoma anomala* (Sond.) extenuates postprandial hyperglycaemia *in vitro* and its modulation on the activities of carbohydrate-metabolizing enzymes in streptozotocin-induced diabetic Wistar rats. *South African journal of botany*, 112, p.102-111.
- Banday, M.Z., Sameer, A.S. and Nissar, S., 2020. Pathophysiology of diabetes: An overview. *Avicenna Journal of Medicine*, 10(04), p.174-188.

- Bano, G., 2013. Glucose homeostasis, obesity and diabetes. *Best Practice and Research Clinical Obstetrics and Gynaecology*, 27(5), p.715-726.
- Bansal, N., 2015. Prediabetes diagnosis and treatment: A review. *World journal of diabetes*, 6(2), p.296.
- Bansode, T.S., Gupta, A. and Salalkar, B.K., 2016. *In silico* and *in vitro* assessment on antidiabetic efficacy of secondary metabolites from *Syzygium cumini* (L.) Skeels. *Plant Science Today*, 3(4), p.360-367.
- Bashary, R., Vyas, M., Nayak, S.K., Suttee, A., Verma, S., Narang, R. and Khatik, G.L., 2020. An insight of alpha-amylase inhibitors as a valuable tool in the management of type 2 diabetes mellitus. *Current Diabetes Reviews*, 16(2), p.117-136.
- Baynes, H.W., 2015. Classification, pathophysiology, diagnosis and management of diabetes mellitus. *Journal of Diabetes and Metabolism*, 6(5), p.1-9.
- Becher, T., Palanisamy, S., Kramer, D. J., Eljalby, M., Marx, S. J., Wibmer, A. G., Butler, S. D., Jiang, C. S., Vaughan, R., Schöder, H., Mark, A., and Cohen, P., 2021. Brown adipose tissue is associated with cardiometabolic health. *Nature medicine*, 27(1), p.58–65.
- Beckles, G.L., Zhu, J., Moonesinghe, R. and Centers for Disease Control and Prevention (CDC), 2011. Diabetes-United States, 2004 and 2008. *MMWR Surveill Summ*, 60(S1), p.90-93.
- Beckman, J.A. and Creager, M.A., 2016. Vascular complications of diabetes. *Circulation research*, 118(11), p.1771-1785.
- Begg, D. P., and Woods, S. C, 2013. The endocrinology of food intake. *Nature Reviews Endocrinology*, 9(10), p.584–597.
- Bellou, V., Belbasis, L., Tzoulaki, I. and Evangelou, E., 2018. Risk factors for type 2 diabetes mellitus: an exposure-wide umbrella review of meta-analyses. *PloS one*, 13(3), p.e0194127.
- Benzie I.F., 2000. Evolution of antioxidant defence mechanisms. *Eur. J. Nutr.* 39 (2), p.53-61.
- Benzie, I.F. and Strain, J.J., 1996. The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: the FRAP assay. *Analytical biochemistry*, 239(1), p.70-76.
- Beppe, G.J., Dongmo, A.B., Foyet, H.S., Dimo, T., Mihasan, M. and Hritcu, L., 2015. The aqueous extract of *Albizia adianthifolia* leaves attenuates 6-hydroxydopamine-induced anxiety, depression and oxidative stress in rat amygdala. *BMC Complementary and Alternative Medicine*, 15(1), p.1-13.
- Bertoluci, M.C. and Rocha, V.Z., 2017. Cardiovascular risk assessment in patients with

- diabetes. *Diabetology and metabolic syndrome*, 9(1), p.1-13.
- Beseni, B.K., Olofinson, K.A., Salau, V.F., Erukainure, O.L. and Islam, M.S., 2022. *Rhus longipes* (Engl.) infusions improve glucose metabolism and mitigate oxidative biomarkers in ferrous sulfate-induced renal injury. *Asian Pacific Journal of Tropical Biomedicine*, 12(11), p.453.
- Bhatnagar, A. and Mishra, A., 2022. α -Glucosidase Inhibitors for Diabetes/Blood Sugar Regulation. In *Natural Products as Enzyme Inhibitors: An Industrial Perspective* (pp. 269-283). Singapore: Springer Nature.
- Blair M., 2016. Diabetes Mellitus Review. *Urologic nursing*, 36(1), p.27-36.
- Blake, S., 2021. Diabetes: is it time to flatten the curve? *The British Journal of General Practice*, 71(705), p.174.
- Blüher, M. and Stumvoll, M., 2020. Diabetes and obesity. In *Diabetes complications, comorbidities and related disorders* (pp. 1-49). Cham: Springer International Publishing.
- Bommer, C., Heesemann, E., Sagalova, V., Manne-Goehler, J., Atun, R., Bärnighausen, T. and Vollmer, S., 2017. The global economic burden of diabetes in adults aged 20–79 years: a cost- of-illness study. *The lancet Diabetes and endocrinology*, 5(6), p.423-430.
- Bommer, C., Sagalova, V., Heesemann, E., Manne-Goehler, J., Atun, R., Bärnighausen, T., Davies, J. and Vollmer, S., 2018. Global economic burden of diabetes in adults: projections from 2015 to 2030. *Diabetes care*, 41(5), p.963-970.
- Borquaye, L.S., Saah, S.A., Adu-Poku, D., Adu-Gyamfi, L., Bitian, K. and Bambil, W., 2020. Anti-inflammatory, antioxidant and total phenolic content of the ethanolic extracts of *Celtis africana* Burm. f. *Current Science Perspectives*, 6(3), p.43-49.
- Boudina, S. and Abel, E.D., 2010. Diabetic cardiomyopathy, causes and effects. *Reviews in Endocrine and Metabolic Disorders*, 11, p.31-39.
- Bourron, O., Daval, M., Hainault, I., Hajdouch, E., Servant, J. M., Gautier, J. F., Ferre, P., and Foufelle, F., 2010. Biguanides and thiazolidinediones inhibit stimulated lipolysis in human adipocytes through activation of AMP-activated protein kinase. *Diabetologia*, 53, p.768–778.
- Bratovcic, A., 2020. Antioxidant enzymes and their role in preventing cell damage. *Acta Scientifci Nutritional Health*, 4(3), p.1-7.
- Brewer, R.A., Gibbs, V.K. and Smith Jr, D.L., 2016. Targeting glucose metabolism for healthy aging. *Nutrition and healthy aging*, 4(1), pp.31-46.

- Brunerova, L., Rahelić, D., Ceriello, A. and Broz, J., 2018. Use of oral antidiabetic drugs in the treatment of maturity-onset diabetes of the young: A mini review. *Diabetes/metabolism research and reviews*, 34(1), p.e2940.
- Burkill, H.M., 1995. The useful plants of west tropical Africa, Vols. 1-3. *The useful plants of west tropical Africa, Vols. 1-3.*, (2. ed.). Kew: Royal Botanic Gardens.
- Busmann, R.W., 2006. Vegetation zonation and nomenclature of African Mountains—an overview. *Lyonia*, 11(1), p.41-66.
- Busmann, R.W., Paniagua-Zambrana, N.Y. and Njoroge, G.N., 2020. *Celtis africana* Burm. f. C annabaceae. *Ethnobotany of the Mountain Regions of Africa*, p.1-6.
- Buwa-Komoren, L.V., Mayekiso, B., Mhinana, Z. and Adeniran, A.L., 2019. An ethnobotanical and ethnomedicinal survey of traditionally used medicinal plants in Seymour, South Africa: An attempt toward digitization and preservation of ethnic knowledge. *Pharmacognosy Magazine*, 15(60), p.115.
- Cabello-Verrugio, C., Ruiz-Ortega, M., Mosqueira, M. and Simon, F., 2016. Oxidative Stress in Disease and Aging: Mechanisms and Therapies. *Oxidative Medicine and Cellular Longevity*, 2016, p.1-2.
- Capoccia, K., Odegard, P.S. and Letassy, N., 2016. Medication adherence with diabetes medication: a systematic review of the literature. *The Diabetes Educator*, 42(1), p.34-71.
- Catalá, A. and Díaz, M., 2016. Impact of lipid peroxidation on the physiology and pathophysiology of cell membranes. *Frontiers in physiology*, 7, p.423.
- Ceriello, A. and Prattichizzo, F., 2021. Variability of risk factors and diabetes complications. *Cardiovascular Diabetology*, 20(1), p.1-11.
- Cersosimo, E., Triplitt, C., Solis-Herrera, C., Mandarino, L.J. and DeFronzo, R.A., 2018. Pathogenesis of type 2 diabetes mellitus. Oregon (USA): Endotext.
- Chacha, M. and Mbugi, N.O., 2019. Acute Toxicity, Brine Shrimp Lethality and Phytochemical Screening of *Lannea schimperi* and *Searsia longipes*. *Journal of Chemical Health Risks*, 9(2), p.87-95.
- Chadt, A. and Al-Hasani, H., 2020. Glucose transporters in adipose tissue, liver, and skeletal muscle in metabolic health and disease. *Pflügers Archiv-European Journal of Physiology*, 472, p.1273-1298.
- Chandel, N.S., 2021. Carbohydrate metabolism. *Cold Spring Harbor Perspectives in Biology*, 13(1), p.a040568.

- Changlian, P.E.N.G., Shaowei, C., Zhifang, L. and Guizhu, L., 2000. Detection of antioxidative capacity in plants by scavenging organic free radical DPPH. *Sheng wu hua xue yu Sheng wu wu li jin Zhan*, 27(6), p.658-661.
- Chaput, J. P., Klingenberg, L., Rosenkilde, M., Gilbert, J. A., Tremblay, A., and Sjödin, A. (2011). Physical activity plays an important role in body weight regulation. *Journal of obesity*, 2011(1), p.1-11.
- Chatterjee, S., and Davies, M. J., 2015. Current management of diabetes mellitus and future directions in care. *Postgraduate Medical Journal*, 91(1081), p.612–621.
- Chaudhury, A., Duvoor, C., Reddy Dendi, V. S., Kraleti, S., Chada, A., Ravilla, R., Marco, A., Shekhawat, N. S., Montales, M. T., Kuriakose, K., Sasapu, A., Beebe, A., Patil, N., Musham, C. K., Lohani, G. P., and Mirza, W., 2017. Clinical Review of Antidiabetic Drugs: Implications for Type 2 Diabetes Mellitus Management. *Frontiers in endocrinology*, 8(6) p.1-12.
- Chawla, A., Chawla, R., and Jaggi, S., 2016. Microvascular and macrovascular complications in diabetes mellitus: Distinct or continuum? *Indian journal of endocrinology and metabolism*, 20(4), p.546-551.
- Chen, Z., Jin, Z.X., Cai, J., Li, R., Deng, K.Q., Ji, Y.X., Lei, F., Li, H.P., Lu, Z. and Li, H., 2022. Energy substrate metabolism and oxidative stress in metabolic cardiomyopathy. *Journal of Molecular Medicine*, 100(12), p.1721-1739.
- Chikara, G., Sharma, P.K., Dwivedi, P., Charan, J., Ambwani, S. and Singh, S., 2018. A narrative review of potential future antidiabetic drugs: should we expect more?. *Indian Journal of Clinical Biochemistry*, 33(2), p.121-131.
- Chinsembu, K.C., 2019. Diabetes mellitus and nature's pharmacy of putative antidiabetic plants. *Journal of herbal medicine*, 15, p.100230.
- Chiwanga, F.S., Njelekela, M.A., Diamond, M.B., Bajunirwe, F., Guwatudde, D., Nankya-Mutyoba, J., Kalyesubula, R., Adebamowo, C., Ajayi, I., Reid, T.G. and Volmink, J., 2016. Urban and rural prevalence of diabetes and pre-diabetes and risk factors associated with diabetes in Tanzania and Uganda. *Global health action*, 9(1), p.31440.
- Cho, N., Shaw, J.E., Karuranga, S., Huang, Y., da Rocha Fernandes, J.D., Ohlrogge, A.W. and Malanda, B., 2018. IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. *Diabetes research and clinical practice*, 138, p.271-281.
- Chowdhury, A.A. and Islam, M.S., 2004. Antimicrobial activity of *Trema orientalis*. *Dhaka*

University Journal of Pharmaceutical Sciences, 2004(3), p.115–117.

- Chowdhury, P. and Soulsby, M., 2002. Lipid peroxidation in rat brain is increased by simulated weightlessness and decreased by a soy-protein diet. *Annals of Clinical and Laboratory Science*, 32(2), p.188-192.
- Christodoulou, M.-I., Tchoumtchoua, J., Skaltsounis, A.-L., Scorilas, A., and Halabalaki, M., 2019. Natural alkaloids intervening the insulin pathway: new hopes for anti-diabetic agents? *Current Medicinal Chemistry*, 26(32), p.5982–6015.
- Chung, S. S., Kim, M., Lee, J. S., Ahn, B. Y., Jung, H. S., Lee, H. M., and Park, K. S., 2011. Mechanism for antioxidative effects of thiazolidinediones in pancreatic β -cells. *American journal of physiology. Endocrinology and metabolism*, 301(5), p.E912–E921.
- Cooper, R.S., Rotimi, C.N., Kaufman, J.S., Owoaje, E.E., Fraser, H., Forrester, T., Wilks, R., Riste, L.K. and Cruickshank, J.K., 1997. Prevalence of NIDDM among populations of the African diaspora. *Diabetes care*, 20(3), p.343-348.
- Coopoosamy, R.M. and Naidoo, K.K., 2012. An ethnobotanical study of medicinal plants used by traditional healers in Durban, South Africa. *African Journal of Pharmacy and Pharmacology*, 6(11), p.818-823.
- Ćorković, I., Gašo-Sokač, D., Pichler, A., Šimunović, J. and Kopjar, M., 2022. Dietary polyphenols as natural inhibitors of α -amylase and α -glucosidase. *Life*, 12(11), p.1692.
- Craft, B.D., Kerrihard, A.L., Amarowicz, R. and Pegg, R.B., 2012. Phenol-based antioxidants and the *in vitro* methods used for their assessment. *Comprehensive Reviews in Food Science and Food Safety*, 11(2), p.148-173.
- Craft, L.L., Zderic, T.W., Gapstur, S.M., VanIterson, E.H., Thomas, D.M., Siddique, J. and Hamilton, M.T., 2012. Evidence that women meeting physical activity guidelines do not sit less: an observational inclinometry study. *International Journal of Behavioral Nutrition and Physical Activity*, 9(1), p.1-9.
- Craig, M.E., Hattersley, A. and Donaghue, K.C., 2009. Definition, epidemiology and classification of diabetes in children and adolescents. *Pediatric diabetes*, 10, p.3-12.
- Dabur, R., Sharma, B., and Mittal, A., 2018. Mechanistic approach of anti-diabetic compounds identified from natural sources. *Chemical Biology Letters*, 5(2), p.63-99.
- Dağışan, S. and Erbaş, O., 2020. Insulin structure, function and diabetes models in animals. *Journal of Experimental and Basic Medical Sciences*, 1(3), pp.096-101.
- Daisy, P., Jasmine, R., Ignacimuthu, S. and Murugan, E., 2009. A novel steroid from *Elephantopus scaber* L. an ethnomedicinal plant with antidiabetic

- activity. *Phytomedicine*, 16(2-3), p.252-257.
- Dallakyan, S., and Olson, A.J. ,2015. Small-Molecule Library Screening by Docking with PyRx. *Methods in Molecular Biology*, vol 1263. New York: Humana Press.
- Dar, R.A., Shahnawaz, M. and Qazi, P.H., 2017. General overview of medicinal plants: A review. *The Journal of Phytopharmacology*, 6(6), p.349-351.
- Das, K. and Gezici, S., 2018. Secondary plant metabolites, their separation and identification, and role in human disease prevention. *Ann. Phytomedicine*, 7, p.13-24.
- Dastjerdi, Z.M., Namjoyan, F. and Azemi, M.E., 2015. Alpha amylase inhibition activity of some plants extract of *Teucrium* species. *European Journal of Biological Sciences*, 7(1), p.26- 31.
- de Paulo Farias, D., de Araujo, F.F., Neri-Numa, I.A. and Pastore, G.M., 2021. Antidiabetic potential of dietary polyphenols: A mechanistic review. *Food Research International*, 145, p.110383.
- De Wet, H., Nzama, V.N. and Van Vuuren, S.F., 2012. Medicinal plants used for the treatment of sexually transmitted infections by lay people in northern Maputaland, KwaZulu–Natal Province, South Africa. *South African Journal of Botany*, 78, p.12-20.
- DeFronzo, R.A., 2009. From the triumvirate to the ominous octet: a new paradigm for the treatment of type 2 diabetes mellitus. *Diabetes*, 58(4), p.773-795.
- Degu, S., Abebe, A. and Gemed, N., 2022. Review of ethnobotanical studies on medicinal plants that used to treat diarrhea and dysentery in Ethiopia. *Discovery Phytomedicine*, 9(3), p.233-246.
- Deka, H., Choudhury, A. and Dey, B.K., 2022. An overview on plant derived phenolic compounds and their role in treatment and management of diabetes. *Journal of Pharmacopuncture*, 25(3), p.199.
- Dendup, T., Feng, X., Clingan, S. and Astell-Burt, T., 2018. Environmental risk factors for developing type 2 diabetes mellitus: a systematic review. *International journal of environmental research and public health*, 15(1), p.78.
- Deng, D. and Yan, N., 2016. GLUT, SGLT, and SWEET: Structural and mechanistic investigations of the glucose transporters. *Protein Science*, 25(3), p.546-558.
- Deng, L., Du, C., Song, P., Chen, T., Rui, S., Armstrong, D. G., and Deng, W., 2021. The Role of Oxidative Stress and Antioxidants in Diabetic Wound Healing. *Oxidative medicine and cellular longevity*, 2021(1), p.1-11.
- Dessie, G., Mulugeta, H., Amare, D., Negesse, A., Wagnew, F., Getaneh, T., Endalamew, A.,

- Adamu, Y.W., Tadesse, G., Workineh, A. and Lebu, S., 2020. A systematic analysis on prevalence and sub-regional distribution of undiagnosed diabetes mellitus among adults in African countries. *Journal of Diabetes and Metabolic Disorders*, 19(2), p.1931-1941.
- Deutschländer, M. S., van de Venter, M., Roux, S., Louw, J., and Lall, N., 2009. Hypoglycaemic activity of four plant extracts traditionally used in South Africa for diabetes. *Journal of ethnopharmacology*, 124(3), p.619–624.
- Dijoux-Franca, M.G., Tchamo, D.N., Cherel, B., Cussac, M., Tsamo, E. and Mariotte, A.M., 2001. New Dihydrophenanthrene and Phenyl-dihydro-iso-coumarin constituents of *Trema orientalis*. *Journal of natural products*, 64(6), p.832-835.
- Dimo, T., Nguenim, F.T., Kamtchouing, P., Dongo, E. and Tan, P.V., 2006. Glucose lowering efficacy of the aqueous stem bark extract of *Trema orientalis* (Linn) Blume in normal and streptozotocin diabetic rats. *Die Pharmazie-An International Journal of Pharmaceutical Sciences*, 61(3), p.233-236.
- Dirir, A.M., Daou, M., Yousef, A.F. and Yousef, L.F., 2022. A review of α -glucosidase inhibitors from plants as potential candidates for the treatment of type-2 diabetes. *Phytochemistry Reviews*, 21(4), p.1049-1079.
- Donnier-Maréchal, M. and Vidal, S., 2016. Glycogen phosphorylase inhibitors: a patent review (2013-2015). *Expert opinion on therapeutic patents*, 26(2), p.199-212.
- Dowarah, J. and Singh, V.P., 2020. Anti-diabetic drugs recent approaches and advancements. *Bioorganic and medicinal chemistry*, 28(5), p.115263.
- Drewes, S.E. and Fletcher, I.P., 1974. Polyhydroxystilbenes from the heartwood of *Schotia brachypetala*. *Journal of the Chemical Society, Perkin Transactions 1*, p.961-962.
- Drucker, D.J., Sherman, S.I., Gorelick, F.S., Bergenstal, R.M., Sherwin, R.S. and Buse, J.B., 2010. Incretin-based therapies for the treatment of type 2 diabetes: evaluation of the risks and benefits. *Diabetes care*, 33(2), p.428-433.
- Du, K., 2011. *Isolation of bioactive constituents from seeds of Schotia brachypetala (Fabaceae) and Colophospermum mopane (Fabaceae)*. Doctoral dissertation. University of the Free State (South Africa).
- Dwivedi, M. and Pandey, A.R., 2020. Diabetes mellitus and its treatment: an overview. *Journal of Advancement in Pharmacology*, 1(1), pp.48-58.
- Eguchi, N., Vaziri, N.D., Dafoe, D.C. and Ichii, H., 2021. The role of oxidative stress in pancreatic β cell dysfunction in diabetes. *International journal of molecular sciences*,

22(4), p.1509.

Eid, S., Sas, K.M., Abcouwer, S.F., Feldman, E.L., Gardner, T.W., Pennathur, S. and Fort, P.E., 2019. New insights into the mechanisms of diabetic complications: role of lipids and lipid metabolism. *Diabetologia*, 62, p.1539-1549.

Einarson, T.R., Acs, A., Ludwig, C. and Panton, U.H., 2018. Prevalence of cardiovascular disease in type 2 diabetes: a systematic literature review of scientific evidence from across the world in 2007–2017. *Cardiovascular diabetology*, 17(1), p.1-19.

El Khatabi, K., El-Mernissi, R., Hajji, H., Singh, A.K., Aziz, M., Lakhlifi, T., Kumar, S. and Bouachrine, M., 2022. Identification of novel indole derivatives as potent α -amylase inhibitors for the treatment of type-II diabetes using in-silico approaches. *Bio interface Research in Applied Chemistry*, 13(1), p.17.

Eldeen, I.M.S., Elgorashi, E.E. and Van Staden, J., 2005. Antibacterial, anti-inflammatory, anti-cholinesterase and mutagenic effects of extracts obtained from some trees used in South African traditional medicine. *Journal of Ethnopharmacology*, 102(3), p.457-464.

Elekofehinti, O. O., 2015. Saponins: Anti-diabetic principles from medicinal plants—A review. *Pathophysiology*, 22(2), 95–103.

Ellman, G.L., 1959. Tissue sulfhydryl groups. *Archives of biochemistry and biophysics*, 82(1), p.70-77.

Erukainure, O.L., Msomi, N.Z., Beseni, B.K., Salau, V.F., Ijomone, O.M., Koorbanally, N.A. and Islam, M.S., 2021. Cola nitida infusion modulates cardiometabolic activities linked to cardiomyopathy in diabetic rats. *Food and Chemical Toxicology*, 154, p.112335.

Erukainure, O.L., Oyebode, O.A., Sokhela, M.K., Koorbanally, N.A. and Islam, M.S., 2017. Caffeine-rich infusion from Cola nitida (kola nut) inhibits major carbohydrate catabolic enzymes; abates redox imbalance; and modulates oxidative dysregulated metabolic pathways and metabolites in Fe^{2+} -induced hepatic toxicity. *Biomedicine and Pharmacotherapy*, 96, p.1065-1074.

Evert, A.B., Dennison, M., Gardner, C.D., Garvey, W.T., Lau, K.H.K., MacLeod, J., Mitri, J., Pereira, R.F., Rawlings, K., Robinson, S. and Saslow, L., 2019. Nutrition therapy for adults with diabetes or prediabetes: a consensus report. *Diabetes care*, 42(5), p.731.

Farag, Y.M. and Gaballa, M.R., 2011. Diabetes: an overview of a rising epidemic. *Nephrology Dialysis Transplantation*, 26(1), p.28-35.

Farah, C., Michel, L.Y. and Balligand, J.L., 2018. Nitric oxide signalling in cardiovascular

- health and disease. *Nature Reviews Cardiology*, 15(5), p.292-316.
- Feinman, R.D., Pogozelski, W.K., Astrup, A., Bernstein, R.K., Fine, E.J., Westman, E.C., Accurso, A., Frassetto, L., Gower, B.A., McFarlane, S.I. and Nielsen, J.V., 2015. Dietary carbohydrate restriction as the first approach in diabetes management: critical review and evidence base. *Nutrition*, 31(1), p.1-13.
- Feldman, E.L., Callaghan, B.C., Pop-Busui, R., Zochodne, D.W., Wright, D.E., Bennett, D.L., Bril, V., Russell, J.W. and Viswanathan, V., 2019. Diabetic neuropathy. *Nature reviews Disease primers*, 5(1), p.1-18.
- Feldman, H.A., 2020. When crisis strikes: the acute complications of diabetes. *Physician Assistant Clinics*, 5(2), p.191-211.
- Ferdosi, M.F., Khan, I.H., Javaid, A., Nadeem, M. and Munir, A., 2021. Biochemical profile of *Calotropis procera* flowers. *Pakistan Journal of Weed Science Research*, 27(3), p.341.
- Fernandes, S.M., Cordeiro, P.M., Watanabe, M., Fonseca, C.D.D. and Vattimo, M.D.F.F., 2016. The role of oxidative stress in streptozotocin-induced diabetic nephropathy in rats. *Archives of endocrinology and metabolism*, 60, pp.443-449.
- Filippatos, T., Tsimihodimos, V., Pappa, E. and Elisaf, M., 2017. Pathophysiology of Diabetic Dyslipidaemia. *Current vascular pharmacology*, 15(6), p.566-575.
- Forbes, J.M. and Thorburn, D.R., 2018. Mitochondrial dysfunction in diabetic kidney disease. *Nature Reviews Nephrology*, 14(5), p.291-312.
- Forouhi, N.G. and Wareham, N.J., 2019. Epidemiology of diabetes. *Medicine*, 47(1), p.22-27.
- Forrester, S.J., Kikuchi, D.S., Hernandez, M.S., Xu, Q. and Griendling, K.K., 2018. Reactive oxygen species in metabolic and inflammatory signaling. *Circulation research*, 122(6), p.877- 902.
- Fowler, M.J., 2011. Microvascular and macrovascular complications of diabetes. *Clinical diabetes*, 29(3), p.116-122.
- Gadde, K.M., Martin, C.K., Berthoud, H.R. and Heymsfield, S.B., 2018. Obesity: pathophysiology and management. *Journal of the American College of Cardiology*, 71(1), p.69- 84.
- Galicia-Garcia, U., Benito-Vicente, A., Jebari, S., Larrea-Sebal, A., Siddiqi, H., Uribe, K.B., Ostolaza, H. and Martín, C., 2020. Pathophysiology of type 2 diabetes mellitus. *International journal of molecular sciences*, 21(17), p.6275.
- Gaschler, M.M. and Stockwell, B.R., 2017. Lipid peroxidation in cell death. *Biochemical and*

- biophysical research communications*, 482(3), p.419-425.
- Gaschler, M.M. and Stockwell, B.R., 2017. Lipid peroxidation in cell death. *Biochemical and biophysical research communications*, 482(3), p.419-425.
- Gbekley, H.E., Karou, S.D., Katawa, G., Tchacondo, T., Batawila, K., Ameyapoh, Y. and Simpure, J., 2018. Ethnobotanical survey of medicinal plants used in the management of hypertension in the maritime region of Togo. *African Journal of Traditional, Complementary and Alternative Medicines*, 15(1), p.85-97.
- Gee, P. and Davison, A.J., 1989. Intermediates in the aerobic autoxidation of 6-hydroxydopamine: relative importance under different reaction conditions. *Free Radical Biology and Medicine*, 6(3), p.271-284.
- Genuth S.M., Palmer J.P., Nathan D.M., 2018. Classification and Diagnosis of Diabetes. In: Diabetes in America. 3rd ed. Bethesda: National Institute of Diabetes and Digestive and Kidney Diseases.
- Gerber, P.A. and Rutter, G.A., 2017. The role of oxidative stress and hypoxia in pancreatic β -cell dysfunction in diabetes mellitus. *Antioxidants and redox signaling*, 26(10), p.501-518.
- Ghasemi-Dehnoo, M., Amini-Khoei, H., Lorigooini, Z. and Rafieian-Kopaei, M., 2020. Oxidative stress and antioxidants in diabetes mellitus. *Asian Pacific Journal of Tropical Medicine*, 13(10), p.431.
- Gheith, O., Farouk, N., Nampoory, N., Halim, M.A. and Al-Otaibi, T., 2016. Diabetic kidney disease: world wide difference of prevalence and risk factors. *Journal of nephropharmacology*, 5(1), p.49.
- Ghimire, K., Altmann, H.M., Straub, A.C. and Isenberg, J.S., 2017. Nitric oxide: what's new to NO?. *American Journal of Physiology-Cell Physiology*, 312(3), pp.C254-C262.
- Giacco, F., and Brownlee, M., 2010. Oxidative stress and diabetic complications. *Circulation Research*, 107(9), p.1058–1070.
- Gilbert, E.R. and Liu, D., 2013. Anti-diabetic functions of soy isoflavone genistein: mechanisms underlying its effects on pancreatic β -cell function. *Food and function*, 4(2), p.200-212.
- Ginzberg, M.B., Kafri, R. and Kirschner, M., 2015. On being the right (cell) size. *Science*, 348(6236), p.1245075.
- Glovaci, D., Fan, W. and Wong, N.D., 2019. Epidemiology of diabetes mellitus and cardiovascular disease. *Current cardiology reports*, 21(4), p.1-8.

- Gohari, A.R., Saeidnia, S., Hadjiakhoondi, A., Abdoullahi, M. and Nezafati, M., 2009. Isolation and Quantificative Analysis of Oleanolic Acid from *Satureja mutica* Fisch. and CA Mey. *Journal of Medicinal Plants*, 8(29), p.65-69.
- Goldenberg, R. and Punthakee, Z., 2013. Definition, classification and diagnosis of diabetes, prediabetes and metabolic syndrome. *Canadian journal of diabetes*, 37, p.S8-S11.
- Governa, P., Baini, G., Borgonetti, V., Cettolin, G., Giachetti, D., Magnano, A.R., Miraldi, E. and Biagi, M., 2018. Phytotherapy in the management of diabetes: a review. *Molecules*, 23(1), p.105.
- Goyal, R. and Jialal, I., 2018. Diabetes mellitus type 2. Treasure Island: StatPearls Publishing.
- Goyal, Y., Verma, A.K., Bhatt, D., Rahmani, A.H. and Dev, K., 2020. Diabetes: perspective and challenges in modern era. *Gene Reports*, 20, p.100759.
- Grace, O.M., Prendergast, H.D.V., Van Staden, J. and Jäger, A.K., 2002. The status of bark in South African traditional health care. *South African Journal of Botany*, 68(1), p.21-30.
- Grace, O.M., Prendergast, H.D.V., Van Staden, J., Jäger, A.K. and Eloff, J.N., 2003. The suitability of Thin Layer Chromatography for authenticating bark medicines used in South African traditional healthcare. *South African journal of botany*, 69(2), p.165-169.
- Gregg, E.W., Hora, I. and Benoit, S.R., 2019. Resurgence in diabetes-related complications. *Jama*, 321(19), p.1867-1868.
- Grotzke, M. and Jones, R.E., 2013. Acute and chronic complications of diabetes. In *Endocrine Secrets* (pp. 16-27). WB Saunders.
- Gulcin, İ., 2020. Antioxidants and antioxidant methods: An updated overview. *Archives of toxicology*, 94(3), p.651-715.
- Gutiérrez-Grijalva, E.P., Ambriz-Pérez, D.L., Leyva-López, N., Castillo-López, R.I. and Heredia, J.B., 2016. Dietary phenolic compounds, health benefits and bioaccessibility. *Archivos latinoamericanos de nutrición*, 66(2), pp.87-100.
- Gylfe, E. and Gilon, P., 2014. Glucose regulation of glucagon secretion. *Diabetes research and clinical practice*, 103(1), pp.1-10.
- Hadwan, M.H. and Abed, H.N., 2016. Data supporting the spectrophotometric method for the estimation of catalase activity. *Data in brief*, 6, p.194-199.
- Hajiaghaalipour, F., Khalilpourfarshbafi, M. and Arya, A., 2015. Modulation of glucose transporter protein by dietary flavonoids in type 2 diabetes mellitus. *International journal of biological sciences*, 11(5), p.508.

- Halim, M. and Halim, A., 2019. The effects of inflammation, aging and oxidative stress on the pathogenesis of diabetes mellitus (type 2 diabetes). *Diabetes and Metabolic Syndrome: Clinical Research and Reviews*, 13(2), p.1165-1172.
- Hameed, I.H., Hussein, H.J., Kareem, M.A. and Hamad, N.S., 2015. Identification of five newly described bioactive chemical compounds in methanolic extract of *Mentha viridis* by using gas chromatography-mass spectrometry (GC-MS). *Journal of Pharmacognosy and Phytotherapy*, 7(7), p.107-125.
- Hameed, S., Khan, K.M., Taslimi, P., Salar, U., Taskin-Tok, T., Kisa, D., Saleem, F., Solangi, M., Ahmed, M.H.U. and Rani, K., 2022. Evaluation of synthetic 2-aryl quinoxaline derivatives as α -amylase, α -glucosidase, acetylcholinesterase, and butyrylcholinesterase inhibitors. *International Journal of Biological Macromolecules*, 211, p.653-668.
- Hamilton, M.T., Hamilton, D.G. and Zderic, T.W., 2014. Sedentary behavior as a mediator of type 2 diabetes. In *Diabetes and Physical Activity* (Vol. 60, p. 11-26). Karger Publishers.
- Haminiuk, C.W., Maciel, G.M., Plata-Oviedo, M.S. and Peralta, R.M., 2012. Phenolic compounds in fruits—an overview. *International Journal of Food Science and Technology*, 47(10), p.2023-2044.
- Han, H.S., Kang, G., Kim, J.S., Choi, B.H. and Koo, S.H., 2016. Regulation of glucose metabolism from a liver-centric perspective. *Experimental and molecular medicine*, 48(3), p.e218-e218.
- Harding, J.L., Pavkov, M.E., Magliano, D.J., Shaw, J.E. and Gregg, E.W., 2019. Global trends in diabetes complications: a review of current evidence. *Diabetologia*, 62(1), p.3-16.
- Harris, E.H., 2005. Elevated liver function tests in type 2 diabetes. *Clinical diabetes*, 23(3), p.115-119.
- Hassan, Z., Yam, M.F., Ahmad, M. and Yusof, A.P.M., 2010. Antidiabetic properties and mechanism of action of *Gynura procumbens* water extract in streptozotocin-induced diabetic rats. *Molecules*, 15(12), p.9008-9023.
- Hatting, M., Tavares, C.D., Sharabi, K., Rines, A.K. and Puigserver, P., 2018. Insulin regulation of gluconeogenesis. *Annals of the New York Academy of Sciences*, 1411(1), p.21-35.
- Hayes, J.M., Kantsadi, A.L. and Leonidas, D.D., 2014. Natural products and their derivatives as inhibitors of glycogen phosphorylase: potential treatment for type 2 diabetes. *Phytochemistry Reviews*, 13, p.471-498.

- He, Y., Wang, Q., Ye, Y., Liu, Z. and Sun, H., 2020. The ethnopharmacology, phytochemistry, pharmacology and toxicology of genus *Albizia*: a review. *Journal of ethnopharmacology*, 257, p.112677.
- He, Z.X., Zhou, Z.W., Yang, Y., Yang, T., Pan, S.Y., Qiu, J.X. and Zhou, S.F., 2015. Overview of clinically approved oral antidiabetic agents for the treatment of type 2 diabetes mellitus. *Clinical and experimental pharmacology and physiology*, 42(2), p.125-138.
- Hemalatha, T., Pulavendran, S., Balachandran, C., Manohar, B. M., and Puvanakrishnan, R., 2010. Arjunolic acid: A novel phytomedicine with multifunctional therapeutic applications. *Indian Journal of Experimental Biology*, 48(3), p.238-247.
- Herman, W.H., 2017. The global burden of diabetes: an overview. In *Diabetes mellitus in developing countries and underserved communities* (p. 1-5). Springer, Cham.
- Himanshu, D., Ali, W. and Wamique, M., 2020. Type 2 diabetes mellitus: pathogenesis and genetic diagnosis. *Journal of Diabetes and Metabolic Disorders*, 19(2), p.1959-1966.
- Hines, D.A. and Eckman, K., 1993. *Indigenous multipurpose trees of Tanzania: uses and economic benefits for people* (p. 39). Ottawa, Canada: Cultural Survival Canada.
- Ho, G.T.T., Kase, E.T., Wangenstein, H. and Barsett, H., 2017. Phenolic elderberry extracts, anthocyanins, procyanidins, and metabolites influence glucose and fatty acid uptake in human skeletal muscle cells. *Journal of Agricultural and Food Chemistry*, 65(13), p.2677-2685.
- Hostalek, U., 2019. Global epidemiology of prediabetes-present and future perspectives. *Clinical diabetes and endocrinology*, 5(1), p.1-5.
- Hovind, P., Rossing, P., Johnson, R.J. and Parving, H.H., 2011. Serum uric acid as a new player in the development of diabetic nephropathy. *Journal of Renal Nutrition*, 21(1), pp.124-127.
- Hu, B., Cui, F., Yin, F., Zeng, X., Sun, Y. and Li, Y., 2015. Caffeoylquinic acids competitively inhibit pancreatic lipase through binding to the catalytic triad. *International Journal of Biological Macromolecules*, 80, p.529-535.
- Hu, C. and Jia, W., 2019. Therapeutic medications against diabetes: what we have and what we expect. *Advanced Drug Delivery Reviews*, 139, p.3-15.
- Hu, F.B., 2003. Sedentary lifestyle and risk of obesity and type 2 diabetes. *Lipids*, 38(2), p.103-108.

- Hua, Q. X., Shoelson, S. E., Kochoyan, M., and Weiss, M. A., 1991. Receptor binding redefined by a structural switch in a mutant human insulin. *Nature*, 354(6350), p.238–241.
- Huang E.S., 2016. Management of diabetes mellitus in older people with comorbidities. *Bmj*. 353, p.2200.
- Huang, D., 2018. Dietary antioxidants and health promotion. *Antioxidants*, 7(1), p.9.
- Huang, D.W. and Shen, S.C., 2012. Caffeic acid and cinnamic acid ameliorate glucose metabolism via modulating glycogenesis and gluconeogenesis in insulin-resistant mouse hepatocytes. *Journal of Functional Foods*, 4(1), p.358-366.
- Hung, H.Y., Qian, K., Morris-Natschke, S.L., Hsu, C.S. and Lee, K.H., 2012. Recent discovery of plant-derived anti-diabetic natural products. *Natural product reports*, 29(5), p.580-606.
- Hutchings, A., 1996. *Zulu medicinal plants: An inventory*. South Africa: University of Natal press.
- Huyut, Z., Beydemir, Ş. and Gülçin, İ., 2017. Antioxidant and antiradical properties of selected flavonoids and phenolic compounds. *Biochemistry research international*, 2017 p.1-10.
- Iacobini, C., Vitale, M., Pesce, C., Pugliese, G. and Menini, S., 2021. Diabetic complications and oxidative stress: A 20-year voyage back in time and back to the future. *Antioxidants*, 10(5), p.727.
- Ibrahim, N., Saleh, A., Usman, A., Yahaya, S. and Isa, H., 2020. Uses and toxicological potentials of *Trema orientalis* linn blume in livestock: a review. *Nigerian Journal of Scientific Research*, 19(2), p.124-132.
- Ighodaro, O.M., 2018. Molecular pathways associated with oxidative stress in diabetes mellitus. *Biomedicine and Pharmacotherapy*, 108, p.656-662.
- Igley, K., Hannachi, H., Joseph Howie, P., Xu, J., Li, X., Engel, S.S., Moore, L.M. and Rajpathak, S., 2016. Prevalence and co-prevalence of comorbidities among patients with type 2 diabetes mellitus. *Current medical research and opinion*, 32(7), p.1243-1252.
- Ike, D.C., Ibezim-Ezeani, M.U. and Akaranta, O., 2021. Cashew nutshell liquid and its derivatives in oil field applications: an update. *Green Chemistry Letters and Reviews*, 14(4), p.620-633.
- Imam, K., 2013. Clinical features, diagnostic criteria and pathogenesis of diabetes mellitus. *diabetes*, p.340-355.
- International Diabetes Federation. IDF Diabetes Atlas, 10th edn. Brussels, Belgium: 2021. Available at: <https://www.diabetesatlas.org>

- International Diabetes Federation. IDF Diabetes Atlas, 9th edn. Brussels, Belgium: 2019. Available at: <https://www.diabetesatlas.org>
- Ito, K. and Ito, M., 2013. The sedative effect of inhaled terpinolene in mice and its structure–activity relationships. *Journal of natural medicines*, 67, p.833-837.
- Iwu, M.M., 1993. *African medicinal plants* (pp. 109-110). Maryland: CRC Press.
- Jacob, B. and Narendhirakannan, R.T., 2019. Role of medicinal plants in the management of diabetes mellitus: a review. 3 *Biotech*, 9, p.1-17.
- Jain, C., Khatana, S. and Vijayvergia, R., 2019. Bioactivity of secondary metabolites of various plants: a review. *International Journal of Pharmaceutical Sciences and Research*, 10(2), p.494- 498.
- Jakubczyk, K., Dec, K., Kałduńska, J., Kawczuga, D., Kochman, J. and Janda, K., 2020. Reactive oxygen species-sources, functions, oxidative damage. *Polski merkuriusz lekarski: organ Polskiego Towarzystwa Lekarskiego*, 48(284), p.124.
- Jayachitra, J. and Janani, B., 2019. GC-MS Analysis Of Methanolic Extract Of Boerhaviadiffusa L. *Think India Journal*, 22(14), p.2853-2862.
- Jenkins, D.W. and Jenks, A., 2017. Exercise and diabetes: a narrative review. *The Journal of Foot and Ankle Surgery*, 56(5), p.968-974.
- Jeong, J.B., Hong, S.C., Jeong, H.J. and Koo, J.S., 2011. Anti-inflammatory effect of 2-methoxy-4-vinylphenol via the suppression of NF- κ B and MAPK activation, and acetylation of histone H3. *Archives of pharmacal research*, 34, p.2109-2116.
- Johannesen, C.O., Dale, H.F., Jensen, C. and Lied, G.A., 2020. Effects of plant-based diets on outcomes related to glucose metabolism: a systematic review. *Diabetes, Metabolic Syndrome and Obesity*, 13(2020), p.2811-2822.
- Joshi, S.G., 2000. *Medicinal plants*. Oxford: IBH publishing.
- Joshi, S.R., Standl, E., Tong, N., Shah, P., Kalra, S. and Rathod, R., 2015. Therapeutic potential of α -glucosidase inhibitors in type 2 diabetes mellitus: an evidence-based review. *Expert opinion on pharmacotherapy*, 16(13), p.1959-1981.
- Jud, P. and Sourij, H., 2019. Therapeutic options to reduce advanced glycation end products in patients with diabetes mellitus: A review. *Diabetes research and clinical practice*, 148, p.54- 63.
- Kabera, J.N., Semana, E., Mussa, A.R. and He, X., 2014. Plant secondary metabolites: biosynthesis, classification, function and pharmacological properties. *J Pharm*

Pharmacol, 2(7), p.377-392.

Kaiser, A.B., Zhang, N. and Der Pluijm, W.V., 2018. Global prevalence of type 2 diabetes over the next ten years (2018-2028). *Diabetes*, 67(S1), p.202-LB.

Kalsbeek, A., la Fleur, S. and Fliers, E., 2014. Circadian control of glucose metabolism. *Molecular metabolism*, 3(4), p.372-383.

Kathuria, D., Bankar, A.A. and Bharatam, P.V., 2018. "What's in a structure?" The story of biguanides. *Journal of Molecular Structure*, 1152, p.61-78.

Katsiki, N., Mikhailidis, D.P. and Mantzoros, C.S., 2016. Non-alcoholic fatty liver disease and dyslipidemia: an update. *Metabolism*, 65(8), p.1109-1123.

Katsiki, N., Tentolouris, N. and Mikhailidis, D.P., 2017. Dyslipidaemia in type 2 diabetes mellitus: bad for the heart. *Current Opinion in Cardiology*, 32(4), p.422-429.

Katzmarzyk, P.T. and Staiano, A.E., 2012. New race and ethnicity standards: elucidating health disparities in diabetes. *BMC medicine*, 10(1), p.42.

Kaul, K., Tarr, J.M., Ahmad, S.I., Kohner, E.M. and Chibber, R., 2013. Introduction to diabetes mellitus. *Diabetes*, p.1-11.

Kaur, R., Dahiya, L. and Kumar, M., 2017. Fructose-1, 6-bisphosphatase inhibitors: a new valid approach for management of type 2 diabetes mellitus. *European journal of medicinal chemistry*, 141, p.473-505.

Kazeem, M.I. and Davies, T.C., 2016. Anti-diabetic functional foods as sources of insulin secreting, insulin sensitizing and insulin mimetic agents. *Journal of functional foods*, 20, p.122- 138.

Kelly, G.S., 1999. Squalene and its potential clinical uses. *Alternative medicine review: a journal of clinical therapeutic*, 4(1), p.29-36.

Kelly, S.D. and Neary, S.L., 2020. Ominous octet and other scary diabetes stories: The overview of pathophysiology of type 2 diabetes mellitus. *Physician Assistant Clinics*, 5(2), p.121-133.

Kendall, D. and Alexander, S., 2017. *Cannabinoid pharmacology*. Academic Press: USA

Kerner, W. and Brückel, J., 2014. Definition, classification and diagnosis of diabetes mellitus. *Experimental and clinical endocrinology and diabetes*, 122(07), p.384-386.

Khalidi, N., Atallah, B., Alkatheri, A.M., Albekairy, A. and Qandil, A.M., 2014. Post market Research of Oral Anti-Diabetic Drugs: Metformin, Glipizide, and Pioglitazone. *Journal of Applied Pharmaceutical Science*, 4(6), p.61.

- Khan, M.A.B., Hashim, M.J., King, J.K., Govender, R.D., Mustafa, H. and Al Kaabi, J., 2020. Epidemiology of Type 2 Diabetes–Global Burden of Disease and Forecasted Trends. *Journal of epidemiology and global health*, 10(1), p.107.
- Kharroubi, A.T. and Darwish, H.M., 2015. Diabetes mellitus: The epidemic of the century. *World journal of diabetes*, 6(6), p.850.
- Khazan, M. and Hedayati, M., 2015. The role of nitric oxide in health and diseases. *Scimetr*, 3(1), p.1-10.
- Khumalo, G.P., Sadgrove, N.J., Van Vuuren, S.F. and Van Wyk, B.E., 2021. South Africa's Best BARK Medicines Prescribed at the Johannesburg Muthi Markets for Skin, Gut, and Lung Infections: MIC's and Brine Shrimp Lethality. *Antibiotics*, 10(6), p.681.
- Khursheed, R., Singh, S.K., Wadhwa, S., Kapoor, B., Gulati, M., Kumar, R., Ramanunni, A.K., Awasthi, A. and Dua, K., 2019. Treatment strategies against diabetes: Success so far and challenges ahead. *European journal of pharmacology*, 862, p.172625.
- Kim, Y.S., Lee, Y.M., Kim, H., Kim, J., Jang, D.S., Kim, J.H. and Kim, J.S., 2010. Anti-obesity effect of *Morus bombycis* root extract: anti-lipase activity and lipolytic effect. *Journal of ethnopharmacology*, 130(3), p.621-624.
- Kirwan, J.P., Sacks, J. and Nieuwoudt, S., 2017. The essential role of exercise in the management of type 2 diabetes. *Cleveland Clinic journal of medicine*, 84(7), p.S1-S15.
- Kirwan, J.P., Solomon, T.P., Wojta, D.M., Staten, M.A. and Holloszy, J.O., 2009. Effects of 7 days of exercise training on insulin sensitivity and responsiveness in type 2 diabetes mellitus. *American Journal of Physiology-Endocrinology and Metabolism*, 297(1), p. E151- E156.
- Koduru, S., Grierson, D.S. and Afolayan, A.J., 2007. Ethnobotanical information of medicinal plants used for treatment of cancer in the Eastern Cape Province, South Africa. *Current Science*, p.906-908.
- Kooti, W., Farokhipour, M., Asadzadeh, Z., Ashtary-Larky, D. and Asadi-Samani, M., 2016. The role of medicinal plants in the treatment of diabetes: a systematic review. *Electronic physician*, 8(1), p.1832.
- Kosmalski, M., Ziółkowska, S., Czarny, P., Szemraj, J. and Pietras, T., 2022. The coexistence of nonalcoholic fatty liver disease and type 2 diabetes mellitus. *Journal of Clinical Medicine*, 11(5), p.1375.
- Krief, S., Hladik, C.M. and Haxaire, C., 2005. Ethnomedicinal and bioactive properties of plants ingested by wild chimpanzees in Uganda. *Journal of ethnopharmacology*, 101(1-3), p.1-

15.

- Kruszynska, Y.T., Ciaraldi, T.P. and Henry, R.R., 2010. Regulation of glucose metabolism in skeletal muscle. *Comprehensive Physiology*, 21 p.579-607.
- Kumar, N. and Goel, N., 2019. Phenolic acids: Natural versatile molecules with promising therapeutic applications. *Biotechnology Reports*, 24, p.e00370.
- Kumar, S., Narwal, S., Kumar, V. and Prakash, O., 2011. α -glucosidase inhibitors from plants: A natural approach to treat diabetes. *Pharmacognosy reviews*, 5(9), p.19.
- Kuo, W.L., Huang, Y.L., Wang, S.T., Ni, C.L., Shien, B.J. and Chen, C.C., 2007. Chemical constituents of *Trema orientalis*. *Chinese Medical Journal*, 18(1), p.2.
- Kurdekar, S. and Sushma, S., 2020. The study of reactive oxygen species (ROS) production in type II diabetes mellitus and its relation with glycemic control. *Int J Basic Appl Physiol*, 9(1), p.100.
- Kwon, D.Y., Kim, Y.S., Ryu, S.Y., Choi, Y.H., Cha, M.R., Yang, H.J. and Park, S., 2012. Platyconic acid, a saponin from *Platycodi radix*, improves glucose homeostasis by enhancing insulin sensitivity *in vitro* and *in vivo*. *European Journal of Nutrition*, 51(5), p.529-540. 51(5):529-40
- Lacroix, D., Prado, S., Kamoga, D., Kasenene, J., Namukobe, J., Krief, S., Dumontet, V., Mouray, E., Bodo, B. and Brunois, F., 2011. Antiplasmodial and cytotoxic activities of medicinal plants traditionally used in the village of Kiohima, Uganda. *Journal of Ethnopharmacology*, 133(2), p.850-855.
- Lakhtakia, R., 2013. The history of diabetes mellitus. *Sultan Qaboos University Medical Journal*, 13(3), p.368.
- Lam, D.W. and LeRoith, D., 2012. The worldwide diabetes epidemic. *Current Opinion in Endocrinology, Diabetes and Obesity*, 19(2), p.93-96.
- Lambrinou, E., Hansen, T.B. and Beulens, J.W., 2019. Lifestyle factors, self-management and patient empowerment in diabetes care. *European journal of preventive cardiology*, 26(2_suppl), p.55-63.
- Lankatillake, C., Huynh, T. and Dias, D.A., 2019. Understanding glycaemic control and current approaches for screening antidiabetic natural products from evidence-based medicinal plants. *Plant Methods*, 15(1), p.1-35.
- Larger, E., Philippe, M. F., Barbot-Trystram, L., Radu, A., Rotariu, M., Nobécourt, E., and Boitard, C., 2012. Pancreatic exocrine function in patients with diabetes. *Diabetic Medicine*, 29(8), p.1047–1054.

- Laryea, M.K. and Sheringham Borquaye, L., 2021. Antimalarial, antioxidant, and toxicological evaluation of extracts of *Celtis africana*, *Grosseria vignei*, *Physalis micrantha*, and *Stachytarpheta angustifolia*. *Biochemistry Research International*, 2021, p.1-10.
- Lattanzio, V., 2013. Phenolic compounds: introduction 50. *Nat. Prod*, p.1543-1580.
- Lawal M, Verma A K, Umar I A, Gadanya A M, Umar B, Yahaya N, Auwal, B.,2020. Analysis of New Potent Anti-Diabetic Molecules from Phytochemicals of PistiaStrateotes with Sglt1 and G6pc Proteins of Homo Sapiens For Treatment Of Diabetes Mellitus. An *In silico*Approach. *J. Pharm. Biol. Sci.* 15, p.59-73.
- Lebbie, A.R. and Guries, R.P., 1995. Ethnobotanical value and conservation of sacred groves of the Kpaa Mende in Sierra Leone. *Economic Botany*, 49(3), pp.297-308.
- Lee, Y.N. and Huda, M.S., 2021. Uncommon forms of diabetes. *Clinical Medicine*, 21(4), p.e337.
- Lewis, G.P. and Rico Arce, M.D.L., 2005. Tribe Ingeae: Legumes of the world. Kew:
- Li, M., Chi, X., Wang, Y., Setrerrahmane, S., Xie, W. and Xu, H., 2022. Trends in insulin resistance: Insights into mechanisms and therapeutic strategy. *Signal Transduction and Targeted Therapy*, 7(1), p.216.
- Li, S., Wang, J., Zhang, B., Li, X. and Liu, Y., 2019. Diabetes mellitus and cause-specific mortality: a population-based study. *Diabetes and metabolism journal*, 43(3), p.319-341.
- Liguori, I., Russo, G., Curcio, F., Bulli, G., Aran, L., Della-Morte, D., Gargiulo, G., Testa, G., Cacciatore, F., Bonaduce, D. and Abete, P., 2018. Oxidative stress, aging, and diseases. *Clinical interventions in aging*, p.757-772.
- Liu, M., Weiss, M.A., Arunagiri, A., Yong, J., Rege, N., Sun, J., Haataja, L., Kaufman, R.J. and Arvan, P., 2018. Biosynthesis, structure, and folding of the insulin precursor protein. *Diabetes, Obesity and Metabolism*, 20, pp.28-50.
- Liu, Q. and Yao, H., 2007. Antioxidant activities of barley seeds extracts. *Food chemistry*, 102(3), p.732-737.
- Liu, Q., Wang, S. and Cai, L., 2014. Diabetic cardiomyopathy and its mechanisms: role of oxidative stress and damage. *Journal of diabetes investigation*, 5(6), p.623-634.
- Liu, T.T., Liu, X.T., Chen, Q.X. and Shi, Y., 2020. Lipase inhibitors for obesity: A review. *Biomedicine and Pharmacotherapy*, 128, p.110314.
- Lo, S., Russell, J. and Taylor, A., 1970. Determination of glycogen in small tissue samples. *Journal of applied physiology*, 28(2), p.234-236.

- Lobo, V., Patil, A., Phatak, A. and Chandra, N., 2010. Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacognosy reviews*, 4(8), p.118.
- Long A.N, and Dagogo-Jack S., 2011. Comorbidities of diabetes and hypertension: mechanisms and approach to target organ protection. *J. Clin. Hypertens.* 13 (4), p. 244-251.
- Lottenberg, A.M., da Silva Afonso, M., Lavrador, M.S.F., Machado, R.M. and Nakandakare, E.R., 2012. The role of dietary fatty acids in the pathology of metabolic syndrome. *The Journal of nutritional biochemistry*, 23(9), p.1027-1040.
- Lovic, D., Piperidou, A., Zografou, I., Grassos, H., Pittaras, A. and Manolis, A., 2020. The growing epidemic of diabetes mellitus. *Current vascular pharmacology*, 18(2), p.104-109.
- Lucchesi, A.N., Cassettari, L.L. and Spadella, C.T., 2015. Alloxan-induced diabetes causes morphological and ultrastructural changes in rat liver that resemble the natural history of chronic fatty liver disease in humans. *Journal of diabetes research*, 2015, p.1-11.
- Ma X, Chen Z, Wang L, Wang G, Wang Z, Dong X, Wen B, Zhang Z., 2018. The pathogenesis of diabetes mellitus by oxidative stress and inflammation: its inhibition by berberine. *Front. Pharmacol.* 9, pp.782.
- Mabogo, D.E.N., 1990. The ethnobotany of the Vhavenda. Magister Scientiae in the Faculty of Science (Department of Botany) Thesis, University of Pretoria. (1990).
- Maestri, D.M., Nepote, V., Lamarque, A.L. and Zygodlo, J.A., 2006. Natural products as antioxidants. *Phytochemistry: advances in research*, 37(661), p.105-135.
- Mahato, A.K., Bhattacharya, S. and Shanthi, N., 2011. Design, synthesis and glucose-6-phosphatase inhibitory activity of diaminoguanidine analogues of 3-guanidinopropionic acid and amino substituted (Pyridin-2-Yl) thiourea derivatives. *Journal of Pharmaceutical Sciences and Research*, 3(1), p.896.
- Mahmood, N., 2016. A review of α -amylase inhibitors on weight loss and glycemic control in pathological state such as obesity and diabetes. *Comparative Clinical Pathology*, 25(6), p.1253- 1264.
- Maiese, K., 2015. New insights for oxidative stress and diabetes mellitus. *Oxidative medicine and cellular longevity*, 2015, p.1.
- Malam, S., Auwalu, G., Hafsat, A.M., Shafi'u, M., Hussaina, N.N., Hasiya, A. and Sani, A., 2012. Comparative *in vitro* antioxidant studies of ethanolic extracts of Psidium guajava stem bark and Telfairia occidentalis leaf. *Int. J. Modern Biochem*, 1(1), p.18-26.
- Malone, J.I. and Hansen, B.C., 2019. Does obesity cause type 2 diabetes mellitus (T2DM)? Or

- is it the opposite?. *Pediatric diabetes*, 20(1), p.5-9.
- Malone-Povolny, M.J., Maloney, S.E. and Schoenfisch, M.H., 2019. Nitric oxide therapy for diabetic wound healing. *Advanced healthcare materials*, 8(12), p.1801210.
- Malviya, N., Jain, S. and Malviya, S.A.P.N.A., 2010. Antidiabetic potential of medicinal plants. *Acta pol pharm*, 67(2), p.113-118.
- Mane, P.B., Antre, R.V. and Oswal, R.J., 2012. Antidiabetic drugs: An overview. *Int. J. Pharm. Chem. Sci*, 1(1), p.301-6.
- Manukumar, H.M., Shiva Kumar, J., Chandrasekhar, B., Raghava, S. and Umesha, S., 2017. Evidences for diabetes and insulin mimetic activity of medicinal plants: present status and future prospects. *Critical reviews in food science and nutrition*, 57(12), p.2712-2729.
- Mapa-Tassou, C., Katte, J.C., Maadjhou, C.M. and Mbanya, J.C., 2019. Economic impact of diabetes in Africa. *Current diabetes reports*, 19(2), p.5.
- Marín-Peñalver, J.J., Martín-Timón, I., Sevillano-Collantes, C. and del Cañizo-Gómez, F.J., 2016. Update on the treatment of type 2 diabetes mellitus. *World journal of diabetes*, 7(17), p.354.
- Maroyi A., 2017. Diversity of use and local knowledge of wild and cultivated plants in the Eastern Cape province, South Africa. *J. Ethnobiol. Ethnomed.* 13(1), p. 1-16.
- Maroyi, A., 2011. An ethnobotanical survey of medicinal plants used by the people in Nhema communal area, Zimbabwe. *Journal of ethnopharmacology*, 136(2), p.347.
- Maroyi, A., 2018. *Albizia adianthifolia*: Botany, medicinal uses, phytochemistry, and pharmacological properties. *The Scientific World Journal*, 2018, p.1-18.
- Mashimbye, N.N., 2019. *Ethnobotanical uses, anatomical features, phytochemical properties, antimicrobial activity, and cytotoxicity of the Sotho medicinal plant Searsia erosa (Anacardiaceae)*. Masters' dissertation. University of Johannesburg (South Africa).
- Mathabe, M.C., Nikolova, R.V., Lall, N. and Nyazema, N.Z., 2006. Antibacterial activities of medicinal plants used for the treatment of diarrhoea in Limpopo Province, South Africa. *Journal of ethnopharmacology*, 105(1-2), p.286-293.
- Matheus, A.S.D.M., Tannus, L.R.M., Cobas, R.A., Palma, C.C.S., Negrato, C.A. and Gomes, M.D.B., 2013. Impact of diabetes on cardiovascular disease: an update. *International journal of hypertension*, 2013, p.1-15.
- Matough, F.A., Budin, S.B., Hamid, Z.A., Alwahaibi, N. and Mohamed, J., 2012. The role of oxidative stress and antioxidants in diabetic complications. *Sultan Qaboos University*

Medical Journal, 12(1), p.5.

- Mbanya, J.C.N., Motala, A.A., Sobngwi, E., Assah, F.K. and Enoru, S.T., 2010. Diabetes in sub-saharan africa. *The lancet*, 375(9733), p.2254-2266.
- Mbugi, N.O., and Chacha, M., 2019. Acute Toxicity, Brine Shrimp Lethality and Phytochemical Screening of *Lannea schimperi* and *Searsia longipes*. *Journal of Chemical Health Risks*, 9(2), p.1-9.
- McGaw, L., Jäger, A., Grace, O., Fennel, C. and van Staden, J., 2005. Medicinal plants. In *Ethics in Agriculture—An African Perspective* (pp. 67-83). Dordrecht: Springer.
- McGaw, L.J., Jäger, A.K. and Van Staden, J., 2000. Antibacterial, anthelmintic and anti-amoebic activity in South African medicinal plants. *Journal of ethnopharmacology*, 72(1-2), p.247-263.
- McGaw, L.J., Jäger, A.K. and Van Staden, J., 2002. Isolation of antibacterial fatty acids from *Schotia brachypetala*. *Fitoterapia*, 73(5), p.431-433.
- McMurray, F., Patten, D.A. and Harper, M.E., 2016. Reactive oxygen species and oxidative stress in obesity—recent findings and empirical approaches. *Obesity*, 24(11), p.2301-2310.
- Meneses, M., M Silva, B., Sousa, M., Sa, R., F Oliveira, P. and G Alves, M., 2015. Antidiabetic drugs: mechanisms of action and potential outcomes on cellular metabolism. *Current pharmaceutical design*, 21(25), p.3606-3620.
- Mihaylova, D. and Popova, A., 2023. Phytochemicals of Natural Products: Analysis and Biological Activities. *Horticulturae*, 9(2), p.167.
- Miki, T., Yuda, S., Kouzu, H. and Miura, T., 2013. Diabetic cardiomyopathy: pathophysiology and clinical features. *Heart failure reviews*, 18, pp.149-166.
- Mirończuk-Chodakowska, I., Witkowska, A.M. and Zujko, M.E., 2018. Endogenous non-enzymatic antioxidants in the human body. *Advances in medical sciences*, 63(1), p.68-78.
- Misra, A., Gopalan, H., Jayawardena, R., Hills, A.P., Soares, M., Reza-Albarrán, A.A. and Ramaiya, K.L., 2019. Diabetes in developing countries. *Journal of diabetes*, 11(7), p.522-539.
- Mistry, K.N., Dabhi, B.K. and Joshi, B.B., 2020. Evaluation of oxidative stress biomarkers and inflammation in pathogenesis of diabetes and diabetic nephropathy. *Indian Journal of Biochemistry and Biophysics*, 57(1), p.45-50.
- Modolo, L.V., de Souza, A.X., Horta, L.P., Araujo, D.P. and de Fátima, Â., 2015. An overview on the potential of natural products as ureases inhibitors: A review. *Journal of Advanced Research*, 6(1), p.35-44.

- Moffett, R.O., 2007. Name changes in the Old World *Rhus* and recognition of *Searsia* (Anacardiaceae). *Bothalia*, 37(2), p.165-175.
- Mohamed, A.I., Salau, V.F., Erukainure, O.L. and Islam, M.S., 2022. Hibiscus sabdariffa L. polyphenolic-rich extract promotes muscle glucose uptake and inhibits intestinal glucose absorption with concomitant amelioration of Fe²⁺-induced hepatic oxidative injury. *Journal of Food Biochemistry*, 46(12), p.e14399.
- Mohamed, J., Nafizah, A.N., Zariyantey, A.H. and Budin, S., 2016. Mechanisms of diabetes-induced liver damage: the role of oxidative stress and inflammation. *Sultan qaboos university medical journal*, 16(2), p.e132.
- Mueckler, M. and Thorens, B., 2013. The SLC2 (GLUT) family of membrane transporters. *Molecular aspects of medicine*, 34(2-3), p.121-138.
- Mukhtar, Y., Galalain, A. and Yunusa, U., 2020. A modern overview on diabetes mellitus: a chronic endocrine disorder. *European Journal of Biology*, 5(2), p.1-14.
- Mushtaq, S., Abbasi, B.H., Uzair, B. and Abbasi, R., 2018. Natural products as reservoirs of novel therapeutic agents. *EXCLI journal*, 17, p.420.
- Nagy, L., Docsa, T., Szanto, M., Brunyanszki, A., Hegedűs, C., Marton, J., Kónya, B., Virág, L., Somsák, L., Gergely, P. and Bai, P., 2013. Glycogen phosphorylase inhibitor N-(3, 5-dimethyl-benzoyl)-N'-(β-D-glucopyranosyl) urea improves glucose tolerance under normoglycemic and diabetic conditions and rearranges hepatic metabolism. *PloS one*, 8(7), p.e69420.
- Naresh, G., Jaiswal, N., Sukanya, P., Srivastava, A.K., Tamrakar, A.K. and Narender, T., 2012. Glucose uptake stimulatory effect of 4-hydroxypipericolic acid by increased GLUT 4 translocation in skeletal muscle cells. *Bioorganic and medicinal chemistry letters*, 22(17), p.5648-5651.
- Navale, A.M. and Paranjape, A.N., 2016. Glucose transporters: physiological and pathological roles. *Biophysical reviews*, 8(1), p.5-9.
- Naveen, Y.P., Urooj, A. and Byrappa, K., 2021. A review on medicinal plants evaluated for anti-diabetic potential in clinical trials: Present status and future perspective. *Journal of Herbal Medicine*, 28, p.100436.
- Nazarian-Samani, Z., Sewell, R.D., Lorigooini, Z. and Rafieian-Kopaei, M., 2018. Medicinal plants with multiple effects on diabetes mellitus and its complications: a systematic review. *Current diabetes reports*, 18, p.1-13.
- Nazir, M.A., AlGhamdi, L., AlKadi, M., AlBeajan, N., AlRashoudi, L. and AlHussan, M., 2018.

- The burden of diabetes, its oral complications and their prevention and management. *Open access Macedonian journal of medical sciences*, 6(8), p.1545.
- Nchabeleng, E.K., 2017. *Determination of biological activity of Celtis africana extracts and its endophytic microflora and mycoflora*. Masters' dissertation. University of Johannesburg (South Africa).
- Necyk, C. and Zubach-Cassano, L., 2017. Natural health products and diabetes: a practical review. *Canadian journal of diabetes*, 41(6), p.642-647.
- Neha, K., Haider, M.R., Pathak, A. and Yar, M.S., 2019. Medicinal prospects of antioxidants: A review. *European journal of medicinal chemistry*, 178, p.687-704.
- Nellaiappan, K., Preeti, K., Khatri, D.K. and Singh, S.B., 2022. Diabetic complications: an update on pathobiology and therapeutic strategies. *Current diabetes reviews*, 18(1), p.5-18.
- Nemkul C.M., Bajracharya G B, Maeda H, Shrestha I., 2021. *Pharmacogn. J.* 13 (1), p.37-43.
- Newsholme, P., Cruzat, V.F., Keane, K.N., Carlessi, R. and de Bittencourt Jr, P.I.H., 2016. Molecular mechanisms of ROS production and oxidative stress in diabetes. *Biochemical Journal*, 473(24), p.4527-4550.
- Nie, J.P., Qu, Z.N., Chen, Y., Chen, J.H., Jiang, Y., Jin, M.N., Yu, Y., Niu, W.Y., Duan, H.Q. and Qin, N., 2020. Discovery and anti-diabetic effects of novel isoxazole based flavonoid derivatives. *Fitoterapia*, 142, p.104499.
- Nirupama, R., Devaki, M., Nirupama, M. and Yajurvedi, H., 2014. *In vitro* and *in vivo* studies on the hypoglycaemic potential of Ashwagandha (*Withania somnifera*) root. *Pharma Sci Monitor*, 5(3), p.45-58.
- Notelovitz, M., 1970. Milestones in the History of diabetes-A brief survey. *South African Medical Journal*, 44(40), p.1158-1161.
- Noungoué Tchamo, D., Cartier, G., Dijoux-Franca, M.G., Tsamo, E. and Mariotte, A.M., 2001. Xanthones and other constituents of *Trema orientalis*. *Pharmaceutical biology*, 39(3), p.202- 205.
- Nowotny, K., Jung, T., Höhn, A., Weber, D. and Grune, T., 2015. Advanced glycation end products and oxidative stress in type 2 diabetes mellitus. *Biomolecules*, 5(1), p.194-222.
- Nyagumbo, E., Pote, W., Shopo, B., Nyirenda, T., Chagonda, I., Mapaya, R.J., Maunganidze, F., Mavengere, W.N., Mawere, C., Mutasa, I. and Kademeteme, E., 2022. Medicinal plants used for the management of respiratory diseases in Zimbabwe: Review and perspectives potential management of COVID-19. *Physics and Chemistry of the Earth, Parts A/B/C*, p.103232.

- O'Neill, B.J., 2020. Effect of low-carbohydrate diets on cardiometabolic risk, insulin resistance, and metabolic syndrome. *Current Opinion in Endocrinology, Diabetes and Obesity*, 27(5), p.301-307.
- Oboh, G. and Ademosun, A.O., 2011. Shaddock peels (*Citrus maxima*) phenolic extracts inhibit α -amylase, α -glucosidase and angiotensin I-converting enzyme activities: A nutraceutical approach to diabetes management. *Diabetes and Metabolic Syndrome: Clinical Research and Reviews*, 5(3), p.148-152.
- Ogunkoya, L., Olubajo, O.O. and Sondha, D.S., 1977. A new triterpenoid alcohol from *Trema orientalis*. *Phytochemistry*, 16(10), p.1606-1608.
- Oguntibeju, O.O., 2019. Type 2 diabetes mellitus, oxidative stress and inflammation: examining the links. *International journal of physiology, pathophysiology and pharmacology*, 11(3), p.45.
- Ogurtsova, K., da Rocha Fernandes, J.D., Huang, Y., Linnenkamp, U., Guariguata, L., Cho, N.H., Cavan, D., Shaw, J.E. and Makaroff, L.E., 2017. IDF Diabetes Atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. *Diabetes research and clinical practice*, 128, p.40-50.
- Ogurtsova, K., Guariguata, L., Barengo, N.C., Ruiz, P.L.D., Sacre, J.W., Karuranga, S., Sun, H., Boyko, E.J. and Magliano, D.J., 2022. IDF diabetes Atlas: Global estimates of undiagnosed diabetes in adults for 2021. *Diabetes Research and Clinical Practice*, 183, p.109118.
- Oh, Y.S., 2015. Plant-derived compounds targeting pancreatic beta cells for the treatment of diabetes. *Evidence-Based Complementary and Alternative Medicine*, 2015.
- Ohiagu, F.O., Chikezie, P.C. and Chikezie, C.M., 2021. Pathophysiology of diabetes mellitus complications: Metabolic events and control. *Biomedical Research and Therapy*, 8(3), p.4243- 4257.
- Ojha, A., Ojha, U., Mohammed, R., Chandrashekar, A. and Ojha, H., 2019. Current perspective on the role of insulin and glucagon in the pathogenesis and treatment of type 2 diabetes mellitus. *Clinical pharmacology: advances and applications*, 11, p.57.
- Ojo, O., 2016. An overview of diabetes and its complications. *Diabetes Research Open Journal*, 2(2), p.e4-e6.
- Olabanji, S.O., Omobuwajo, O.R., Ceccato, D., Buoso, M.C., De Poli, M. and Moschini, G., 2006. Analysis of some medicinal plants in South-western Nigeria using PIXE. *Journal of radioanalytical and nuclear chemistry*, 270(3), p.515-521.
- Olasunkanmi, A.A., Fadahunsi, O.S. and Adegbola, P.I., 2022. Gas chromatography-Mass

- spectroscopic, high-performance liquid chromatographic and In-silico characterization of antimicrobial and antioxidant constituents of *Rhus longipes* (Engl). *Arabian Journal of Chemistry*, 15(2), p.103601.
- Olofinisan K A, Erukainure O L, Msomi N Z, Islam M.S., 2022. Senna petersiana inhibits key digestive enzymes and modulates dysfunctional enzyme activities in oxidative pancreatic injury. *Asian Pac. J. Trop. Biomed.* 12 (7), p.300-311.
- Olorunnisola, O.S., Adetutu, A., Owoade, A.O., Adesina, B.T. and Adegbola, P., 2017. Toxicity evaluation and protective effect of *Rhus longipes* Engl. leaf extract in paracetamol induced oxidative stress in wister rats. *J Phytopharmacol*, 6(2), p.73-77.
- Olson, A.L. and Humphries, K., 2020. Recent advances in understanding glucose transport and glucose disposal. *F1000Research*, 9, p.F1000 Faculty Rev–639.
- Oršolić, N., Sirovina, D., Odeh, D., Gajski, G., Balta, V., Šver, L. and Jazvinščak Jembrek, M., 2021. Efficacy of caffeic acid on diabetes and its complications in the mouse. *Molecules*, 26(11), p.3262.
- Osadebe, P.O., Odoh, E.U. and Uzor, P.F., 2014. Natural products as potential sources of antidiabetic drugs. *British Journal of Pharmaceutical Research* 4(17), p.2075-2095.
- Oshima, M., Shimizu, M., Yamanouchi, M., Toyama, T., Hara, A., Furuichi, K. and Wada, T., 2021. Trajectories of kidney function in diabetes: a clinicopathological update. *Nature Reviews Nephrology*, 17(11), p.740-750.
- Ota, T., 2014. Obesity-induced inflammation and insulin resistance. *Frontiers in endocrinology*, 5, p.204.
- Ozougwu, J.C., 2017. Physiology of the liver. *International Journal of Research in Pharmacy and Biosciences*, 4(8), p.13-24.
- Ozougwu, J.C., Obimba, K.C., Belonwu, C.D. and Unakalamba, C.B., 2013. The pathogenesis and pathophysiology of type 1 and type 2 diabetes mellitus. *Journal of physiology and pathophysiology*, 4(4), p.46-57.
- Padhi, S., Nayak, A.K. and Behera, A., 2020. Type II diabetes mellitus: A review on recent drug based therapeutics. *Biomedicine and Pharmacotherapy*, 131, p.110708.
- Palmer, E. and Pitman, N., 1972. Trees of Southern Africa: covering all known indigenous species in the Republic of South Africa, South-West Africa, Botswana, Lesotho and Swaziland. Volumes 1 and 2. *Trees of Southern Africa: covering all known indigenous species in the Republic of South Africa, South-West Africa, Botswana, Lesotho and Swaziland. Volumes 1 and 2*. A. A. Balkema:USA.
- Pamplona R. and Costantini D., 2011. Molecular and structural antioxidant defenses against

- oxidative stress in animals. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 301 (4), p.R843-R863
- Pan J, Xin L, Wang D, Liao Z, Lin J H, Li B R., Du T T, Ye B, Zou W B, Chen H, Ji JT.,2016. Risk factors for diabetes mellitus in chronic pancreatitis: a cohort of 2011 patients. *Medicine.* 95 (14), p. e3251.
- Pant, P., Pandey, S. and Dall'Acqua, S., 2021. The influence of environmental conditions on secondary metabolites in medicinal plants: a literature review. *Chemistry and Biodiversity*, 18(11), p.e2100345.
- Papatheodorou, K., Banach, M., Bekiari, E., Rizzo, M. and Edmonds, M., 2018. Complications of diabetes 2017. *Journal of diabetes research*, 2018, (3086167), p.1-4.
- Papatheodorou, K., Papanas, N., Banach, M., Papazoglou, D. and Edmonds, M., 2016. Complications of diabetes 2016. *Journal of diabetes research*, 2016, p.1-3.
- Parvez, A., Azad, M.A., Islam, M.Z., Munna, M.M.R., Shaheen, S.M., Rahman, M.M. and Azad, A.K., 2019. A phytochemical and pharmacological review on *Trema orientalis*: a potential medicinal plant. *Pharmacology Online*, 3, p.103-119.
- Patel, D.K., Kumar, R., Laloo, D. and Hemalatha, S., 2012. Diabetes mellitus: an overview on its pharmacological aspects and reported medicinal plants having antidiabetic activity. *Asian Pacific Journal of Tropical Biomedicine*, 2(5), p.411-420.
- Peer, N., Kengne, A.P., Motala, A.A. and Mbanya, J.C., 2014. Diabetes in the Africa Region: an update. *Diabetes research and clinical practice*, 103(2), p.197-205.
- Pereira, A.S., Den Haan, H., Peña-García, J., Moreno, M.M., Pérez-Sánchez, H. and Apostolides, Z., 2019. Exploring African medicinal plants for potential anti-diabetic compounds with the DIA-DB inverse virtual screening web server. *Molecules*, 24(10), p.2002.
- Perveen, S., Al-Taweel, A.M., Fawzy, G.A., El-Shafae, A.M., Khan, A. and Proksch, P., 2015. Cytotoxic glucosphingolipid from *Celtis africana*. *Pharmacognosy magazine*, 11(S1), p.S1.
- Perveen, S., El-Shafae, A.M., Al-Taweel, A., Fawzy, G.A., Malik, A., Afza, N., Latif, M. and Iqbal, L., 2011. Antioxidant and urease inhibitory C-glycosylflavonoids from *Celtis africana*. *Journal of Asian natural products research*, 13(9), p.799-804.
- Perveen, S., Malik, A. and Tareen, R.B., 2009. Phytochemical studies on *Perovskia atriplicifolia*. *Journal of the chemical society of Pakistan*, 31(2), p.314-318.
- Petersen, M.C. and Shulman, G.I., 2018. Mechanisms of insulin action and insulin resistance. *Physiological reviews*.98(4), p.2133–2223.
- Petersen, M.C., Vatner, D.F. and Shulman, G.I., 2017. Regulation of hepatic glucose

- metabolism in health and disease. *Nature reviews endocrinology*, 13(10), p.572-587.
- Petersmann, A., Müller-Wieland, D., Müller, U.A., Landgraf, R., Nauck, M., Freckmann, G., Heinemann, L. and Schleicher, E., 2019. Definition, classification and diagnosis of diabetes mellitus. *Experimental and Clinical Endocrinology and Diabetes*, 127(S 01), p.S1-S7.
- Petersmann, A., Nauck, M., Müller-Wieland, D., Kerner, W., Müller, U.A., Landgraf, R., Freckmann, G. and Heinemann, L., 2018. Definition, classification and diagnosis of diabetes mellitus. *Experimental and clinical endocrinology and diabetes*, 126(07), p.406-410.
- Phiri, F., Kanengoni, A.T., Hattas, D. and Mbatha, K.R., 2022. Seasonal effects of *Rhus lancea* and *Celtis africana* on intake, preference, and physiological responses in South African indigenous goats. *South African Journal of Animal Science*, 52(6), p.900-913.
- Pico, J. and Martínez, M.M., 2019. Unraveling the inhibition of intestinal glucose transport by dietary phenolics: A review. *Current Pharmaceutical Design*, 25(32), p.3418-3433.
- Picot, C., Subratty, A.H. and Mahomoodally, M.F., 2014. Inhibitory potential of five traditionally used native antidiabetic medicinal plants on α -amylase, α -glucosidase, glucose entrapment, and amylolysis kinetics *in vitro*. *Advances in Pharmacological Sciences*, 2014, p.1- 7.
- Pippitt, K., Li, M. and Gurgle, H.E., 2016. Diabetes mellitus: screening and diagnosis. *American family physician*, 93(2), p.103-109.
- Pisoschi, A.M. and Pop, A., 2015. The role of antioxidants in the chemistry of oxidative stress: A review. *European journal of medicinal chemistry*, 97, p.55-74.
- Pitocco, D., Tesauro, M., Alessandro, R., Ghirlanda, G. and Cardillo, C., 2013. Oxidative stress in diabetes: implications for vascular and other complications. *International journal of molecular sciences*, 14(11), p.21525-21550.
- Pop-Busui, R., Boulton, A.J., Feldman, E.L., Bril, V., Freeman, R., Malik, R.A., Sosenko, J.M. and Ziegler, D., 2017. Diabetic neuropathy: a position statement by the American Diabetes Association. *Diabetes care*, 40(1), p.136-154.
- Poznyak, A., Grechko, A.V., Poggio, P., Myasoedova, V.A., Alfieri, V. and Orekhov, A.N., 2020. The diabetes mellitus–atherosclerosis connection: The role of lipid and glucose metabolism and chronic inflammation. *International journal of molecular sciences*, 21(5), p.1835.

- Prasath, G.S. and Subramanian, S.P., 2013. Fisetin, a tetra hydroxy flavone recuperates antioxidant status and protects hepatocellular ultrastructure from hyperglycemia mediated oxidative stress in streptozotocin induced experimental diabetes in rats. *Food and chemical toxicology*, 59, pp.249-255.
- Preiser, J.C., 2012. Oxidative stress. *Journal of Parenteral and Enteral Nutrition*, 36(2), p.147-154.
- Proença, C., Ribeiro, D., Freitas, M. and Fernandes, E., 2022. Flavonoids as potential agents in the management of type 2 diabetes through the modulation of α -amylase and α -glucosidase activity: a review. *Critical Reviews in Food Science and Nutrition*, 62(12), p.3137-3207.
- Pujol, J., 1990. *Natur Africa*. Jean Pujol Natural Healers Foundation: USA.
- Punthakee, Z., Goldenberg, R. and Katz, P., 2018. Definition, classification and diagnosis of diabetes, prediabetes and metabolic syndrome. *Canadian journal of diabetes*, 42, p.S10-S15.
- Quianzon, C.C. and Cheikh, I., 2012. History of insulin. *Journal of community hospital internal medicine perspectives*, 2(2), p.18701.
- Radosinska, J. and Vrbjar, N., 2016. The role of red blood cell deformability and Na, K-ATPase function in selected risk factors of cardiovascular diseases in humans: focus on hypertension, diabetes mellitus and hypercholesterolemia. *Physiological Research*, 65(S1), p.S43-S54.
- Raj, R.R.R., Sahay, S.S.S. and Tripathi, J.T.J., 2016. Medications of diabetes mellitus and antidiabetic medicinal plants: a review. *International Journal of Indigenous Herbs and Drugs*, p.19-28.
- Rajas, F., Gautier-Stein, A. and Mithieux, G., 2019. Glucose-6 phosphate, a central hub for liver carbohydrate metabolism. *Metabolites*, 9(12), p.282.
- Ramachandran, A., Snehalatha, C. and Nanditha, A., 2017. Classification and diagnosis of diabetes. *Textbook of diabetes*, p.23-28.
- Ramathal, D.C. and Ngassapa, O.D., 2001. Medicinal plants used by Rwandese traditional healers in refugee camps in Tanzania. *Pharmaceutical Biology*, 39(2), p.132-137.
- Ramya, B., Malarvili, T. and Velavan, S., 2015. GC-MS analysis of bioactive compounds in Bryonopsis laciniosa fruit extract. *International Journal of Pharmaceutical Sciences and Research*, 6(8), p.3375.

- Rana, M.G., Katbamna, R.V., Padhya, A.A., Dudhrejiya, A.D., Jivani, N.P. and Sheth, N.R., 2010. *In vitro* antioxidant and free radical scavenging studies of alcoholic extract of *Medicago sativa* L. *Romanian Journal of Biology-Plant Biology*, 55(1), p.15-22.
- Rani, V., Deep, G., Singh, R.K., Palle, K. and Yadav, U.C., 2016. Oxidative stress and metabolic disorders: Pathogenesis and therapeutic strategies. *Life sciences*, 148, p.183-193.
- Raveendran, A.V., Chacko, E.C. and Pappachan, J.M., 2018. Non-pharmacological treatment options in the management of diabetes mellitus. *European endocrinology*, 14(2), p.31.
- Recknagel, R.O., Glende, E.A. and Britton, R.S., 2020. Free radical damage and lipid peroxidation. In *Hepatotoxicology* (pp. 401-436). London: CRC press.
- Reddy, L.H. and Couvreur, P., 2009. Squalene: A natural triterpene for use in disease management and therapy. *Advanced drug delivery reviews*, 61(15), p.1412-1426.
- Rehman, K. and Akash, M.S.H., 2017. Mechanism of generation of oxidative stress and pathophysiology of type 2 diabetes mellitus: how are they interlinked?. *Journal of cellular biochemistry*, 118(11), p.3577-3585.
- Richter, E.A. and Hargreaves, M., 2013. Exercise, GLUT4, and skeletal muscle glucose uptake. *Physiological reviews*, 93(3), p.993-1017.
- Rieg, T. and Vallon, V., 2018. Development of SGLT1 and SGLT2 inhibitors. *Diabetologia*, 61(10), p.2079-2086.
- Rieg, T. and Vallon, V., 2018. Development of SGLT1 and SGLT2 inhibitors. *Diabetologia*, 61(10), pp.2079-2086.
- Rines, A.K., Sharabi, K., Tavares, C.D. and Puigserver, P., 2016. Targeting hepatic glucose metabolism in the treatment of type 2 diabetes. *Nature reviews Drug discovery*, 15(11), p.786- 804.
- Rivadeneyra-Domínguez, E., Becerra-Contreras, Y., Vázquez-Luna, A., Díaz-Sobac, R. and Rodríguez-Landa, J.F., 2018. Alterations of blood chemistry, hepatic and renal function, and blood cytometry in acrylamide-treated rats. *Toxicology reports*, 5, p.1124-1128.
- Rix, I., Nexøe-Larsen, C., Bergmann, N.C., Lund, A. and Knop, F.K., 2015. Glucagon physiology. South Dartmouth: Endotext.
- Rizvi, S.I. and Mishra, N., 2013. Traditional Indian medicines used for the management of diabetes mellitus. *Journal of diabetes research*, 2013, p.1-11.
- Rizzo, A.M., Berselli, P., Zava, S., Montorfano, G., Negroni, M., Corsetto, P. and Berra, B., 2010. Endogenous antioxidants and radical scavengers. In *Bio-farms for nutraceuticals*:

functional food and safety control by biosensors (pp. 52-67). USA: Springer.

- Robson, R., Kundur, A.R. and Singh, I., 2018. Oxidative stress biomarkers in type 2 diabetes mellitus for assessment of cardiovascular disease risk. *Diabetes and Metabolic Syndrome: Clinical Research and Reviews*, 12(3), p.455-462.
- Röder, P. V, Wu, B., Liu, Y., and Han, W. (2016). Pancreatic regulation of glucose homeostasis. *Experimental and Molecular Medicine*, 48(3), e219–e219.
- Roglic, G. and Unwin, N., 2010. Mortality attributable to diabetes: estimates for the year 2010. *Diabetes research and clinical practice*, 87(1), p.15-19.
- Roglic, G., 2016. WHO Global report on diabetes: A summary. *International Journal of Noncommunicable Diseases*, 1(1), p.3.
- Roglic, G., Unwin, N., Bennett, P.H., Mathers, C., Tuomilehto, J., Nag, S., Connolly, V. and King, H., 2005. The burden of mortality attributable to diabetes: realistic estimates for the year 2000. *Diabetes care*, 28(9), p.2130-2135.
- Rtibi, K., Selmi, S., Balti, R., Marzouki, L. and Sebai, H., 2019. Natural Bioactive Compounds with Small-Bowel Glucose/Anti-absorption and Sugar Digestion Enzymes' Inhibition Actions: New Strategy to Relieve Hyperglycemia and Diabetes. *Recent Adv Biol Med*, 5(2019), p.10437.
- Rubab, M., Chelliah, R., Saravanakumar, K., Barathikannan, K., Wei, S., Kim, J.R., Yoo, D., Wang, M.H. and Oh, D.H., 2020. Bioactive Potential of 2-Methoxy-4-vinylphenol and Benzofuran from Brassica oleracea L. var. capitata f, rubra (Red Cabbage) on Oxidative and Microbiological Stability of Beef Meat. *Foods*, 9(5), p.568.
- Rubino, F., Amiel, S.A., Zimmet, P., Alberti, G., Bornstein, S., Eckel, R.H., Mingrone, G., Boehm, B., Cooper, M.E., Chai, Z. and Del Prato, S., 2020. New-onset diabetes in Covid-19. *New England Journal of Medicine*, 383(8), pp.789-790.
- Rui, L., 2014. Energy metabolism in the liver. *Comprehensive physiology*, 4(1), p.177.
- Rupasinghe, H. P. V., Arumuggam, N., Amararathna, M., and De Silva, A., 2018. The potential health benefits of haskap (*Lonicera caerulea* L.): Role of cyanidin-3-O-glucoside. *Journal of Functional Foods*, 44, p.24–39.
- Saeedi, P., Petersohn, I., Salpea, P., Malanda, B., Karuranga, S., Unwin, N., Colagiuri, S., Guariguata, L., Motala, A. A., and Ogurtsova, K. (2019). Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas. *Diabetes Research and Clinical*

- Practice*, 157, p.107843. Salau, V.F., Erukainure, O.L., Koorbanally, N.A. and Islam, M.S., 2022. Ferulic acid promotes muscle glucose uptake and modulate dysregulated redox balance and metabolic pathways in ferric-induced pancreatic oxidative injury. *Journal of food biochemistry*, 46(4), p.e13641.
- Salehi, B., Ata, A., V. Anil Kumar, N., Sharopov, F., Ramirez-Alarcon, K., Ruiz-Ortega, A., Abdulmajid Ayatollahi, S., Valere Tsouh Fokou, P., Kobarfard, F., Amiruddin Zakaria, Z. and Iriti, M., 2019. Antidiabetic potential of medicinal plants and their active components. *Biomolecules*, 9(10), p.551.
- Salehi, B., Ata, A., V. Anil Kumar, N., Sharopov, F., Ramírez-Alarcón, K., Ruiz-Ortega, A., Abdulmajid Ayatollahi, S., Valere Tsouh Fokou, P., Kobarfard, F., Amiruddin Zakaria, Z. and Iriti, M., 2019. Antidiabetic potential of medicinal plants and their active components. *Biomolecules*, 9(10), p.551.
- Saltiel, A.R., 2015. Insulin signaling in the control of glucose and lipid homeostasis. *Metabolic control*, p.51-71.
- Sam, S., 2019. Importance and effectiveness of herbal medicines. *Journal of pharmacognosy and phytochemistry*, 8(2), p.354-357.
- Sanni, O., Erukainure, O.L., Oyeboode, O.A. and Islam, M.S., 2020. Fractions from *Annona muricata* attenuate oxidative stress in pancreatic tissues, inhibits key carbohydrate digesting enzymes and intestinal glucose absorption but enhances muscle glucose uptake. *Journal of food biochemistry*, 44(6), p.e13211.
- Santos-Sánchez, N.F., Salas-Coronado, R., Villanueva-Cañongo, C. and Hernández-Carlos, B., 2019. Antioxidant compounds and their antioxidant mechanism. *Antioxidants*, 10, p.1-29.
- Saranraj, P., Behera, S.S. and Ray, R.C., 2019. Traditional foods from tropical root and tuber crops: Innovations and challenges. In *Innovations in traditional foods* (pp. 159-191). UK: Woodhead publishing.
- Sargsyan A, and Herman M.A., 2019. Regulation of glucose production in the pathogenesis of type 2 diabetes. *Curr. Diabetes Rep.* 19 (9), p.77.
- Schalla, M.A. and Stengel, A., 2019. Pharmacological modulation of ghrelin to induce weight loss: successes and challenges. *Current diabetes reports*, 19, p.1-11.
- Scheepers, A., Joost, H.G. and Schurmann, A., 2004. The glucose transporter families SGLT and GLUT: molecular basis of normal and aberrant function. *Journal of parenteral and enteral nutrition*, 28(5), p.364-371.

- Schwarz, P.E., Greaves, C.J., Lindström, J., Yates, T. and Davies, M.J., 2012. Nonpharmacological interventions for the prevention of type 2 diabetes mellitus. *Nature Reviews Endocrinology*, 8(6), p.363-373.
- Senkoro, A.M., Barbosa, F.M., Moiane, S.F., Albano, G. and de Barros, A.I.R., 2014. Bark Stripping from forest tree species in Madjadjane, Southern Mozambique: medicinal uses and implications for conservation. *Natural Resources*, 05(05), p.192–199.
- Shah, M.S. and Brownlee, M., 2016. Molecular and cellular mechanisms of cardiovascular disorders in diabetes. *Circulation research*, 118(11), pp.1808-1829.
- Shahidi, F. and Peng, H., 2018. Bioaccessibility and bioavailability of phenolic compounds. *Journal of Food Bioactives*, 4(2018), pp.11-68.
- Shahwan, M., Alhumaydhi, F., Ashraf, G.M., Hasan, P.M. and Shamsi, A., 2022. Role of polyphenols in combating Type 2 Diabetes and insulin resistance. *International Journal of Biological Macromolecules*, 206, p.567-579.
- Shai, L.J., Masoko, P., Mokgotho, M.P., Magano, S.R., Mogale, A.M., Boaduo, N. and Eloff, J.N., 2010. Yeast alpha glucosidase inhibitory and antioxidant activities of six medicinal plants collected in Phalaborwa, South Africa. *South African Journal of Botany*, 76(3), p.465-470.
- Shao, J.-W., Jiang, J.-L., Zou, J.-J., Yang, M.-Y., Chen, F.-M., Zhang, Y.-J., and Jia, L., 2020. Therapeutic potential of ginsenosides on diabetes: From hypoglycemic mechanism to clinical trials. *Journal of Functional Foods*, 64, p.103630.
- Sharabi, K., Tavares, C.D., Rines, A.K. and Puigserver, P., 2015. Molecular pathophysiology of hepatic glucose production. *Molecular aspects of medicine*, 46, p.21-33.
- Sharif, H.B., Mukhtar, M.D., Mustapha, Y. and Lawal, A.O., 2015. Preliminary investigation of bioactive compounds and bioautographic studies of whole plant extract of *Euphorbia pulcherrima* on *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, and *Pseudomonas aeruginosa*. *Advances in Pharmaceutics*, 2015, p. 1-14.
- Shelke, A. and Zilate, S., 2021. Recent Trends in the Therapeutic Approach of Type 2 Diabetes Mellitus. *Journal of Pharmaceutical Research International*, 33(60B), p.3662-3668.
- Shorr, R. I., Ray, W. A., Daugherty, J. R., and Griffin, M. R. (1997). Incidence and risk factors for serious hypoglycemia in older persons using insulin or sulfonylureas. *Archives of Internal Medicine*, 157(15), 1681–1686.
- Shrivastava, S.R., Shrivastava, P.S. and Ramasamy, J., 2013. Role of self-care in management of diabetes mellitus. *Journal of diabetes and Metabolic disorders*, 12(1), p.1-5.

- Siebert, S.J., Struwig, M., Knoetze, L. and Komape, D.M., 2018. *Celtis sinensis* Pers.(Ulmaceae) naturalised in northern South Africa and keys to distinguish between *Celtis* species commonly cultivated in urban environments. *Bothalia-African Biodiversity and Conservation*, 48(1), p.1-9.
- Silas, S.S. and Alfred, M., 2019. A review of plants used against diabetes mellitus by Bapedi and Vhavenda ethnic groups in the Limpopo province, South Africa. *Asian J. Pharm. Clin. Res*, 12, p.44-50.
- Skyler, J.S., 2004. Diabetes mellitus: pathogenesis and treatment strategies. *Journal of medicinal chemistry*, 47(17), p.4113-4117.
- Smith, C.A., 1966. Common names of South African plants (No. 35). South Africa: Government Printer.
- Sobeh, M., ElHawary, E., Peixoto, H., Labib, R.M., Handoussa, H., Swilam, N., El-Khatib, A.H., Sharapov, F., Mohamed, T., Krstin, S. and Linscheid, M.W., 2016. Identification of phenolic secondary metabolites from *Schotia brachypetala* Sond.(Fabaceae) and demonstration of their antioxidant activities in *Caenorhabditis elegans*. *PeerJ*, 4, p.e2404.
- Sola, D., Rossi, L., Schianca, G. P. C., Maffioli, P., Bigliocca, M., Mella, R., Corlianò, F., Fra, G. P., Bartoli, E., and Derosa, G. (2015). State of the art paper Sulfonylureas and their use in clinical practice. *Archives of Medical Science*, 11(4), 840–848.
- Solis-Herrera, C., Triplitt, C., Cersosimo, E., and DeFronzo, R. A., 2021. Pathogenesis of Type 2 Diabetes Mellitus. Oregon (USA): Endotext.
- Song, P., Onishi, A., Koepsell, H. and Vallon, V., 2016. Sodium glucose cotransporter SGLT1 as a therapeutic target in diabetes mellitus. *Expert opinion on therapeutic targets*, 20(9), p.1109- 1125.
- Sonibare, M.A., Ayoola, I.O. and Elufioye, T.O., 2017. Antioxidant and acetylcholinesterase inhibitory activities of leaf extract and fractions of *Albizia adianthifolia* (Schumach) WF Wright. *Journal of basic and clinical physiology and pharmacology*, 28(2),p.143-148.
- Sonkamble, V.V., Wagh, N.S. and Pai, S.R., 2019. Role of plant secondary metabolites as antidiabetic agents. *Natural Bio-active Compounds: Volume 1: Production and Applications*, p.529-550.
- Soran, H., Schofield, J.D., Adam, S. and Durrington, P.N., 2016. Diabetic dyslipidaemia. *Current opinion in lipidology*, 27(4), p.313-322.
- Sridhar, S.N.C., George, G., Verma, A. and Paul, A.T., 2019. Natural products-based pancreatic lipase inhibitors for obesity treatment. *Natural Bio-active Compounds: Volume 1:*

Production and Applications, p.149-191.

Stagos, D., 2019. Antioxidant activity of polyphenolic plant extracts. *Antioxidants*, 9(1), p.19.

Stahnke, A.M., Isaacs, D., Capoccia, K.L., Cox, M., Johnson, T. and Shealy, K.M., 2020. Comprehensive Diabetes Medication Resource. *ADCES in Practice*, 8(2), p.27-34.

Stanford, K.I., Middelbeek, R.J., Townsend, K.L., An, D., Nygaard, E.B., Hitchcox, K.M., Markan, K.R., Nakano, K., Hirshman, M.F., Tseng, Y.H. and Goodyear, L.J., 2012. Brown adipose tissue regulates glucose homeostasis and insulin sensitivity. *The Journal of clinical investigation*, 123(1), p.215-223.

Stein, S.A., Lamos, E.M. and Davis, S.N., 2013. A review of the efficacy and safety of oral antidiabetic drugs. *Expert opinion on drug safety*, 12(2), p.153-175.

Sterrett, J.J., Bragg, S. and Weart, C.W., 2016. Type 2 diabetes medication review. *The American Journal of the Medical Sciences*, 351(4), p.342-355.

Stinkens, R., Goossens, G.H., Jocken, J.W. and Blaak, E.E., 2015. Targeting fatty acid metabolism to improve glucose metabolism. *Obesity Reviews*, 16(9), p.715-757.

Stitt, A.W., Curtis, T.M., Chen, M., Medina, R.J., McKay, G.J., Jenkins, A., Gardiner, T.A., Lyons, T.J., Hammes, H.P., Simo, R. and Lois, N., 2016. The progress in understanding and treatment of diabetic retinopathy. *Progress in retinal and eye research*, 51, p.156-186.

Stöckli, J., Fazakerley, D.J. and James, D.E., 2011. GLUT4 exocytosis. *Journal of cell science*, 124(24), p.4147-4159.

Stravodimos, G., A Chetter, B., Kyriakis, E., L Kantsadi, A., SM Chatzileontiadou, D., T Skamnaki, V., Kato, A., M Hayes, J. and D Leonidas, D., 2017. Phytogenic polyphenols as glycogen phosphorylase inhibitors: the potential of triterpenes and flavonoids for glycaemic control in type 2 diabetes. *Current medicinal chemistry*, 24(4), p.384-403.

Sun, C., Zhao, C., Guven, E.C., Paoli, P., Simal-Gandara, J., Ramkumar, K.M., Wang, S., Buleu, F., Pah, A., Turi, V. and Damian, G., 2020. Dietary polyphenols as antidiabetic agents: Advances and opportunities. *Food Frontiers*, 1(1), p.18-44.

Sun, H., Saeedi, P., Karuranga, S., Pinkepank, M., Ogurtsova, K., Duncan, B.B., Stein, C., Basit, A., Chan, J.C., Mbanya, J.C. and Pavkov, M.E., 2022. IDF Diabetes Atlas: Global, regional and

country-level diabetes prevalence estimates for 2021 and projections for 2045. *Diabetes research and clinical practice*, 183, p.109119.

- Sun, L. and Miao, M., 2020. Dietary polyphenols modulate starch digestion and glycaemic level: A review. *Critical reviews in food science and nutrition*, 60(4), p.541-555.
- Tahrani, A.A., Barnett, A.H. and Bailey, C.J., 2013. SGLT inhibitors in management of diabetes. *The lancet Diabetes and endocrinology*, 1(2), p.140-151.
- Tamokou, J.D.D., Simo Mpetga, D.J., Keilah Lunga, P., Tene, M., Tane, P. and Kuiate, J.R., 2012. Antioxidant and antimicrobial activities of ethyl acetate extract, fractions and compounds from stem bark of *Albizia adianthifolia* (Mimosoideae). *BMC Complementary and Alternative Medicine*, 12(1), p.1-10.
- Tangvarasittichai, S., 2015. Oxidative stress, insulin resistance, dyslipidemia and type 2 diabetes mellitus. *World journal of diabetes*, 6(3), p.456.
- Tatipamula, V.B., Killari, K.N., Prasad, K., Rao, G.S.N.K., Talluri, M.R., Vantaku, S., Bilakanti, D. and Srilakshmi, N., 2019. Cytotoxicity Studies of the Chemical Constituents from Marine Algae *Chara baltica*. *Indian Journal of Pharmaceutical Sciences*, 81(5), p.815-823.
- Tayyab, F., Lal, S.S., Mishra, M. and Kumar, U., 2012. A review: Medicinal plants and its impact on diabetes. *World J Pharm Res*, 1(4), p.1019-1046.
- Teoh, E.S. ,2016. Secondary Metabolites of Plants. In: Medicinal Orchids of Asia. Springer, Cham.
- Thakur, A., Chun, Y.S., October, N., Yang, H.O. and Maharaj, V., 2019. Potential of South African medicinal plants targeting the reduction of A β 42 protein as a treatment of Alzheimer's disease. *Journal of ethnopharmacology*, 231, pp.363-373.
- The Royal Botanic Gardens.pp.193-213.
- Thilagam, E., Parimaladevi, B., Kumarappan, C. and Mandal, S.C., 2013. α -Glucosidase and α -amylase inhibitory activity of *Senna surattensis*. *Journal of acupuncture and meridian studies*, 6(1), p.24-30.
- Thiruvoipati, T., Kielhorn, C.E. and Armstrong, E.J., 2015. Peripheral artery disease in patients with diabetes: Epidemiology, mechanisms, and outcomes. *World journal of diabetes*, 6(7), p.961.
- Thomas R.L, Halim S, Gurudas S, Sivaprasad S, Owens D.R, 2019. IDF Diabetes Atlas: A review of studies utilising retinal photography on the global prevalence of diabetes related retinopathy between 2015 and 2018. *Diabetes Research and Clinical Practice*,157, pp.107840. Thomas, V., 2019. *Investigating intervention strategies for the management of*

diabetes in South Africa: A system dynamics approach. Doctoral dissertation. Stellenbosch: Stellenbosch University.

- Thomford, N.E., Dzobo, K., Chopera, D., Wonkam, A., Skelton, M., Blackhurst, D., Chirikure, S. and Dandara, C., 2015. Pharmacogenomics implications of using herbal medicinal plants on African populations in health transition. *Pharmaceuticals*, 8(3), p.637-663.
- Thorens, B. and Mueckler, M., 2010. Glucose transporters in the 21st Century. *American Journal of Physiology-Endocrinology and Metabolism*, 298(2), p.E141-E145.
- Thorens, B., 2015. GLUT2, glucose sensing and glucose homeostasis. *Diabetologia*, 58(2), p.221-232.
- Thrasher, J., 2017. Pharmacologic management of type 2 diabetes mellitus: available therapies. *The American journal of cardiology*, 120(1), p. S4-S16.
- Timson, D.J., 2019. Fructose 1, 6-bisphosphatase: getting the message across. *Bioscience reports*, 39(3), p.1-5.
- Tiwari, B.K., Pandey, K.B., Abidi, A.B. and Rizvi, S.I., 2013. Markers of oxidative stress during diabetes mellitus. *Journal of biomarkers*, 2013, p.1-8.
- Tiwari, R., and Rana, C.S., 2015. Plant secondary metabolites: a review. *International Journal of Engineering Research and General Science*, 3(5), p.661-670.
- Tokarz, V.L., MacDonald, P.E. and Klip, A., 2018. The cell biology of systemic insulin function. *Journal of Cell Biology*, 217(7), pp.2273-2289.
- Tran, N., Pham, B. and Le, L., 2020. Bioactive compounds in anti-diabetic plants: From herbal medicine to modern drug discovery. *Biology*, 9(9), p.252.
- Trifani, R., Rabinowitz, O., Abdillah, S. and Sinaga, E., 2022. GC-MS and LC-MS/MS Analysis of Bouea macrophylla Fruit Juice. *International Journal of Biological, Physical and Chemical Studies*, 4(2), p.01-10.
- Trifani, R., Rabinowitz, O., Abdillah, S. and Sinaga, E., 2022. GC-MS and LC-MS/MS Analysis of Bouea macrophylla Fruit Juice. *International Journal of Biological, Physical and Chemical Studies*, 4(2), p.01-10.
- Ts'ehlana, M.T.E., 2005. *Comparative evaluation of Celtis Africana in Lesotho with that in KwaZulu-Natal, South Africa*. Doctoral dissertation. University of KwaZulu Natal.
- Tsalamandris, S., Antonopoulos, A.S., Oikonomou, E., Papamikroulis, G.A., Vogiatzi, G., Papaioannou, S., Deftereos, S. and Tousoulis, D., 2019. The role of inflammation in diabetes: current concepts and future perspectives. *European cardiology review*, 14(1),

p.50.

- Tschöpe, D., Hanefeld, M., Meier, J.J., Gitt, A.K., Halle, M., Bramlage, P. and Schumm-Draeger, P.M., 2013. The role of co-morbidity in the selection of antidiabetic pharmacotherapy in type-2 diabetes. *Cardiovascular Diabetology*, 12(1), p.1-10.
- Tuei, V.C., Maiyoh, G.K. and Ha, C.E., 2010. Type 2 diabetes mellitus and obesity in sub-Saharan Africa. *Diabetes/metabolism research and reviews*, 26(6), p.433-445.
- Twaij, B.M. and Hasan, M.N., 2022. Bioactive secondary metabolites from plant sources: Types, synthesis, and their therapeutic uses. *International Journal of Plant Biology*, 13(1), p.4- 14.
- Uddin, S.N., Uddin, K.M.A. and Ahmed, F., 2008. Analgesic and antidiarrhoeal activities of *Trema orientalis* Linn. in mice. *Advances in Traditional Medicine*, 8(2), p.187-191.
- Ullah, S., Khan, M.A., Zafar, H., Younus, M., Choudhary, M.I. and Basha, F.Z., 2021. Dibenzazepine-linked isoxazoles: New and potent class of α -glucosidase inhibitors. *Bioorganic and Medicinal Chemistry Letters*, 40, p.127979.
- Umanath, K. and Lewis, J.B., 2018. Update on diabetic nephropathy: core curriculum 2018. *American journal of kidney diseases*, 71(6), p.884-895.
- Usman, B., Sharma, N., Satija, S., Mehta, M., Vyas, M., Khatik, G.L., Khurana, N., Hansbro, P.M., Williams, K. and Dua, K., 2019. Recent developments in α -glucosidase inhibitors for management of type-2 diabetes: An update. *Current pharmaceutical design*, 25(23), p.2510- 2525.
- Van Greevenbroek, M.M., Schalkwijk, C.G. and Stehouwer, C.D., 2013. Obesity-associated low-grade inflammation in type 2 diabetes mellitus: causes and consequences. *Neth J Med*, 71(4), p.174-87.
- van Poelje, P.D., Dang, Q. and Erion, M.D., 2007. Fructose-1, 6-bisphosphatase as a therapeutic target for type 2 diabetes. *Drug Discovery Today: Therapeutic Strategies*, 4(2), p.103-109.
- Van Wyk, A.S. and Prinsloo, G., 2018. Medicinal plant harvesting, sustainability and cultivation in South Africa. *Biological conservation*, 227, p.335-342.
- Van Wyk, B.E. and Gericke, N., 2000. *People's plants: A guide to useful plants of Southern Africa*. South Africa: Riza publications.
- Vardanyan, R. and Hruby, V., 2006. *Synthesis of essential drugs*. USA: Elsevier.
- Velagic, A., Qin, C., Woodman, O.L., Horowitz, J.D., Ritchie, R.H. and Kemp-Harper, B.K., 2020. Nitroxyl: a novel strategy to circumvent diabetes associated impairments in nitric

- oxide signaling. *Frontiers in Pharmacology*, 11, p.727.
- Velderrain-Rodríguez, G.R., Palafox-Carlos, H., Wall-Medrano, A., Ayala-Zavala, J.F., Chen, C.O., Robles-Sánchez, M., Astiazaran-García, H., Alvarez-Parrilla, E. and González-Aguilar, G.A., 2014. Phenolic compounds: their journey after intake. *Food and function*, 5(2), p.189- 197.
- Velho, A.M. and Velho, R.M., 2013. Anatomy and physiology series: infrastructure of the kidney. *Journal of renal nursing*, 5(5), p.228-231.
- Venkatesan, S.K., 2021. Classification of Diabetes Mellitus: A Review. *International Journal of Diabetes Management*, 1(1), p.20-22.
- Venter, F. and Venter, J.A., 1996. *Making the most of indigenous trees*. South Africa: Briza publications.
- Vergès, B., 2015. Pathophysiology of diabetic dyslipidaemia: where are we?. *Diabetologia*, 58(5), p.886-899.
- Verma, S. and Hussain, M.E., 2017. Obesity and diabetes: an update. *Diabetes and Metabolic Syndrome: Clinical Research and Reviews*, 11(1), p.73-79.
- Verspohl, E.J., 2012. Novel pharmacological approaches to the treatment of type 2 diabetes. *Pharmacological reviews*, 64(2), p.188-237.
- Vinayagam, R., Jayachandran, M., and Xu, B., 2016. Antidiabetic effects of simple phenolic acids: A comprehensive review. *Phytotherapy Research*, 30(2), p.184–199.
- Visnagri, A., Kandhare, A.D., Chakravarty, S., Ghosh, P. and Bodhankar, S.L., 2014. Hesperidin, a flavanoglycone attenuates experimental diabetic neuropathy via modulation of cellular and biochemical marker to improve nerve functions. *Pharmaceutical biology*, 52(7), pp.814-828.
- Volpe, C.M.O., Villar-Delfino, P.H., Dos Anjos, P.M.F. and Nogueira-Machado, J.A., 2018. Cellular death, reactive oxygen species (ROS) and diabetic complications. *Cell death and disease*, 9(2), p.119.
- Wang, J. K., Walaas, S. I., Sihra, T. S., Aderem, A., and Greengard, P. (1989). Phosphorylation and associated translocation of the 87-kDa protein, a major protein kinase C substrate, in isolated nerve terminals. *Proceedings of the National Academy of Sciences*, 86(7), 2253–2256. Wang, W. and Lo, A.C., 2018. Diabetic retinopathy: pathophysiology and treatments. *International journal of molecular sciences*, 19(6), p.1816.
- Wang, Y.W., He, S.J., Feng, X., Cheng, J., Luo, Y.T., Tian, L. and Huang, Q., 2017. Metformin: a review of its potential indications. *Drug design, development and therapy*, 11, p.2421.

- Wang, Z., Xiong, H. and Ren, T.Y.S., 2021. Repair of damaged pancreatic β cells: New hope for a type 2 diabetes reversal?. *Journal of Translational Internal Medicine*, 9(3), p.150-151.
- Watt, J.M. and Breyer-Brandwijk, M.G., 1962. The Medicinal and Poisonous Plants of Southern and Eastern Africa being an Account of their Medicinal and other Uses, Chemical Composition, Pharmacological Effects and Toxicology in Man and Animal. *The Medicinal and Poisonous Plants of Southern and Eastern Africa being an Account of their Medicinal and other Uses, Chemical Composition, Pharmacological Effects and Toxicology in Man and Animal.*, (Edn 2). UK: E. and S. Livingstone Ltd.
- Wei, Z.J. and Prasad, C., 2015. Carbohydrates as functional food. *Current Topics in Nutraceutical Research*, 13(4), p.7.
- Whalen, K., Miller, S. and Onge, E.S., 2015. The role of sodium-glucose co-transporter 2 inhibitors in the treatment of type 2 diabetes. *Clinical therapeutics*, 37(6), p.1150-1166.
- Whiting, D. R., Guariguata, L., Weil, C., and Shaw, J., 2011. IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. *Diabetes Research and Clinical Practice*, 94(3), 311–321.
- Wilson, R.D. and Islam, M., 2012. Fructose-fed streptozotocin-injected rat: an alternative model for type 2 diabetes. *Pharmacological reports*, 64(1), p.129-139.
- Wink, M., 2015. Modes of action of herbal medicines and plant secondary metabolites. *Medicines*, 2(3), p.251-286.
- Woisky RG, Salatino A. Analysis of propolis: some parameters and procedures for chemical quality control.,1998. *Journal of apicultural research*,37(2), p.99-105.
- Woldemariam, T. and Van Winkle, J., 2015. *In vitro* Hypoglycemic Effect of Salvia hispanica Using a Yeast Glucose Uptake Model. *Journal of Pharmaceutical Sciences and Pharmacology*, 2(2), p.119-122.
- World Health Organization, 2013. State of health financing in the African Region.
- Wu, Y., Ding, Y., Tanaka, Y. and Zhang, W., 2014. Risk factors contributing to type 2 diabetes and recent advances in the treatment and prevention. *International journal of medical sciences*, 11(11), p.1185.
- Xiao, J.B. and Hogger, P., 2015. Dietary polyphenols and type 2 diabetes: current insights and future perspectives. *Current medicinal chemistry*, 22(1), p.23-38.
- Xiaoxing, Y., Yindi, Z., Junxian, Y., Pei, Z., Jianping, S., Jun, Q., Haiwei, W. and Xiang, Z., 2006. The antioxidative effects of astragalus saponin I protect against development of

- early diabetic nephropathy. *Journal of pharmacological sciences*, 101(2), p.166-173.
- Yagasaki, K. and Muller, C.J., 2022. The Effect of Phytochemicals and Food Bioactive Compounds on Diabetes. *International Journal of Molecular Sciences*, 23(14), p.7765.
- Yamanouchi, T., 2010. Concomitant therapy with pioglitazone and insulin for the treatment of type 2 diabetes. *Vascular health and risk management*, 6, p.189.
- Yang, S., Wang, S., Yang, B., Zheng, J., Cai, Y. and Yang, Z., 2016. Weight loss before a diagnosis of type 2 diabetes mellitus is a risk factor for diabetes complications. *Medicine*, 95(49), p.e5618.
- Yang, Z., Kulkarni, K., Zhu, W. and Hu, M., 2012. Bioavailability and pharmacokinetics of genistein: mechanistic studies on its ADME. *Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents)*, 12(10), p.1264-1280.
- Yaribeygi, H., Farrokhi, F.R., Butler, A.E. and Sahebkar, A., 2019. Insulin resistance: Review of the underlying molecular mechanisms. *Journal of cellular physiology*, 234(6), p.8152-8161.
- Yaribeygi, H., Sathyapalan, T., Atkin, S.L. and Sahebkar, A., 2020. Molecular mechanisms linking oxidative stress and diabetes mellitus. *Oxidative medicine and cellular longevity*, 2020, p.1-13.
- Yattoo, M.I., Saxena, A., Gopalakris, A., Alagawany, M. and Dhama, K., 2017. Promising antidiabetic drugs, medicinal plants and herbs: An update. *International Journal of Pharmacology*, 13(7), p.732-745.
- Yin, X., Zhang, Y., Wu, H., Zhu, X., Zheng, X., Jiang, S., Zhuo, H., Shen, J., Li, L. and Qiu, J., 2004. Protective effects of Astragalus saponin I on early stage of diabetic nephropathy in rats. *Journal of pharmacological sciences*, 95(2), p.256-266.
- Yuan, Q., Cheng, Y., Sheng, R., Yuan, Y., and Hu, M., 2022. A Brief Review of Natural Products with Urate Transporter 1 Inhibition for the Treatment of Hyperuricemia. *Evidence- Based Complementary and Alternative Medicine*, 2022, p.1-16.
- Yuan, T., Yang, T., Chen, H., Fu, D., Hu, Y., Wang, J., Yuan, Q., Yu, H., Xu, W. and Xie, X., 2019. New insights into oxidative stress and inflammation during diabetes mellitus-accelerated atherosclerosis. *Redox biology*, 20, p.247-260.
- Zeb, A., 2020. Concept, mechanism, and applications of phenolic antioxidants in foods. *Journal of Food Biochemistry*, 44(9), p.e13394.
- Zhang, H. and Tsao, R., 2016. Dietary polyphenols, oxidative stress and antioxidant and anti-inflammatory effects. *Current Opinion in Food Science*, 8, p.33-42.

- Zhang, H., Ma, J., Tang, K. and Huang, B., 2021. Beyond energy storage: roles of glycogen metabolism in health and disease. *The FEBS Journal*, 288(12), p.3772-3783.
- Zhang, P., Li, T., Wu, X., Nice, E.C., Huang, C. and Zhang, Y., 2020. Oxidative stress and diabetes: antioxidative strategies. *Frontiers of medicine*, 14(5), p.583-600.
- Zhang, P., Zhang, X., Brown, J., Vistisen, D., Sicree, R., Shaw, J. and Nichols, G., 2010. Global healthcare expenditure on diabetes for 2010 and 2030. *Diabetes research and clinical practice*, 87(3), p.293-301.
- Zhao, C., Yang, C., Wai, S.T.C., Zhang, Y., P. Portillo, M., Paoli, P., Wu, Y., San Cheang, W., Liu, B., Carpené, C. and Xiao, J., 2019. Regulation of glucose metabolism by bioactive phytochemicals for the management of type 2 diabetes mellitus. *Critical Reviews in Food Science and Nutrition*, 59(6), p.830-847.
- Zheng, Y., Ley, S.H. and Hu, F.B., 2018. Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. *Nature Reviews Endocrinology*, 14(2), p.88-98.
- Zhou, Y. and Xu, B., 2022. New insights into anti-diabetes effects and molecular mechanisms of dietary saponins. *Critical Reviews in Food Science and Nutrition*, 63(33), p.12372-12397.
- Zhu, Y. and Zhang, C., 2016. Prevalence of gestational diabetes and risk of progression to type 2 diabetes: a global perspective. *Current diabetes reports*, 16(1), p.1-11.
- Zimmet, P., Alberti, K., and Shaw, J., 2001. Global and societal implications of the diabetes epidemic. *Nature*, 414(6865), p.782–787.
- Zimmet, P., Alberti, K.G., Magliano, D.J. and Bennett, P.H., 2016. Diabetes mellitus statistics on prevalence and mortality: facts and fallacies. *Nature Reviews Endocrinology*, 12(10), p.616.

APPENDIX A ETHICS APPROVAL



School of Laboratory Medicine and Medical Sciences
Westville Campus
Private Bag X54001
Durban
4000
Tel: 031 260 7671
Fax: 031 260 7730

Biomedical Resource Unit

November 28, 2018

RE: ATTENDANCE OF LAS COURSE

This letter certifies that Beseni Brian K: 218088125 has attended the Laboratory Animal Course that was hosted by the Biomedical Resource Unit, School of Laboratory Medicine & Medical Sciences, CHS. The course was held on the 17 – 10 October 2018 and entailed the following:

- Introduction to laboratory animal species
- Bioethics and animal experimentation
- Animal research methodology
- Experimental design
- Environmental enrichment
- Occupational safety.

The course was completed satisfactorily, and the student may be allowed to initiate research if the relevant practical procedures were done to a level of competency that was signed off by the veterinarian in charge.

Kind Regards



Dr L A Bester
Course Coordinator

28/11/18
Date

Mrs Ritta Govender, Room 201, 2nd Floor U- Block, E-mail radeber@ukzn.ac.za

To Reduce Replace and Refine Animal Research

15 September 2021

Mr Brian Kudakwashe Beseni (218088125)
School of Life Sciences
Westville

Dear Mr Beseni,

Protocol reference number: AREC/038/019D

Old Project title: Antioxidant, hypoglycaemic and hypolipidemic effects of Zn(II) poly-phenol complexes and indigenous plants from KwaZulu-Natal province, South Africa.

New Project title: Antioxidant, hypoglycaemic and hypolipidemic effects of Zn(II) poly-phenol complexes and some Sudanese and South African indigenous medicinal plants in a type 2 diabetes model of SD rats.

Approval – Amendment Application

With regard to your first amendment request received on 07 September 2021, the Animal Research Ethics Committee has accepted the documents submitted, and **FULL APPROVAL** for the protocol is granted.

We note your request to amend your study title and protocol by adding extracts from *Cyperus rotundus*, *Hauclea latifolia* and *Hibiscus sabdariffa*. We also note the addition of Idris Mohamed Almahi (219094524) as a researcher to your study.

Please note: There must be adherence to national and institutional COVID-19 regulations and guidelines at all times. Researchers will be personally responsible and liable for non-adherence to national regulations. If in doubt, please contact the Research Ethics Chair and/or the University Dean of Research for advice.

Any alteration/s to the approved research protocol, i.e. Title of Project, Location of the Study, Research Approach and Methods must be reviewed and approved through the amendment/modification prior to its implementation. In case you have further queries, please quote the above reference number.

Please note: Research data should be securely stored in the discipline/department for 5 years.

The ethical clearance certificate is only valid for one year from the date of issue. Renewal for the study must be applied for before 14 September 2022 as a new application on RIG.

Please note: the study renewal in 2022 must be uploaded to the RIG online system as a new application.

Animal Research Ethics Committee (AREC)

Ms Karen Reinertsen (Administrator)

Westville Campus, Govan Mbeki Building

Postal Address: Private Bag X54001, Durban 4000

Telephone: +27 (0) 31 260 8350 Facsimile: +27 (0) 31 260 4609 Email: animalethics@ukzn.ac.za

Website: <http://research.ukzn.ac.za/Research-Ethics/Animal-Ethics.aspx>



Founding Campuses: ■ Edgewood ■ Howard College ■ Medical School ■ Pietermaritzburg ■ Westville

Attached to the Approval letter is a template of the Progress Report required at the end of the study or when applying for Renewal (whichever comes first). An Adverse Event Reporting form has also been attached in the event of any unanticipated event involving the animals' health/wellbeing.

I take this opportunity of wishing you everything of the best with your study.

Yours faithfully



Dr Sanil D Singh, BVSc, MS, PhD
Chair: Animal Research Ethics Committee
/kr

cc Supervisor: Prof Shahidul Islam
cc BRU Manager: Dr Jaca

Animal Research Ethics Committee (AREC)

Ms Karen Reinertsen (Administrator)

Westville Campus, Govan Mbeki Building

Postal Address: Private Bag X54001, Durban 4000

Telephone: +27 (0) 31 260 8350 **Facsimile:** +27 (0) 31 260 4609 **Email:** animalethics@ukzn.ac.za

Website: <http://research.ukzn.ac.za/Research-Ethics/Animal-Ethics.aspx>



100 YEARS OF ACADEMIC EXCELLENCE

Founding Campuses:  Edgewood  Howard College  Medical School  Pietermaritzburg  Westville



UNIVERSITY OF
KWAZULU-NATAL
INYUVESI
YAKWAZULU-NATALI

15 September 2021

Mr Brian Kudakwashe Beseni (218088125)
School of Life Sciences
Westville

Dear Mr Beseni,

Protocol reference number: AREC/038/019D

Old Project title: Antioxidant, hypoglycaemic and hypolipidemic effects of Zn(II) poly-phenol complexes and indigenous plants from KwaZulu-Natal province, South Africa.

New Project title: Antioxidant, hypoglycaemic and hypolipidemic effects of Zn(II) poly-phenol complexes and some Sudanese and South African indigenous medicinal plants in a type 2 diabetes model of SD rats.

Full Approval – Renewal Application

With regards to your renewal application received on 14 September 2021. The documents submitted have been accepted by the Animal Research Ethics Committee and **FULL APPROVAL** for the protocol has been granted.

Please note: Any Veterinary and Para-Veterinary procedures must be conducted by a SAVC registered VET or SAVC authorized person.

Any alteration/s to the approved research protocol, i.e Title of Project, Location of the Study, Research Approach and Methods must be reviewed and approved through the amendment/modification prior to its implementation. In case you have further queries, please quote the above reference number.

Please note: Research data should be securely stored in the discipline/department for a period of 5 years.

The ethical clearance certificate is only valid for a period of one year from the date of issue. Renewal for the study must be applied for before 14 September 2022.

Please note: the study renewal in 2022 must be uploaded to the RIG online system as a new application.

Attached to the Approval letter is a template of the Progress Report that is required at the end of the study, or when applying for Renewal (whichever comes first). An Adverse Event Reporting form has also been attached in the event of any unanticipated event involving the animals' health / wellbeing.

I take this opportunity of wishing you everything of the best with your study.

Yours faithfully



Dr Sanil D Singh, BVSc, MS, PhD
Chair: Animal Research Ethics Committee

/kr

cc Supervisor: Professor Shahidul Islam
cc BRU Manager: Dr Jaca

Animal Research Ethics Committee
Telephone: 031 2608850
Email: animalethics@ukzn.ac.za
University Road
Chiltern Hills
Westville
3629
South Africa



Founding Campuses:

- Edgewood
- Howard College
- Medical School
- Pietermaritzburg
- Westville

APPENDIX B PUBLICATIONS FROM THESIS

Asian Pacific Journal of Tropical Biomedicine 2022; 12(11): 453–465

453



Original Article

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.apjtb.org

doi: 10.4103/2221-1691.360561

Impact Factor: 1.51

Rhus longipes (Engl.) infusions improve glucose metabolism and mitigate oxidative biomarkers in ferrous sulfate–induced renal injury

Brian K. Beseni¹, Kolawole A. Olofinisan¹, Veronica F. Salau¹, Ochuko L. Erukainure^{1,2}, Md. Shahidul Islam¹✉

¹Department of Biochemistry, University of KwaZulu–Natal, Westville Campus, Durban 4000, South Africa

²Department of Pharmacology, University of the Free State, Bloemfontein 9300, South Africa

ABSTRACT

Objective: To explore the antioxidant and antidiabetic activities of *Rhus longipes* (*R. longipes*) leaf and stem bark aqueous infusions.

Methods: *R. longipes* leaf and stem bark infusions were characterized via gas-chromatography mass-spectroscopy (GC-MS) analysis. *In vitro* antioxidant and carbohydrate and lipid digestive enzyme inhibitory activities of *R. longipes* infusions were determined. Additionally, the modulatory effects of *R. longipes* infusions on intestinal glucose absorption, muscle glucose uptake, and biomarkers of renal oxidative injury were evaluated. Molecular docking was performed to determine the binding affinities of the identified compounds from the leaf and stem bark infusions on carbohydrate and lipid digestive enzymes.

Results: GC-MS analysis revealed the presence of several

1. Introduction

Diabetes mellitus (DM) is an enervating metabolic disorder characterized by chronic hyperglycemia, with type 1, type 2, and gestational diabetes being the most prevalent forms of diabetes[1,2]. The risk factors of DM include genetic predisposition, age, ethnicity, and unhealthy lifestyle[1]. Similarly, high-calorie diets and lack of physical activity, which progressively develop obesity, have also been implicated as major risk factors and comorbidity associated with diabetes pathology[2], when obesity is one of the major risk factors behind the development of type 2 diabetes.

In obese individuals, the elevated amounts of pro-inflammatory factors, non-esterified fatty acids, and cytokines lead to an increased cellular concentration of reactive free radicals, which ultimately

Significance

APPENDIX C OTHER RESEARCH PUBLICATIONS

Sequential extracts of red honeybush (*Cyclopia genistoides*) tea: Chemical characterization, antioxidant potentials, and anti-hyperglycemic activities

Xin Xiao, Ochuko L. Erukainure, Brian Beseni, Neil A. Koorbanally, Md. Shahidul Islam 

First published: 27 September 2020 | <https://doi.org/10.1111/jfbc.13478>

[Read the full text >](#)



PDF



TOOLS



SHARE

Abstract

The antioxidant, antidiabetic, and anti-obesogenic potentials of different extracts (dichloromethane, ethyl acetate, ethanol, and aqueous) of the red honeybush (*Cyclopia genistoides*) tea were investigated in vitro and ex vivo. All extracts exhibited significant scavenging and reducing power activities, with the aqueous and ethyl acetate extracts being the most potent. In vitro antidiabetic analysis revealed the extracts to be potent inhibitors of α -glucosidase and lipase activities. All extracts increased catalase and SOD activities, and glutathione level in oxidative pancreatic injury. GC–MS analysis revealed the presence of fatty acids, fatty acid ester, phytols, sterols, saccharide, ketones, and triterpenes. These results imply that the sequential extracts of honeybush tea

tation

Cola nitida infusion modulates cardiometabolic activities linked to cardiomyopathy in diabetic rats

Ochuko L. Erukainure ^{a, b} , Nontokozo Z. Msomi ^a, Brian K. Beseni ^a, Veronica F. Salau ^a, Omamuyovwi M. Ijomone ^c, Neil A. Koorbanally ^d, Md. Shahidul Islam ^a  

Show more 

+ Add to Mendeley  Share  Cite

<https://doi.org/10.1016/j.fct.2021.112335> 

[Get rights and content !\[\]\(6059a5aa8b4ca7bb793408023d6c6e42_img.jpg\)](#)

Abstract

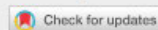
This study investigated the therapeutic mechanism of *Cola nitida* seeds on diabetic cardiomyopathy in hearts of diabetic rats. Type 2 diabetic (T2D) rats were treated with *C. nitida* infusion at 150 or 300mg/kg body weight (bw). The rats were sacrificed after 6 weeks of treatment, and their hearts harvested. There was an upsurge in oxidative stress on induction of T2D as depicted by the depleted levels of glutathione, superoxide dismutase and catalase activities, and elevated malondialdehyde level. The activities of

The antioxidant and antidiabetic potentials of polyphenolic-rich extracts of *Cyperus rotundus* (Linn.)

Almahi I. Mohamed , Brian K. Beseni , Nontokozo Z. Msomi , Veronica F. Salau ,
Ochuko L. Erukainure , Aimen Aljoundi & ...show all

Pages 12075-12087 | Received 09 May 2021, Accepted 08 Aug 2021, Published online: 28 Aug 2021

 Cite this article  <https://doi.org/10.1080/07391102.2021.1967197>



 Full Article

 Figures & data

 References

 Citations

 Metrics

 Reprints & Permissions

Read this article

Abstract

In this study, the rhizome of *Cyperus rotundus* L was investigated for its antioxidant and antidiabetic effects using *in vitro* and *in silico* experimental models. Its crude extracts (ethyl acetate, ethanol and aqueous) were screened *in vitro* for their antioxidant activity using ferric-reducing antioxidant power (FRAP) and 1,1-diphenyl-2-picrylhydrazyl (DPPH), as well as their inhibitory effect on α -glucosidase enzyme.

R



Original Article

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.apjtb.org

doi: 10.4103/2221-1691.333209

Impact Factor: 1.55

Harpephyllum caffrum stimulates glucose uptake, abates redox imbalance and modulates purinergic and glucogenic enzyme activities in oxidative hepatic injury

Kolawole A. Olofinisan¹, Ochuko L. Erukainure², Beseni K. Brian¹, Md. Shahidul Islam¹✉¹Department of Biochemistry, School of Life Sciences, University of KwaZulu-Natal, (Westville Campus), Durban 4000, South Africa²Department of Pharmacology, University of the Free State, Bloemfontein 9300, South Africa

ABSTRACT

Objective: To investigate the antioxidative and antidiabetic effects of *Harpephyllum caffrum* bark infusion as well as its effects on glucogenic and nucleotide hydrolyzing enzyme activities in FeSO₄-induced oxidative stress in rat hepatic tissue.

Methods: *Harpephyllum caffrum* infusion was prepared from dried plant materials (40 g) infused in boiling water (400 mL) for 20 min at room temperature. The antioxidative and inhibitory activities against carbohydrate digestive enzymes of the infusion were determined using established protocols. The liver tissues of rats were used for glucose uptake assay and to evaluate the infusion's effect on endogenous antioxidant, glucogenic, and nucleotide hydrolyzing enzyme activities in FeSO₄-induced hepatic injury.

Results: The *Harpephyllum caffrum* infusion significantly reduced ferric iron (FRAP) and free radicals (OH[•] and DPPH) in a dose-

1. Introduction

Diabetes is one of the diseases of global public health significance, with a continuous annual increase in global prevalence. Epidemiological data released by the International Diabetes Federation in 2019 revealed that nearly 463 million individuals worldwide are affected by diabetes. This value represents an 11.56% increase of 415 million earlier reported in 2015[1]. Moreover, it was projected that by 2030, an estimated 578 million people constituting 5.01% Africans, will suffer from this

Significance

Harpephyllum caffrum is an underutilized wild food plant that is indigenous to South Africa. The plant is valued traditionally due to the nutritional and medicinal properties associated with its parts. More importantly, an aqueous extract of the bark is utilized in diabetes management but without a detailed scientific basis