UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

Morphology, Phytochemistry and medicinal properties of South African *Mangifera indica* L. leaves for summer and winter seasons.

By

Arvish Maharaj

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School of Life Sciences
College of Agriculture, Engineering and Science
University of KwaZulu-Natal
Westville
South Africa

As the candidate's supervisor and co-supervisor, we have approved submission of this thesis.

Supervisor: Prof. Y. Naidoo

Signed: [redacted]

Date: 13th December 2021

Co-supervisor: Prof. Y. H. Dewir

Signed: [redacted]

Date: 13th December 2021

ABSTRACT

Herbal preparations of plants continue to present mankind with novel remedies as many of these plants contain important secondary metabolites. Plant species of the family Anacardiaceae are rich in bioactive phytochemicals. *Mangifera indica* (Anacardiaceae) is an introduced and naturalised species to South Africa. Herbal use of this plant has not been fully documented; however, it is used in traditional medicine. This study aimed at characterizing the morphology, phytochemistry, and biological activity of *Mangifera indica* leaves harvested in winter and summer. The foliar biology of the plant was conducted by various microscopy techniques such as stereo- and Scanning electron microscopy. The length and diameter of the different trichome types were measured using ImageJ. The non-glandular trichome lengths range between 70 - 200 µm. The peltate gland trichomes consist of 2 rows of 8 oblong cells each with a size ranging from 32- 48 µm. Morphological observations using stereo- and SEM revealed the presence of non-glandular trichomes with cuticular warts and glandular peltate trichomes on the leaves of *Mangifera indica*. Transmission electron micrographs showed the presence of numerous mitochondria, starch grains, plastoglobuli, and plastids. The results for summer and winter leaves resembled somewhat similar-to-identical morphological characteristics on all fronts. For the phytochemical and biological assays, this study aimed to investigate some of the phytochemical and biological properties using different solvents (hexane, chloroform, and methanol) for extraction of the leaves of *Mangifera indica* for the summer and winter seasons. Preliminary phytochemical screening for the hexane, chloroform and methanolic extracts was done using a reflux extraction apparatus to uncover the presence of different metabolites and the anti-oxidant screening was done by the radical scavenging activity, which was established using the 2,2-diphenyl-1-picrylhydrazyl assay. Potent radical scavenging activity was exhibited for both summer and winter seasons with hexane and methanolic extracts for summer (IC_{50} of 19.53 µg/mL and 12.71 µg/mL respectively) and winter (22.32 µg/mL and 14.35 µg/mL respectively) in comparison to the control ascorbic acid which produced an IC_{50} of 3.20 µg/mL. The summer extracts had better radical scavenging IC_{50} capacity than winter extracts. The antibacterial activity of the methanolic leaf extracts for summer and winter of *Mangifera indica* were evaluated against the bacterial species: Gram-negative *Escherichia coli* (ATCC 25922) and Gram-positive: *Staphylococcus aureus* (ATCC ATCC 43300). For *S. aureus* (ATTC 43300), the summer crude extract displayed lower antibacterial activity than the control streptomycin, the summer extracts had a zone of inhibition of 14.17 mm while streptomycin had a 16.67 mm zone of inhibition. winter extracts had a zone of inhibition of 12 mm while streptomycin had a 13.67 mm zone of inhibition. For *E. coli* (ATCC 25922), the summer crude extract displayed higher antibacterial activity than the control gentamycin; the summer extract had a zone of inhibition of 18.05 mm while gentamycin had a 17.5 mm zone of inhibition. The winter extracts had a zone of inhibition of 8.5 mm

while gentamycin had a 14.5 mm zone of inhibition. Between seasons, summer had better antibacterial activity compared to winter for both Gram-positive and Gram-negative bacteria.
Phytochemical screening showed the presence of phenols, flavonoids, tannins, and terpenoids, alkaloids, phytosterols, saponins, steroids, and carbohydrates. Potent radical scavenging activity was exhibited for the hexane and methanolic extracts for summer and winter, indicating that *Mangifera indica* is a potential source of medicinally important compounds. Antibacterial screening showed positive results with antibacterial properties for both summer and winter samples revealing its valuable biological activities. Summer overall performed better than the winter season. Future studies on this plant species are recommended to advance the use of indigenous herbal medicine or produce novel drug leads. To our knowledge, this study represents the first recent investigation in South Africa describing key foliar micromorphological features, phytochemicals, and biological activities of *Mangifera indica* L.

Keywords: *Mangifera indica*; Morphology; Bioactive compounds; Antibacterial; Anti-oxidant.

PREFACE

The research described in this dissertation was completed by the candidate while based in the Discipline of Biological Sciences, School of Life Sciences of the College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Westville, South Africa. The study was financially supported by the National Research Foundation.

The contents of this study represent the original work by the author and have not been submitted in any form to another tertiary institution. Where use has been made of the work of others it is appropriately acknowledged in the text.

As the candidate's supervisor(s) we have approved this dissertation for submission.

Signed: [redacted]

Professor Y. Naidoo

Supervisor

Date: 13th December 2021

Signed: [redacted]

Professor Y. H. Dewir

Co-supervisor

Date: 13th December 2021

DECLARATION 1- PLAGIARISM

I, Arvish Maharaj, declare that:

The research reported in this thesis, except where otherwise indicated, is my original work.

This thesis has not been submitted for any degree or examination at any other university.

This thesis does not contain other person's data, pictures, graphs, or other information, unless specifically acknowledged as been source from other persons.

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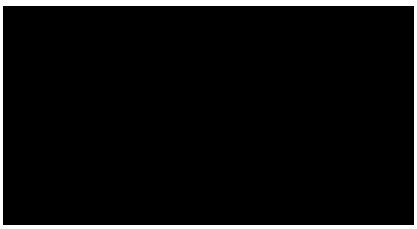

Signed: Arvish Maharaj
Date: 15 December 2021
Declaration Plagiarism 22/05/08 FHDR Approved

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LIST OF ABBREVIATIONS AND SYMBOLS

AD	Anno Domini, Latin For “In The Year Of The Lord”
AgNPs	Silver- Nanoparticles
ATP	Adenosine Triphosphate
BC	Before Christ
C	Cortex
C_6H1_4	Hexane
CH_3OH	Methanol
$CHCl_3$	Chloroform
COVID-19	Coronavirus Disease 2019
CU	Cuticle
CuW	Cuticular Warts
CVB	Central Vascular Bundle
CW	Cell Wall
DPPH	2,2-diphenyl-1-picrylhydrazyl
$C_4H_8O_2$	Ethyl acetate
GC–MS	Gas Chromatography–Mass Spectrometry
GSTs	Glandular Secreting Trichomes
GT	Glandular Trichome
HPLC-ESI-qTOF-MS/MS	High-Performance Liquid Chromatography Coupled to Electrospray Ionization and Quadrupole Time-Of-Flight Mass Spectrometry
IC	Inner Cortex
LE	Lower Epidermis
LI	Lipid Inclusion
LV	Large Vacuole
M	Mitochondria
MDR	Multi-Drug Resistant
MHA	Müller-Hinton Agar
MIC	Minimum Inhibitory Concentration
MX	Meta Xylem
NCT	Non-Glandular Trichome
NMR	Nuclear Magnetic Resonance
OC	Outer Cortex
OM	Outer Membrane

OsO_4	Osmium Tetroxide
P	Phloem
PG	Plastoglobuli
PGT	Peltate Gland Trichome
PhRC	Phloem Resin Canal
PRC	Pith Resin Canal
PX	Phloem Xylem
RC	Resin Canal
S	Stomata
SEM	Scanning Electron Microscopy
SG	Starch Grain
SLP	Single Layer Palisade
SP	Spongy Parenchyma
STs	Simple Trichomes
TEM	Transmission Electron Microscopy
TFC	Total Flavonoid Content
TPC	Total Phenolic Compounds
UE	Upper Epidermis
V	Vesicle
VB	Vascular Bundle
WHO	World Health Organization

DEDICATION

This dissertation is dedicated to my mom whose love, guidance, and sacrifice is why I am here today.

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CHAPTER 1:

INTRODUCTION

1.1 Rationale for the research (nature and scope)

The practice of using plants for curative purposes is deeply engrained in the history of mankind and is an essential part of most civilisations across the world (Unuofin and Lebelo, 2020). The pillars for the use of medicinal plants rely on the experience-based knowledge of indigenous tribes and communities (de Lucena *et al.,* 2020). The ancient knowledge of using plants for curative purposes is supported by modern-day science by conducting pharmacological research to understand and verify the efficacy of the chemical constituents and cellular principles of medicinal plants (Efferth *et al.*, 2020). The unequivocal importance of medicinal plants has been advocated in the successful treatment and prevention of various diseases (Krishnaprabu, 2019; Unuofin and Lebelo, 2020).

South Africa possesses a diverse population of flowering plants species; many of these plants have not yet been explored for their phytotherapeutic properties (Erhabor *et al*., 2019; Mahavy *et al*., 2020). This highlights a knowledge gap and the potential discovery of new phytochemicals. Furthermore, popular medicinal plants are under increasing pressure from climate change, development, and over-exploitation in response to increasing demand (Xiao *et al*., 2019). Hence there is an urgent need to source new lead compounds (de Lucena *et al.,* 2020). This premise instituted the primary rationale for this dissertation, which focused on unravelling the phytochemical and pharmacological properties of a local South African plant species, *Mangifera indica* L.

Mangifera indica belongs to the family Anarcardiaceae, consisting of 83 genera and approximately 873 known species distributed worldwide (Ramessur *et al*., 20011; Uddin *et al*., 2011). Although there are several species in this genus, most studies have focused on a few key species (Shah *et al*., 2010; Kole, 2011; Kalita 2014). One such economically important plant is *Mangifera indica*. *Mangifera indica* is an Asian species, which has a growing as a medicinal plant and is commonly used in Ayurvedic practices to treat abscesses, rabid dog or jackal bite, snakebite, stings, datura poisoning, heatstroke, anthrax, blisters, wounds in the mouth, tympanitis, colic, diarrhea, glossitis, indigestion, bacillosis, bloody dysentery, liver disorders, excessive urination, tetanus, and asthma (Kalita 2014; Erhabor *et al*., 2019; Elizabeth *et al.,* 2021). In addition, there are also ethnobotanical studies documented using this plant, however, all outside South Africa (Kalita 2014; Erhabor *et al*., 2019; Togue *et al*., 2020; Elizabeth *et al.,* 2021). In contrast, there is a lack of studies that have been reported on *Mangifera indica* and it's possible phytomedicinal properties. Thus, an attempt was made to elucidate the medically essential phytochemicals present in *Mangifera indica* with the intent of adding knowledge to this vast genus.

Natural anti-oxidants from plants counteract and attenuate cellular oxidative stress, reducing various diseases (Xu *et al*., 2017; Jamshidi-kia *et al*., 2020; Muñoz-Ramírez *et al*., 2020). Despite these established studies and advancements made, diseases such as cardiovascular diseases (CVD), inflammatory and metabolic disorders are prevalent and widespread in society. From a global perspective, as the Coronavirus disease 2019 (COVID-19) pandemic has spread rampantly across continents, a high mortality rate has been associated with COVID-19 patients with underlying comorbidities such as cardio- vascular disease (CVD) (Diniz *et al.,* 2020; Nishiga *et al*., 2020). In light of such developments, there is an urgent need to find novel compounds that can scavenge free radicals and serve as reducing agents to prevent the onset of underlying conditions. In this regard, this study aimed to establish the radical scavenging and anti-oxidant capacity of *Mangifera indica.*

The rise in antibiotic resistance has resulted in an intense drive to search for novel antibacterial agents as alternatives to conventional antibiotics, with researchers exploring the efficacy of various antibacterial compounds derived from plant sources (Guo *et al*., 2020). The present study intends to support this inquest by investigating the antibacterial potential of the extracts of *Mangifera indica*.

Even though many studies do exist on *Mangifera indica* investigations, it is important to note that all the studies were conducted outside of South Africa. Since South Africa has rich biodiversity (Bellocchi *et al*., 2021), it would be highly beneficial to add local *Mangifera indica,* a medicinal plant, to South Africa's ethnobotanical knowledge. This present study aims to add to the South African ethnobotany by considering the effect of seasonal changes on the foliar morphological characteristics through microscopic and macroscopic evaluation, as well as conduct phytochemical, antibacterial, and anti-oxidant screening of local *Mangifera indica* L. leaves.

1.2 Aims and Objectives of this study

The aims and objectives of each chapter are listed below:

Chapter 2:

Aim

To provide an overview of the genus *Mangifera* with relevance to its ethnopharmacological uses and current research developments.

Objectives

- Produce a literature overview that establishes the traditional uses of the species within the genus *Mangifera*.

- Provide an account of the current research trends performed using the genus *Mangifera*.

- Generate an overview and description of the plant species *Mangifera indica L.*

Chapter 3:

Aims

- To provide insight into the macro-morphology using a stereomicroscope and micro-morphology
- To provide histochemical staining on the leaf of *Mangifera indica* leaves for summer and winter.

Objectives

- Ascertain the differences in macro- and micro- morphology of *Mangifera indica* leaves for summer and winter to determine whether there are any morphological differences.
- Determine the presence of metabolites in the leaves of *Mangifera indica.*

Chapter 4:

Aim

To analyse the phytochemical profile as well as investigate the antibacterial and anti-oxidant activity of different solvents extracts of *Mangifera indica* leaves for summer and winter seasons.

Objectives

- Ascertain the phytochemical classes present in the crude extracts of *Mangifera indica* leaves using preliminary phytochemical tests.
- Assess the antibacterial potential of the methanolic extracts of *Mangifera indica* leaves for summer and winter using the agar-well method.
- Assess the anti-oxidant activities of the different solvent extracts of *Mangifera indica* leaves for summer and winter by measuring the radical scavenging ability of extracts by employing the 2, 2-diphenyl 1-picryl hydrazyl radical (DPPH) assay.

1.3 Overview methodology

1.3.1 Collection and identification of plant material

The leaves of *Mangifera indica* were collected from Durban, KwaZulu- Natal, South Africa. The summer samples were collected from December 2019 to March 2020, and the winter samples were collected from June-August 2020. Professor Y. Naidoo confirmed the species identity. A voucher specimen (accession number: NU0092176) was deposited in the Ward Herbarium, School of Life Sciences (Biology), University of KwaZulu-Natal, Durban, South Africa.

1.3.2 Preparation of leaf extracts

Fresh leaves of *Mangifera indica* were air-dried and ground into powder form using a Waring blender. Approximately 10 g powder of *Mangifera indica* leaves for summer and winter was formed after grounding. Ten grams for each season were subjected to a sequential extraction by increasing polarity using hexane (C_6H_{14}), chloroform ($CHCl_3$), and methanol (CH_3OH) using a reflux extraction apparatus. The extracts were cooled at room temperature, after which they went through filtration using Whatman no. 1 filter paper (Whatman Limited, UK) until no precipitate was seen in the filtrate. The extract was subjected to drying at room temperature and then stored in labelled air-tight jars the dark at 4°C until further use. This was done to prevent the extracts from reacting with atmospheric humidity. This was done to the leaves for both summer and winter seasons. Thereafter, the thoroughly dried plant materials were determined by the formula below:

$$\text{Extract Yield (\%)} = \frac{Weight\ of\ dried\ extract\ (g)}{Weight\ of\ leaf\ material\ (g)} \times 100$$

1.3.3 Phytochemical analysis

1.3.3.1 Qualitative phytochemical analysis

The qualitative phytochemical constituents screening of the hexane, chloroform and methanol leaves extracts for summer and winter for *Mangifera indica* was conducted using standard qualitative protocols (Harborne *et al.*, 1973; Trease and Evans, 1978; Sofowora, 1993).

1.3.4 Biological evaluation: Antibacterial and anti-oxidant assays of local leaves of Mangifera Indica L.

1.3.4.1 *In vitro* antibacterial assay

The antibacterial activity was assayed on Gram-negative, *Escherichia coli* (ATCC 25922) and Gram-positive *Staphylococcus aureus* (ATCC 43300) bacterial strains. The strains were provided by Professor Johnson Lin, Department of Microbiology, University of KwaZulu- Natal, and maintained in 75% glycerol at -80°C.

1.3.4.2 Sample preparation

The crude extracts (methanol) from the summer and winter leaves of *Mangifera indica* were dissolved in 10% Dimethylsulfoxide (DMSO) to different concentrations of 10, 5, 2.5, 1.25, and 0.625 mg/mL.

1.3.4.3 Antibacterial activity assay

The *in vitro* antibacterial assay of the methanolic crude leaf extract for summer and winter was carried out using the agar well diffusion method described by Perez *et al*, (1990). Bacteria was swabbed onto Mueller-Hinton agar (MHA) plates, a 6 mm diameter improvised sterile cork-borer was used to bore wells in the Petri plates, thereafter 100 µL each of the prepared different concentrations (0.625, 1.25, 25, 5, and 10 mg/ mL) of the crude extract which were pipetted into the wells. The plates were incubated at 36°C and the growth inhibition zones around the bored wells were taken as positive results, and the diameters were measured within 18-24 h after incubation. Autoclaved sterile 10% Dimethylsulfoxide (DMSO) was used as the negative control, while 10 µg/mL of gentamicin (for Gram-negative) and streptomycin (for Gram-positive) were used as the positive controls. The tests were conducted in triplicates, and data were expressed as mean ± standard deviation.

1.3.4.4 *In vitro* anti-oxidant assay

1.3.4.4.1 DPPH free radical scavenging activity

The hydrogen donating ability of solvents was determined using a modified 2,2-diphenyl-1-picrylhydrazyl (DPPH) spectrophotometric assay as described by Braca *et al.* (2002). Briefly, 1 mL of plant extract with concentrations of a range of 15, 30, 60, 120, and 240 µg/mL was pipetted into a 96-well microplate in triplicate. After that, 150 µL of 0.3 mM DPPH solution was added. The microplate was incubated in the dark at 25°C for 30 minutes. The absorbance of the solvents was measured at 517

nm using the spectrophotometer, and the percentage of the free radical inhibition was used to express the free radical scavenging activity. Ascorbic acid was used as the standard. The IC_{50} was derived from the inhibition curves by plotting the percentage inhibition against the concentration logarithmic scale. The free radical scavenging ability of pure compound was calculated using the equation below:

$$\text{DPPH scavenging activity (\%)} = \left(\frac{Abs\ control - Abssample}{Abs\ control}\right) \times 100$$

Where;
Abs control is the absorbance of DPPH and methanol
Abs sample is the absorbance of DPPH radical + sample

Dose-response curves were plotted between the percentage of scavenging activity (y-axis) and the logarithmic transformation of the concentrations (x-axis), using Microsoft Office Excel, 2016. The IC_{50} value was calculated from the antilogarithmic values of the linear regression analysis. The IC_{50} is the concentration of extract that inhibits the formation of DPPH radicals by 50%.

1.3.5 Statistical analysis

Statistical, analyses were conducted using Statistical Package for the Social Sciences (SPSS) versions 26 and 27. Data are indicated as a mean of triplicate replicates (n=3). For anti-oxidants, the mean values were determined using two-way analysis of variance (ANOVA) with $p \leq 0.05$ considered significant with Tukey honestly significant difference (HSD) *post hoc* test (Moradi *et al*., 2020).

1.3.6 Micro and Macro- Morphology

1.3.6.1 Leaf collection

Leaves of *Mangifera indica* were harvested from Durban, KwaZulu-Natal, South Africa. The summer samples were collected from December 2019 to March 2020 and the winter samples were collected from June-August 2020. Professor Y. Naidoo confirmed the species identity. A voucher specimen (accession number: NU0092176) was deposited in the Ward Herbarium, School of Life Sciences (Biology), University of KwaZulu-Natal, Durban, South Africa.

1.3.6.2 Stereomicroscopy

Fresh leaves were examined using the Nikon AZ100 stereomicroscope (Nikon Corporation, Yokohama, Japan) equipped with a Nikon Fiber Illuminator and photographed using the NIS-Elements Software (NIS-elements D 3.00). The adaxial and abaxial surfaces of fresh leaves at the three developmental stages (emergent, young and mature) were imaged with an emphasis on surface detail.

1.3.6.3 Histochemistry

Hand-cut sections of the fresh *Mangifera indica* leaves were subjected to various histochemical tests to reveal the presence and location of certain phyto- metabolites such as tannins, lipids, alkaloids, polyphenols, lignin aldehydes, unesterified pectins and mucilage, terpenoids, total proteins, and lipids. The following tests were conducted: Wagner's and Dittmar reagents for alkaloids (Furr and Mahlberg 1981; Tiwari *et al*., 2011); ruthenium red for acidic polysaccharides, e.g. unesterified pectins and mucilage (Johansen 1940); NADI reagent for terpenoids of essential oils (Baskaran and Karthikeyan, 2019); ferric trichloride for phenolic compounds (Johansen 1940; Sofowora, 1993); toluidine blue for carboxylated polysaccharides and polyphenol, bromophenol blue for total proteins; phloroglucinol for lignin aldehydes; III and IV (Sudan red) for lipids (Lison 1960) and Nile blue for lipids (Harborne *et al.*, 1973). The unstained sections served as controls (results not shown). The sections were examined and photographed using a Nikon Eclipse 80i Compound Light Microscope (Nikon, Japan).

1.3.7 Scanning electron microscopy (SEM)

The fresh leaves from the three developmental stages (emergent, young, and mature) of growth were cut into small pieces approximately 2-3 x 4.0 mm^2 and a similar technique of scanning electron microscopy (SEM) described by Naidoo *et al*., (2013) was used with slight modifications in the use of 2.5% glutaraldehyde and 0.5% osmium tetroxide (OsO_4) for primary and secondary fixation respectively.

1.3.7.1 Freeze drying

Another set of fresh leaves from the three developmental (emergent, young, and mature) of *Mangifera indica* were placed in liquid nitrogen (-196°C), after which the sections were further frozen in an Edwards Modulyo freeze dryer (Edwards High Vacuum International Ltd., UK), at -40 to -60°C in a vacuum of 10^{-1} Torr for 72 h. The samples were stuck to aluminium stubs with carbon conductive tape, gold sputter coated twice using a Polaron SC500 Sputter Coater (Quorum Technologies Ltd., UK) in a

0.1 Torr vacuum. The prepared leaf segments were viewed using a Zeiss Ultra-Plus FEG-Scanning electron microscope operating at 20 kV.

1.3.7.2 Morphometric analysis of trichomes

Selected images of trichomes obtained from SEM were analysed using the ImageJ software program (Schindelin *et al*., 2012). The diameter (μm) of the trichome head, length, and width of the stalk for each type was measured.

1.3.7.3 Transmission electron microscopy (TEM)

Fresh leaves of *Mangifera indica*, at different stages of development (emergent, young, and old) were cut into pieces of approximately 2–3 x 5 m^2. A modified version of the technique described by Naidoo *et al*. (2011) was employed. The modifications were in the use of 2.5% glutaraldehyde and 0.5% osmium tetroxide (OsO_4) for the primary and secondary fixation, respectively.

1.3.8 Statistical analysis

The data were analysed using EXCEL and SPSS 25 for Windows, IBM Corporation, New York, USA. Data obtained were expressed as mean ± standard deviation. P- values of <0.05 were considered significant. All analyses were performed in triplicate. (n=3).

1.4 Outline of dissertation structure

To achieve the aims and objectives, the dissertation has been outlined below:

Chapter 1: Discusses the rationale of the study, the overview of the aims and objectives. It also includes the general outline of the dissertation methodology.
Chapter 2: The literature review gives an insight into the genus *Mangifera Indica L.* including an overview of traditional uses and recent scientific research developments.
Chapter 3: The macromorphological and micromorphological seasonal characteristics of local *Mangifera indica* L. leaves were investigated.
Chapter 4: In this chapter, the preliminary phytochemistry, antibacterial and anti-oxidant activities of *Mangifera indica* leaves extracts were determined for summer and winter seasons.
Chapter 5. This chapter concludes the study stating the major findings, challenges that were faced, and future research plans.

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CHAPTER 2: LITERATURE REVIEW

Review of Mangifera indica Linn.: morphology, phytochemistry, biological activities of Mangifera indica Linn. Leaves.

Arvish Maharaj[a], Yougasphree Naidoo[a], Yaser Hassan Dewir[b,c]

[a]School of Life Sciences, University of KwaZulu-Natal,Westville Campus, Private Bag X5400, Durban 4001, South Africa

[b]Plant Production Department, P.O. Box 2460, College of Food and Agriculture Sciences, King Saud University, Riyadh 11451, Saudi Arabia

[c]Kafrelsheikh University, Faculty of Agriculture, Kafr El-Sheikh 33516, Egypt

Abstract

Plant species belonging to the family Anacardiaceae are globally known to possess various medicinal properties and have cultural and economic importance in traditional medicine and horticulture. The plant kingdom contains various novel biologically active compounds, which could potentially be of great medicinal value. They are important to both animals and humans and are used as food or for ornamental purposes worldwide. *Mangifera indica* is from the family Anacardiaceae and is an important tropical fruiting tree with ~30 species used in Ayurvedic and indigenous medicine for more than 4000 years. The flowers, leaves, stems, roots, and seed extracts of plants belonging to this genus are rich in bioactive compounds and have exhibited significant medicinal potential for treating various ailments and infections. Evidence derived from several studies has demonstrated the anti-oxidant, antibacterial, antifungal, anti-inflammatory, anticancer, antidiabetic, antiulcer, hepatoprotective, analgesic, antiamoebic, antihelminthic, antiarthritic, antihypertensive, and antiviral properties of this plant. The pharmacological and medicinally important chemical mangiferin which is a polyphenolic anti-oxidant and a C-glucosyl-xanthone, has strong anti-lipid peroxidation, anti-oxidant, and immunomodulation effects. Important biological activities found in the *Mangifera indica* extracts can be linked to mangiferin. Based on the traditional knowledge of the properties of mangiferin, plant-based medicine using this compound should be adequately standardised as it has been suggested to have no side effects. Traditionally, the genus *Mangifera* has significant medicinal potential; however, there is a scarcity of information on various species that are yet to be evaluated. This review provides a comprehensive report on existing literature concerning the general taxonomy, phytochemistry, biological activities, and morphological studies of *Mangifera indica*.

Keywords: Anacardiaceae; Bioactive compounds; *Mangifera indica*; Mangiferin; Trichomes.

2.1 Introduction

Since prehistoric times, man has used herbal products, such as plants, in medicines to improve and treat diseases (Bombard and Schnell, 1997). Fossil records traced the usage of plants in medicine at least 60 000 years back (Fabricant and Farnsworth, 2001; Shi *et al*., 2010). Evidence of mankind's dependence on medicinal plants and their uses are found in ancient, documented pharmacopeia (Page *et al.,* 1997; Cragg and Newman, 2001). The use of herbal medicines has presented a great challenge to early humans (Kim *et al.,* 2021). It is highly likely that when pursuing food, early humans consumed poisonous plants, which led to diarrhoea, vomiting, or other toxic reactions and possibly even death (Kim *et al.,* 2021). However, in this manner, early humans acquired knowledge about natural medicines and edible materials (Gao *et al*., 2007).

The increasing use of medicinal plants in various cultures has prompted scientific studies into natural products (Fabricant and Farnsworth, 2001; Shi *et al*., 2010). These studies are aimed at evaluating whether their traditional practices are supported with pharmacological effects or if their use is simply based on folklore (Sparg *et al*., 2002). Due to the growing interest in the use of traditional medicine, it is essential to meet some of the concerning challenges such as the overall lack of research, evidence of safety, efficiency and good quality of natural products, patenting rights and, the need to maximize and integrate natural products as possible sources of remedies in primary health care (Fabricant and Farnsworth, 2001; Shi *et al*., 2010). These challenges need to be quantified for the appropriate use of traditional medicine in modern therapeutics (Gamaniel and Jsselmuiden, 2004; Muhammad and Awaisu, 2008).

Plants have been extensively used globally for medicine due to their availability (>250 000 higher plant species), there is a great likelihood of discovering useful bioactive compounds in the plant kingdom (Newman and Cragg, 2007). A single plant in traditional medicine may contain several phytochemical compounds, such as alkaloids, flavonoids, phenols, terpenoids, lignin, steroids, saponins, proteins, carbohydrates, triterpenoids, fats and oils. These chemicals can act alone or in combination with one another to deliver the desired pharmacological impact (Medina *et al*., 2013; Knop, 2019). Through advances in therapeutic principles, theoretical background, associated technologies, and knowledge of life sciences, a deeper understanding of the role of bioactive compounds in traditional medicine has become possible (Bussmann and Sharon, 2006).

Anacardiaceae (cashew family) is a large family of dicotyledonous flowering plants. Many plant species of Anacardiaceae possess great therapeutic potential, whilst some are unexplored to date (Bussmann and Sharon, 2006). Plant species of this family play an important role to both man and animals as they

are used for food, medicine, or as ornamentals (Shah *et al.*, 2010) and contain many essential secondary metabolites; some include alkaloids, terpenoids, phenols, tannins, quinones and flavonoids (Kumar *et al.,* 2021). Several plant species are being utilised for their ethnomedicinal properties based on the phytocompounds they acquire, with *Mangifera* (Anacardiaceae) being one of such genera.

Mangifera indica Linn. is a member of the plant family Anacardiaceae (vascular plants) (Illon and Olovode, 1991; Doughari and Manzara, 2008; Shah *et al.*, 2010). *Mangifera indica* is a tropical fruiting tree and an important herb in Ayurvedic and indigenous medicine for over 4000 years (Shah *et al.*, 2010). Several species within *Mangifera* possess various pharmacological properties including, antimicrobial (Shah *et al*., 2010, Rakholiya and Chanda, 2012), anti-inflammatory (Talita and Synara, 2012; Das *et al.*, 2015), antiviral (Shah *et al.*, 2010; Das *et al.*, 2015) and anticancer (Shah *et al.*, 2010; Talita and Synara, 2012; Ganogpichayagrai *et al.,* 2017) activities.

The most active biological constituent of *Mangifera indica* is mangiferin, followed by phenolic acids, benzophenones, and other anti-oxidants (Manzara and Doughari, 2008; Ribeiro *et al*., 2010; Masud, 2016; Lauricella *et al.,* 2017; Kabir *et al*., 2017; Dineshkumar *et al.,* 2018; Kumar *et al*., 2021). Mangiferin is the main contributor to most of the biological activities of *Mangifera indica* leaf methanolic extracts (Ribeiro *et al*., 2010; Lauricella*.,* 2017). The benzophenone derivatives in *Mangifera indica* leaves possess significant α-glucosidase inhibitory and immunosuppressive activities (Ediriweera *et al*., 2017). Several reviews have been developed to discuss the bioactive compounds and health-promoting effect of *Mangifera indica* fruit/pulp (Ribeiro *et al.*, 2010; Masud, 2016; Lauricella *et al*., 2017; Kabir *et al*., 2017).

There is very limited documented information on South African *Mangifera indica* leaves, and there is no formal pharmacopoeia outlining the ethnobotanical use of the plant distribution, and the ethnobotanical records are either scarce or inconsistent. The current study aims at reviewing the taxonomy, phytochemistry, ethnopharmacology, pharmacology, and morphology studies of the family Anacardiaceae, genus *Mangifera* and species *indica* using existing literature.

2.2 History, origin, domestication, and geographical distribution

Mangifera indica is a diploid fruiting tree with 20 pairs of chromosomes and a small genome size of 439 Mbp (Arumuganathan and Earle, 1991). Historians and horticulturists agreed that the cultivated *Mangifera indica* tree originated in India (Hooker, 1876; Mukherjee, 1949a, 1951, 1953,1972; Woodrow, 1904). Based on the level of genetic diversity observed, Vavilov (1926) reported that the Assam-Burma region is the center of the origin of *Mangifera indica*. Mukherjee (1951) considered the origin of the genus *Mangifera* in Southeast Asia and the origin of cultivated *Mangifera indica* in the

Assam-Burma region (Mukherjee, 1949). Scientists of the Birbal Sahni Institute of Palaeobotany, Lucknow, India, have traced the origin of the genus *Mangifera* to 60-million-year-old fossil compressions of carbonized *Mangifera indica* leaves in Palaeocene sediments near Damalgiri, West Garo Hills, Meghalaya, India and named them *Eomangiferophyllum damalgiriensis* (Mehrotra *et al*., 1998).

Extensive comparisons of the anatomy and morphology of several modern-day species of *Mangifera* with fossil samples have reinforced the view that Northeast India is the center of origin of *Mangifera indica* (Hooker, 1876; Mukherjee, 1949, 1951, 1953, 1972; Woodrow, 1904). From here, it spread into the neighbouring areas of Southeast Asia after the formation of a land connection due to a collision of the Indian plate with the Asian plate (Mukherjee, 1949, 1951). Thereafter, the species diversified extensively in the Malaysian and Sumatran rain forests (Kostermans and Bompard, 1992; Kostermans, 1993; Salma, 2010; Williams, 2012). Today, the highest diversity of wild *Mangifera indica* is found in Malaysia and Indonesia, especially peninsular Malaya, Borneo, and Sumatra (Kostermans and Bompard, 1993). The natural occurrence of *Mangifera* species extends as far north and as far east as the Caroline Islands (Bompard and Schnell, 1997). The wild *Mangifera indica* tree is also found in India, Sri Lanka, Bangladesh, Myanmar, Thailand, Kampuchea, Vietnam, Laos, Southern China, Singapore, Brunei, the Philippines, Papua New Guinea, and the Solomon and Caroline Islands (Sreekumar, 1996; Orwa *et al.*, 2009; Litz, 2009; Kole, 2011).

The English word "*Mangifera indica*" originated from the Malayalam "manga" and the Tamil "mangai" (Arumuganathan and Earle, 1991). After *Mangifera indica* was domesticated in India more than 4000 years ago, rulers, traders, and travelers took the species to different regions of the world for plantation (Arumuganathan and Earle, 1991). During the 4th and 5th centuries BC, Buddhist monks took the *Mangifera indica* to the Malaya Peninsula and East Asia (Arumuganathan and Earle, 1991). In addition, *Mangifera indica* was first introduced in China from India during the middle of AD 7th century, when a Chinese traveller Hwen T'sang returned from India to China with the seedling (Litz, 2009; Gao *et al*., 2011), and in AD 10th century, the Persians carried it to East Africa (Arumuganathan and Earle, 1991).

During AD 16th century, the Portuguese took the *Mangifera indica* to West Africa and Brazil (Litz, 2009), from where it was carried to the West Indies, being first planted in Barbados around 1742 and later in the Dominican Republic. It reached Jamaica in about 1782. As early as the 19th century, *Mangifera indica* was moved to Mexico from the Philippines and the West Indies (Morton, 1987), and then reached Miami in 1862 or 1863 from the West Indies (Litz, 2009). It is believed that the seedling was polyembryonic and from the "No. 11" parent (Litz, 2009). In the same decade, after post-European colonisation, ~40 varieties of *Mangifera indica* from India were initially planted in 1875 in North

Queensland Australia (Morton, 1987). Figure 2.1 shows *Mangifera indica* production in different regions of the world.

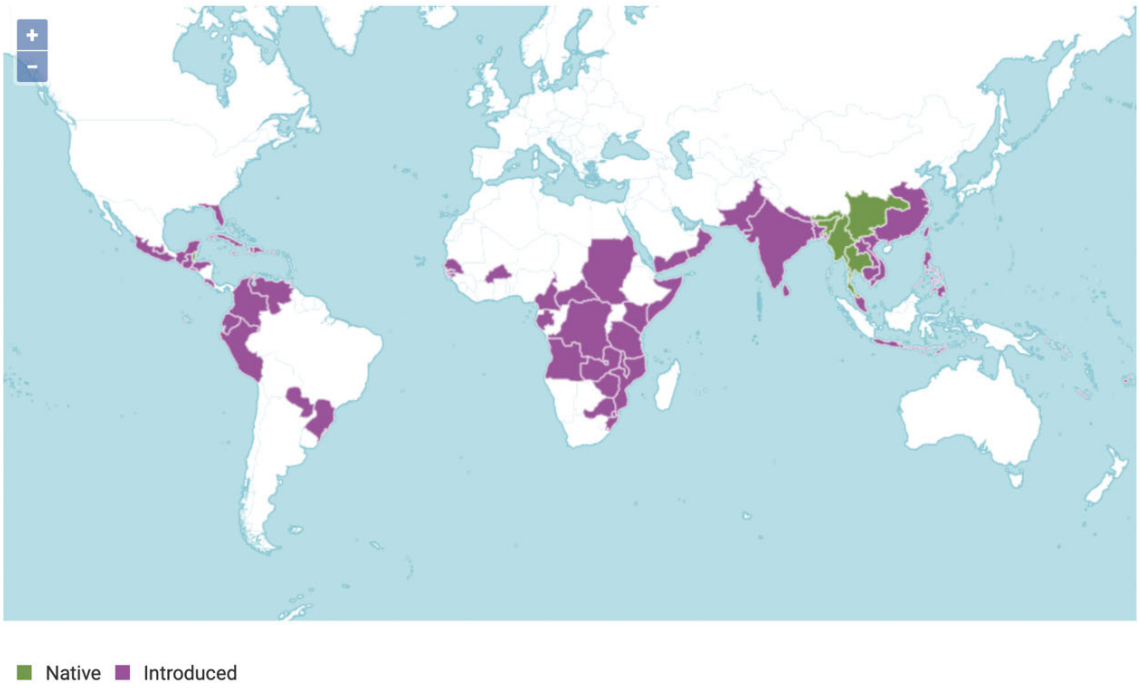

Figure 2.1: Geographical distribution of *Mangifera indica* L. plants in different regions of the world. (Adapted from Royal Botanic Gardens Vilarroel *et al.*, 2021).

Table 2.1 Taxonomic classification of *Mangifera indica* L.

Classification	Name
Domain	Eukaryota
Kingdom	Plantae
Subkingdom	Tracheobionta
Division	Angiospermae
Sub-division	Dicotyledons
Class	Magnoliopsida
Order	Sapindales
Family	Anarcardiaceae
Genus	*Mangifera*
Species	*M. indica* L.
Scientific name	*Mangifera indica* L

2.3 Botanical description

2.3.1 Tree morphology

The *Mangifera indica* tree reaches 40 m in height and can live for several hundred years (Doughari and Manzara, 2008). It bears a rosette of evergreen leaves (red or yellow at first) and contains up to 30 cm long dense panicles of small (5–10 mm) reddish or yellowish flowers (Illon and Olovode, 1991). *Mangifera indica* has a taproot system of a 6 m length. (Illon and Olovode, 1991; Doughari and Manzara, 2008). The stout trunk is ~90 cm in diameter, and the bark is brown, with many thin and some thick fissures, which become darker, rough, and scaly or furrowed (Doughari and Manzara, 2008; Shah *et al.*, 2010). Branchlets are rather stout, pale green, and hairless, and the inner bark is light brown and brittle (Figure 2.2) (Illon and Olovode, 1991; Doughari and Manzara, 2008; Shah *et al.*, 2010).

Figure 2.2: (Left) Tree of *Mangifera indica* L.; (Right) The bark of *Mangifera indica* L. (Original image by author A. Maharaj)

2.3.2 The fruit

The fruit of the *Mangifera indica* tree is a drupe, green when young and yellow on ripening, with a solitary seed (Doughari and Manzara, 2008). There is variation in the fruit, such as the size, form, quality, and color (Illon and Olovode, 1991; Doughari and Manzara, 2008). The fruit is 5-10 cm long and 3-4.5 cm wide with a fleshy, bright yellow mesocarp and a compressed endocarp (Illon and Olovode, 1991; Doughari and Manzara, 2008). The drupes may be nearly round, oval, ovoid-oblong, or subreniform weighing 1.8–2.26 kg (Illon and Olovode, 1991; Doughari and Manzara, 2008). The skin is leathery, waxy, smooth, thick, and aromatic and is coloured light or dark green to yellow, yellow-orange, and reddish-pink, with dark-red, greenish, or reddish dots, and a grey or purplish bloom when fully ripe (Illon and Olovode, 1991; Doughari and Manzara, 2008). Some have a “turpentine” odour and flavour, while others are richly and pleasantly fragrant (Illon and Olovode, 1991). The mesocarp (flesh) is a pale yellow to deep orange (Illon and Olovode, 1991). It is fibrous and extremely juicy when ripe, with a flavour ranging from very sweet to sub-acid to tart (Illon and Olovode, 1991; Doughari and Manzara, 2008). There is a single, longitudinally ribbed, pale white-yellow, and somewhat woody stone, which can be flattened, oval, or kidney-shaped, sometimes rather elongated (Illon and Olovode, 1991; Doughari and Manzara, 2008). Within the stone is a starchy seed, either monoembryonic (usually single sprouting) or polyembryonic (usually producing more than one seedling) (Illon and Olovode, 1991; Doughari and Manzara, 2008; Shah *et al.,* 2010) (Figure 2.3). The seed is solitary, ovoid, or oblong, and encased in a hard, compressed, fibrous endocarp (Shah *et al.,* 2010).

Figure 2.3: Fruit of *Mangifera indica* L. (Original image by author A. Maharaj)

2.3.3 The leaves

The leaf blade is oblong-lanceolate, 12-30 cm long, and 3.5-8 cm wide (up to 50 cm long on sterile branches) (Doughari and Manzara, 2008; Shah *et al.,* 2010) (Figure 2.4). The leaf arrangement is alternate, and leaves are leathery, deep green adaxially, light green abaxially, glabrous on both sides, with a cuneate to obtuse base, an entire margin, undulate, an acute to long acuminate apex, 20-25 pairs of lateral veins, a prominent midrib on both sides, and obscure reticulate venation (Shah *et al.,* 2010). In addition, the leaves are spirally arranged on branches, elliptical, and pointed at both ends, and the leaf blade is reddish and thinly flaccid when first formed and releases an aromatic odour when crushed (Shah *et al.,* 2010). Full-grown leaves are 12-30 cm long and 3.5-8 cm wide on flowering branches (up to 50 cm long on sterile branches), curved upward from the midrib, and sometimes with slightly wavy edges (Doughari and Manzara, 2008; Shah *et al.,* 2010). Young leaves are red, and old ones are shiny dark green above, with a lighter-below, pale, and conspicuous midrib and yellow or white venation (Shah *et al.,* 2010). The petiole is 2-6 cm long, grooved apically, and striated and swollen at the base (Shah *et al.,* 2010).

Figure 2.4: Whole mature leaf of *Mangifera indica* L. (Original image by author A. Maharaj)

2.3.3 The flowers

Inflorescence occurs in panicles comprising ~3000-4000 tiny, whitish-red, or yellowish-green flowers (Shah *et al.,* 2010). The flowers are 25%-98% male, and the rest are hermaphroditic (Shah *et al.,* 2010). They are borne in profuse, showy, erect, pyramidal, branched clusters 6-40 cm high, radially symmetrical, usually with 5 spreading imbricate petals, 3-5 mm long and 1-1.5 mm broad, streaked with red, with the median petal prolonged like a crest at the base (Shah *et al.,* 2010). They are finely hairy and fragrant, with a stalk short, and each flower has 5 stamens (1 fertile and 4 shorter and sterile) borne in a disc, paniculate, terminal, and glabrous to tomentose-pilose (Doughari and Manzara, 2008; Shah *et al.,* 2010). Bracts are ~1.5 mm long, lanceolate, and pubescent, and the pedicel is 1.5-3 mm long and articulate (Doughari and Manzara, 2008; Shah *et al.,* 2010). The five sepals are ovate-lanceolate, 2-3 mm long and ~1.5 mm wide, glabrous to pubescent, acuminate, and green or yellowish-green with a whitish margin (Doughari and Manzara, 2008; Shah *et al.,* 2010). Petals are oblong or oblong-lanceolate, light yellow with a prominent red, tree-shaped pattern adaxially, 3.5-4 mm × ~1.5 mm in size, glabrous, and recurved at anthesis (Shah *et al.,* 2010). There is one ~2.5 mm fertile stamen, with an ovate anther; four 0.7-1 mm staminodes. The ovary is oblique, ovate, ~1.5 mm in diameter at the anthesis, and the style is 2.5 mm and eccentric (Shah *et al.,* 2010). The flowers have a conspicuous five-lobed, inflated, fleshy disc between the petals and stamens (Shah *et al.,* 2010). The calyx is yellow green, very short, deeply fiveilobed (Doughari and Manzara, 2008; Shah *et al.,* 2010) (Figure 2.5).

Figure 2.5: Flowers of *Mangifera indica* L. (Original image by author A. Maharaj)

2.4 Trichome development

Trichomes are multicellular or unicellular appendages derived from cells found on the aerial epidermis (Werker, 2000). The development of a trichome begins when single protodermal cells cease to divide and grow outward in the plane of the leaf surface (Szymanski *et al.*, 1998, 1999; Tian *et al.*, 2015). Committed cells then proceed through a series of distinguishable stages of trichome differentiation. The first stage is categorized by the radial expansion in the plane of the leaf; whereas the second stage is the growth out of the plane of the leaf, the third stage includes the development of branches, the fourth stage is where branch expansion occurs, additionally the fifth stage includes diffuse expansion, and finally the last stage includes the process of cell wall maturation (Szymanski *et al*., 1998, 1999; Marks *et al*., 2008).

Scientific interest in trichomes is based on their taxonomic and functional importance as well as on the economic efficacy of trichome secreted substances (Choi and Kim, 2013). Trichomes vary significantly in location, morphology, mode of secretion, and ability to secrete (Serna and Martin, 2006). Though the morphology of trichomes differs greatly, they can be classified into two types: glandular secreting trichomes (GSTs) and simple trichomes (STs) also referred to as "non-glandular" (presumably non-secreting) (Wagner *et al*., 2004; Huchelmann *et al*., 2017). Leaves of various plants are heavily protected with glandular and non-glandular trichomes which are derived from the epidermal cells (Valkama *et al.,* 2004; Huchelmann *et al*., 2017). Trichomes occur in various forms, being straight, tortuous, hooked, spiral, stellate, simple, peltate or capitate (Levin, 1973a and b). In the leaves of Anacardiaceae (two types of trichomes occur: (a) glandular and (b) non-glandular (Valkama *et al.,* 2004; Huchelmann *et al*., 2017).

2.4.1 Glandular trichomes

Glandular trichomes comprise a variety of glands that synthesise, accumulate, and secrete active compounds (Gershenzon *et al*., 1989; Huchelmann *et al*., 2017). The structure of glandular trichomes usually consists of a unicellular or a multicellular stalk (Shaheen *et al.,* 2009), with the glandular head being either unicellular or multicellular (Werker, 2000). Glandular trichomes comprise a stalk, base, and a terminal secretory head, which all may consist of one or many cells (Werker, 2000). A neck cell can be found occasionally, between the secretory head and stalk cells, and is morphologically different from the two (Werker, 2000). Glandular trichomes can be found throughout dicotyledonous angiosperms (Metcalfe and Chalk, 1950; Levin, 1973a and b; Bhatt *et al*., 2010). Glandular trichomes of 39 genera (109 species) of Anacardiaceae are known (Seyed and Ahmad, 1978; Bhatt *et al*., 2010).

Glandular capitate trichomes are variable in structure among different species and several trichomes are seen in *Mangifera taipa* (Bhogaonkar and Lande, 2012). Two general types of capitate trichomes were identified by Werker (2000) and Bhogaonkar and Lande (2012). The first comprised of a uni-, bi- or multicellular head cell with a subcuticular space for the storage of exudates, a neck cell, unicellular stalk, and a basal epidermal cell (Bhogaonkar and Lande, 2012). The second is similar to the first type, except that it has a unicellular, bulbous head cell with a subcuticular space and with one or two basal epidermal cells (Maleci-Bini and Giuliani, 2006; Jia *et al*., 2012). These trichomes are considered as short-term trichomes, as the duration of secretion is brief, and is only active during the early development of organs (Maleci-Bini and Giuliani, 2008).

2.4.2 Non-glandular trichomes

Non-glandular trichomes may be unicellular, multicellular, or branched (Gairola *et al*., 2009; Kryvych *et al*., 2011; Osman, 2012). Non-glandular trichomes of 39 genera (109 species) of Anacardiaceae are illustrated by Hou (1978). Certain species of *Mangifera* have characteristic non-glandular trichomes which are unicellular (Hou, 1978; Rocha *et al.,* 2015). Multicellular trichomes can be classified further as uniseriate (single row of cells) (Werker, 2000), as seen in *Mangifera altissima* and *Mangifera casturi* of Anacardiaceae (Hou, 1978; Rocha *et al.,* 2015); or multiseriate, (several rows of cells) as noted in *Mangifera applanate* (Balkwill and Balkwill, 1997). Non-glandular trichomes in plant species found within Anacardiaceae (vary in density in the different developmental stages of leaves (Rocha *et al.,* 2015). Trichome diversity observed in *Mangifera* includes the occurrence of sunken glands present on the abaxial surface of leaves, stellate, dendroid, anvil-shaped, and biramous trichomes, as well as adpressed non-glandular trichomes with multiseriate bases (Balkwill and Balkwill, 1997).

2.5 Trichome function

2.5.1 Plant defence

Plants can negatively affect insect survival and fertility, thus indicating a practical need for trichome research, especially in economically important crop species (Levin, 1973a and b). The success and proliferation of trichomes throughout various plant families lie in its ability to perform several functions (Wagner *et al.*, 2004; Figueiredo *et al*., 2013). Some of these functions can include plant defence (Wagner *et al.*, 2004; Figueiredo *et al*., 2013), and the ability to modify trichome type and density through eco-geographically and environmentally driven selection (Wellso and Hoxie, 1982). Plants may be protected against herbivores and destructive insects both by morphological structures, such as trichomes and thorns and by secondary compounds ("chemical defences") (Shaheen *et al.,* 2009; Tooker *et al.,* 2010; Santos *et al.,* 2016). Trichome secretions can poison some pests, whilst others are rendered harmless by immobilisation in the secretion (Levin, 1973).

In *Mangifera laurina*, phytochemical constituents in leaves and stems such as tannins, alkaloids, and flavonoids are secondary metabolites that serve as defence mechanisms against the predation of herbivores, insects, and microorganisms (Singh *et al*., 2010). Some plants produce trichomes constitutively, while others react to damage by increasing trichome density in emergent leaves (Dalin *et al.,* 2008). Trichome defences (both glandular and non-glandular) can detrimentally affect natural enemies by hindering movement and causing their entrapment (Keller, 1987; Krips *et al.,* 1999).

Glandular trichomes can differ in structure and secrete a variety of secondary substances (Levin, 1973). These trichomes are involved in the sequestration, production, and accumulation of specific phytochemicals that often contain anti-oxidant and antimicrobial properties (Duke, 1994; Schilmiller *et al*., 2008). Glandular trichomes can be involved in both physical and chemical defence (Duke, 1994; Soroka *et al.,* 2011; Oksanen, 2018). Several compounds such as essential oils, resins, tannins, and mucilage substances can be found in the glandular exudates of numerous plant genera and families (Soroka *et al.,* 2011; Oksanen, 2018).

The restricted movement of insects on leaf surfaces can be further inhibited by the presence of sticky exudate associated with various glandular trichomes (Elsey, 1974; Belcher and Thurston, 1982; Obrycki and Tauber, 1984; Jakoby *et al*., 2008). Glandular trichomes exudate can affect natural enemies by reducing their dwelling time on plants (Obrycki and Tauber, 1984; Romeis *et al.,* 1999; Lovinger *et al.*, 2000; Serna and Martin, 2006), entrapping small-bodied individuals (Obrycki and Tauber, 1984; Perring *et al.*, 1999), and can act as a toxin (Boughton *et al.,* 2005). In addition to poisoning and trapping insects, non-volatile exudates from trichomes such as simple or complex phenolics and alkaloids may serve as gustatory repellents. This is proposed by the feeding response of insects to intact tissue or specific chemicals (Levin, 1973).

Non-glandular trichomes are known exclusively for their physical protection in plants against biotic and abiotic stresses (Werker, 2000; Wagner *et al*., 2004), as well as discouraging feeding and ovipositing insects (Levin, 1973; Baur *et al.,* 1991; Szyndler *et al.,* 2013). These trichomes are presumed to arise early in leaf development and deteriorate with maturity (Wagner *et al.,* 2004). This may suggest that non-glandular trichomes may play a role in the protection of emergent leaves until there is a build-up of defence chemicals upon maturation (Johnson, 1975). Expression of genes involved in anthocyanin, glucosinolate, and flavonoid pathways can nevertheless be detected in non-glandular trichomes, indicating the roles of these trichomes in defence and the biosynthesis of secondary compounds (Wangner *et al.,* 2004; Jakoby *et al*., 2008). The density of non-glandular trichomes in young *Mangifera laurina* (L.) trees after the attack of *Mangifera altissima* (L.) beetles was observed by

Baur *et al*. (1991). The number of trichomes increased in the leaves after the attack. The non-glandular trichomes were positively correlated with the amount of defoliation caused to the plant.

2.5.2 Pollination and attraction

In addition to plant defence, trichomes play a role in plant-insect interactions (Oelschlägel *et al*., 2009). Several trichome-derived compounds are used as attractants for species-specific pollination (Gutiérrez-Alcalá *at al*., 2005). Trichomes are also involved in specialised mechanisms of insect capture for pollination (Oelschlägel *et al*., 2009). *Mangifera altissima* flowers trap their pollinators using various mechanisms (Oelschlägel *et al*., 2009). These include a waxy surface, narrowing of the utricle, and directional trichome (Gutiérrez-Alcalá *at al*., 2005). These mechanisms guide insects into the entrance of the flowers but hinder their escape until post-pollination when flowers modify their inner surface (Oelschlägel *et al*., 2009). A study conducted by Hesse *et al.* (2000) reported on trichomes that mimicked pollen and attracted pollinators by deceit.

2.5.3 Protection against water loss, ultraviolet-B, and light damage

In cases of elevated temperatures or drought, trichomes can reflect light (Abdulrahaman and Oladele, 2011). This lowers the temperature over the leaf surface, thus reducing water loss through transpiration (Abdulrahaman and Oladele, 2011). Dense trichomes, thick leaf cuticles, and surface waxes in plants (Van der Merwe *et al.,* 1994) are believed to have evolved in response to water stress (Karaboumiotis *et al.,* 1999). This progression is known to be beneficial in improving ultraviolet-B (UV) stress (Abdulrahaman and Oladele, 2011). Leaf trichomes are known to accumulate flavonoids and UV-B-absorbing phenolic compounds (Karaboumiotis *et al*., 1993[a]; Karaboumiotis *et al.,* 1999; Skaltsa et al., 1994; Yan *et al*., 2012).

Trichomes prominent on the leaf apex and veins are commonly seen in angiosperms (Oppenheimer, 1959). This adaptation may limit incoming UV light and hence protect vascular tissue (Abdulrahaman and Oladele, 2011). Several South African plants have high foliar alkaloid levels (Van de Watt and Pretorius, 2001), which contain UV-B screening properties. This development can protect the plant against UV-B radiation (Yan *et al*., 2012) and limit photosynthetic damage to the leaf (Yan *et al*., 2012). Research indicated that UV-B radiation can produce an increase in trichome density in *Arabidopsis thaliana* and *Nicotiana tabacum* (Abdulrahaman and Oladele, 2011). This is important in the protection of the underlying plant tissues against the damaging effects of UV-B radiation (Barnes *et al*., 1996; Yan *et al*., 2012).

2.6 Ethnomedicinal uses of *Mangifera indica*

According to this system of traditional medicine, different parts of the *Mangifera indica* tree have varied medicinal properties, both as food and as medicine (Barnes et al., 1996; Yan *et al*., 2012). The plant is an antidiuretic, antidiarrheal, antiemetic, and cardiac herb (Bompard, 1992; Kostermans, 1993; Yan *et al*., 2012; Bhatt *et al*., 2010; Abdulrahaman and Oladele, 2011). Various parts of *Mangifera indica* (bark, leaves, roots, fruits, and flowers) have been used in traditional medicine for the treatment of various diseases and conditions (Bompard, 1992; Kostermans, 1993). Ethnomedicinal uses of various parts of *Mangifera indica* in different countries of the world have been summarised in Table 2.2.

Table 2.2: Ethnomedicinal use of different parts of *Mangifera indica* in the world.

Country	Part(s) used	Ethnomedicinal use	References
Bangladesh	Bark	Diarrhoea, gastric disorders, asthma, mouth sores, liver diseases, urinary tract infections, diabetes, rheumatism, leukorrhea, bleeding haemorrhoids, lung haemorrhage, nerve disorders, syphilis, cough, and jaundice. Resins of the *Mangifera indica* bark have been used for the treatment of cracked skin and feet	Mukherjee (1953); Tirtha, (2007); Khare (2008); Parvez (2016); Ediriweera *et al.,* (2017); Zapaterio (2021).
	Seeds, fruit, and kernel	Urethrorrhagia, vaginopathy, dysentery, diarrhoea, ophthalmia, and haemorrhage in lungs, uterus, and intestine	
	Roots	Ulcers, syphilis, and leukorrhea.	

	Flowers	Ulcers, diarrhoea, haemorrhage, anaemia, dyspepsia, and dysentery, Haemorrhages, diarrhoea, ulcers, dysentery, cough, gall bladder and kidney diseases, wounds, throat diseases, and hiccups.	
	leaves	Burns, scalds, and diabetes.	
Benin	Bark	Hypotension and anaemia	Wauthoz, *et al*., (2007); Parvez (2016); Ediriweera *et al.,* (2017).
Brazil	Bark	Scabies/itch	
Canary Islands	Bark	Diarrhoea	
Cuba	Bark	Mouth sores, tooth pain, cancer, diabetes, asthma, gastric disorders, and lupus	
Fiji	Bark	Syphilis	
Ghana	Bark	Hypertension and diabetes	Mukherjee (1953); Tirtha, (2007); Khare (2008); Wauthoz *et al.*,

			(2007); Bekoe *et al.,* (2017); Ediriweera *et al.,* (2017).
Guyana	Bark	Gastric disorders and diarrhoea	Wauthoz *et al*., (2007); Parvez (2016).
Haiti	Bark	Hepatic disorders.	
India	Bark	Diabetes, gastric disorders, asthma, mouth sores, leukorrhea, bleeding haemorrhoids, lung haemorrhage, nerve disorders, syphilis, cough, and jaundice.	Mukherjee (1953); Tirtha, (2007); Khare (2008); Agarwal *et al.,* (2008); Khandare (2016).
	Seeds, fruit, and kernel	Ophthalmia, haemorrhage in lungs, uterus, and intestine, urethrorrhagia, vaginopathy, dysentery, and diarrhoea.	
	Roots	Ulcers, syphilis, and leukorrhea.	
	Flowers	Ulcers, diarrhoea, haemorrhage, anemia, dyspepsia, and dysentery Diarrhoea, ulcers, diabetes, dysentery, cough, gall bladder, and kidney diseases.	
	Leaves	Haemorrhages, wounds, diseases in throat and hiccups, burns, and scalds.	

Madagascar	Bark	Liver obstruction	Mukherjee (1953); Tirtha, (2007); Khare (2008); Wauthoz, *et al.*, (2007); Zapaterio (2021).
Mali	Bark	For vomiting	
Nicaragua	Bark	Wounds	
Nigeria	Leaves	Leaf decoctions have been commonly used to treat diabetes and malaria	Mukherjee (1953); Tirtha, (2007); Khare (2008); Wauthoz *et al.*, (2007); Etuk *et al.,* (2010); Ene *et al*., (2012); Jahurul *et al.,* (2015); Omar (2015); Zapaterio (2021).
Pakistan	Bark	Asthma, bronchitis, cough, and throat problems	Memon *et al.,* (2008); Nisar *et al*., (2011); Zapaterio (2021).
	Leaves and seeds	Earache and vomiting	
Peru	leaves	Bronchitis, colds, and inflammation	Wauthoz *et al.,* (2007); Nisar *et al*., (2011); kumar et al., (2021).
Senegal	Leaves	Mouth sores, toothache, dysentery, and diarrhoea	Jayaweera (1980); Bussmann *et al*., (2006); Wauthoz *et al*., (2007);
Sri Lanka	Bark	Menorrhagia, leucorrhea, piles, and hemorrhages of the lungs and intestine	

			Juhaimi *et al.,* (2015); Lauricella *et al.,* (2017); Zapaterio (2021).
	Leaves	Diseases of the lungs, coughs, and asthma	
	Flowers	Diarrhoea, dysentery, and gleet	
Tanzania	Bark	Toothache	Wauthoz *et al*., (2007); Nisar *et al*., (2011); Majumder *et al.,* (2020); Zapaterio (2021).
Tonga	Bark	Dysmenorrhoea	

2.7 Phytochemical profile

Generally, *Mangifera indica* leaves that are burnt or discarded are considered as agricultural crop waste (Akbar, *et al.* 1991). However, the medicinal properties of *Mangifera indica* leaves make them a useful ingredient in traditional folk tea preparation (Shah *et al.*, 2010; Zhang *et al*., 2019). As described in previous studies by Akbar *et al.* (1991); Alberts *et al*. (2002); Fafiolu *et al*. (2006); Dayal *et al.* (2016) and Laulloo *et al*. (2018); *Mangifera indica* leaves contain a good quality of bioactive polysaccharides, proteins, lipids, vitamins, and minerals (Zhang *et al*., 2019). Bioactive phytochemicals present in *Mangifera indica* leaves extracts exhibit high pharmacological and biological activities viz. antiparasitic, anti-inflamatory, anti-tumour, anti-oxidant, antidiarrheal, antimicrobial, antifungal, anti-obesity, antidiabetic, antiviral, immunomodulatory, antiallergic, and antipyretic activities (Dayal *et al.,* 2016; Batool *et al*., 2018; Laulloo *et al*., 2018). Phytochemicals present in *Mangifera indica* leaves can be broadly categorised as polyphenols, terpenoids, carbohydrates, sterols, carotenoids, vitamins, fatty acids, and amino acids (Dayal *et al.,* 2016; Batool *et al*., 2018; Laulloo *et al*., 2018). Amongst the phytochemicals present in *Mangifera indica* leaves, the total phenolic compounds (TPC), including phenolic acids, tannins, benzophenones, xanthones, flavonoids, and terpenoids, are most abundant (Batool *et al*., 2018; Mirza *et al*., 2013). Studies by Mirza *et al*. (2013); Rasouli *et al*. (2017); and Batool *et al*. (2018) proved *Mangifera indica* activities of TPC against chronic diseases viz. diabetes, cancer, and neurodegenerative and cardiovascular diseases. A study by Swaroop *et al.* (2019) reported the total phenolic compounds and total flavonoid content in crude, MeOH, and EtOAc extracts of *Mangifera indica* leaves. The phytochemical investigation of MeOH, EtOAc crude extracts of *Mangifera indica* leaves using ultra-high-pressure liquid chromatography (UPLC)-MS/MS that identified several secondary metabolites, including ten benzophenones, eleven phenols, seven terpenoids, nine flavanols, four xanthones, and four derivatives of gallotannins. The EtOAc extract showed higher TPC, and total flavonoids (TFC) compared to the MeOH extract. Ouf *et al*. (2020) identified 83 compounds in *Mangifera indica* leaf essential oils of five cultivars using gas chromatography-mass spectrometry (GC–MS). Among these compounds, α-selinene (4.33–16.92%), trans-caryophyllene (8.06–18.88%), and α-humulene (8.48–25.98%) were found in the higher concentrations (Mirza *et al*., 2013; Batool *et al*., 2018; Ouf *et al*., 2020). Gu *et al.* (2019) isolated and characterised four benzophenone derivatives, manindicin A, manindicin B, norathyriol, and mangiferin from *Mangifera indica* leaf extract by nuclear magnetic resonance (NMR) spectroscopic technique (Mirza *et al*., 2013; Batool *et al*., 2018; Gu *et al.,* 2019). Some of these compounds exhibited significant anti-oxidant, immunosuppressive and α-glucosidase inhibitory activities (Mirza *et al*., 2013; Batool *et al*., 2018; Gu *et al.,* 2019). These studies suggests that *Mangifera indica* leaves can be served as a potential source of food supplement for improving human health. A list of various phytochemicals present in *Mangifera indica* leaves is depicted in Table 2.3, and structures are presented in Figure 2.6.

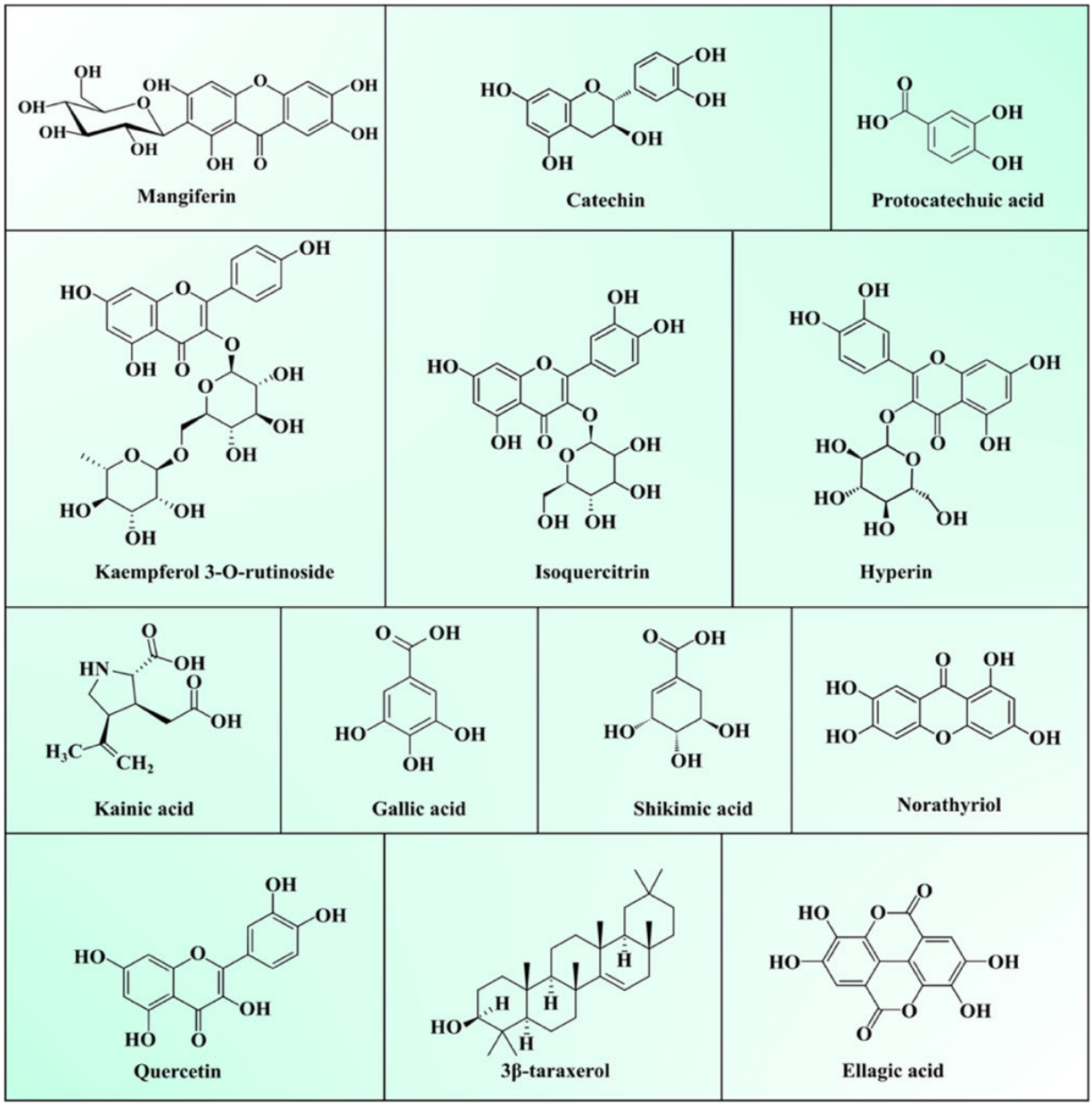

Figure 2.6: Structure of major compounds present in *Mangifera indica* L. leaves. (Adapted from Kumar *et al*., 2020)

Table 2.3: Phytochemical profile and Biological activities of the *Mangifera indica* leaves.

Variety	Type of extract	Bioactive compounds identified	References
Mangifera indica leaves	Crude, Methanol, Hexane, Ethyl acetate	Phenolic compounds (gallic acid; derivative of gallic acid; sodium gallate; ellagic acid; protacatechuic acid; methyl gallate; theogallin; derivative of theogallin; tetrahydroxy sodium benzoate), xanthones (mangiferin; isomangiferin; mangiferin-6′-O-gallate; mangiferin 3-methyl ether), Flavonols (kaemferol; quercetin; quercetin 3-O-glucoside; quercetin pentoside; quercetin carboxylic acid; epicatechin gallate hexamalonate; quercetin 3-O-rhamnoside; rhamnetin; rhamnetin hexoside), Benzophenones [3-glucosylmaclurin; maclurin 3-C-β-D-glucoside, maclurin di-O-galloylglucoside, maclurin 3-C-(6′-O-phydroxybenzoyl)β-D-glucoside, maclurin mono-O-galloylglucoside, maclurin, iriflophenone tri-O-galloylglucoside; riflophenone 3-C-β-D-glucopyranoside; maclurin 3-C-(6”-O-p-hydroxybenzoyl)β-D-glucoside; iriflophenone-di-O-galloyl glucoside;	Litz (2009); Kole (2011); Kalita (2014); Laulloo *et al*., (2018); Heinrich *et al.,* (2020).

		iriflophenone glucoside derivative], Terpenoids (3,27-dihydroxycycloart-24-en-26-oic acid; 3β-cycloartane-3,29-diol; cycloartane-3,24,25-triol; mangiferonic acid; lupeol; cycloart-25-ene-3,24,27-triol; manglanostenoic acid), Gallotannins (digalloyl glucoside;tri-O-galloyl glucoside; tetra-O-galloyl glucoside; pentagalloyl glucose), Other compound (ferulic acid hexoside)	
	70% ethanol extract	Gallic acid; quercetin; protocatechuic acid; mangiferin; isovitexin; vitexin; Iriflophene; isoswertisin; taxifolin; amentoflavone; hypericin; 2,4,4′,6-tetrahydroxybenzophenone-3-β-D-glucoside; gvajaverin; 4′,6-dihydroxy-4-methoxybenzophenone-2-O-β-D-glucoside; 2,4′,6-trihydroxy-4- methoxybenzophenone-3-C-β-D-glucopyranoside; hyperoside; 2,4,4′ ,6-tetrahydroxybenzophenone-3-C-(2-O-p-hydroxybenzoyl-p-hydroxybenzoyl)- β-D-glucoside; methyl-2-O-β-D-glucopyranosylbenzoate; foliamangiferoside A1; soquercitrin; 4′,6-dihydroxy-4-methoxybenzophenone-2-O-(2”),3-C-(1”)-1”-	Oluwafemi (2015); Pan *et al*., (2018); Heinrich *et al*., (2020)

		desoxy- β-fructopyranoside; quercitrin; quercetin-3-O-β-D-xylopyranoside; quercetin-4′-O-β-D-glucoside; 3′,5′-dimethoxy-4′,5,7-trihydroxyflavone; 4′-O-p-hydroxybenzoylmangiferin; 2,4′,6-trihydroxy-4-methoxybenzophenone-3-C- (2-O-p-hydroxybenzoyl-p-hydroxybenzoyl)-α-D-galactoside; 4,4′ ,6-trihydroxybenzophenone-2-O-(2”),3-C-(1”)-1”-desoxy-β-fructofuranoside; luteolin-7-O-β-D-glucoside; 4,4′ ,6-trihydroxybenzophenone-2-O-(2”),3-C-(1”)-1”-desoxy-β-fructopyranoside; 4′,6-dihydroxy-4-methoxybenzophenone-2-O-(2”),3-C-(1”)-1”-desoxy-β- fructopyranoside	
	Aqueous extract	Acarbose; manindicin A; manindicins B; mangiferin; norathyriol	Kole (2011); Kalita (2014); Ouf *et al*., (2020); Heinrich *et al.,* (2020).

	Aqueous extract	cetaldehyde; 2-hydroxyacetophenone; 2-furanmethanol; furfural; phenol; 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one; oleic acid; o-catechol; hydroquinone; pyrogallol	Ronchi *et al*., (2015); Martínez-Bernett *et al*., (2016); Gu *et al*., (2019).

2.7.1 Uses of *Mangiferin*

In recent years, diets enriched with bioactive compounds have garnered much attention due to their potential to minimize the risks of several chronic diseases' development (Batool *et al*., 2018). Pan *et al*. (2018) stated mangiferin (7.43%) is a major constituent in *Mangifera indica* leaf extract, whereas other compounds that were reported to be of high concentration include quercetin-3- *O*-β-D-galactoside (0.86%), isoswertisin (1.25%), and quercetin-3-*O*-β-Dglucoside (0.82%).

Mangiferin is a polyphenolic anti-oxidant and a glucosyl xanthone, which suggests it has strong anti-oxidant, anti-lipid peroxidation, immunomodulatory, cardiotonic, hypotensive, wound-healing, antidegenerative, and antidiabetic activities (Litz, 2009; Shah, *et al.*, 2010; Kole, 2011; Kalita, 2014). Various parts of *Mangifera indica* are used as a dentifrice, antiseptic, astringent, diaphoretic, stomachic, vermifuge, tonic, laxative, diuretic and to treat diarrhea, dysentery, anemia, asthma, bronchitis, cough, hypertension, insomnia, rheumatism, toothache, leucorrhea, haemorrhage, and haemorrhoids (Litz, 2009; Shah, *et al*., 2010; Kole, 2011; Kalita, 2014). According to Shah *et al.*, (2010), all parts of *Mangifera indica* are used to treat abscesses, broken horn, rabid dog or jackal bite, tumours, snakebite, stings, datura poisoning, heatstroke, anthrax, blisters, wounds in the mouth, tympanitis, colic, diarrhea, glossitis, indigestion, bacillosis, bloody dysentery, liver disorders, excessive urination, tetanus, and asthma (Litz, 2009; Shah, *et al*., 2010; Kole, 2011; Kalita, 2014).

According to Musud, (2016), fumes from the burning of *Mangifera indica* leaves can be inhaled for relief from hiccups and throat infections. The bark is also an astringent, it is used in diphtheria and rheumatism cases, and it is believed to show a tonic action on the mucous membrane (Litz, 2009; Kole, 2011). *Mangifera indica* gum is used in dressings for cracked feet and scabies and is also considered antisyphilitic (Litz, 2009; Kole, 2011). According to Wu (1979); Litz (2009); Orwa (2009), and Kole (2011) most parts of *Mangifera indica* are used medicinally.

2.8 Biological and Pharmacological Activities of the *Mangifera indica* Leaves Extracts

2.8.1 Anti-oxidant Activities

Many studies recently, have shown that free radicals generated during the metabolic process that contribute to many degenerative diseases such as neurological disorders, acquired immunodeficiency syndrome, ischemic diseases, and many more (Barreto *et al.,* 2008). Anti-oxidant substances, provide a high level of anti-oxidant activity to lessen the adverse effects of free radical (Kumar *et al*., 2020). *Mangifera indica* leaves were reported to have free radical anti-oxidant capacity due to the presence of phenolics and flavonoids in different studies (Kumar *et al*., 2020). High-performance liquid chromatography coupled to electrospray ionisation and quadrupole time-of-flight mass spectrometry (HPLC-ESI-qTOF-MS/MS) analysis of *Mangifera indica* leaves extract had identified neomangiferin, mangiferin, kaempferol-3-O-rutinoside, isoquercitrin, and quercetin as the main compounds and reported that these compounds contributed directly to the anti-oxidant activity of *Mangifera indica* leaves (Wu *et al*., 2020). The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and superoxide dismutase (SOD)-like activity had shown that *Mangifera indica* leaves serve as a moderate anti-oxidant with an IC_{50} value of ~9 and 117 µg/mL (Itoh *et al.*, 2020). In another analysis, *Mangifera indica* leaves methanol extract provided radical scavenging activity with an IC_{50} value of 13.37 µg/mL (Mohan *et al.,* 2013). Fraction analysis of *Mangifera indica* leaf extract with ethylacetate, hexane, and *n*-butanol demonstrated that the ethylacetate fraction had the highest anti-oxidant capacity of 1226 and 2817.99 mg trilox/g, which was estimated by using 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) and DPPH assay, respectively, and reducing the power of 10172.59 µmol $FeSO_4.7H_2O$/g extracts analysed through ferric reducing anti-oxidant power (FRAP) assay (Kitbumrungsart *et al*., 2011). Similarly, subcritical water extracts of *Mangifera indica* leaves had anti-oxidant activity index (AAI) values of 7.92 ± 0.16 and demonstrated superior activity to (+)-α-tocopherol (AAI = 3.65 ± 0.07) (Itoh *et al.*, 2020). Trolox equivalent anti-oxidant ability (TEAC) study of *Mangifera indica* leaf extracts was observed to be 2.13 and 2.59 mmol TE/g DW, for mangiferin pentoside and benzophenones, respectively (Prommajak *et al*., 2014). The efficacy of the *Mangifera indica* leaves was also studied in the chitosan-based films, and it was found that the anti-oxidant capacity of the *Mangifera indica* leaf supplemented chitosan films improved in a dose-dependent manner (Rambabu *et al*., 2019). The anti-oxidant activity of hydroalcoholic *Mangifera indica* leaf extract fermented with either *Lactobacillus casei* or effective microorganisms had higher anti-oxidant activity (Rambabu *et al*., 2019). They observed that the quantity of lipopolysaccharide-generated reactive oxygen species was reduced by the

fermented extracts of *Mangifera indica* (Park *et al*., 2015). In summary, many interesting results indicated the potential of *Mangifera indica* extract as an anti-oxidant with wider applicability in food, food packaging, and many more industries (Neuana *et al.,* 2020).

2.8.2 Antimicrobial activities

There is great interest in understanding the bioactive compounds present in nature (Dzotam, *et al*., 2017). Some medicinal plants with antimicrobial properties can evade the activity of multi-drug resistant (MDR) microbes, which helps in withstanding antimicrobial resistance (Dzotam, *et al*., 2017). Distinct morphological parts of the *Mangifera indica* plant, such as the stems, kernel, leaves, bark and seeds have shown antimicrobial activities against microbes like *Bacillus subtilis, Staphylococcus* sp., *Escherichia coli, Proteus vulgaris, Candida albicans, Shigella flexneri, Pseudomonas fluorescens, Salmonella typhi, Klebsiella pneumoniae* (Ouf *et al*., 2020). *Mangifera indica* leaf extract is the most studied part for antibacterial effects. Dzotam, *et al*. (2017) observed that hexane and hexane/ethyl acetate extracts of *Mangifera indica* leaves exhibited favourable antibacterial effects against *Enterobacter aerogene, and Mycobacterium tuberculosis*. Ouf *et al*., (2020), showed that the essential oils from the leaves of the five *Mangifera indica* cultivars obtained from Egypt, exhibited antibacterial activity against, *Pseudomonas aeruginosa* (Minimum Inhibitory Concentration (MIC): 500 µg/mL), *Staphylococcus* sp. (MIC): 62.5 µg/mL), *Escherichia coli* (MIC: 125 µg/mL), *Salmonella typhi* (MIC: 1000 µg/mL, *Aspergillus flavus* (MIC: 1000 µg/mL), and *Bacillus subtilis* (MIC: 125 µg/mL). The major phytochemicals responsible for the antimicrobial activity in *Mangifera indica* leaves include phenolics, alkaloids, saponins, glycosides, terpenes, and tannins. The concentration of the mentioned compounds was measured as follows: flavonoid content was the highest at 11.25 mg/100 g; tannins content was at 0.46 mg/100 g, phenolic content was 0.08 mg/100 g; and saponins conent was 3.23 mg/100 (Okwu *et al*., 2008). Polyphenols and phenolic acids present in *Mangifera indica* leaf extract include quercetin, gallic acid, protocatechuic acid, hyperin, , kaninic acid, ellagic acid, catechin, ethyl digallate, and shikimic acid, which can inhibit the growth of pathogens (Islam *et al*., 2010). The mechanism of exertion of antimicrobial activity by these compounds involves the depletion of intracellular ATP levels, cytoplasm leakage, depolarisation of plasma membrane, declining the concentration of microbial protein, and damaging genetic material (Guo *et al*., 2020). Additionally, an adequate level of antibacterial activity of leaf extract was found against Gram-positive bacteria, while no activity against Gram-negative bacteria was observed (Islam *et al*., 2010). According to Islam *et al.* (2010), no inhibition was observed against

Gram-negative bacteria *Salmonella* spp. However, inhibition was observed against Gram-positive bacteria *Bacillus* spp. and *Staphylococcus* spp. with zone of inhibition range of 7.0-11.5 mm. Additionally, Mangiferin, a xanthone C-glycosyl compound extracted from *Mangifera indica* leaf extract, has also been shown to possess strong iron-chelating activity that favours antimicrobial activity (Guo *et al*., 2020). They also observed that fungal attack on this species also initiates the biosynthesis of these five flavonoid compounds including (5-hydroxy-3-(4-hydroxyl phenyl) pyrano chromene-4 (8H)-one, epicatechin(2-(3,4dihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol,epicatechin-3-*O*-β glucopyranoside, 6-(phydroxybenzyl) taxifolin-7-*O*-β-D-glucoside, and quercetin-3-O-α-glucopyranosyl-(1-2)-β-D-glucopyranoside, and at 1000 ppm the growth of *Aspergillus* and *Alternaria* fungus was reduced. Konishi, *et al.* (1993) also stated that gallotannins have antimicricbial properties which is associated to their abaiity for chelation of metal irons, which prevent enxymatic pothengenic reaction and lip-bilayer membrane disintergration. A myriad of terpenes identified from *Mangifera indica* leaves extracts including friedelin, α-pinene, γ–terpinene, taraxerol, β-pinene, γ–cadinene, δ-elemene, , β-elemene, camphene, lupeol, α-guaiene, linalool, α-farnesene, humulene, myrcene, limonene, α-terpinolene, and β-ocimene exhibit bactericidal and bacteriostatic effects against different pathogens (Kanwal *et al*., 2010; Ediriweera *et al.*, 2017; Guo *et al*., 2020).

The mentioned phytocompounds of the leaf extracts of *Mangifera indica* indicates the metabolites can be used not only for antimicrobial and anti-oxidant applications but can also be used to improve the shelf life of food when used as additives. This is due to their broad pharmacological and biological activities (Karthiken *et al*., 2020).

2.9 Conclusion and future perspectives

This review describes a comprehensive account of general taxonomy, the phytochemical constituent's, ethnopharmacology, biological activities, and morphology of plants belonging to the genus *Mangifera*. There are several bioactive compounds isolated from *Mangifera indica*, such as iridoids, phenolics, flavonoids, terpenoids, phytosterols, phenylethanoid glycosides, and aromatic compounds, possess various biological properties of medicinal importance. Moreover, these bioactive compounds have demonstrated several biological activities, including anti-oxidant, antibacterial, antifungal, anti-inflammatory, anticancer, antidiabetic, antiulcer, hepatoprotective, analgesic, antiamoebic, antihelmintic, antiarthritic, antihypertensive, antiviral, and acetylcholinesterase activity inhibition. Further investigations are recommended to explore more about the species within *Mangifera* to identify new therapeutic compounds or drug leads,

as most of them have not yet been subjected to chemical and biological assessment. Therefore, further research on the bioactive compounds and pharmacological activities of plants within this genus will provide a basic understanding of the importance of these species as medicinal plants and a potential source of novel and useful drugs.

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CHAPTER 3

Macromorphological and Micromorphological seasonal differences studies of local *Mangifera indica* L. leaves

Arvish Maharaj[a], Yougasphree Naidoo[a], Yaser Hassan Dewir[b,c]

[a]School of Life Sciences, University of KwaZulu-Natal,Westville Campus, Private Bag X5400, Durban 4001, South Africa

[b]Plant Production Department, P.O. Box 2460, College of Food and Agriculture Sciences, King Saud University, Riyadh 11451, Saudi Arabia

[c]Kafrelsheikh University, Faculty of Agriculture, Kafr El-Sheikh 33516, Egypt

Abstract

Mangifera indica (Anacardiaceae), is a species known as a valuable medicinal plant with a broad spectrum of antibacterial and anti-inflammatory activities. Herbal uses of this plant have not been fully documented; however, several *Mangifera indica* species are used in traditional medicine. This study aimed at characterising the morphology, histo-phytochemical screening of *Mangifera indica* leaves using various microscopy and standard staining techniques. Foliar biology of the plant was conducted using stereomicroscopy, Scanning electron microscopy (SEM) and Transmission electron microscopy (TEM) techniques. ImageJ was used to measure the length and diameter of the different trichome types. Morphological observations using stereo- and scanning electron microscopy (SEM) revealed the presence of non-glandular trichomes with cuticular warts and glandular peltate trichomes on the leaves of *Mangifera indica*. The Transmission electron micrographs showed that numerous mitochondria, starch grains, plastoglobuli, and plastids were present. Histo-phytochemical leaf sections indicated the presence of alkaloids and phenolics, which are major medicinal compounds. The results for summer and winter leaves resembled somewhat similar-to-identical morphological characteristics on all fronts; this indicates that the leaves of *Mangifera indica* can be harvested throughout the year for the qualitative extraction of useful compounds. This study aims to investigate South African *Mangifera indica* L. by describing key micromorphological features of the foliar structures of *Mangifera indica* L.

Keywords: Mangifera indica; Anacardiaceae*;* Bioactive compound*;* Morphology; Trichomes

3.1 Introduction

The Anacardiaceae family has approximately 83 genera and 873 species, present mainly in tropical and subtropical regions around the world (Herrera *et al*., 2018). It is a family intensively studied, due to its economic importance in countries that export the fruits, such as mango, cashew nut, pistachio, ambarella, yellow mombin, and red mombin (Lorenzi *et al*., 2015; Coelho *et al*., 2019).

Mangifera indica L., popularly known as mango, is a perennial tree, 8-18 m in height, originating in India and Myanmar (Lorenzi *et al*., 2015). Different parts of the plant, such as root, bark, leaves, flowers, fruits, and seeds, are usually used for the treatment of diseases, such as diabetes, anaemia, diarrhoea, haemorrhoids, indigestion, asthma, bronchitis, and influenza (Shah *et al.,* 2010; Santos *et al.,* 2012; Ghuniyal, 2015; Jahurul *et al.,* 2015; Parvez, 2016;Ribeiro *et al.,* 2017; Ediriweera *et al.,* 2017; Lauricella *et al.,* 2017).

Even though many studies do exist when investigating *Mangifera indica*, it is important to note that to our knowledge, all the studies were investigated outside of South Africa (Shah *et al.,* 2010; Ghuniyal, 2015; Jahurul *et al.,* 2015; Parvez, 2016; Santos; Ribeiro *et al.,* 2017; Ediriweera *et al.,* 2017; Lauricella *et al.,* 2017). Since South Africa has rich biodiversity (Bellocchi *et al*., 2021), it would be highly beneficial to add local *Mangifera indica*, a highly medicinal plant, to the South African ethnobotanical knowledge. The present study aims to add to the South African ethnobotany by considering the seasonal changes of morphological characteristics through macro- and microscopic evaluation and ascertaining the location and presence of metabolites (bioactive compounds) in the leaves of *Mangifera indica* L.

The are many studies on the presence of bioactive compounds in *Mangifera indica* suggesting the presence of several metabolites with proven pharmacological activities, such as antidiabetic, anticancer, analgesic, antipyretic, anti-inflammatory, anti-ulcer, and antibacterial (Shah *et al.,* 2010; Ghuniyal, 2015; Jahurul *et al.,* 2015; Parvez, 2016; Ribeiro *et al.,* 2017; Ediriweera *et al.,* 2017; Lauricella *et al.,* 2017). This study will illustrate the differences in the presence and location of these bioactive compounds through visual histo-phytochemical staining's, *i.e.,* to decipher any differences in the distributions of phytocompounds in the summer and winter leaves.

3.3.1 Seasonal change

Seasons were introduced to this study to factor in that global climate change since it may lead to elevated temperatures (Intergovernmental Panel on Climate Change, 2021). High temperatures

may result in heat stress, which not only affects plant morphology and causes leaf etiolation and wilting but also alters the anatomy, physiology, photosynthetic capability, and genetic expression of plants (Chen *et al.*, 2014). Furthermore, heat stress also changes primary and secondary plant metabolism (Macedo, 2012). Among the deleterious, the overgeneration and reactions of reactive oxygen species (ROS), such as singlet oxygen ($^1O_2$), superoxide radicals ($\bullet O^-_2$), hydrogen peroxide (H_2O_2), and hydroxyl radicals (•OH), are common under heat stress and may damage chloroplasts and cells by attacking membrane lipids, DNA, and proteins (Chen *et al*., 2014). Conversely, plants have developed different physiological mechanisms at the transcriptomic, proteomic, and metabolomic levels to counteract ROS and adjust to or avoid prevailing oxidative damage (Dobra *et al*., 2015; Waqas *et al*., 2016). The factors that lead the photosynthesis under heat stress include the structural and functional disruptions of chloroplasts, degradation, or fewer accumulations of photosynthetic pigments. Therefore, scavenging ROS, maintaining cell membrane stability, and/or enhancing photosynthesis is effective ways to harvest light and sustain normal growth (Dobra *et al*., 2015; Waqas *et al*., 2016). The accumulation of osmotic proline, total soluble sugars, and total soluble protein is helpful to protect the structure of enzymes and proteins and maintain cell membrane integrity in the way of low-molecular-weight chaperones (Huve *et al.,* 2006; Hameed *et al.,* 2012; Manaa *et al.,* 2014). In addition, plants have developed complex anti-oxidative defence systems consisting of an enzymatic system and a nonenzymatic system to counteract the injurious effects of ROS (Xu *et al*., 2006).

An overall tendency exists to survive under heat stress by reducing cell size, enlarging the xylem vessel diameter, increasing stomatal density to benefit water transport, and reducing transpiration (Banon *et al.,* 2004; Chen *et al*., 2012). High-temperature stress strongly influences cell ultrastructure, especially chloroplasts, which are often assessed for evidence of stress (Banon *et al.,* 2004; Chen *et al*., 2012). Any heat-related damage to thylakoid membranes in chloroplasts is expected to result in chlorophyll loss (Vacha *et al*., 2007).

Research on abiotic stress of *Mangifera indica* was studied earlier, which mainly focused on drought and cold stress (Anisko and Lindstrom, 1996; Lipp and Nilsen, 1997; Cordero and Nilsen, 2002). However, fewer studies about heat stress were reported (Ranney *et al*., 1995; Banon *et al.,* 2004; Chen *et al*., 2012). Heat stress studies of many other plants have recently focused on physiological effects (Gupta *et al.*, 2013). Little is known about how heat stress affects anatomical structures, such as stoma, mesophyll tissue, and epidermal cells, and ultrastructure, such as chloroplasts (Machado *et al,.* 2002; Medina *et al.,* 2002). The objective of this study was to preliminarily ascertain the differences for summer and winter *Mangifera indica* leaves through

macro- and micromorphological ultrastructural anatomy as well as ascertain the location and presence of metabolites in the *Mangifera indica* leaves.

In *Mangifera indica* plants, some authors have reported a decrease in root permeability and in-plant hydraulic conductance due to low temperatures (Syvertsen *et al,.* 1983; Moreshet and Green 1984). As a consequence of low temperature in plant water relations, the reduction of stomatal conductance of mango plants was noticed during winter (Ribeiro and Machado 2007). The reduced stomatal aperture may impair leaf photosynthesis by decreasing CO_2 availability to Rubisco (Jones 1985; Machado *et al,.* 2002; Medina *et al,.* 2002). Cool temperatures also modify the biochemical reactions underlying CO_2 fixation (Allen and Ort, 2001), with mango plants showing the reduction in Rubisco carboxylation and regeneration during winter (Ribeiro *et al.*, 2007).

Based on this supposition, this study was directed to evaluate summer and winter leaves by viewing the macro-and micro-morphology to ascertain if there are any morphological differences seasonally and detect the presence of trichomes.

3.1.2 Trichomes

Specialised hair-like epidermal cells are known as trichomes (Kariyat *et al*., 2018). Trichomes play a role in a plants defence against biotic threats such as predators acting as both a chemical mediator and physical barrier, furthermore acting as protection mechanisms from abiotic factors such as sunlight by reflecting excess radiation (Valverde *et al*., 2001; Kariyat *et al*., 2018). The location of, these structures differ with species and can be found on the leaves, stems, roots, and even seed coats (Levin, 1973b; Naidoo *et al*., 2014). Trichomes are known to be classified as either glandular or non-glandular. This classification depends on their shape and function (Choi and Kim, 2013). The absence of a glandular head in non-glandular trichomes is the most distinctive morphological difference (Werker, 2000). Although trichomes can be used for taxonomic purposes, these can be subdivided further relatively to their morphological characteristics (de Vargas *et al*., 2018).Non-glandular trichomes are considered to act exclusively as mechanical barriers, compared to glandular trichomes are responsible for the storage and/or exudation of biologically active phytocompounds (Levin, 1973a; Werker, 2000; Naidoo *et al*., 2011).

Mangifera indica trichomes have been described as non-glandular (Lizarraga *et al*., 2017) with seemingly no consensus. Based on this supposition, this study was directed to evaluate summer

and winter leaves by viewing the micro-and macro-morphology to ascertain if there are any morphological differences seasonally as well as to determine the trichome type using stereo- and scanning microscopes. Histo-phytochemical analyses were also performed to elucidate the chemical classes of phytocompounds present in *Mangifera indica* leaves for the summer and winter seasons.

3.2 Materials and Methods

3.2.1 Collection and identification of plant materials

The fresh leaves of *Mangifera indica* L. were collected from Durban, KwaZulu-Natal, South Africa (24° 49'05" S 30°56'46" E). The summer samples were collected from December 2019 to March 2020, and the winter samples were collected from June-August 2020. Professor Y. Naidoo confirmed the species identity. A voucher specimen (accession number: NU0092176) was deposited in the Ward Herbarium, School of Life Sciences (Biology), University of KwaZulu-Natal, Durban, South Africa.

3.2.2 Stereomicroscopy

Fresh leaves were examined using the Nikon AZ100 stereomicroscope (Nikon Corporation, Yokohama, Japan) equipped with a Nikon Fiber Illuminator and photographed using the NIS-Elements Software (NIS-elements D 3.00). The adaxial and abaxial surfaces of fresh leaves at the three developmental stages (emergent, young and mature) were imaged with an emphasis on surface detail.

3.2.3 Histochemistry

Hand-cut sections of the fresh *Mangifera indica* leaves were histochemically stained as described below. The stained sections were viewed and photographed with the Nikon Eclipse 80i compound light microscope equipped with the Nikon DS-Fi1 compound microscope (Nikon).

3.2.3.1 Alkaloids

Ditmars and Wagners: Sections were stained separately for 10 minutes each with both Ditmars and Wagners staining reagents, rinsed with distilled water, mounted and viewed. A deep brown-orange colour indicated a positive reaction (Furr and Mahlberg, 1981).

3.2.3.2 Cellulose

Fast green: Sections were dipped in the stain for 1 minutes and rinsed thoroughly with distilled water. A bright green colour indicated a positive reaction (cell walls) (Tos *et al*., 1980).

3.2.3.3 Lipid, lignin cutin and suberin

Sudan Black B: Sections were stained for 30 minutes, rinsed with 70% ethanol and washed in distilled water. Sections were mounted onto a slide with glycerol. A blue-black staining of tissues indicated a positive reaction (Pearse, 1985; Demarco, 2017).

Nile blue: Sections were immersed in 1% Nile Blue at 60°C for 5 minutes and then in 1% acetic acid for an additional 1 minutes. Sections were rinsed with distilled water, mounted and viewed. Acidic lipids stained blue (Cain, 1947; Demarco, 2017).

3.2.3.4 Monochromatic staining

Toluidine Blue: Sections were immersed in the stain for 1 minutes and thereafter rinsed with distilled water, mounted and viewed. A bright pink-purple colour indicated positively for carboxylated polysaccharides and polyphenols stained blue to green. Phosphate groups present on macromolecules stained purple to blue (O'Brien *et al*., 1964; Sridharan and Shankar, 2012).

3.2.3.5 Mucilages and polysaccharides

Ruthenium red: Sections were placed in 0.1% of the solution for 5 minutes, washed twice in distilled water, mounted in glycerol and viewed. A pink to red colour indicated a positive reaction (Gregory and Baas, 1989; Demarco, 2017).

3.2.3.6 Phenolic compounds

Ferric trichloride: Sections were placed in 10% Ferric trichloride stain and a drop of aqueous sodium carbonate was added to the section for 15 minutes at room temperature. Deep black deposits produced, indicated a positive reaction (Johansen, 1940).

3.2.6.7 Total proteins

Coomassie Blue: Sections were immersed in 0.25% Coomassie blue for 15 minutes and differentiated in 7% acetic acid. Sections were then rinsed briefly in distilled water and mounted in glycerol. A blue staining of tissues indicated a positive reaction (Fisher, 1968).

3.2.4 Scanning electron microscopy (SEM)

A scanning electron microscope was used to reveal the morphology and distribution of the trichomes present on the leaf surfaces (adaxial and abaxial sides).

The micromorphology of the chemically-fixed samples of both leaf surfaces for each developmental stage was examined in detail. The initial preparation step involved the primary fixation of fresh leaf sections (± 5 mm^2) in 2.5% glutaraldehyde for 18-24 h. Thereafter, the samples were rinsed thrice (for 5 minutes each) with 0.1 M sodium phosphate buffer (pH 7.2) and were subjected to post-fixation in 0.5% osmium tetroxide for 3 h at 24°C. The samples were again washed thrice (5 minutes each) using the sodium phosphate buffer and were dehydrated by exposing them to increasing concentrations of ethanol (30%, 50%, 75%, 100%) for two sessions, each of 5 minutes, followed by exposure to 100% ethanol for two sessions, each of 10 minutes. The dehydrated samples were critically point-dried using the Quorum K850 Critical Point Dryer (Quorum Technologies Ltd., Laughton, East Sussex, UK) with a vertical chamber. The samples were then mounted onto small aluminium stubs using double-sided adhesive carbon tape and sputter coated with a layer of gold using the Quorum 150 RES (Quorum Technologies Ltd.), a combined system for carbon and sputter coating. The samples were viewed and photographed using the LEO 1450 SEM at a working distance (WD) of 12-15 mm. Images were captured using the SmartSEM image software (Zeiss, Jena, Germany). (Protocol adapted from the microscopy and microanalysis unit).

3.2.4.1Freeze drying

Another set of fresh leaves from the three developmental (emergent, young and mature) of *Mangifera indica* were placed in liquid nitrogen (-196 °C), after which the sections were further frozen in an Edwards Modulyo freeze dryer (Edwards High Vacuum International Ltd., UK), at -40 to -60 °C in a vacuum of 10^{-1} Torr for 72 h. The samples were adhered to aluminium stubs with carbon conductive tape, gold sputter coated twice using a Polaron SC500 Sputter Coater (Quorum Technologies Ltd., UK) in a 0.1 Torr vacuum. The time for sputter coating and thickness of sputter coat is standardised by the Polaron SC500 Sputter Coater equipment. The prepared leaf

segments were viewed using a Zeiss Ultra-Plus FEG-Scanning electron microscope operating at 20 kV.

3.2.5 Morphometric analysis of trichomes

Ten selected images of trichomes obtained from SEM were analysed using the ImageJ software program (Schindelin *et al*., 2015). The diameter (μm) of the trichome head, length, and width of the stalk for each type was measured.

3.2.6 Transmission electron microscopy (TEM)

The ultrastructure of leaf tissue was viewed, analysed and imaged using TEM. Leaf sections from different developmental stages (±2 mm^2) were excised and primarily fixed in 2.5% glutaraldehyde for 24 h. The sections were rinsed thrice in a 0.1 M phosphate buffer (pH 7.2) and thereafter post-fixed in 0.5% osmium tetroxide for 3 h. Samples were rinsed thrice for 5 minutes; using the phosphate buffer. The samples were then dehydrated using a graded acetone series (for 5 minutes each in 30%, 50%, 75%) and two sessions of 10 minutes each in 100% acetone. After dehydration, the samples were transferred to the clearing agent, propylene oxide, for 15 minutes and then gradually infiltrated using a graded series of Spurr's low-viscosity epoxy resin in propylene oxide solution (25%, 50%, 75% and 100%) (Spurr, 1969). The samples were embedded in equal parts of Spurr's resin and acetone for 4 h and then in 100% resin for 24 h at 70°C (Spurr, 1969). Subsequently, the samples were transferred to silicon moulds and polymerised for 8 h at 70°C.

Glass knives were prepared on the LKB Knifemaker 7801A (Elekta, Stockholm, Sweden) and were used to section the resin blocks. Ultrathin resin-embedded sections were obtained using the Reichert Jung ultra- microtome (Leica, Wetzlar, Germany). The sections were surveyed in order to determine the regions of interest. They were stained with 1% Toluidine Blue, placed on slides, and viewed using the Nikon Eclipse 80i light microscope (Nikon Corporation), equipped with a Nikon DS-Fi1 camera and the NIS-Elements imaging software package. The ultrathin sections were then excised at 90–110 nm using the Reichert Jung ultra-microtome and placed on copper grids. The sections were stained with 2.5% uranyl acetate for 10 minutes at 23°C, rinsed with distilled water and then stained with lead citrate for 10 minutes. The copper grids were rinsed with distilled water, viewed, and photographed using the JEOL 1010 TEM (JEOL, Tokyo, Japan) equipped with the iTEM software. (The protocol was adapted from the microscopy and microanalysis unit).

3.3 Results and Discussion

Indian systems of medicine use the majority of the crude drugs that are of plant origin (Jagetia and Baliga, 2005; Okwu and Ezenagu, 2008; Rakholiya and Chanda, 2012; Joona *et al.,* 2013). Acoording to Samkuwar and Kamble (2013) and Nwankwo and Osaro-Mathew (2014), It is necessary to catogorize and determine the plant's identity and ascertain its quality before use. Therefore, a detailed pharmacognostic evaluation is an essential prerequisite (Somkuwar and Kamble, 2013; Nwankwo and Osaro-Mathew, 2014). According to World Health Organization (WHO) (2002), the macroscopic and microscopic description of a medicinal plant is the first step towards establishing its identity and purity and should be carried out before any tests are undertaken.

Although there are already some studies about this species (Okwu and Ezenagu, 2008; Helen *et al.,* 2013; Somkuwar and Kamble, 2013; Nwankwo and Osaro-Mathew, 2014; Dhital, 2017; Diso *et al*., 2017; Divyalashmi and Sharmili, 2017), to the best of our knowledge this is the first study to report on *Mangifera indica* in South Africa. The present study provided new anatomical valuable information for the correct identification of *Mangifera indica* as well as aims to provide insight on macro-morphology using the stereomicroscope and micro-morphology using the Scanning electron microscopy (SEM) and Transmission electron microscopy (TEM).

3.3.1. Steriomicroscopy

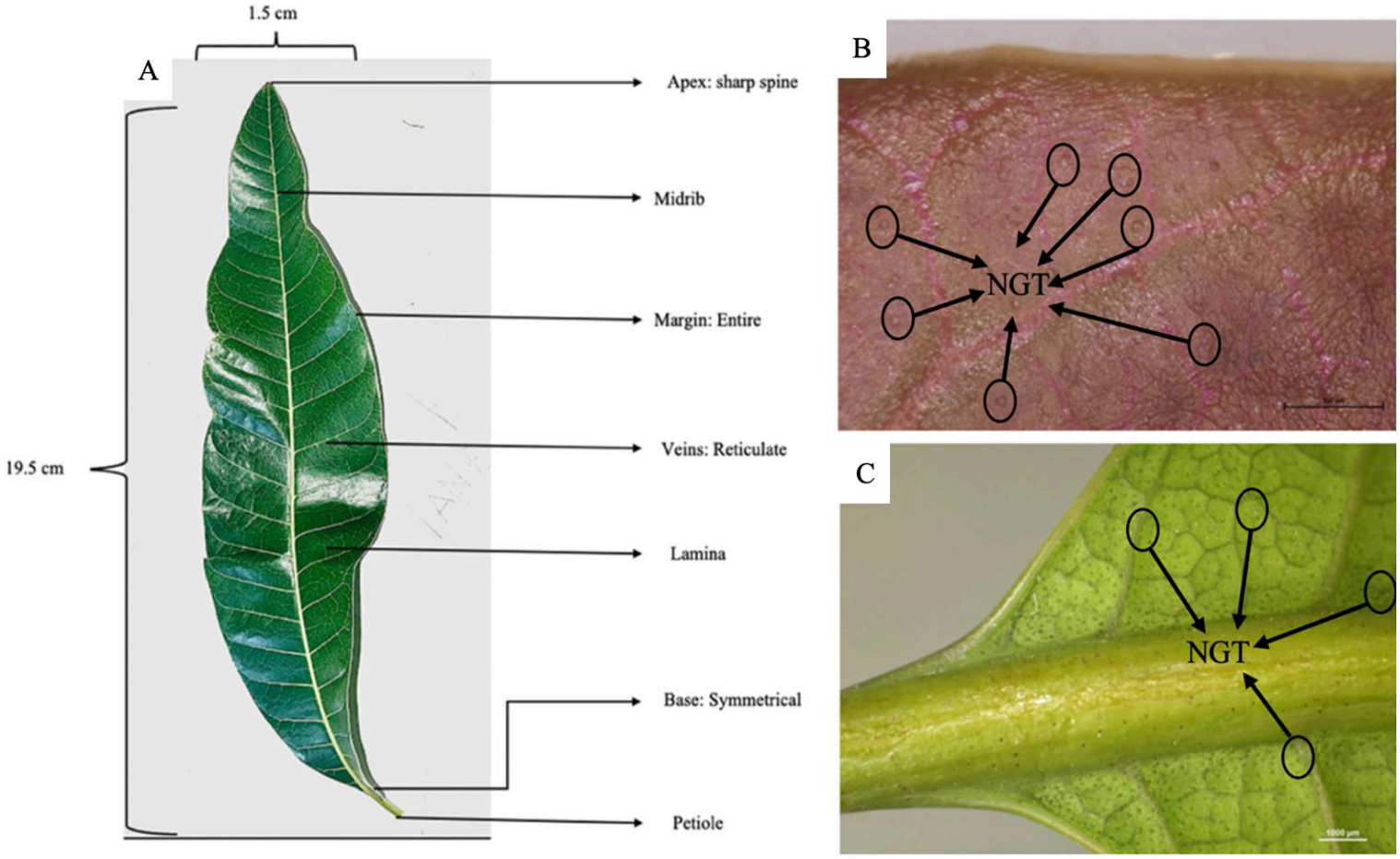

Figure 3.1: Stereomicroscopic micrographs showing characteristics of *Mangifera indica* leaf. A- Adaxial mature entire leaf; B- Adaxial emergent leaf showing non- glandular peltate glands; C- Abaxial young leaf showing non- glandular peltate glands. Abbreviations: NCT= non-glandular trichome.

Stereomicroscopy images showing the adaxial surfaces of an emergent leaf (reddish in colour) (Figure 3.1 B), a young leaf (green-yellow colour) (Figure 3.1 C), and a mature leaf that is dark green (Figure 3.1 A). The presence of non-glandular trichomes was evident on the adaxial and abaxial surfaces, with a denser cover on the abaxial leaf surface (Figure 3.1 B-C). In addition, there are considerably fewer trichomes on mature leaves in comparison to the emergent and young leaves. According to Werker (2000), this is due to the surface area increasing in mature leaves, which disperse the peltate glands.

3.3.2 Compound light microscopy

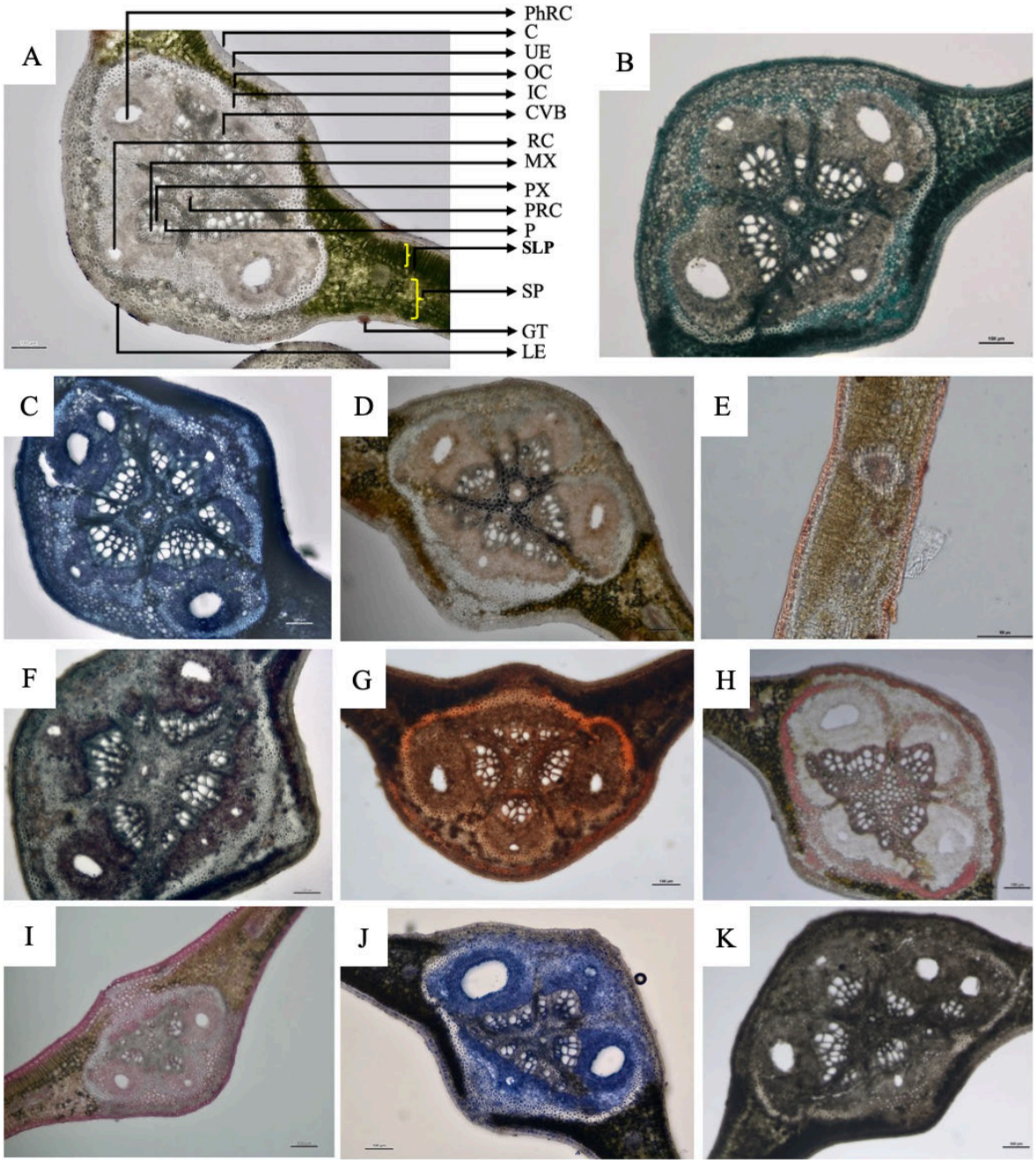

Figure 3.2A: Cross-sectional histochemical staining micrographs of *Mangifera indica* L. leaf for Summer. A- Control; B- Sudan Black; C-Toluidine blue; D- Wagners reagent; E- Sudan III and IV; F- Bromophenol blue; G- NADI; H- Phloroglucinol; I- Ruthenium Red; J- Coomassie blue; K- Ferric trichloride. Abbreviations: PhRC= Phloem resin canal; C= Cortex; UE= Upper epidermis; OC= Outer cortex; IC= Inner cortex; CVB= Central vascular bundle; RC= Resin canal; MX= Meta xylem; PX= Phloem xylem; PRC: = Pith resin canal; P= Phloem; SLP= Single layer palisade; SP= Spongy parenchyma; GT= Glandular trichome; LE= Lower epidermis.

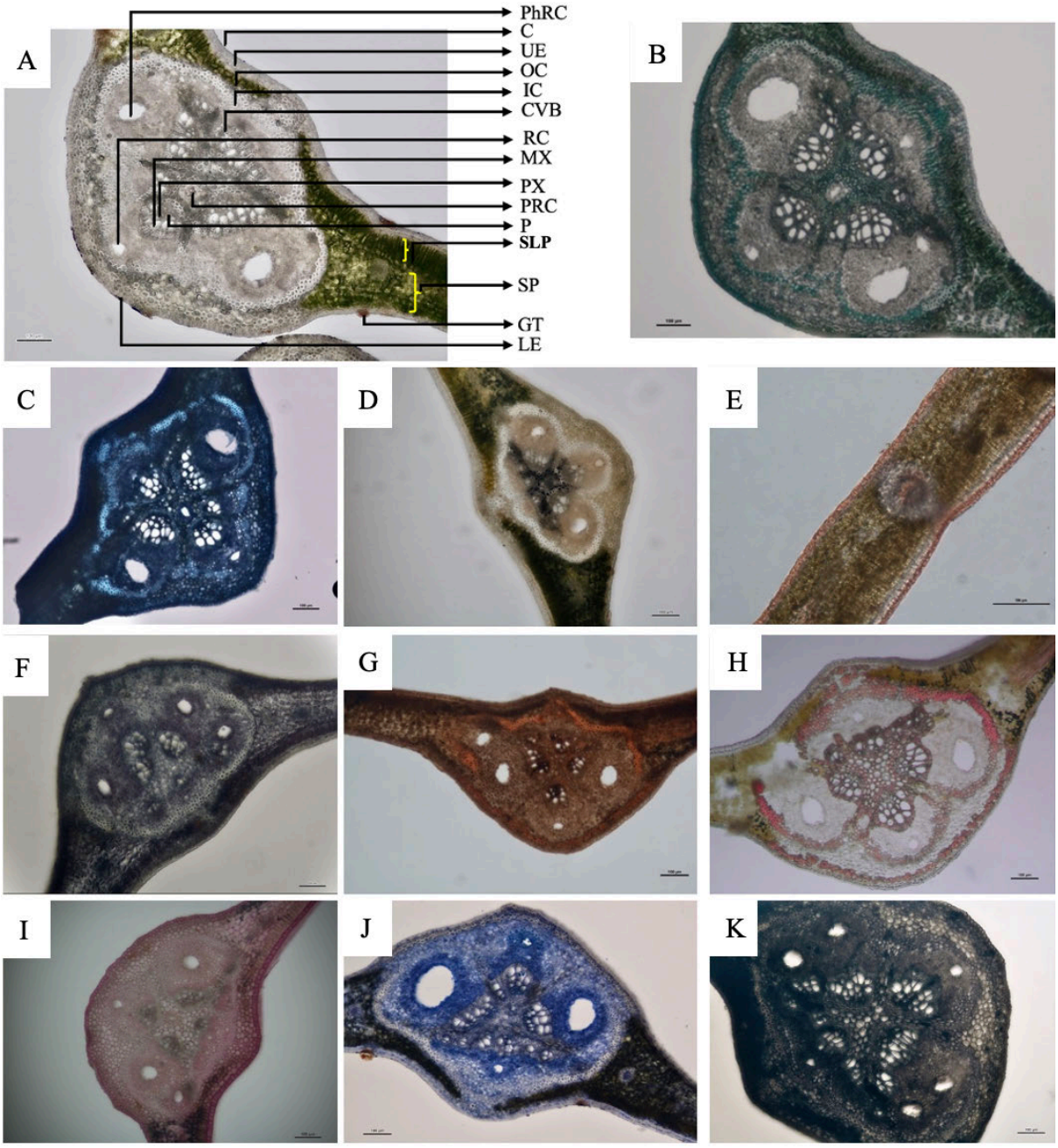

Figure 3.2B: Cross-sectional histochemical staining micrographs of *Mangifera indica* L. leaf for Winter. A- control; B-Sudan Black; C-Toluidine blue; D- Wagners reagent; E- Sudan III and IV; F- Bromophenol blue; G- NADI; H- Phloroglucinol; I- Ruthenium red; J- Coomassie blue; K- Ferric trichloride. J- Abbreviations: PhRC= Phloem resin canal; C= Cortex; UE= Upper epidermis; OC= Outer cortex; IC= Inner cortex; CVB= Central vascular bundle; RC= Resin canal; MX= Meta xylem; PX= Phloem xylem; PRC: = Pith resin canal; P= Phloem; SLP= Single layer palisade; SP= Spongy parenchyma; GT= Glandular trichome; LE= Lower epidermis.

In the transverse section of the leaf, the epidermal cells show slightly sinuous and thick walls on both adaxial and abaxial sides for summer and winter (Figure 3.3A- A and Figure 3.4B- A). The leaf blade is hypostomatic, with anomocytic stomata (Figure 3.4A- D and Figure 3.4B- D). According to Metcalfe and Chalk (1950), the family Anacardiaceae is characterised by hypostomatic or amphistomatic leaf blades. Multicellular glandular trichomes were found on both leaf surfaces (Figure 3.1A; Figure 3.4A and B). Rocha *et al*. (2015) mentioned the presence of trichomes in a study on the leaf of *Mangifera indica*. The authors also found non-glandular trichomes in the leaf of *Mangifera laurina*. The presence of non-glandular trichomes has also been described in the leaf of *Mangifera altissima* (Vasconcelos and Randau, 2016), the same trichome which is found in *Mangifera indica* (Figure 3.4A- F, F and Figure 3.4B- E, F).

The midrib, in the cross-section, is biconvex (Figure 3.1A). Rocha *et al*. (2015) found a similar shape of the midrib. The epidermis is uniseriate, covered by a thick cuticle (Figure 3.3B). The vascular bundle has a biconvex shape and is collateral, surrounded by sclerenchyma (Figure 3.2A- A and Figure 3.2B- A), corroborating with some studies that indicated the presence of a thick cuticle, uniseriate epidermis and the vascular bundle having a biconvex shape (Santhan, 2014; Rocha *et al.,* 2015). The presence of these secretory structures such as laticifers and idio-blasts is one of the family Anacardiaceae (Metcalfe and Chalk, 1950). In vegetative organs, secretory structures are found mainly in the phloem and pith (Lacchia and Carmello- Guerreiro, 2009); however, in this study, secretory structures were not indicated as no laticifers and idio-blasts were found. This observation may be due to some geographical conditions. However, further studies should be conducted with an increased sample size and have samples from different locations; this may improve may reveal the presence of these secretory structures (laticifers and idio-blasts).

The mesophyll presents an organisation of the dorsiventral type, with one-two layer of palisade parenchyma and around six-eight layers of spongy parenchyma (Figure 3.2A- A, B and Figure 3.2B- A, B). Santhan (2014) observed two-three layers of palisade parenchyma in the *Mangifera indica* species. Lignin was observed in xylem sclerenchyma (Figure 3.2A- D and Figure 3.2B- D). The tests for alkaloids, proteins, suberin, lipids, resin acids, mucilage and gums, and phenolics were all positive for the summer and winter seasons as depicted in Figures 3.2A and 3.2B, which agrees with literature (Okwu and Ezenagu, 2008; Helen *et al.,* 2013; Somkuwar and Kamble, 2013; Nwankwo and Osaro-Mathew, 2014; Dhital, 2017; Diso *et al*., 2017; Divyalashmi and Sharmili, 2017). The summer and winter leaves did not show any variation, as the histochemical tests revealed positive results for both seasons. This suggests that *Mangifera indica* L. can be used throughout the year for qualitative metabolites extraction, however further studies are necessary.

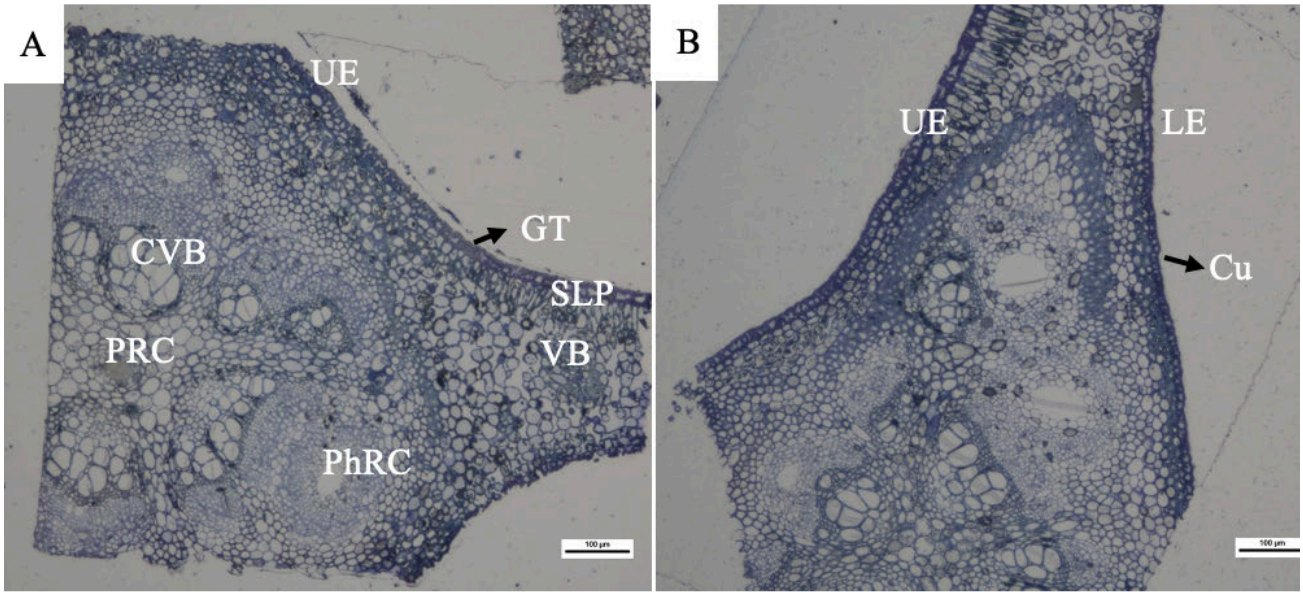

Figure 3.3 A and B: Transverse section micrographs of *Mangifera indica* L. leaves (Left: A- Summer) (Right: B- Winter). A-B Toluidine blue stain. Abbreviations: CVB= Central Vascular bundle; PRC= Pith resin canal; VB= Vascular bundle; SLP= Singly layer palisade; Cu= Cuticle; LE= Lower epidermis; UE= Upper epidermis; GT= Glandular trichome; PhRC= Phloem resin canal; S= Stomata.

The transverse section through midrib for summer and winter showed the same dorsiventral with upper palisade and lower spongy parenchyma cells (Figure 3.3- A, B) of each season. The midrib showed a centrally located vascular bundle (Figure 3.3- A, B) for summer and winter. The detailed transverse section showed upper and lower single-layered compactly arranged barrel-shaped epidermal cells with a cuticle. Some of the epidermal cells appear to be interrupted by stomatal openings (Figure 3.3- A). Mesophyll cells consisting of an upper compactly arranged group of cells without any air spaces. There are 1-2 layers of the elongated palisade and lower 5-6 layers of an oval to rounded shaped with intercellular spaces (Figure 3.3 A and B). Characteristics from the midrib transverse sections in this study are in acceptance with a previous study that coincides with this study's findings (Norfaizal and Latiff, 2013). The transverse section indicates that there is no distinct anatomical differences between the summer and winter leaves, as depicted in Figure 3.3.

3.3.3. Scanning electron microscopy

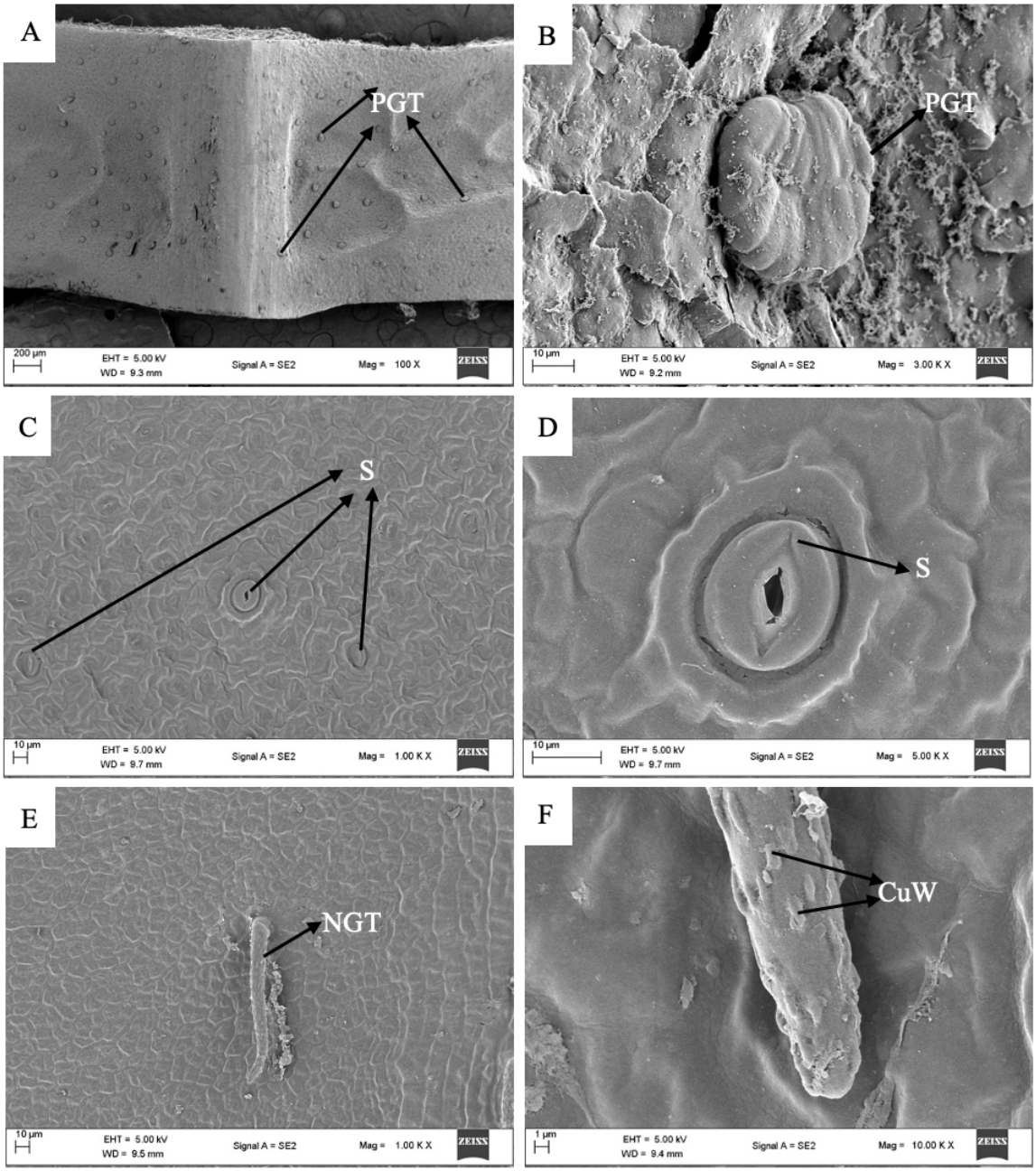

Figure 3.4A: Scanning electron micrographs of *Mangifera indica* L. leaf for Summer. A- Abaxial surface peltate gland trichome; B- Adaxial surface peltate gland trichome consisting of 2 rows of 8 oblong cells each; C and D- Abaxial surface showing anomocytic stomata; E and F- Adaxial surface of non-glandular trichome with cuticular warts. Abbreviations: PGT= Peltate gland trichome; S= Stomata; NCT= non-glandular trichome; CuW= Cuticular warts.

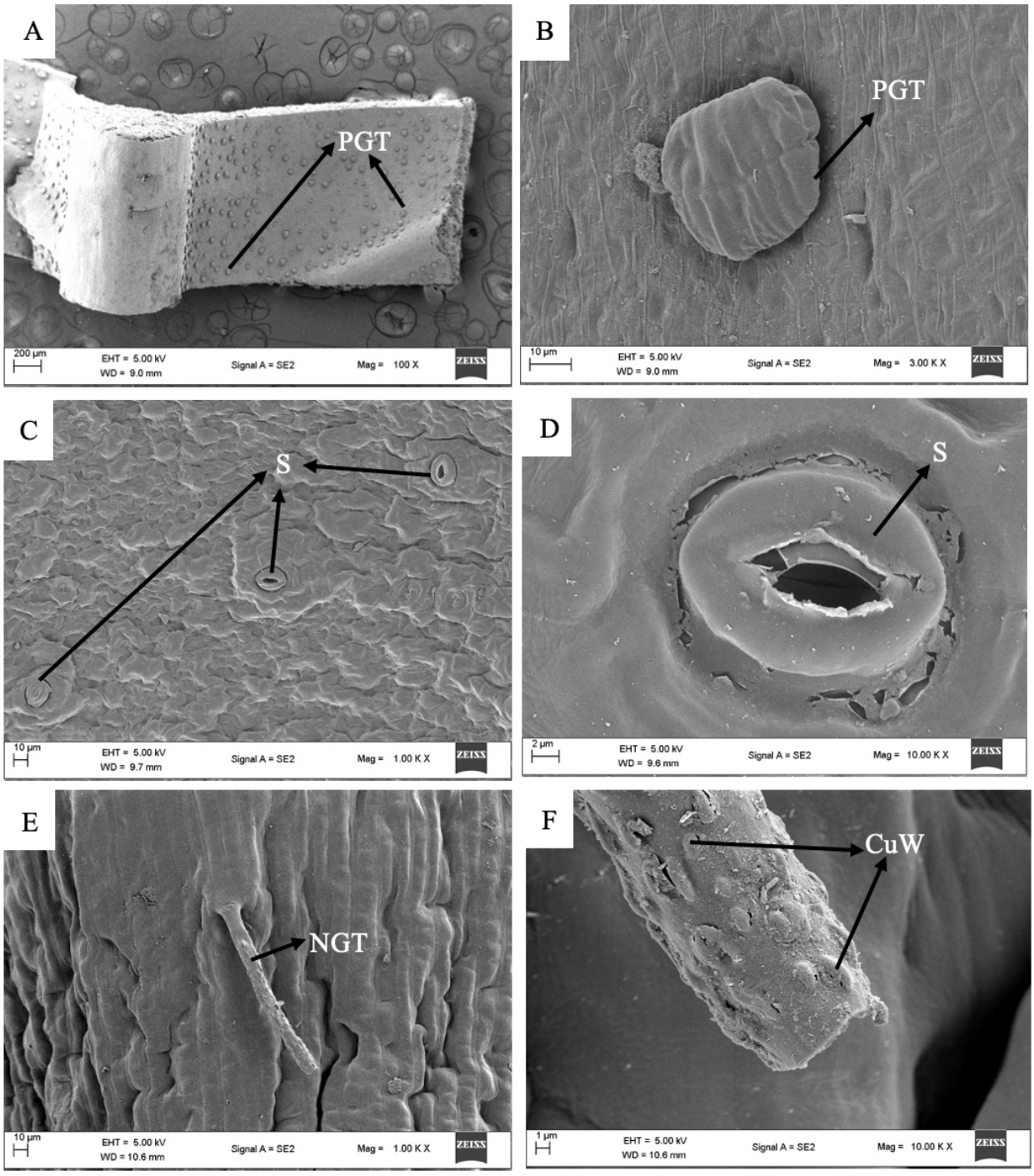

Figure 3.4B: Scanning electron micrographs of *Mangifera indica* L. leaf for Winter. A- Abaxial surface peltate gland trichome; B- Adaxial surface peltate gland trichome consisting of 2 rows of 8 oblong cells each; C and D- Abaxial surface showing anomocytic stomata; E and F- Adaxial surface of non-glandular trichome with cuticular warts. Abbreviations: PGT= Peltate gland trichome; S= Stomata; NCT: non-glandular trichome; CuW= Cuticular warts.

3.3.4 Transmission electron microscopy.

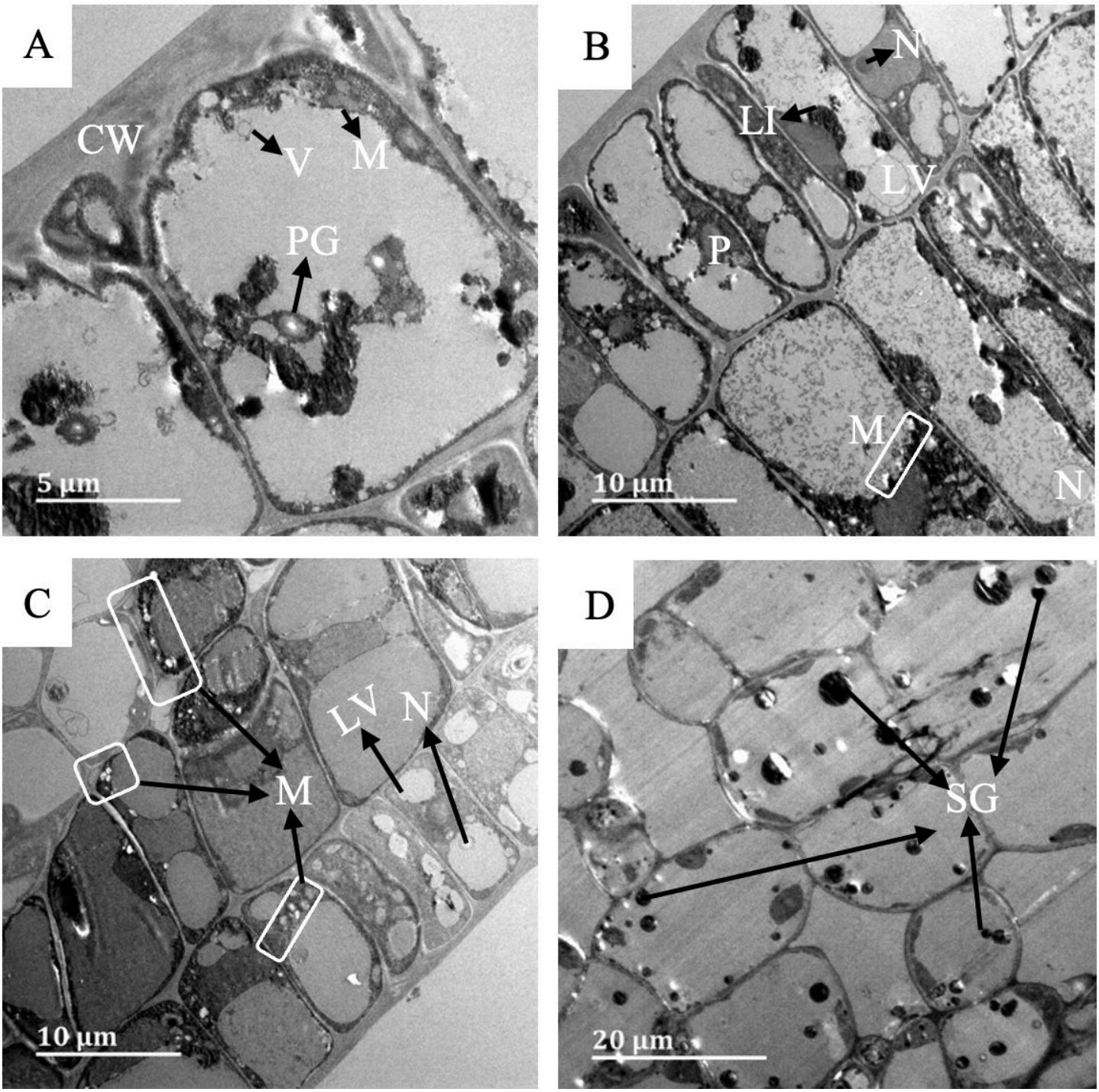

Figure 3.5 A: Transmission electron micrographs of *Mangifera indica* L. leaf for Summer. A- Upper epidermis; B- Upper epidermis and palisade cells; C- Lower epidermis and spongy mesophyll; D- spongy mesophyll. Abbreviations: CW= Cell wall; LV= Large vacuole; PG= Plastoglobuli; M= Mitochondria; SG= Starch grain; V= Vesicle; LI= Lipid inclusion.

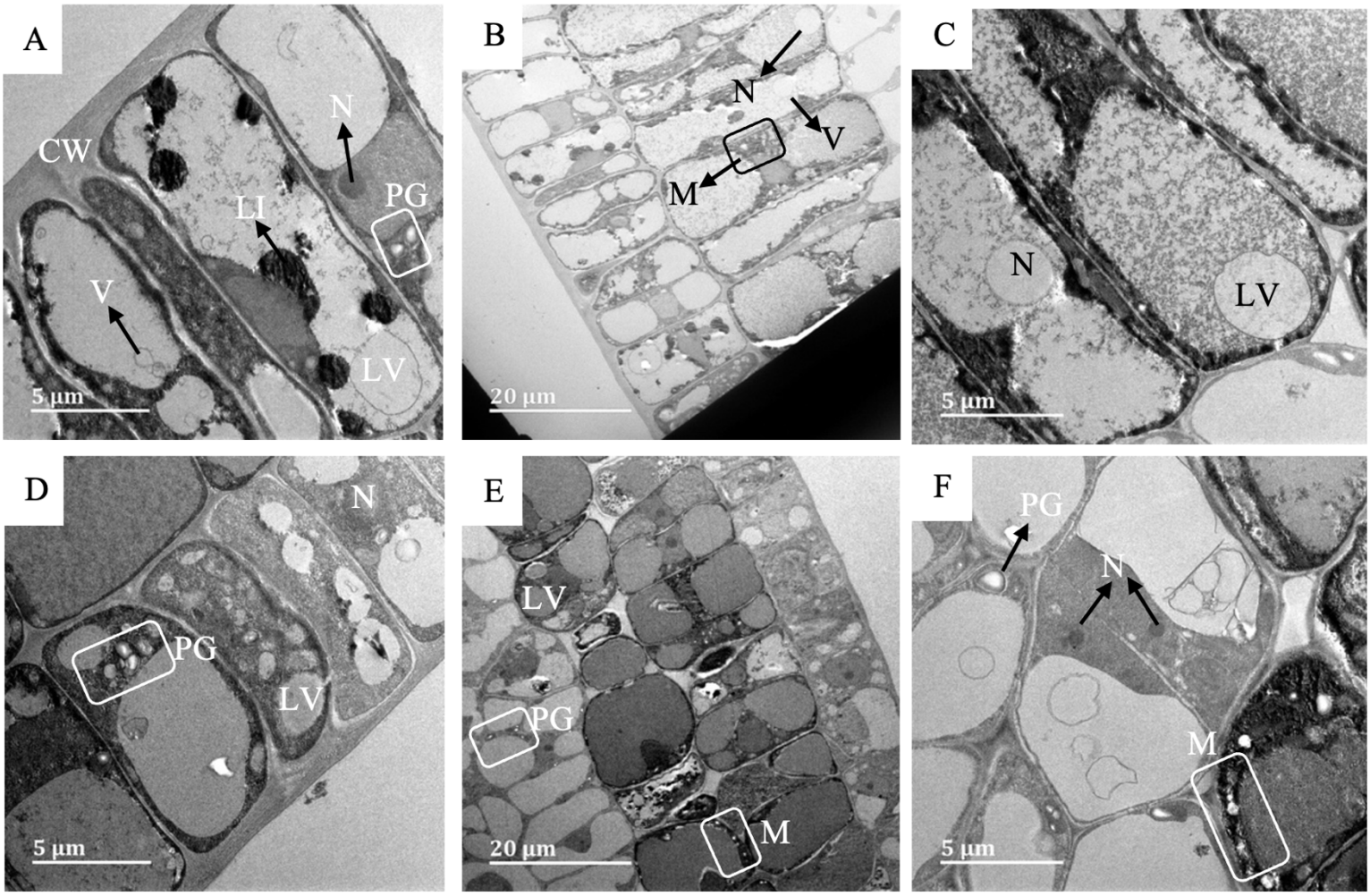

Figure 3.5 B: Transmission electron micrographs of *Mangifera indica* L. leaf for Winter. A- Upper epidermis; B- Upper epidermis and palisade cells; C- Palisade cells; D- Lower epidermis; E- Lower epidermis and spongy mesophyll; F- Spongy mesophyll. Abbreviations: CW= Cell wall; LV= Large vacuole; PG= Plastoglobuli; M= Mitochondria; SG= Starch grain; V= Vesicle; LI= Lipid inclusion.

Similar anatomical characteristics among *Mangifera* species are helpful for the division of genera in Anacardiaceae (Norfaizal and Latiff, 2013). The typical anatomical characteristics of all *Mangifera* species are as follows: i) the typical cyclocytic and anomocytic stomata with a limited number of subsidiary cells in adaxial and abaxial surfaces, respectively; ii) the amphistomatic leaves; iii) the jigsaw shape with deeply undulate cell wall in adaxial epidermal cell; iv) the presence of sunken peltate trichomes on lamina and midrib; v) the presence of bundle sheath extension to both surfaces; vi) the presence of fibre at the apex of leaf margin, midrib and petiole; vii) the presence of resin ducts; and vii) the presence of mucilaginous cells in the epidermis and

midrib (Mckay *et al.,* 2003; Sharma *et al.*, 2012; Norfaizal and Latiff, 2013; Ferrenberg, 2014; Cahyanto *et al.*, 2017).

The deeply undulate cell walls are of jigsaw shape. The irregular shape with slightly undulate and straight cell walls is found on adaxial and abaxial surfaces, respectively (Figure 3.5A- A, B, C and figure 3.5B- A, B, D, E). Although this finding corresponded partially to Norfaizal and Latiff (2013) investigations, the oval and round shapes of epidermal cells in Ganogpichayagrai *et al*. (2016) disagree with the epidermal features of this study. According to Sharma *et al.* (2012) and Cahyanto *et al.* (2017), *Mangifera indica* leaves have a 1-layered epidermis, parenchymatous cortex, closed vascular system, and resin ducts that corresponded with this study. As the function of the resin canal, the complex mixtures of substances in the resin duct resist the herbivorous pest, bacterial invasion, and wounding (Mckay *et al.,* 2003; Ferrenberg, 2014). Noticeably, starch grains (Figures 3.5A and 3.5B) in parenchyma cells of *Mangifera indica* leaf sections have not been reported in previous studies but have been reported in the present study for both the summer and winter seasons. Starch grains are used to store energy/ food for the plant (Lacchia and Carmello- Guerreiro, 2009). These findings may indicate why the summer and winter seasons show very little to no visible seasonal morphological differences. Further studies should be conducted to quantify the phytochemicals present in the seasonal leaves, indicating any variation between the seasons.

The pattern of stomatal apparatus varies in each plant group, so these characteristics enhance the species identification (Cotthem, 1970). *Mangifera indica* generally has anomocytic stomata (Metcalfe and Chalk, 1957; Norfaizal and Latiff, 2013; Ganogpichayagrai *et al*., 2016), which correspond with this study; however, work done by Cahyanto *et al*. (2017) noticed the presence of actinocytic stomata. The results from this study disagree with the results found by Cahyanto *et al*. (2017). Moreover, the anomocytic stomata are different from the anisocytic stomata of *Mangifera odorata, Mangifera pentandra,* and *Mangifera quadrifida* in Norfaizal and Latiff (2013).

Trichomes are highly diverse structures that are in contact with the external environment whose function is in response to different biotic and abiotic stimuli (Tooker *et al.,* 2010; Li *et al*., 2018). Trichomes, therefore, serve as the first line of defence against predators and with some producing bioactive compounds that may attract and guide pollinators (Wagner, 1991; Hegebarth *et al*., 2016).

The trichomes of *Mangifera indica* densely cover the leaf surface (Figures 3.4A- A and Figure 3.4B- A), occurring more frequently on the adaxial surfaces of the emergent and young leaves Figure 3.1 B and C). Two types of trichomes were observed on the leaves of *Mangifera indica,* and it appears to be peltate glandular trichomes and non-glandular trichomes with cuticular warts. The non-glandular trichome is uniseriate and multicellular with a tapering end (Metcalfe and Chalk, 1957; Norfaizal and Latiff, 2013; Ganogpichayagrai *et al*., 2016) as shown in (Figures 3.4A- E, F and 3.4B- E, F). The non-glandular trichome lengths are inconsistent, ranging between 70 - 200 µm. The peltate gland trichome is multicellular, consisting of 2 rows of 8 oblong cells, each with a size ranging from 32- 48 µm.

Non-glandular trichomes enhance plant defence systems by reducing UV radiation through surface reflectance and providing drought tolerance by reducing leaf temperatures and preventing photo-inhibition stress (Levin, 1973; Wagner, 1991; Werker, 2000). Szyndler *et al.,* (2013) demonstrated that trichomes might also limit the movement of herbivores, such as insects, thereby restricting plant tissue damage. Kariyat *et al.* (2017) proved that non-glandular trichomes deter insects by causing post-ingestive gut damage since some trichomes are reinforced by silica, which damage the peritrophic matrix (PM), a protective sheath that lines the guts of most insects and which serves to prevent mechanical damage to the gut epithelium, inhibit pathogen invasion and assist in digestion and nutrient absorption. In general, trichomes provide mechanical and chemical barriers against herbivores (Terra, 2001). The functional properties of glandular trichomes' secretory metabolites have led to commercial applications in the cosmetic, food, and pharmaceutical industries, e.g. The glandular trichomes that secrete essential oils, which give those leaves their distinctive fragrance. Natural essential oils have great commercial value. Many species of *Mangifera* are aromatic and are used as spices, herbs, medicines, and a source of fragrance (Valkama *et al.,* 2003; Balcke *et al*., 2017). Trichomes are highly diverse, and thus, their morphological traits have been key characteristics in plant taxonomic studies (Ko *et al.,*2007; Huang *et al.,* 2008; Luo *et al*., 2010).

Non-glandular trichomes are metabolically active during the earliest stages of development (Levin, 1973; Mayekiso *et al.,* 2008) and are thought to play a minor role throughout the remaining lifespan of the plant (Levin, 1973; Mayekiso *et al.,* 2008). Santos *et al.* (2016) showed that while the traditional roles of non-glandular trichomes were to protect plant materials from predators, UV radiation, and abiotic factors, they also have the potential to produce, store, and liberate bioactive substances (Levin, 1973; Mayekiso *et al.,* 2008).

Previous investigations highlighted the presence or absence, size, colour, distribution pattern, and type of trichomes, which can be used as taxonomic characteristics for plant classification (Cooper, 1932; Navarro and Oualidi, 2000; Shaheen *et al*., 2009). The presence of peltate trichomes agrees with the previous study by Metcalfe and Chalk (1957) (Figures 3.4A- B and 3.4B- B). The possession of trichomes is in contrast with the work of Norfaizal and Latiff (2013), which indicated the appearance of trichomes *in Mangifera indica* epidermis. The features of sunken peltate trichomes are considered significant characteristics and may be involved in ecological adaptations. (Johnson, 1975; Bibi *et al*., 2014).

According to Ganong (1895) and Bibi *et al*. (2014), the limited supply of water induces modification of the anatomy of plants, such as more cuticular thickness, more trichome density (Figures 3.4A- A and 3.4B- A), and presence of cuticular warts on the trichome (Ganong, 1895; Werker, 2000; Bibi *et al.,* 2014). This suggests that water availability may play a role in the presence or absence of glandular trichomes (Figures 3.4A-B and 3.4B- B) and non-glandular trichomes with cuticular warts (Figures 3.4A- E, F and 3.4B- E, F). This may vary based on the plant's environment (Ganong, 1895; Werker, 2000; Bibi *et al.,* 2014). This study does not agree with that as the summer and winter leaves resembled similar-to-identical morphological characteristics on all fronts. This may be due to the sample size of this study being too small to see distinct or any differences. Another reason may be due to climate change, that the plant has adapted itself to survive in any climatic condition. Further research is needed in this case to prove the statement true.

3.4 Conclusion and future recommendations

The present study provided new anatomical information that is useful for identifying *Mangifera indica*, such as the presence of glandular and non-glandular trichomes in the leaf. The histochemical tests allowed the determination of the sites of accumulation and synthesis of the metabolites. The results contribute to the pharmaco-botanical standardisation of this species with phytocompounds like alkaloids and phenols that exhibit strong medicinal properties. The present study demonstrates the anatomical features: i) the shape of epidermal cell; ii) the outline of leaf margin midrib; iii) the shape of epidermal; iv) the layer of hypodermis; v) the number of palisade cell layers; vi) presence of peltate gland trichome and non-glandular trichomes with cuticular warts; vii) the distribution of peltate trichome; viii) the inclusion in each organelle e.g., vacuole, starch grains, etc). The combination of stereo- and scanning electron microscopy facilitated the identification of the leaf morphology of *Mangifera indica*. Our knowledge of the leaf morphology of *Mangifera indica* using microscopy techniques is lacking. Leaf structures identified were

compared to previously reported information on the species. Thus, the results presented in this study contribute significantly to our growing understanding of the leaf anatomy of this species. In this regard, the study is novel. Furthermore, results from this study will also assist taxonomists in identifying *Mangifera indica* using the SEM micrographs of their distinct leaf structures. Additionally, ultrastructural studies on leaf structures should be conducted to further examine the internal features of the cells and organelles, specifically the trichomes. Further studies may also focus on evaluating the micromorphology of the seeds and roots of *Mangifera indica*.

3.5 References

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CHAPTER 4

Phytochemical, antibacterial, and anti-oxidants screening of local *Mangifera indica* L. leaves

Arvish Maharaj[a], Yougasphree Naidoo[a], Yaser Hassan Dewir[b,c]

[a]School of Life Sciences, University of KwaZulu-Natal,Westville Campus, Private Bag X5400, Durban 4001, South Africa

[b]Plant Production Department, P.O. Box 2460, College of Food and Agriculture Sciences, King Saud University, Riyadh 11451, Saudi Arabia

[c]Kafrelsheikh University, Faculty of Agriculture, Kafr El-Sheikh 33516, Egypt

Abstract

Medicinal plants and their bio-constituents play a pivotal role in the synthesis and formulation of new and novel therapeutic drugs. There is an urgent need to source, evaluate and document these phytomedicinal agents. Consequently, this study aimed to investigate some of the phytochemical and biological properties using different solvent extracts (hexane, chloroform, and methanol) of the leaves of *Mangifera indica* for summer and winter. The preliminary phytochemical screening was done to uncover the presence of different metabolites using standard phytochemical procedures, the antimicrobial analysis was conducted using the agar well diffusion assay, and the anti-oxidants screening was conducted using 2, 2-diphenyl 1-picryl hydrazyl radical (DPPH) assay. The extract of *Mangifera indica* leaves for both summer and winter showed the presence of phenols, flavonoids, tannins, and terpenoids, alkaloids, phytosterol, saponins, steroids, and carbohydrates. The antibacterial activity of the methanolic leaf extracts for summer and winter of *Mangifera indica,* were evaluated against the bacterial species *Staphylococcus aureus* (ATCC 43300) and *Escherichia coli* (ATCC 25922) and. For *S. aureus* (ATTC 43300), the summer crude extract displayed lower antibacterial activity than the control streptomycin, the summer extracts had a zone of inhibition of 14.17 mm while streptomycin had a 16.67 mm zone of inhibition. winter extracts had a zone of inhibition of 12 mm while streptomycin had a 13.67 mm zone of inhibition. For *E. coli* (ATCC 25922), the summer crude extract displayed higher antibacterial activity than the control gentamycin; the summer extract had a zone of inhibition of 18.05 mm while gentamycin had a 17.5 mm zone of inhibition. The winter extracts had a zone of inhibition

of 8.5 mm while gentamycin had a 14.5 mm zone of inhibition. Between seasons, summer had better antibacterial activity compared to winter for both Gram-positive and Gram-negative bacteria. Potent radical scavenging activity was exhibited for both summer and winter seasons with hexane and methanolic extracts for summer (IC_{50} of 19.53 µg/mL and 12.71 µg/mL respectively) and winter (22.32 µg/mL and 14.35 µg/mL respectively) in comparison to the control ascorbic acid which produced an IC_{50} of 3.20 µg/mL. The summer extracts had better radical scavenging IC_{50} capacity than winter extracts. The findings provided evidence that *Mangifera indica* is a possible source of important medicinal compounds. Antibacterial screening showed positive results for both summer and winter samples. The present study concludes that the hexane and methanolic extracts have significant anti-oxidants activity, while methanolic extracts exhibited good antibacterial activity. Future studies can be done against more strains of bacteria and cancer cell lines to test its potency.

Keywords: Antimicrobial, Anti-oxidant, *Mangifera indica*, Medicinal plants, Phytochemicals.

4.1 Introduction

Herbal remedies from medicinal plants to cure and prevent several ailments differ between communities (Sharif and Banik, 2006, Kubmarawa *et al.,* 2007). The advent of science into the search for antibiotics largely depends on some of these medicinal plants as raw materials (Lamboro *et al.,* 2016). For many years, medicine had depended exclusively on flowers, barks, and leaves of plants; only recently the use of these carbon copy chemicals, which are identified in plants, have been used to make synthetic drugs (Chatterji, 2017; Tower *et al*., 2020; Streicher, 2021). According to the World health organisation (WHO) any plant is considered medicinal if it contains substances in one or more of its organs that can be used for any therapeutic purposes or precursors for the synthesis of useful drugs (Junaid *et al*., 2006). At present, medicinal plants have assumed a central stage in public health care as a natural alternative to contemporary drugs, with an estimated 65-80% of the world population living in developing countries relying on plant-based traditional medicine (Dwivedy *et al*., 2019).

Due to the increase in human infections that are caused by bacteria, researchers have thus focused on medicinal plants for cheap and effective modes of therapy (Chatterji, 2017; Tower *et al*., 2020; Streicher, 2021). Medicinal plants represent a rich source of antimicrobial agents (Asma'a Al-Rifai *et al*., 2017). Due to microorganisms having developed resistance to many antibiotics, the use of medicinal plant extracts and their isolated compounds as resistance against microorganisms

has been increased (Dzotam *et al.,* 2017). Medicinal plants produce secondary metabolites, which are a rich source of antibacterial and anti-oxidants properties (Diso *et al.,* 2017; Kumar *et al*., 2021). The biologically active metabolites in medicinal plants and their possible therapeutic potential have become a subject of active investigation (Khoushika *et al*., 2016). Plant-based medicines are becoming popular because of their lesser toxicity and side effects (Kumar *et al*., 2021). Anti-oxidants significantly delay or prevent oxidation of oxidisable substrates when present at lower concentrations than the substrate (Mohammed *et al.,* 2016). Since the anti-oxidants potential of plants has received a great deal of attention because increased oxidative stress has been identified as a major causative factor in the development and progression of several life-threatening diseases in humans (Deepak *et al*., 2015; Yoo *et al*., 2018).

Many South Africans (up to 80%) rely on local medicines, mostly from plants, to manage their diseases and general healthcare needs (Erhabor *et al.,* 2019). Low-income communities are predicted to be hit the hardest by drug-resistant infections (Tan *et al.,* 2020). The proportion of people succumbing to such infections is escalating, with the current pace of drug development insufficient to mitigate the surge in antibiotic resistance (Chatterji and Petchiappan, 2017; Tower *et al*., 2020; Streicher, 2021). To address this knowledge gap, the plant species *Mangifera indica* L. belonging to the family Anacardiaceae was investigated to identify phytochemicals that could promote antibacterial and anti-oxidants activities. The medicinal properties of other species from the genus *Mangifera* have been reported to include antidiabetic (Verma *et al*., 2017), antiviral (Guha *et* al., 2021; Tegen *et al.,* 2021), antibacterial (Verma *et al.,* 2017), anti-Alzheimer agent (Nuthakki *et al*., 2019), anti-oxidants (Nafiqoh *et al.*, 2020) and anti-cancer (Tegen *et al.,* 2021).

The discovery of antibiotics has revolutionised modern medicine, transforming the methods used to treat many infectious diseases of human and animal origin (Aslam *et al.,* 2018). Primarily, due to their indiscriminate use, antibiotic therapy has been globally jeopardised by the marked increase in antibacterial resistance among common bacterial pathogens (Alam *et al.*, 2020). This has led to an intense drive to find novel agents that can alleviate the rise of antibiotic resistance (Rupasinghe *et al.,* 2017). Research exploring the mechanistic of medicinal plants as an alternative to conventional medicine has dramatically increased in recent years (Erhabor *et al*., 2019; Mulat *et al.,* 2019). Since plants do not possess a developed cellular and biochemical immune system, it is suggested that they would have evolved intrinsic methods to overcome bacterial infections (Lamboro *et al.,* 2016). Thus, the inherent phytochemicals in medicinal plants may represent a valuable reservoir of therapeutic products that display varying effectiveness against bacteria, sometimes even at low concentrations (Bouyahya *et al*., 2017). In addition to having antimicrobial properties, plants have been examined for their role in disrupting the

bacterial infection process (Khan *et al*., 2018; Erhabor *et al*., 2019). Conventional antibiotics produce bactericidal and mutagenic action, an attribute that has culminated in the expansion of multi-resistant pathogens that are difficult to treat (Qais *et al*., 2019). The approach of interfering with bacterial pathogenesis provides an attractive alternative that advantageously does not necessarily favour the evolution of bacterial resistance (Swaroop *et al*., 2018; Saeki *et al.,* 2020).

To date, little is known about the phytochemical and pharmacological activities of *Mangifera indica* in South Africa. Thus, to incept the investigations in the current study, the phytochemical classes were identified as well as anti-oxidant screening was done using 3 solvents (Hexane, chloroform, and methanol) of *Mangifera indica* leaves for summer and winter, followed by the analyses of the antibacterial properties of one solvent (methanolic extract) of *Mangifera indica* leaves for summer and winter using a Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria.

4.2 Materials and Methods

4.2.1 Collection and identification of plant materials

The leaves of *Mangifera indica* were collected from Durban, KwaZulu-Natal, South Africa (24° 49’05” S 30°56'46” E). The summer samples were collected from December 2019 to March 2020, and the winter samples were collected from June-August 2020. Professor Y. Naidoo confirmed the species identity. A voucher specimen (accession number: NU0092176) was deposited in the Ward Herbarium, School of Life Sciences (Biology), University of KwaZulu-Natal, Durban, South Africa.

4.2.2 Preparation of leaf extracts

Fresh leaves of *Mangifera indica* were air-dried to a constant weight and ground into powder form using a Waring blender. Approximately 10 g of powdered *Mangifera indica* leaves for summer and winter was formed after grounding. Ten grams for each season were subjected to a sequential extraction by increasing polarity using hexane (C_6H_{14}), chloroform ($CHCl_3$), and methanol (CH_3OH) using a reflux extraction apparatus. The extracts were cooled at room temperature, after which they went through filtration using Whatman no. 1 filter paper (Whatman Limited, UK) until no precipitate was seen in the filtrate. The extract was subjected to drying at room temperature and then stored in labelled, airtight jars the dark at 4°C until further use. This was done to prevent the extracts from reacting with atmospheric humidity. This was done to the

leaves for both the summer and winter seasons. Thereafter, the thoroughly dried plant materials were determined by the formula below (Akwu *et al.,* 2019):

$$\text{Extract yield (\%)} = \frac{Weight\ of\ dried\ extract\ (g)}{Weight\ of\ leaf\ material\ (g)} \times 100$$

4.2.3 Phytochemical analysis

4.2.3.1 Qualitative phytochemical analysis

The intensity of the colour reactions was illustrated by symbols, (-) for no observed changes and therefore negative result, (+) for low-intensity positive result, (++) for mildly intensity positive, and (+++) for very high-intensity positive result. Preliminary phytochemical screening was carried out on the powdered material and chemically tested for the presence of various constituents using standard methods (Harborne *et al.*, 1973; Trease and Evans, 1978; Sofowora, 1993; Tiwari *et al*., 2011; Karthikeyan *et al.*, 2020) as described below.

4.2.3.1.1 Test for alkaloids

Two drops of Mayer's chemical reagent (potassium iodide was added to mercury (II) chloride solution) was added were transferred to 1 mL of extract in a test tube. The formation of a yellow precipitate indicated a positive reaction.

Two drops of Wagner's chemical reagent (iodine was added to potassium iodide) were transferred to 1 mL of extract in a test tube. A positive reaction was indicated by the formation of an orange-brown precipitate.

Two drops of Dragendorff reagent (potassium iodide solution was added to bismuth subnitrate and glacial acetic acid) were mixed with 1 mL of extract in a test tube. The formation of a reddish precipitate indicated a positive reaction.

4.2.3.1.2 Test for amino acids and proteins

Biuret test (Harborne, 1998)

In a test tube containing 1 mL of crude extract, 1 mL of 10% NaOH was added. The contents of the test tube were mixed thoroughly, and the addition of 95% ethanol followed. Thereafter, 1 mL

of 0.5% copper sulphate was inserted by the side of the test tube. The formation of a purplish - violet or pink- violet colour confirms the presence of a protein.

4.2.3.1.3 Tests for carbohydrates

One drop of Molisch reagent (α-naphthol solution) was transferred to 1 mL of extract in a test tube. After the solution was thoroughly mixed, 0.5 mL of concentrated sulphuric acid was decanted slowly along the sides of the test tube to settle above the solution. The development of violet or deep purple ring indicated a positive reaction.

Approximately 1 mL of each extract was mixed with 1 mL of Fehling's solution A (aqueous solution of copper sulphate) and B (potassium sodium tartrate solution in sodium hydroxide) and left undisturbed in a water bath to boil. The formation of a red precipitate indicated a positive reaction.

Approximately 1 mL of Benedict's reagent (solution of sodium citrate, sodium carbonate, and copper sulphate pentahydrate) was added to a test tube containing 1 mL of extract, the solution was mixed and boiled for 2 min in a water bath. The formation of a precipitate varying in colour from yellow to red indicated a positive reaction.

4.2.3.1.4 Test for fixed oils and fats

One drop of the extract was dispensed onto a filter paper (Whatman No. 1). The appearance of an oil stain on the filter paper indicated a positive reaction.

4.2.3.1.5 Test for flavonoids

One mL of 5% lead acetate solution was added to 5 mL extract. The formation of a white precipitate indicated a positive reaction.

4.2.3.1.6 Tests for mucilage

Precipitation test (Trease and Evans, 1985)

In a test tube containing 1.5 mL of crude extract, 2 mL of distilled water was added. To this diluted solution, 2 mL of absolute ethanol was added with continuous stirring. The formation of a white or cloudy precipitate confirms the presence of gums or mucilage.

Ruthenium red test (Trease and Evans, 1985)

In a test tube containing 2 mL of crude extract, two drops of ruthenium red were added. The colour change of the solution into a pink coloured solution confirms the presence of mucilage.

4.2.3.1.7 Tests for phenolics

Two drops of 10% ferric trichloride were transferred to 1 mL of extract in a test tube. The appearance of a green or black colour indicated a positive reaction.

4.2.3.1.8 Test for saponins

Foam test (Bargah, 2015)

In a test tube containing 5 mL of crude extract, 20 mL of distilled water was added. The test tube was then vigorously shaken for 15 minutes. The appearance of a froth layer is indicative of the presence of saponins (triterpene glycosides). The observed results were recorded as negative for no froth formed, positive for the presence of froth formed 1.2 cm high, strongly positive for froth formation greater than 2cm high, and weakly positive for froth formed less than 1 cm high.

Olive oil test (Trease and Evans, 1985)

In a test tube containing 2 mL of crude extract, two drops of olive oil were added. The test tube was shaken vigorously for 5 minutes. Thereafter, a soluble emulsion confirms the presence of saponins.

4.2.3.1.9 Test for terpenoids

Two mL of chloroform was added to 5 mL plant extract, followed by 3 mL of concentrated sulphuric acid along the side of the test tube to form a layer. A reddish-brown colour indicated a positive reaction.

4.2.3.1.10 Test for sterols

Three mL of chloroform was mixed with 2 mL of extract, followed by the addition of 2-3 drops of sulphuric acid down the side of the test tube. The appearance of a red ring between the solvent layers and a fluorescent green ring below indicated a positive reaction.

4.3 Biological evaluation: Antibacterial and anti-oxidants assays of local *Mangifera indica* L. leaves

4.3.1 *In vitro* antibacterial assay

4.3.1.1 Bacterial strains

The prepared crude extracts were subjected to antibacterial assays. The antibacterial activity of leaf samples where tested against 2 strains, the Gram-negative, *Escherichia coli* (ATCC 25922) and Gram-positive *Staphylococcus aureus* (ATCC 43300) bacterial strains. The strains were provided by Professor Johnson Lin, Department of Microbiology, University of KwaZulu- Natal, and maintained in 75% glycerol at -80 °C.

4.3.1.2 Preparation of media and bacterial culture

The crude extracts (methanol) from the summer and winter leaves of *Mangifera indica* were dissolved in 10% Dimethylsulfoxide (DMSO) to different concentrations of 10, 5, 2.5, 1.25, and 0.625 mg/mL.

Mueller-Hinton agar (MHA) (Biolab, South Africa) was prepared by suspending 38 g of the agar into 1 L of distilled water. The medium was mixed on a stirrer (15 min), and autoclaved (Model: HL-320) at 121°C for 1 h. The agar was poured into sterile Petri plates (90 mm) and was allowed to set at room temperature (23°C).

Both Gram-positive and Gram–negative bacteria from stock cultures were sub-cultured onto fresh nutrient broth (Merck, Germany) and incubated overnight (24 h) at 37°C, after which the bacterial cultures (inoculum) were further diluted with sterile nutrient broth to an OD of 0.08-0.1 at 625 nm using a UV-vis spectrophotometer (Agilent Cary 60 Spectrophotometer, USA) to yield a final concentration of approximately 1 x 108^{-1} x 10^{9} bacterial cells /mL and the standardised cell culture was then swabbed evenly onto the Müller-Hinton agar (MHA) plates and allowed to dry at room temperature.

4.3.2 Antibacterial activity assay

The *in vitro* antibacterial assay of the methanolic crude leaf extract for summer and winter was carried out using the agar well diffusion method described by Perez *et al*, (1990) with slight

modifications. Each bacterial strain was separately smeared uniformly over the surface of the MHA plates with a sterile cotton swab. A 6 mm diameter improvised sterile cork-borer was used to bore wells in the Petri plates, thereafter 100 µL each of the prepared different concentrations (0.625, 1.25, 25, 5, and 10 mg/ mL) of the crude extract, which were pipetted into the wells. The plates were incubated at 36°C, and the growth inhibition zones around the bored wells were taken as positive results, and the diameters were measured within 18-24 hours after incubation. Autoclaved sterile 10% Dimethylsulfoxide (DMSO) was used as the negative control (Akwu *et al.,* 2019; Mani *et al.,* 2021), while 10 µg/mL of gentamicin (for Gram-negative) and streptomycin (for Gram-positive) were used as the positive controls. The tests were conducted in triplicates, and data were expressed as mean ± standard deviation.

4.3.2.1 *In vitro* anti-oxidants assay: DPPH free radical scavenging activity

The hydrogen donating ability of solvents was determined using a modified 2,2-diphenyl-1-picrylhydrazyl (DPPH) spectrophotometric assay as described by Braca *et al.* (2002) with slight modifications. Briefly, 1 mL of plant extract with concentrations of a range of 15, 30, 60, 120, and 240 µg/mL was pipetted into a 96- well microplate in triplicate. After that, 150 µL of 0.3 mM DPPH solution was added. The microplate was incubated in the dark at 25°C for 30 min. The absorbance of the solvents was measured at 517 nm using the Synergy HTX Multi-mode reader, BioTek Instruments Inc., (Winooski, USA), and the percentage of the free radical inhibition was used to express the free radical scavenging activity. Ascorbic acid was used as the standard. The IC_{50} value (the concentration of the anti-oxidant agent that gives rise to 50% inhibition of the oxidant) was derived from the inhibition curves by plotting the percentage inhibition against the concentration logarithmic scale. The free radical scavenging ability of pure compound was calculated using the equation below:

$$\text{DPPH scavenging activity (\%)} = \left(\frac{Abs\ control - Abssample}{Abs\ control}\right) \times 100$$

Where;

$Abs_{control}$ is the absorbance of DPPH and methanol

Abs_{sample} is the absorbance of DPPH radical + sample

Dose-response curves were plotted between the percentage of scavenging activity (y-axis) and the logarithmic transformation of the concentrations (x-axis), using Microsoft Office Excel, 2016. The IC_{50} value was calculated from the antilogarithmic values of the linear regression analysis. The IC_{50} is the concentration of extract that inhibits the formation of DPPH radicals by 50%.

4.4 Statistical analysis

Statistical, analyses were conducted using Statistical Package for the Social Sciences (SPSS) versions 26 and 27. Data are indicated as a mean of triplicate replicates (n=3). For anti-oxidants, the mean values were determined using two-way analysis of variance (ANOVA) with $p \leq 0.05$ considered significant with Tukey honestly significant difference (HSD) *post hoc* test (Moradi *et al*., 2020).

4.5 Results and discussion

Globally, the emphasis has been placed on identifying new strategies to circumvent the escalation of antibiotic resistance (Krishnananda and Shabaraya, .2017). As the efficacy of the current antibiotic therapy decreases and the discovery of new antibiotic drugs stagnates, exploring alternative therapies could offer a much-needed solution (Streicher *et al.*, 2021). Renewed interest has been invigorated towards utilising medicinal plants for the advancement of modern medicinal practices (Casciaro *et al.,* 2019; Mickymaray, 2019; Streicher *et al*., 2021). In the current study, *Mangifera indica* leaves were evaluated through preliminary screening for their potentiality to be eventually utilised in western medicine.

4.5.1 Percentage yield

Table 4.1: Percentage yield of the crude extract of leaves for summer and winter of *Mangifera indica* L.

Crude extracts	**Summer leaves**	**Winter leaves**	**Summer leaves**	**Winter leaves**
	Dried extract yield (g)		**Percentage yield (%)**	
Hexane	0.82	0.45	8.2	5.5
Chloroform	0.98	0.77	9.8	7.7
Methanol	2.7	2.33	27	23.3

The crude methanol extract of the leaves for summer and winter gave the highest recovery yield (27 and 23.3 %, respectively), while the lowest yield was recorded from the crude hexane extract(8.2 and 5.5 %, respectively) (Table 4.1), this implies that *Mangifera indica* leaves have more polar compounds then non-polar compounds. Phyto-metabolites such as phenols, flavonoids, alkaloids, tannins, terpenes, terpenoids (steroids), triterpenes (cardiac glycosides),

carbohydrates, proteins and gums and mucilage were present in the crude hexane, chloroform, and methanol extracts (Table 4.2).

Table 4.2: Phytochemical screening of *Mangifera indica L.* leaf crude extracts with different polarities: Hexane, chloroform, and methanol for the summer and winter season.

Test	Summer extracts			Winter extracts		
	Hexane	**Chloroform**	**Methanol**	**Hexane**	**Chloroform**	**Methanol**
Alkaloid Test						
(i)Mayers Test	+	+	+	+	+	+
(ii)Wagners Test	+	+	+	+	+	+
Phenols, Tannins, Flavonoids, Quinones Test						
(i) Ferric Chloride Test	++	+	+	++	+	+
(ii) Lead acetate test	+	++	+++	+	++	+++
(iii) Gelatin Test	+++	++	–	+++	+++	–
Flavonoid Test						
(i) Alkaline reagent test (Alkaline hydrolysis)	++	+	+++	++	+	+++
(ii) Acid hydrolysis (Sulphuric Acid)	+++	+	+	+++	+	+

Terpene Test						
(a) Saponins						
(i) Foam test	–	–	+	–	–	+
(ii) Olive oil test	–	+	+	–	+	+
(b) Steroids						
(i) Liebermann-Burchard test or acetic anhydride *test*	+	–	+	+	–	+
(ii) Salkowski's test	+	+	+	+	+	+
Carbohydrate Test						
(i) Molisch's test	+	+	+	+	+	+
Protein Test						
(i) Ninhydrin test	–	–	–	–	–	–
(ii) Biuret test	+++	–	+	+++	–	+
Gum and Mucilage *Test*						
(i) Precipitation test	+	+	+	+	+	+
(ii) Ruthenium red test	–	+++	+	–	++	+
(iii) Detection of Resins	+	–	–	+	–	–

key: - Negative result; + Mildly present; ++ Distinctly present; +++ Very strongly present

4.5.2 Phytochemical activity

Medicinal plants and their phyto-therapeutic components have an integral role in the advancement of modern medicine (Aslam *et al.,* 2018). Over recent years, there has been a renewal of interest in traditionally used medicinal plants in southern Africa, propelling the exploration of these plants for new therapeutically active compounds (Omokhua *et al.*, 2018). This premise formed the foundation for the current study, i.e., to initialise investigations into the potential curative properties of the plant species *Mangifera indica.*

Medicinal plants and their phytocompounds dictate their therapeutic effectiveness (Oladeji, 2016). The polarity and solvent used during the extraction process have an intense effect on the medicinal properties, including the yield of the resultant extracts due to the solubility of the phytocompounds in the various solvents (Dowlath *et al.,* 2020).

The therapeutic potential of extracts was assessed by performing an array of phytochemical tests, which confirmed the presence of different compounds (Table 4.2) known to impart medicinal characteristics of the plant. The extract of *Mangifera indica* leaves for both summer and winter showed the presence of phenols, flavonoids, tannin, terpenoids, alkaloids, phytosterol, saponins, steroids, and carbohydrates. These findings are similar to a study of phytochemicals done by Mohammed *et al*. (2016) which showed the presence of flavonoids, steroids, alkaloids, and tannins in their aqueous, ethanol, and chloroform extracts (Mohammed *et al.,* 2016).

In nature, carbohydrates are potentially the most common organic substance (Ruther et *al*., 2010). Carbohydrates are utilised in pharmacy for the preparation of sucrose, as binders for the preparation of tablets (lactose), in anti-diarrhoea drugs (pectin), antacids, diuretic drugs (mannitol and sorbitol), etc. (Ruther *et al*., 2010). Gums are formed by the breakdown of plants cell walls and occur as a result of injury to the plant due to unfavourable conditions, such as drought (Geetha et *al*., 2009). Mucilage are formed within the cells of plants and are usually normal metabolism products (Geetha et *al*., 2009). Therefore, gums and mucilage are regarded as pathological products and physiological products of plants, respectively (Geetha et *al*., 2009). Both gums and mucilage have several applications in pharmacy. They are utilised in medicine for their anti-inflammatory and anti-irritant activity in cough suppression. These hydrophilic (soluble in water) polymers are useful as tablet binders, coating agents in capsules, and disintegrants (are agents added to tablet formulations to help break up the tablet) (Sungthongjeen *et al.*, 1999).

In this study, flavonoids, steroids, terpenoids, and gums and mucilage were shown to be present in all extracts for both summer and winter. Due to their anti-inflammatory properties, these phytochemicals

are known to alleviate chest pains that are caused by respiratory diseases or infections (Babu and Savithramma, 2014). Terpenoids have been discovered to be useful in preventing, treating several diseases, and management of cancer. They are well known to possess antimicrobial, antiparasitic, antifungal, antiviral, anti-inflammatory, and anti-allergenic properties. It is also used to help in the regulation of immune systems (Rabi and Bishayee, 2009; Yadav *et al*., 2014). Steroids help calm airway inflammation in asthma (Krishnaiah *et al*., 2009) and possess cholesterol-reducing properties (Shah *et al*., 2009).

The leaf methanol extract tested positive for majority of the phytochemicals tested for both summer and winter, whereas hexane and chloroform did not. This is because hexane is only regarded as a good solvent when dissolving a non-polar compound such as oils; however, using hexane to dissolve a polar compound would be highly ineffective (Doss, 2009). On the other hand, methanol would be a much better option than hexane for dissolving polar compounds as methanol is polar and would interact with the polar compounds more easily (Doss, 2009).

From this study, it can be deduced that *Mangifera indica* extracts have various medicinal values due to the presence of a variety of phytochemicals. As reported from previous studies Joona *et al.* (2013); Rakholiya *et al.* (2012); Masud (2016); Kabir *et al.* (2017), all the phytochemical compounds found in the leaves of this plant has been reported to have beneficial health effects on humans.

4.5.3 Antibacterial assays

The antimicrobial activity of the methanolic leaf extracts for summer and winter of *Mangifera indica* were evaluated against the bacterial species *Staphylococcus aureus* and *Escherichia coli* using the agar well diffusion method described by (Perez *et al.,* 1990). 10 µg/mL of gentamicin (Gram-negative) and streptomycin (Gram-positive) were used as the positive controls. The tests were conducted in triplicates and data were expressed as mean ± standard deviation. The evaluation of the antibacterial activity of the tested plant extracts was recorded in Table 4.3.

Table 4.3: Antibacterial screening of the crude methanolic leaf extracts of *Mangifera indica* L. for summer and winter seasons against Gram-negative, *E. coli* (ATCC 25922), and Gram-positive *S. aureus* (ATCC 43300) bacterial strains. Gentamicin (Gram-negative) and Streptomycin (Gram-positive) were the positive controls.

Bacterial strain	Concentrations (mg/mL) and diameter of zone of inhibition (mm)					
	0.625	1.25	2.5	5	10	Control
Summer						
Staphylococcus aureus (ATCC 43300)	7.5 ± 0.5	8.83 ± 0.76	9.83 ± 076	11.50 ± 1.32	14.17 ± 0.29	13.67 ± 0.76
Escherichia coli (ATCC 25922)	9.17 ± 1.76	11.17 ± 1.04	14.33 ± 0.58	16.33 ± 1.15	18.5 ± 0.5	17.5 ± 1.5
Winter						
Staphylococcus aureus (ATCC 43300)	6.83 ± 0.29	7.67 ± 0.58	8.83 ± 0.76	10.67 ± 0.58	12.33 ± 0.58	13.33 ± 0.76
Escherichia coli (ATCC 25922)	6.25 ± 0.35	6.67 ± 1.15	7.17 ± 1.15	7.67 ± 1.61	8.5 ± 1.32	14.5 ± 3.04

The methanolic solvent extracts of *Mangifera indica* leaf demonstrated promising antibacterial activity against Gram-positive bacteria (Table 4.3). *Staphylococcus aureus* strains are associated with a plethora of diseases, such as mild skin and soft tissue infections, infective endocarditis, osteomyelitis, bacteraemia, and fatal pneumonia, with the multidrug-resistant strains presenting a severe challenge in hospital-acquired infections (Guo *et al*., 2020; Sengstock *et al.,* 2021). Consistent with the results obtained from the current investigations, ethnobotanical studies have documented that tribal groups of India, especially in the northern and eastern areas of the country, use the fruits and leaves of *Mangifera indica* to treat skin diseases, including carbuncles, which are generally caused by *S. aureus* infections (India *et al.,* 2013; Jeeva *et al.,* 2011; Choudhary and Swarnkar, 2011; Shukla, *et al*., 2021). Scientific studies carried out by Rondevaldova *et al.* (2015) showed the anti-staphylococcal capacity of *Mangifera indica* leaves and bark (which have the most concentrated amount of mangiferin) synergistically enhanced the susceptibility of *S. aureus*. These findings were further supported by Mazlan *et al*. (2019), where authors reported an increase in the antimicrobial activity with the combinatory effect of mangiferin with antibiotics, nalidixic acid, ciprofloxacin, vancomycin, and tetracycline against *S. aureus*.

Interestingly, for *S. aureus* (ATCC 43300), the summer crude extract displayed higher antibacterial activity than the control streptomycin. The summer extract had a zone of inhibition of 14.17 mm while streptomycin had a 13.67 mm zone of inhibition. Whereas, the winter extracts had a zone of inhibition of 12.33 mm while streptomycin had a 13,33 mm zone of inhibition. Correspondingly, concentration plays a role in effectiveness as the concentration of crude extract decreased, so did the zones of inhibition. This was shown for both summer and winter samples. The results are in accordance with the comparison of antibacterial activity of leaves extracts of *Mangifera laurina, Mangifera indica,* and *Mangifera altissima* investigated by Krishnananda and Ramakrishna, (2017), which showed that extracts of *Mangifera indica* were effective against all test concentrations, however the potency was dependent on the concentration of crude extract. It came as no surprise that for *E. coli* (ATCC 25922), the summer crude extract displayed higher antibacterial activity than the control gentamycin: the summer extract had a zone of inhibition of 18.05 mm while gentamycin had a 17.05 mm zone of inhibition. The winter extracts had a zone of inhibition of 8,5 mm while gentamycin had a 14.05 mm zone of inhibition. The weak activity of both the control gentamycin may indicate that this commercial antibiotic drug is slowly losing potency and no longer capable of exerting an effect against *E. coli* (ATCC 25922) bacteria (Diso *et al.,* 2017).

A study by Krishnananda and Ramakrishna (2017) did a comparative study of the antibacterial activity of different solvent extracts of each plant organ that showed the methanolic leaf, stem bark, and seed extracts had the most pronounced effect against the Gram-positive strains compared to the other solvent extracts of the respective plant organs (Krishnananda and Ramakrishna, 2017). It can be deduced that

methanol is an effective extracting solvent which effectively solubilised phytocompounds with antibacterial activity (Sanrawal and Sushil, 2013). This can be supported by other studies that acquired similar results (Verma and Verma *et al.,* 2017; Seleshe and Kang, 2019; Cudjoe *et al.,* 2020). Overall, the summer extracts performed the best in the antibacterial assays as these extracts showed notable antibacterial activity against Gram-negative and Gram-positive bacteria.

Alternatively, the different solvent extracts of *Mangifera indica* were only mildly effective against the tested Gram-negative pathogens from an antibacterial perspective. It is widely known that Gram-negative bacteria possess a formidable outer membrane (OM) that is composed of an asymmetric bilayer of phospholipids and lipopolysaccharides (Delcour, 2009; Flaxman *et al*., 2020). The OM of Gram-negative bacteria is the main reason for resistance to a wide range of antibiotics, including β-lactams, quinolones, colistins, and other antibiotics (Breijyeh *et al.,* 2020). To circumvent such challenges, alternative strategies such as conjugating antibacterial agents to siderophore molecules that chelate iron (Fe^{3+}) ions have been investigated (Dauner and Skerra, 2020). Bacteria require Fe^{3+} for proliferation and may take up the conjugates of the siderophores and the antimicrobial agents into its periplasm or cytoplasm (Tavassoli-Kafrani *et al*., 2019). The mangiferin compound possesses a long, non-polar, hydrophobic alkyl saturated chain (Zhang *et al.,* 2016), potentially useful for membrane insertion (Qais *et al*., 2020). This characteristic might afford the mangeferin compound to be an ideal siderophore conjugating agent that could bypass the protective OM bilayer of the Gram-negative bacteria (Sridharan *et al.,* 2020). Future studies exploring these aspects together with the isolation of lead compounds from *Mangifera indica* fruit and bark extracts may result in a greater potency against Gram-negative bacteria.

The antibacterial activity in the leaves of *Mangifera indica* leaves can be attributed to the presence of steroids, tannins, flavonoids and phenols. These compounds are good antifungal and antibacterial agents which may play a role in the antibacterial properties in the leaves of *Mangifera indica* (Verma and Verma *et al.,* 2017; Seleshe and Kang, 2019; Cudjoe *et al.,* 2020). Though this study suggests antibacterial potential, future studies should include more bacterial strains which will highlight the antibacterial activity on a broader scale and possibly motivate for the use of *Mangifera indica* in future drug developments.

4.5.4 *In vitro* anti-oxidants assay

Anti-oxidants analysis is done by intercepting reactive oxygen species to prevent or lessen the radicals and to produce less aggressive chemical species that are likely to cause tissue damage (Kumar *et al.,* 2020). The use of anti-oxidants has garnered much attention due to their protective effect against

damage from reactive oxygen species (Mohan *et al.,* 2014). These solutions had discoloured from purple to a faded solution. A purple-coloured solution visible in the DPPH assay accepts electrons, which then converts to a discoloured solution (Bouyahya *et al.,* 2017; Breijyeh *et al.,* 2020; Itoh *et al.,* 2020). The point of colour change is linked to the effectiveness and concentration of anti-oxidants present (Herrera-Calderon *et al.,* 2018). The amount of discolouration indicates the free radical scavenging action (Kumar *et al*., 2014).

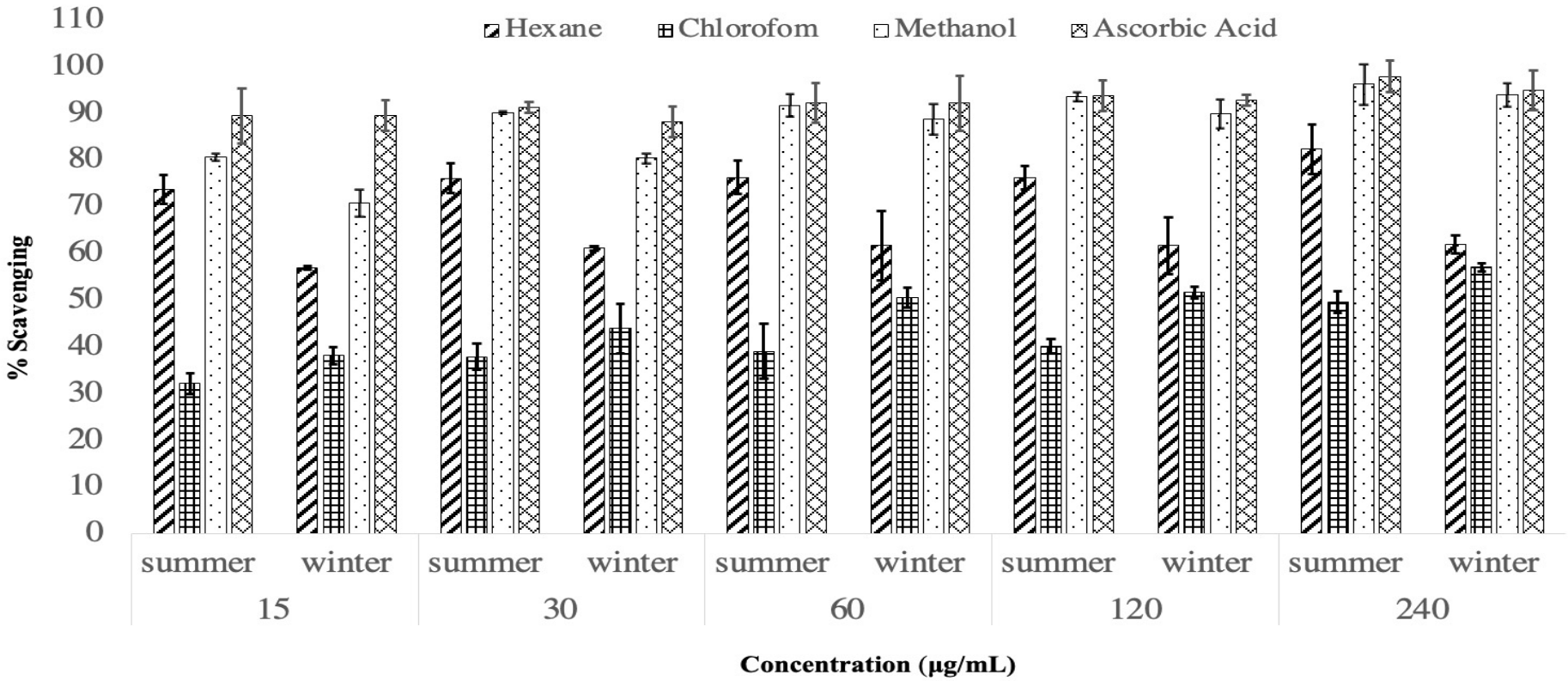

Figure 4.1: Mean and standard deviation of DPPH Free radical scavenging activity of hexane, chloroform, and methanol from leaves of *Mangifera indica L* at various concentrations for summer and winter seasons. (Two-way-ANOVA multivariate Tukey's honest significant difference multiple range post hoc test P< 0. 05 IBM SPSS version 25. Each bar was considered statistically significant when comparing each extract to the ascorbic acid at different concentrations, 15-240 µg/mL). Data are presented as means ± SD, *n* = 3 and displayed as a percentage of the control sample.

The DPPH free radical scavenging activity was evaluated by the decrease in absorbance at 516 nm, which is induced by anti-oxidants (Manjula and Ganthi, 2018). This assay is not specific to any precise class of anti-oxidants and therefore provides the general anti-oxidants capacity of the extract (Tepe *et al*., 2007). The percentage free radical scavenging activity of crude extracts of the leaves of *Mangifera indica* for summer and winter is presented in Figure 4.1. The radical scavenging activity of the crude extracts was studied by its ability to reduce DPPH (stable radical) and any molecule that may donate hydrogen or electron to DPPH (Patel *et al*., 2012). The electron-donating ability of *Mangifera indica* is most determined using DPPH free radical scavenging tests due to its reliability (Dowlath *et al.,* 2020). There was a dose-dependent change in radical scavenging activities for all crude extracts *i.e.,* in general all extracts, with increasing concentration, showed an increase in the DPPH radical scavenging activity (Figure 4.1). Statistical analysis showed all extracts had significantly different activity across all concentrations (P <0.05) when compared to the ascorbic acid, 15-240 µg/mL.

The methanolic leaf extracts for summer and winter displayed more effective radical scavenging activity than the hexane and chloroform plant extracts, with inhibition at 96.17 and 93.89%, respectively. Between summer and winter, summer exhibited the better radical scavenging activity. Dose-response radical scavenging activities were also observed in the methanolic extracts of different parts of *Mangifera caesia*, *Mangifera pajang* and *Mangifera linearifolia* (Amoo *et al*., 2011). The scavenging activity of the methanolic extracts compared with the standard ascorbic acid suggests that the leaves of *Mangifera indica* are also an effective scavenger of free radicals (Nithitanakool *et al.,* 2009; Joona *et al.,* 2013; Dhital *et al.,* 2017). Free-radical reactions are linked to the pathology of several diseases such as cancer, Alzheimer's, and inflammation (Houghton *et al*., 2007). Kumari *et al.* (2017) observed the DPPH radical-scavenging activity of the methanolic leaf extracts of *Mangifera linearifolia*. The authors observed IC_{50} values of the methanol leaves as 48.86 µg/mL and hexane 60.82 µg/mL. This trend corresponds with the current study where methanol extracts have a better radical-scavenging activity than hexane. Overall, the results obtained in this study indicated that the hexane and methanolic extracts displayed good radical scavenging activity, which was a lower amount compared to the standard ascorbic acid (97.86%).

Table 4.4: IC_{50} values of hexane, chloroform, and methanolic extracts from the leaves of *Mangifera indica*, ascorbic acid used for the DPPH assay.

	Extraction solvents (IC_{50} - µg/mL)		
Seasons	**Hexane**	**Chloroform**	**Methanol**
Summer	19.53	70.13	12.71
Winter	22.32	93.08	14.35
Ascorbic acid		3.20	

Note: IC_{50} value represents the concentration of sample required to inhibit 50% of DPPH free radical. The IC_{50} of the DPPH assay of isolated from the crude hexane, chloroform, and methanolic extract of the leaves of *Mangifera indica.* Data are presented as mean, *n*= 3, of triplicate determinates.

The methanolic and hexane crude extracts for summer and winter showed the most convincing radical scavenging capacity with an IC_{50} value for summer extracts being 19.53 and 70.23 µg/mL and 12.71 µg/mL for hexane, chloroform, and methanol, respectively. The winter extracts IC_{50} value was 22.3 µg/mL, 93.08 µg/mL and 14.35 µg/mL for hexane, chloroform, and methanol respectively when compared to ascorbic acid, which had an IC_{50} of 3.202 µg/mL (Table 4.4). For the summer and winter extracts, hexane and methanol showed the better scavenging capacity than chloroform. Overall, summer has a better scavenging capacity than winter with respect to the control. Studies done by Born *et al.* (1996); Mohan *et al*. (2013), and Itoh *et al*. (2020), all ran tests for methanolic and hexane extracts with results that correspond with this study where anti-oxidants activity was more potent for hexane and methanol extracts. Born *et al.* (1996) had IC_{50} values for hexane and methanol of 13.45 and 11.31 µg/mL, respectively, compared to ascorbic acid, which had an IC_{50} of 29 µg/mL. Mohan *et* al. (2013) had IC_{50} of 35.05 µg/mL and 32.45 µg/mL respectively when compared to ascorbic acid, which had an IC_{50} of 22.11 µg/mL, and Itoh *et al*. (2020) had IC_{50} of 6.87 and 5.76 µg/mL respectively when compared to ascorbic acid, which had an IC_{50} of 1.30 µg/mL. A study by Ocampo *et al*. (2020) also revealed that the other parts of *Mangifera indica* also exhibit significant anti-oxidants activity as well as the leaves, which supports the findings in this study (Ocampo *et al*., 2020).

The anti-oxidant activity in the leaves of *Mangifera indica* can be attributed to the presence flavonoids, terpenes and phenols. Phenolic compounds are strong anti-oxidants that can prevent the tissue damage caused by the free radicals (Rupasinghe *et al*., 2016). Since the extracts contain a significant amount of phenol content, it might be responsible for their anti-oxidants property (Xu *et al.,* 2008; Kumar *et al.*, 2014; Ocampo *et al*., 2020). The flavonoids act through the scavenging or chelating process and help

in reducing the effect of anti-oxidants (Kumar *et al.*, 2014). The flavonoid content present in the extracts may also contribute to their anti-oxidants activity.

Though this study suggests anti-oxidants potential, it is suggested that at least three anti-oxidants activity assays should be conducted to determine the anti-oxidants activity of a compound to increase the validity of the experiment (Ocampo *et al*., 2020). Further investigations are also required to reveal other active constituents present.

4.6 Conclusion and future recommendation

There are some advantages of using antimicrobial compounds of medicinal plants that include fewer side effects, better patient tolerance, relatively less expensive, and its acceptance due to its long history of use and is renewable in nature (Vermani and Garg, 2002). Based on this, the search for new antibiotics continues with great urgency.

Various parts of *Mangifera indica* have been reported to have medicinal benefits and are used in traditional medicine. *Mangifera indica* has shown important biological properties that can be used to treat various diseases. The present study showed that the hexane and methanol extracts have significant anti-oxidants and antibacterial activities. The presence of important phytoconstituents such as alkaloids, flavonoids, phenols, saponins, steroids, and many more may be responsible for their potent biological properties. Bioassay-guided fractionation, isolation of specific bioactive compounds from the leaves and the evaluation of their safety will be necessary to further explore this species for potentially new therapeutic drug leads. This could perhaps aid in underpinning the specific compounds responsible for the various pharmacological activities. Findings from this study would significantly contribute to the advancement of natural compounds for potential use in the healthcare sector.

4.7 References

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CHAPTER 5

CONCLUSIONS, CHALLENGES, AND RECOMMENDATIONS FOR FUTURE RESEARCH

5.1 Conclusions

This study was undertaken to characterise the general morphology, phytochemical screening, and biological activity of the leaf extracts of *Mangifera indica* L. SEM micrographs revealed the secretory structures documented within the leaves of *Mangifera indica* which include glandular and non-glandular trichomes. The combination of different microscopic techniques allowed for the identification of one kind of multicellular glandular peltate trichome and one type of non-glandular trichome which had cuticular warts. Glandular and non-glandular trichomes were found on both leaves of summer and winter. Histochemical tests indicated the presence of alkaloids, proteins, suberin, lipids, resin acids, gums and mucilage, and phenolics for both summer and winter leaves. The summer and winter leaves did not show any variation as the histochemical tests revealed positive results for both seasons. The preliminary phytochemical analysis further indicated the presence of several phytocompounds (10 compounds). This study ultimately suggests that leaf extracts of *Mangifera indica* contained numerous biologically active compounds such as alkaloids and phenolics. The major pharmacological compounds found in these extracts were phenols and alkaloids. These compounds identified, possess various biological properties of medicinal importance. Crude methanolic extracts for summer and winter positively inhibited Gram-positive, *S. aureus* (ATCC 43300) and Gram-negative, *E. coli* (ATCC 25922) bacteria. These results are suggestive that the leaves of *Mangifera indica* are rich in bioactive compounds which, could be a possible source of antibacterial agents in treating several diseases linked to the pathogenic bacteria studied. The preliminary phytochemical profile provided in this study could be further exploited for plant-based drug development. The crude extracts also displayed varying degrees of anti-oxidant activities and were dose-dependent. The presence of anti-oxidant activity was exhibited in hexane and methanol extracts for summer and winter for which hexane and methanolic extracts showed the most convincing radical scavenging capacity.

The various preliminary screening conducted in this study provides a basic understanding of the importance of the investigated medicinal plant as a potential source of novel and useful drug leads. Preliminary research achieved in this study will contribute to the growing ethnopharmacological field in South Africa. To our knowledge, this study is the first report combining the morphology, phytochemical, and biological activity of *Mangifera indica* leaves. Thus, there is a wide scope for future research in this field, especially in South Africa since there are very scarce studies done on *Mangifera indica* in the country.

5.2 Challenges

There have been various challenges that had impacted this project during the year 2020-2021. Several instruments required to complete experimental work in this project were not operational at the microscopy microanalysis unit from 2020 to date, specifically the SEM and TEM. In addition, a major setback was due to a fire that had occurred in the Life Science building on the 5th floor at the University of KwaZulu-Natal. This fire had taken place in December 2018 which destroyed the entire west wing of the 5th floor. This resulted in no lab space, equipment, chemicals, or resources were destroyed or contaminated. This made it very difficult to complete work and conduct experiments when it was possible in 2020 (before and after the closure of the University due to COVID-19). To date, the 5th floor is not operational and is under construction. This was a major setback.

Furthermore, the beginning of the academic year for 2020 began with several challenges. Violent student protests commenced from the 27th of January until the 20th of February 2020. This disruption was a major setback as experimental work could not continue and the University had suspended the academic program. Whilst the academic program was suspended, postgraduate students were not allowed to continue their experimental work due to safety issues. For the academic year 2020, nearly 20 working days were lost due to the student protest action. This hindered experimental work as various assays could not be completed. Thereafter, the COVID-19 pandemic devastated South Africa, which resulted in the total suspension of academic activity and the closure of the University of KwaZulu-Natal from the 16th of March 2020 until July 2020. Upon returning to campus during COVID-19 and due to several protocols been in place for social distancing etc., there was limited access to research space and equipment. Strict access to various instruments/ lab space was only allowed to residing students of specific departments (Microbiology, Biochemistry, and Chemistry). The final challenge experienced was due to the looting which occurred from the 9thth of July 2021 to the 18th of July 2021. This stopped all work due to safety as the circumstance was highly stressful and scary. Through these challenges I have tried to stay motivated and have had sleepless nights to complete all lab work and writing to meet my deadlines.

5.3 Future perspectives

The genus *Mangifera* is of great medicinal importance throughout the world. However, there is a lack of knowledge of some species within the family which provides an opportunity for further studies. Ultra-structural studies on the leaf structures should further examine the internal features of cells and organelles present, as well as quantify the phytocompounds present and run toxicological tests on the leaves of *Mangifera indica* such as evaluating the cytotocic and apoptotic effect the leaf extract may have on cancer cell lines. This will provide insight on the effect this species has on human cells. It will be important to investigate other plant parts such as the flowers, roots, and stems of *Mangifera indica* for similar pharmacological activities to ensure proper use of the plant. Phytochemical screening of leaf extracts revealed the presence of various compound classes of medicinal importance emphasising *Mangifera indica* as a suitable candidate for further isolation and purification of medicinally active compounds which can lead to sustainable drug development. The isolation and identification of phytocompounds in other parts of the investigated plant should also be undertaken and subjected to antibacterial, anti-oxidants, and cytotoxicity assays.

Other solvents used in phytochemical extractions such as ethanol and acetone can also be subjected to antibacterial, anti-oxidants assays to evaluate their potency. Further research should be conducted on the mechanisms involved in cell death induced by the extracts from *Mangifera indica* on the various cancer cell lines. Plant extracts of *Mangifera indica* should also be utilised to synthesise silver-nanoparticles (AgNPs) and possibly further be used to synthesise other metals such as gold or copper and subjected to the various pharmacological assays. More detailed antibacterial studies are required to determine minimum inhibitory concentration (MIC) values against the microorganisms by implementing more microbial strains. Overall, further investigations are necessary to fully explore *Mangifera indica* for novel therapeutic compounds or drug leads.

5.4 Closing Remarks

This project has produced relevant data regarding *Mangifera indica* as a medicinal plant. This study will foster a building block into the therapeutic properties of this plant species.

Appendix

Table 5: Anti-oxidant raw data, mean ± standard deviation leaf extracts of *Mangifera indica* L. for summer and winter seasons.

Summer samples (Hexane)					
Concentration	Replicates (nm)				
	1	2	3	Mean	SD
15	0,228	0,213	0,293	0,2447	0,0425
30	0,333	0,326	0,325	0,3280	0,0044
60	0,270	0,352	0,282	0,3013	0,0443
120	0,3271	0,3263	0,3379	0,3304	0,0065
240	0,3992	0,3069	0,3822	0,3628	0,0491
Winter samples (Hexane)					
Concentration	Replicates (nm)				
	1	2	3	Mean	SD
15	0,4869	0,6080	0,6895	0,5948	0,1019
30	0,5620	0,5524	0,4988	0,5377	0,0341
60	0,4707	0,4917	0,6259	0,5294	0,0842
120	0,5333	0,4753	0,6210	0,5432	0,0734
240	0,5251	0,5524	0,4988	0,5254	0,0268
Summer samples (Chloroform)					
Concentration	Replicates (nm)				
	1	2	3	Mean	SD
15	0,9537	0,9538	0,9018	0,9364	0,0300
30	0,8303	0,8643	0,8777	0,8574	0,0244
60	0,7991	0,8750	0,8524	0,8422	0,0390
120	0,8666	0,7417	0,8710	0,8264	0,0734

240	0,6283	0,6754	0,7863	0,6967	0,0811
Winter samples (Chloroform)					
Concentration	Replicates (nm)				
	1	2	3	Mean	SD
15	0,8209	0,8809	0,8605	0,8541	0,0305
30	0,7947	0,7757	0,7508	0,7737	0,0220
60	0,6839	0,6661	0,6986	0,6829	0,0163
120	0,6841	0,6897	0,6305	0,6681	0,0327
240	0,5962	0,6032	0,5809	0,5934	0,0114
Summer samples (Methanol)					
Concentration	Replicates (nm)				
	1	2	3	Mean	SD
15	0,2705	0,2563	0,2780	0,2683	0,0110
30	0,1123	0,1821	0,1136	0,1360	0,0399
60	0,1178	0,1170	0,1103	0,1150	0,0041
120	0,1022	0,0720	0,0934	0,0892	0,0155
240	0,0730	0,0146	0,0709	0,0528	0,0331
Winter samples (Methanol)					
Concentration	Replicates (nm)				
	1	2	3	Mean	SD
15	0,3566	0,4471	0,4056	0,4031	0,0453
30	0,2668	0,2621	0,2854	0,2714	0,0123
60	0,1837	0,1051	0,1770	0,1553	0,0436
120	0,1017	0,2100	0,1085	0,1401	0,0607
240	0,0546	0,1224	0,0760	0,0843	0,0347

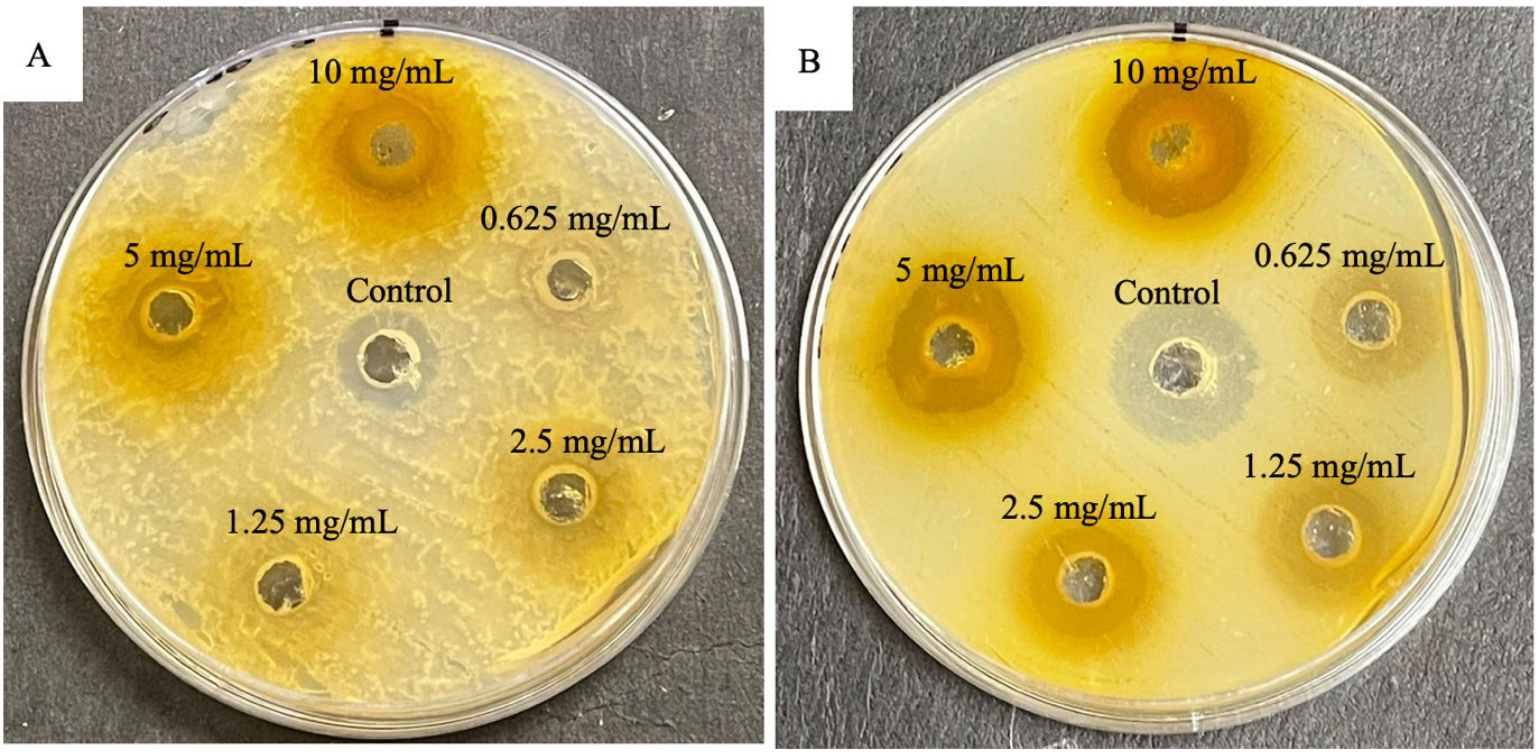

Figure 5: Plates displaying antibacterial activity of methanolic crude extract from the leaves of *Mangifera indica* L. for summer. A: Gram-positive *Staphylococcus aureus* (ATCC 43300) bacteria against control streptomycin; B: Gram-negative *Escherichia coli* (ATCC 25922) bacteria against control gentamycin.

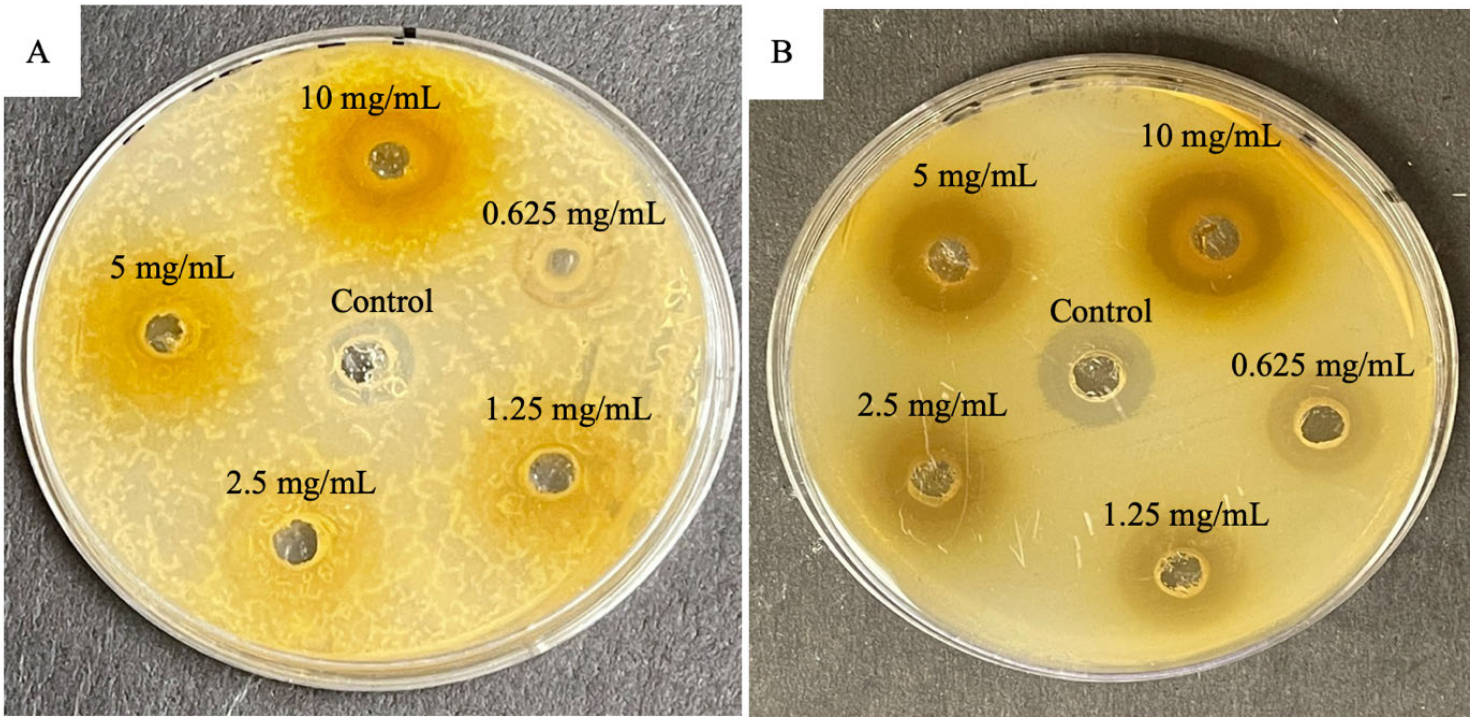

Figure 6: Plates displaying antibacterial activity of methanolic crude extract from the leaves of *Mangifera indica* L. for winter. A: Gram-positive *Staphylococcus aureus* (ATCC 43300) bacteria against control streptomycin; B: Gram-negative *Escherichia coli* (ATCC 25922) bacteria against control gentamycin.