The Role of Traditional Health Practitioners and the Effects of *Salix mucronata* on the Treatment of Negative Symptoms of Schizophrenia in KwaZulu-Natal, South Africa.

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PREFACE

This study, entitled 'The Role of Traditional Health Practitioners and the Effects of *Salix mucronata* on the Treatment of Negative Symptoms of Schizophrenia in KwaZulu Natal, South Africa', is divided into two components. The first component sought to document the role of the traditional health practitioners (THPs) in the treatment of the negative symptoms of schizophrenia. This component of the study was conducted in four municipalities in the Harry Gwala district; *viz.* Greater Kokstad, uMzimkhulu, Buhlebezwe and Dr. Nkosazana Zuma of the KwaZulu-Natal province in South Africa.

The second component sought to highlight traditional medicines used by THPs for the management of mental illness and examine their phytochemistry and cytotoxicity. It also aimed to investigate the effects of traditional medicine (*Salix Mucronata*) in the treatment of the negative symptoms of schizophrenia. This component was conducted in the Disciplines of Human Physiology and Chemistry, and the Biomedical Research Unit of the University of KwaZulu-Natal, South Africa.

This research project was supervised by Dr. Brenda De Gama from the Discipline of Clinical Anatomy while the animal experimental component of this research project was supervised by Prof. Musa Mabandla from the Discipline of Human Physiology. Both Disciplines are located in the School of Laboratory Medicine and Medical Sciences, College of Health Sciences, University of KwaZulu-Natal, South Africa.

DECLARATION

I, **Miss Ntombifuthi Princess Ngubane**, declare as follows:

1. That the work described in this thesis has not been previously submitted to the University of KwaZulu-Natal or any other tertiary institution for purposes of obtaining an academic qualification, whether by myself or any other party. The research work presented in this thesis is original work unless otherwise stated.
2. This thesis does not contain text, data, pictures, tables, graphics or other information from other persons' work or copied and pasted from webpages, unless specifically acknowledged, and the source being detailed in the thesis and in the relevant reference section.
3. This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other sources. Where other written sources have been quoted, then:
   - Their words have been re-written but the general information attributed to authors has been sourced.
   - Where their exact words have been used, their writing has been placed inside quotation marks and clearly referenced.

Candidate: _____ Date: _____

As the candidate's supervisors, we hereby approve this thesis for submission.

Supervisor: _____ Date: _____

(Dr. Brenda De Gama)

Co-Supervisor: _____ Date: _____

(Prof. Musa Mabandla)

DEDICATION

If love could give you a ticket to life, you would still be here, for the love we have for you is the undying kind of love. To my late father Mr. Mxolisi Norris Ngubane, for being a present father and for always going above and beyond your call of duty as a parent. Of all the chapters and sections of this thesis, this part was the hardest to write because I had never imagined that you would not be here to witness the fruits of your hard labour. As I have always said to you when you were still with us, I will repeat, I would not have come this far in life if it hadn't been for you and uMaMchunu. What hurts the most is that you will not be present to witness me wearing that red gown and your constant reminder that you could not wait to call me Doctor. Nonetheless, I can imagine the joy and contentment that completing this journey would have brought you. May you continue to rest in eternal peace and may the love you had for us and the love we have for you continue to comfort and strengthen us. Usebenzile Ngubane, Nomafu, Myaluza, Ngcukumane, Zikode elimhlophe, Matomela, Nzombane, Ngogozabantu, Nomasikisiki, nyon'esindwa isisila sayo, Mbovu! (1964-2020)

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Romans 8:38-39

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LIST OF ABBREVIATIONS AND ACRONYMS

ACh	Acetylcholine
APD/s	Antipsychotic Drug/s
AREC	Animal Research Ethics Committee
CAM	Complementary and Alternative Medicine
$Ctrl^{APD}$	Control group (Antipsychotic Drug)
$Ctrl^{SAL}$	Control group (Saline)
$Ctrl^{TM}$	Control group (Traditional Medicine/Herbal Extract)
D1 receptor	Dopamine receptor D1
D2 receptor	Dopamine receptor D2
DA	Dopamine
DCM	Dichloromethane
DMS	Diagnostic and Statistical Manual of Mental Disorders
DoH	Department of Health
ELISA	Enzyme-linked immunosorbent assay
EtOH	Ethanol
Exp^{APD}	Experimental group (Antipsychotic Drug)
Exp^{SAL}	Experimental group (Saline)
Exp^{TM}	Experimental group (Traditional Medicine/Herbal Extract)
GC-MS	Gas Chromatography Mass Spectrometry
H_2O	Water
HGDM	Harry Gwala District Municipality
HIV	Human Immunodeficiency Virus
HSSREC	Humanities and Social Sciences Research Ethics Committee
MeOH	Methanol
MHPF	Mental Health Policy Framework
MTT Assay	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide Assay
NMDA	N-methyl-D-aspartate
PANSS	Positive and Negative Syndrome (or Severity) Scale
PFC	Prefrontal Cortex

RIS	Risperidone
SP%	Sucrose preference percentage
StatsSA	Statistics South Africa
TAAR-1	Trace Amine-Associated Receptor 1
THPs	Traditional Health Practitioners
TM	Traditional Medicine
VTA	Ventral Tegmental Area
WHO	World Health Organisation
WM	Western Medicine

GLOSSARY

IsiZulu	**English Alternate**
Amafufunyana	culture-specific syndrome characterised as the possession by evil spirits (Niehaus *et al.,* 2004), also classified as "hysterical psychosis" or schizophrenia (Molot, 2017)
Inyanga	herbalist – mainly prescribes herbal remedies for treatment of illness (Zuma *et al.,* 2016)
Isangoma	diviner – uses divination to determine the cause of illness (Zuma *et al.,* 2016)
Ubizo/ukuthwasa	ancestral calling to be a traditional healer (Ogana and Ojong, 2015)
Ukuhlanya/ukuphambana	madness or insanity (Donda, 1997)
Umthandazi	faith healer – mainly uses water and prayer for healing (Zuma *et al.,* 2016)
Umuthi	Medicine or tree (Donda, 1997); herbal medicine (Mbendana *et al.,* 2019)

THESIS OUTLINE

This thesis contains six chapters and is presented in the manuscript/paper format. In accordance with the approved University of KwaZulu-Natal, College of Health Sciences' guidelines for presentation of theses, an interlinking page is included between all of the chapters to ensure a logical presentation and interrelation of the findings of this study. The thesis contains the introduction and literature review, a review paper, quantitative and qualitative papers from the first component of the study, two experimental papers emanating from the second component of the study, synthesis, and also a conclusion, limitations and recommendations.

Chapter One: This chapter provides the background and introduction to the study and includes the aim and objectives of conducting this research. In addition, it presents a review of literature and briefly discusses the methodology.

Chapter Two: This chapter is an expansion of Chapter One and is presented as a systematic review paper. The review paper is titled "Global Perspectives on the Traditional Approaches used in the Treatment of Schizophrenia: A Systematic Review". This paper provides a unique literary perspective on how medicinal plants and other indigenous methods are used in the treatment of schizophrenia which may be referred to as mania, madness, psychosis and *ufufunyana/amafufunyana* in different parts of the world. It was published online in the Asian Journal of Psychiatry on the 22nd of May 2024 (https://doi.org/10.1016/j.ajp.2024.104081).

Chapter Three: A paper reporting on the quantitative data collected in this study is included in this chapter. The paper is titled "A quantitative Evaluation of Traditional Health Practitioners' Perspectives on Mental Disorders in KwaZulu-Natal: Knowledge, Diagnosis, and Treatment Practices". It provides socio-demographic profiles of the traditional health practitioners from the Harry Gwala District and contributes knowledge on how mental disorders are diagnosed and treated in the traditional healing community. This paper was published online in the Journal of Spirituality in Mental Health on the 27th of March 2023 (https://doi.org/10.1080/19349637.2023.2194561).

A paper presenting the qualitative data collected from the focus group discussions in this study is also included in this chapter. The paper is titled: "The Influence of Culture on the Cause, Diagnosis and Treatment of Serious Mental Illness (*Ufufunyana*): Perspectives of Traditional Health Practitioners in the Harry Gwala District, KwaZulu-Natal". It contributes knowledge on how culture and spiritual beliefs influence the explanations used by THPs regarding the causation of schizophrenia, and traditional methods used by the THPs to diagnose and treat this disorder. This paper was published online in the Culture, Medicine, and Psychiatry journal on the 23rd of June 2024 (https://doi.org/10.1007/s11013-024-09863-7).

Chapter Four: This chapter contains an experimental manuscript titled: "Phytochemical Screening and Cytotoxic Effect of Herbal Plants Used by Traditional Health Practitioners in KwaZulu-Natal for the Treatment of Schizophrenia". This manuscript discusses the phenolic contents from the methanol, ethanol and DCM extracts of the seven medicinal plants used to treat schizophrenia and also reports on the cytotoxicity levels in the different extract of these plants. This manuscript is currently under review in the South African Journal of Botany and was submitted on the 5th of February 2024 (Manuscript Number: SAJB-D-24-00451R1).

Chapter Five: This chapter contains an experimental paper titled "The Antipsychotic Potential of *Salix mucronata* on Ketamine-induced Rats". It presents the differences in sociability and anhedonic behaviour between rats that were treated with traditional medicine (i.e. *Salix mucronata*) and others treated with the antipsychotic drug (risperidone). The effect of traditional medicine and the antipsychotic drug in the hippocampus and the prefrontal cortex is also reflected and discussed. This paper was published online in the IBRO Neuroscience Reports journal on the 14th of June 2024 (https://doi.org/10.1016/j.ibneur.2024.06.003).

Chapter Six: This, the final chapter, presents the synthesis of this study which provides a concise summary of the main findings of each of the papers. It also includes how each of the papers addresses the aim and objectives of the study and the limitations experienced in the protocols of the study. Thereafter, it offers associated recommendations for future studies.

ABSTRACT

Introduction: The negative symptoms of schizophrenia overlap with the symptoms presented in conditions associated with witchcraft, evil spirits and ancestors in the African healing setting. The cornerstone biomedical treatment for schizophrenia is antipsychotics which are associated with adverse side effects. Traditional healing employs a holistic approach when managing physical and mental ailments, and encompasses cultural and spiritual beliefs. Hence, its preferability in African communities. Thus, this study aimed to investigate the role of Traditional Health Practitioners (THPs) and traditional medicine in the treatment of negative symptoms of schizophrenia by investigating the THPs' methods of diagnosis and treatment. The herbal plants used by THPs were investigated for phytochemistry and cytotoxicity, and *S. mucronata* was further investigated for its antipsychotic activity which was compared to the activity of the currently used antipsychotic drug, risperidone. **Methodology:** Self-developed questionnaires were administered to 100 randomly selected THPs from the Harry Gwala District Municipality and focus group discussions were conducted with 31 of the THPs with experience in treating mental illness. Phytochemistry and cytotoxicity of herbal plants recommended by the THPs were investigated using GC-MS and the MTT assay, respectively. *S. mucronata*, was further tested for antipsychotic potential on ketamine-induced Sprague-Dawley rats. The animal component of the study consisted of a total of 66 male adult rats (n=21 formed the experimental groups and n=45 formed the experimental groups which received 30 mg/kg of ketamine). During the treatment phase, the animals received either saline, risperidone (6 mg/kg) or the herbal extract of *S. mucronata* (5 mg/kg). This plant was selected since there is a paucity of studies reporting on its potential for the treatment of this disorder. Three-chambered sociability and sucrose preference tests were used to examine asociality and anhedonia, respectively. The concentrations of dopamine and acetylcholine were measured using ELISA as the dysfunction of these neurotransmitters is linked with the pathophysiology of schizophrenia. **Results:** Consultation with ancestors and the administration of a medicinal concoction were the common methods employed by the THPs to diagnose and treat schizophrenia. A variety of bioactive compounds were identified from herbal plants recommended by the THPs for treatment of schizophrenia and these plants showed no cytotoxic effects. *S. mucronata* reversed social withdrawal and promoted social novelty in ketamine-induced rats. **Conclusion:** THPs play a significant role in the treatment of schizophrenia and traditional medicine (TM) had a positive effect in the treatment of this disorder. The herbal plants used by THPs to treat mental disorders in KwaZulu-Natal can be considered safe for use as they have indicated non-cytotoxicity and may have a number of benefits as they possess a variety of beneficial bioactive compounds. *S. mucronata* showed antipsychotic activity similar to the effects of risperidone.

CHAPTER 1

1. INTRODUCTION

1.1 Background

Mental health had been neglected until the establishment of human rights for those with mental disorders. Access to the mental healthcare system was then focused on and transformed by the Mental Health Act of 2002 in South Africa (Foster *et al.,* 1997; Stein, 2014; Raphalalani *et al.,* 2021). It was further improved through the adoption of the Mental Health Policy Framework (MHPF) for South Africa and the Strategic Plan 2013-2020 by the National Health Council (DoH, 2013; Stein, 2014), while the current Strategic Plan 2020/21-2024/25 by the National Department of Health aims to promote mental health in order to reduce premature mortality from chronic or non-communicable diseases as mental health is fundamental in reducing the global burden of these diseases (NDoH, 2020; Stein *et al.,* 2019). Nonetheless, mental and behavioural disorders are increasingly becoming a concern worldwide, as they affect over 400 million individuals, the majority of whom are from developing countries, with fewer than 10% having access to mental healthcare (WHO, 2003; StatsSA, 2017; Bifftu *et al.,* 2018). In the South African population, approximately 27% of individuals who are diagnosed with severe mental disorders do not receive treatment due to the associated stigma which may also be an indication that mental health is still not considered a priority within the healthcare system (StatsSA, 2017; Nguse and Wassenaar, 2021).

South Africa is estimated to have a 16.5% prevalence rate of four mental illnesses or neuropsychiatric disorders (viz. substance abuse, depression, bipolar disorder and schizophrenia) which are reported to contribute to the leading causes of years lived with a disability (Benjamin *et al.,* 2021). In particular, approximately 20 million individuals are diagnosed with schizophrenia globally (WHO, 2019). Of all mental disorders studied by Whiteford *et al.* (2013), schizophrenia received the highest disability weighting, reflecting it as the third leading cause of disability in South Africa (Spedding *et al.,* 2014). Schizophrenia is a neurodegenerative and severe psychiatric disorder which is characterised by positive, negative and cognitive symptoms (Carbon and Correll, 2014; Koster *et al.,* 2014; Schooler, 2020). The positive symptoms of schizophrenia include delusions and hallucinations, while negative symptoms include asociality, alogia and anhedonia, cognitive impairment includes lack of memory and attention (APA, 2013; Koster *et al.,* 2014; Deng and Xu, 2017). There are several theories on the pathophysiology of schizophrenia; however, the basic neurotransmitter marker is thought to be the dysregulation of dopamine (DA), with the four main dopaminergic pathways being the nigrostriatal, mesolimbic, mesocortical, and tuberoinfundibular pathways (Sonnenschein *et al.,* 2020, de Bartolomeis *et al.,* 2023). Positive symptoms of schizophrenia arise in the mesolimbic pathway due to an excess of dopamine being produced from the ventral tegmental area (VTA) to the limbic areas (Patel *et al.,* 2014). Conversely, low levels of dopamine (DA) in the mesocortical pathway from the VTA to the cortex are

linked to negative symptoms (Patel *et al.,* 2014, Shettima *et al.,* 2023, Velligan and Rao, 2023). The negative symptoms can also be worsened by the blockage of the D2 receptor in the mesocortical, nigrostriatal, and tuberoinfundibular pathways (de Bartolomeis *et al.,* 2023). Dopamine D2 partial agonists and antagonists are prescribed for the management of the positive symptoms; however, these have limited beneficial effects for the negative and cognitive symptoms and thus the latter symptoms have a pronounced illness burden (Kahn *et al.,* 2015). Antidepressants and atypical antipsychotic drugs are the currently used medication for the treatment of the negative and cognitive symptoms; however, these leave a majority of patients with persistent disabling side effects; namely, motor impairments, metabolic disturbances and cardiovascular complications (Kantrowitz, 2021). The lack of coherent and effective pharmacological medications for treatment of the negative symptoms of schizophrenia globally presents a seriously neglected necessity. Hence, alternative approaches, such as indigenous medical approaches, are explored.

Western psychiatry tends to not assign causality of mental illness to cultural beliefs; whereas, cultural beliefs, values and concepts play a significant role in influencing health-seeking pathways in traditional medicine, and Traditional Health Practitioners (THPs) play a major role in managing disease, including mental illness, in many cultures (Kaplan and Sadock, 1988; Versola-Russo, 2005). In South Africa, THPs, who include herbalists, diviners and faith healers (commonly referred to as *Izinyanga'*, *'Izangoma'* and/or *'abathandazi'* in IsiZulu, respectively), are individuals who are trained to dispense or apply locally prepared herbal remedies or medicines for treatment of various diseases (WHO, 2002; Ae-Ngibise *et al.,* 2010). Traditional medicine by definition is the combination of expertise and practices originating from indigenous experiences, beliefs and theories of different cultures which are used for diagnosis, treatment and prevention of mental and physical illness as well as overall maintenance of health (WHO, 2002; WHO, 2013; Abbo, 2011; Mokgobi, 2014). In Sub-Saharan Africa, THPs are recognised as having unique skills to manage various conditions involving or caused by spells, spirits, sorcery and social misconduct which allopathic clinicians lack the skill or ability to treat (Kale, 1995; Pinkoane, 2005; Makundi *et al.,* 2006; Audet *et al.,* 2017). Audet *et al.* (2012) suggested that the other advantages held by THPs compared to allopathic clinicians are that THPs speak languages typically preferred by patients, live closer to patients and are often available to meet without any difficulty to discuss causes of illness, diagnoses as well as treatment or management of illnesses.

South Africa is one of the countries with access to both biomedical (public and private) and traditional medical services; hence, the population may choose one of the two or seek both (Puckree *et al.,* 2002; Moshabela *et al.,* 2011). Despite public healthcare being funded by the government, the quality of healthcare is negatively impacted by factors such as old and neglected facilities, poor quality, poor

disease control and prevention practices, and with long waiting periods when compared to private healthcare (Young, 2016; Maphumulo and Bhengu, 2019). Thus, traditional medicine tends to be the preferred option as it is recognised to be more acceptable, affordable and accessible (Oyebode *et al.,* 2016). According to Biswal *et al.* (2017), every society is deeply influenced by its own culture which impacts every individual. As hypothesised by Glick (1967), establishing the principal sources of power (whether religious, political or social) of a society offers an understanding of its culture and beliefs about the cause and treatment of illness. THPs are known to employ a holistic approach to health and illness with a deep connection between the spirit, mind and the body, which makes their treatment approaches effective, with patients rating themselves as cured after treatment (Kapur 1975; Kleinman and Sung, 1979; Incayawar, 2008; Lucana and Elfers, 2020). However, contention surrounds the magical and mystical explanations related to this type of healing and the safety of the traditional medicines remains questionable as little is known and understood in this area. In addition, the safety and efficacy of using both biomedical and traditional medicine concurrently is also questionable (Green and Makhubu, 1984; Freeman and Motsei, 1992; Moshabela *et al.,* 2016).

1.2 Problem Statement

Mental health disorders affect approximately 1 in 8 individuals globally and include anxiety, major depressive, bipolar, post-traumatic stress, eating, disruptive behaviour and dissocial disorders, schizophrenia and neurodevelopmental disorders (WHO, 2022). Approximately 5% of the global population, with the rate estimated to be 5.4% in the African region, and estimates of 4.6% people in South Africa suffer from depression (WHO, 2017; WHO, 2023) while bipolar disorder is experienced by over 1% of people globally (Miola *et al.,* 2022). Substance abuse involving alcohol has led to disability-adjusted life years in 132.6 million people and has contributed 5.3% to overall deaths worldwide (WHO, 2018). Additionally, WHO reports that approximately 24 million or 1 in 300 people are affected by schizophrenia globally (WHO, 2022). Schizophrenia is not as common as other mental disorders, but it has been reported that 40-60% of the individuals diagnosed with schizophrenia are most likely to die prematurely when compared to the general population (van den Heuvel *et al.,* 2022; WHO, 2021; WHO, 2022). Hence, the focus on this disorder in this study. In addition, schizophrenia is ranked as one of the top ten illnesses which contribute to the global burden of disease and is one of the top five (along with HIV, drug use, heart disease and arthritis) leading causes of disability in South Africa and other developing countries (Ahmed and Azam, 2014; Spedding *et al.,* 2014; Wootton *et al.,* 2023).

The pathogenesis of schizophrenia as a mental disorder of focus in this study, is still unclear; however, the factors that have been reported to play a role in the development of this disorder are environmental, genetic and neurodevelopmental factors (Bialon and Wasik, 2022; Sanavi *et al.,* 2022). The cornerstone

treatment for this disorder comprises antipsychotic medications classified into typical (first generation) and atypical (second generation) drugs, with typical drugs primarily targeting the positive symptoms and do not reverse all the categories of the symptoms (Marder and Kirkpatrick, 2014; Brown *et al.,* 2022; Luther *et al.,* 2023). Moreover, the lack of therapeutic interventions which target the negative and cognitive symptoms may explain why patients often show incomplete functional recovery from schizophrenia (Schulz and Murray, 2016). In addition, approximately 40% of patients inadequately respond to the antipsychotic drugs used for the treatment of schizophrenia (Lowe *et al.,* 2018). Further, the side effects associated with these drugs can be severe and unbearable; viz. acute dystonias, weight gain, tardive dyskinesia, myocarditis and agranulocytosis, leading patients to withdraw from this type of treatment (Deng and Xu, 2017; Stroup and Gray, 2018; Crellin *et al.,* 2022). In order to explore new and effective drugs, an appropriate animal model which will simulate the symptoms observed in patients with schizophrenia needs to be explored. Hence, a pharmacological animal model for schizophrenia was included in this study.

The commonly prescribed antipsychotic drugs for the treatment and management of schizophrenia, particularly the negative symptoms, are risperidone and clozapine which are expensive, leading to their unaffordability, and therefore limiting their accessibility (Gilbody *et al.,* 1996; Barbato *et al.,* 1997; Stroup *et al.,* 2000; Sanz-Fuentenebro *et al.,* 2013). Specifically, in South Africa there is a vast majority of people with limited access to psychiatric care, especially in rural areas. Moreover, the psychologist to population ratio of approximately 2.5:100,000 is unlikely to close the existing treatment gap (Padmanabhanunni *et al.,* 2022). Factors such as affordability, location of mental facilities and the number of available and accessible psychologists in the country prove the orphanism of mental health in the healthcare system; hence, alternatives then need to be explored to solve this challenge.

Biomedical treatment approaches are often recognised as the preferred approach. However, traditional healing practices have been available for centuries and most African communities still strongly believe in their traditional religions and prefer these practices (Weru and Nafula, 2022). This is attested to by the over 80% of African populations and approximately 70% of the Black South African population relying on traditional medicine as a treatment option, and the approximately 60% of this population consulting with THPs (Zuma *et al.,* 2016). Between 40 and 60% of the reasons for consultation of THPs include mental health problems; however, there is minimal knowledge on the profiles and outcomes of the traditional approaches for treatment and prevention of mental illnesses or disorders in South Africa and globally (Abbo, 2011). Although studies have been conducted in South Africa focusing on access to traditional healers for mental disorders, the perceptions of indigenous healers and their methods for mental illness treatment (Edwards, 1986; Freeman and Motsei, 1992; Freeman *et al.,* 1994; Mbanga *et*

al., 2002; Nattrass, 2005), there is a paucity of studies which investigate how the THPs classify and diagnose different mental illnesses in South Africa. In addition, there is a scarcity of studies specifically focused on schizophrenia and the use of traditional medicine in its treatment. Further, little is published on THPs and their traditional treatment approaches for schizophrenia. Reports on the phytochemicals, cytotoxicity and effectiveness of herbal plants used in medicinal concoctions that are administered for the treatment of schizophrenia are also lacking.

1.3 Purpose of the Study

The aim of this study was to document the role of THPs in the treatment of schizophrenia by investigating the methods of diagnosis and treatment that they use for mental disorders, particularly the negative symptoms of schizophrenia. The study also aimed to investigate the phytochemicals and cytotoxicity of the herbal plants used by the THPs to treat schizophrenia. Additionally, it aimed to compare the effectiveness of the currently and commonly used pharmacological or biomedical medicine, the antipsychotic drug risperidone with the herbal plant, *Salix mucronata*, of traditional medicine that THPs utilise in the treatment of the negative symptoms of schizophrenia.

1.4 Objectives

The objectives of this study were as follows:

(a) To quantitatively investigate (through the administration of questionnaires) and document the methods used by THPs to diagnose and treat mental illnesses, particularly schizophrenia.

(b) To qualitatively investigate further (through focus group discussions) and document the THPs' perspective on how the explanation of mental disorders (viz. their cause, methods of diagnosis and treatment), particularly schizophrenia, is influenced by cultural beliefs.

(c) To investigate the phytochemicals and cytotoxicity of the medicinal plants used by THPs to treat schizophrenia.

(d) To investigate and compare the effectiveness of the medicinal plants used by THPs with the currently used antipsychotic drug risperidone in the treatment of schizophrenia through a pharmacological animal model, and further, to document behavioural changes, brain mass and volume, and changes in the concentration of dopamine and acetylcholine on the hippocampus and prefrontal cortex.

1.5 Key Research Questions

The following were the key research questions:

(a) Which traditional treatment methods are commonly used globally for the treatment of schizophrenia?
(b) Who are the THPs and what are their classifications (i.e. diviners, herbalists and/or faith healers)?
(c) What are the traditional methods of diagnosis and treatment used by the THPs in KwaZulu-Natal for the treatment of schizophrenia?
(d) How safe and effective are the traditional medicines used by traditional health practitioners to treat schizophrenia compared to the antipsychotic drug risperidone which is currently used for treatment of schizophrenia?

1.6 Conceptual Framework

This study adopted and referred to two healthcare models, namely, the Kleinman's explanatory model of illness framework (Kleinman *et al.,* 1978), and the bio-psycho-social-spiritual model (Cumella, 2002; Koenig, 2008; Leavey, 2010).

1.6.1 Kleinman's explanatory model of illness framework

As observed by Kleinman *et al.* (1978), every society has its own beliefs, approaches and choices of treatment, roles of practitioners and healthcare institutions arranged in the form of a cultural system. Therefore, healing cannot be separated from particular social and cultural contexts. Due to the incomplete comprehension of mental health disorders or illnesses by the scientific community, it is debatable whether explanatory models are sufficient to explain the mental health needs of patients across cultures as they have not been influential in the psychiatric practice routine (Saravanan, 2002). According to Mkabile and Swartz (2022), this model is suitable for the exploration of perspectives as it utilises a specific approach to construct the conceptions of the aetiology and management of illness by the THPs. In this study, this model informed the focus group questions in order to elicit the perspectives of the THPs about appropriate treatment for schizophrenia.

1.6.2 The Biopsychosocial-spiritual model

Individuals from non-Western cultures have been reported to possess a "holistic" concept of health which includes a spiritual aspect on the aetiology of disease (Tirodkar *et al.,* 2011). The main objective of the holistic model is to utilise all accessible diagnostic and treatment methods and procedures to

improve the health (physical, mental, emotional, social and spiritual) of a person at all levels of well-being without causing pain or harm to the person (Klinghardt, 2005). The holistic approach is often considered in traditional healing and in complementary and alternative medicine (CAM). Instead of treating the illness alone, this model attempts to treat the patient holistically. For this reason, the adoption of the biopsychosocial-spiritual model in this study was deemed as appropriate as it acknowledges the intersection between the biological or physical, psychological, social and spiritual aspects of well-being and care for a patient (Beng, 2004). The biopsychosocial-spiritual model is appropriate for capturing these intersecting aspects (Figure 1) (Cumella, 2002; Koenig, 2008; Leavey, 2010).

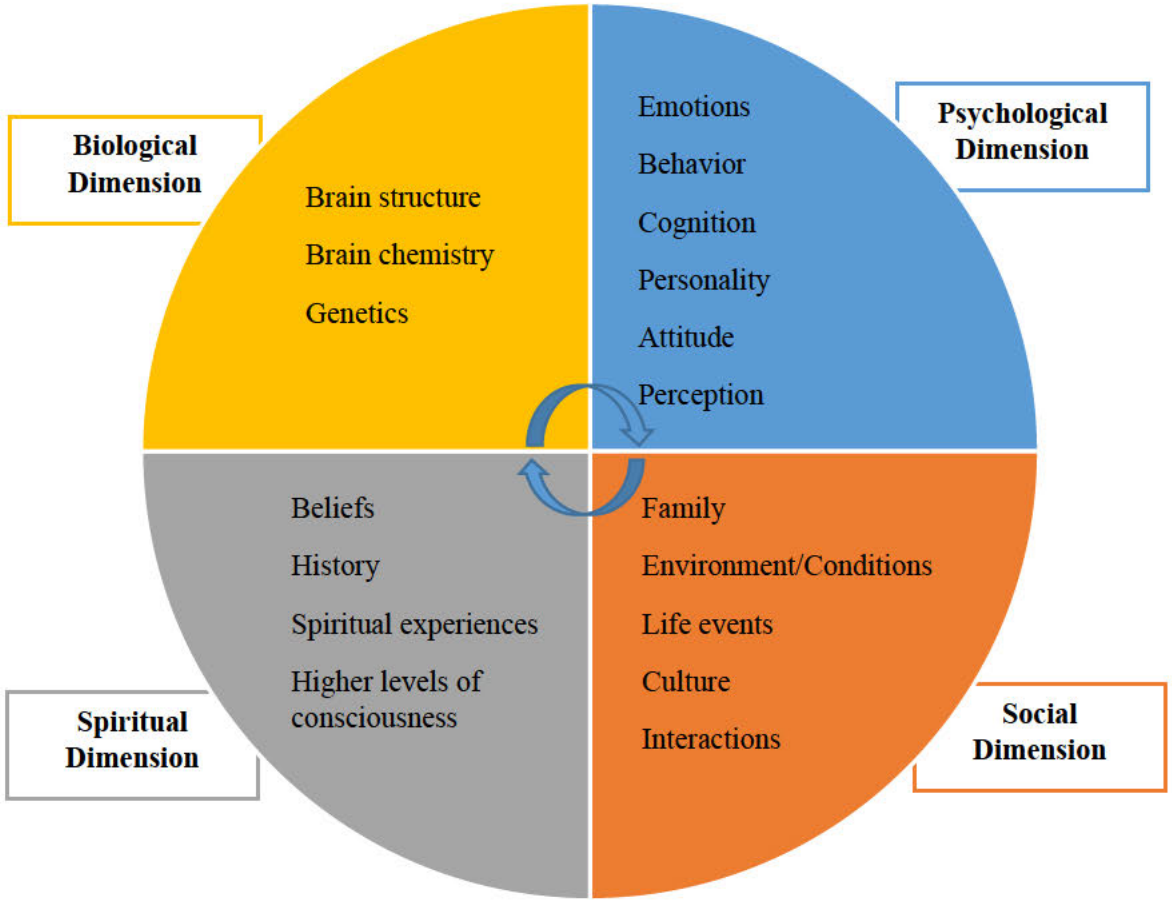


Figure 1: Showing the biopsychosocial-spiritual model *(Adapted from Murambidzi, 2016)*

According to Cumella (2002), spirituality is a crucial component of the 'psychosocial' portion of the biopsychosocial model as its inclusion of spirituality increases the evidence that health perceptions, choices and behaviours are influenced by spiritual beliefs. This approach is beneficial in modern medicine as it contributes to individual-centred care while promoting well-being with a focus on the biopsychosocial-spiritual components (Campos and Rodrigues, 2022). The model also suggests that in order to either increase the susceptibility of a person, or to reduce the person's protection against the

development of a specific mental illness, biological, psychological, social and spiritual factors within or outside the individual must interconnect with each other. Thus, all of these interconnected factors must be considered for a comprehensive treatment package as they all have a connection to the development, experience, explanation and management of specific mental illness (Cumella, 2002). This study investigates schizophrenia, a mental disorder that has common symptoms in observed illnesses that can only be spiritually and culturally explained in an African or traditional healing setting, hence the inclusion of this model. Social models of health take into consideration the patterns of health and ill-health within a society and variables such as age, sex and population group (Rumbold, 2007). Hence, by utilising this model it afforded understanding patterns between male and female THPs of different ages regarding their perspectives on the aetiology and treatment approaches for mental health within the Harry Gwala District.

It can be concluded that there is no healthcare model that can completely meet the different needs of individuals at the time of disease or illness on its own (Klinghardt, 2005). For this reason, this study utilises the various intersecting components of the two models in order to offer a comprehensive perspective on traditional medicine and schizophrenia. The Kleinman's explanatory model offers the society and cultural context of aetiology and treatment of this illness, while the Biopsychosocial-spiritual model adds insights into physical, mental, emotional and spiritual aspects for diagnostic and treatment methods used in traditional medicine approaches for the treatment of schizophrenia.

1.7 Literature Review

1.7.1 Mental Health

Mental health is defined as "a condition of well-being in which the person understands his or her own abilities, can cope with the usual demands of life, can work successfully and fruitfully, and is able to contribute to his or her community" (WHO, 2005). And by definition, health is "a state of excellent or perfect physical, mental, and social wellbeing, and not merely the absence of disease or infirmity" (WHO, 2019). According to the World Health Organization (2005), the concept of 'health' cannot exist without mental well-being, as a disturbance in mental health or the development of a mental disorder may be associated with an increased risk of communicable and non-communicable diseases, intentional and/or unintentional injury and sometimes premature death (Prince *et al.,* 2007; Plana-Ripoll *et al.,* 2019; WHO, 2019). Contrarily, the risk of developing a mental disorder may also be increased by various health conditions or diseases. The chronic illnesses that are more likely to lead to the development of a mental disorder include diabetes, heart disease, respiratory diseases and cancer (Prince *et al.,* 2007; Stein *et al.,* 2019). Young people (individuals between 10-24 years of age) in South

Africa are at risk of mental illness due to the high rates of exposure to HIV infection and other factors such as substance abuse and violence (Mindu *et al.,* 2023). The novel coronavirus disease 2019 (COVID-19) has also contributed to the burden of mental illness globally as people affected by this disease have been reported as developing various mental health issues; such as stress, anxiety disorders, emotional disturbance, depression, somatization disorder, suicidal behaviour and sleep disorder (Hossain *et al.,* 2020).

In low and middle-income countries mental health remains a low priority and over 75% of individuals diagnosed with mental health conditions do not receive treatment as developing countries prioritise controlling and eradicating other health concerns such as; infectious diseases and reproductive, maternal and child health (Prince *et al.,* 2007; Grant *et al.,* 2023). Approximately 16.5% of the adult population in South Africa are diagnosed with a mental disorder and in 3% of cases the mental illness is severe (Shange and Ross, 2022). It has also been reported that 90% of these people do not receive treatment (Shange & Ross, 2022) and only 7.5% of the uninsured (not covered under any health or medical insurance) South African population receives outpatient mental healthcare (Grant *et al.,* 2023). Additionally, in 100 000 people with severe mental illness in South Africa, there are approximately only 1.5 psychiatrists and 2.5 psychologists available, reflecting a clear indication of the lack of allopathic mental healthcare providers, particularly in the public sector to address this gap (WHO, 2019; Padmanabhanunni *et al.,* 2022). In an attempt to reduce this gap, the South African Mental Health Care Policy Framework and Strategic Plan 2013-2020 has underwritten the integration of mental health into primary health care (DoH, 2013).

Efforts to reinforce the mental health system in South Africa have also been strengthened by the reformation of the Mental Health Care Act 17 of 2002 and through the adoption of the National Health Insurance Policy (2017) (Docrat *et al.,* 2019). However, these only look good on paper since mental health remains a challenge in South Africa and the progress in service delivery is crippled by a number of factors such as the lack of resource tracking, inadequate communication, promotion and implementation of mental health policies, and the limited knowledge on the accurate burden of mental disorders and access to mental health services (Docrat *et al.,* 2019). Another factor that may impede the efforts of the Mental Health policies in South Africa is how these policies may ignore the complexity of the contribution of non-Western culture in mental health as these policies, and the theory and practice of mental health emanated from a Western understanding of the human condition and Western cultural traditions (Gopalkrishnan, 2018).

1.7.1.1 Mental Disorders

By definition, mental disorders, also referred to as mental health conditions, are "conditions that are characterized by a clinically significant disturbance in an individual's cognition, emotional regulation, or behaviour" (WHO, 2022). Mental disorders fall under mental illness which is also defined as "conditions that affect an individual's thinking, feeling, mood, or behaviour" (Marzukhi *et al.,* 2023). According to the Global Burden of Diseases, Injuries, and Risk Factors Study (GBD) 2019 (2022), there has been a noticeable increase of 48.1% of cases of mental disorders globally from the year 1990 (654·8 million cases) to 2019 (970·1 million cases).

Most mental disorders are reported to have emerged by the age of 14 years in both males and females (Solmi *et al.,* 2022). Women are more affected by mental disorders compared to men, with comparable rates of between 10-19% of women who are pregnant or post-partum and non-pregnant women (Halim *et al.,* 2018). A study conducted among South African medical insurance beneficiaries confirmed that there were 31% of cases of females diagnosed with mental health conditions compared to 22% of cases in males (Ruffieux *et al.,* 2023). Since South Africa has the highest global rate of sexual offences against women and girls, factors such as intimate partner violence may also increase the risk of mental disorders in women. Other factors contributing to this include social networks, poverty, infant sex and poor health during the perinatal period (Halim *et al.,* 2018). The prevalence of mental disorders may also be increased by sexual assault as it is reported to be higher in victims or survivors of sexual assault, with posttraumatic and depressive disorders being common in these cases (Dworkin, 2020).

1.7.1.2 Diagnosis of Mental Disorders

Unlike in most areas of medicine where diagnosis is fundamentally influenced by biomarkers indicating disease, symptoms are still exclusively used for the diagnosis of mental disorders (Marquand *et al.,* 2019). Diagnostic criteria for each mental disorder are provided by the primary diagnostic tools called the Diagnostic and Statistical Manual of Mental Disorders (DSM), with the fifth version currently being used, and the International Classification of Diseases (ICD) developed by the World Health Organisation (WHO) with recent version being the 11th revision. These tools list and describe all mental disorders; however, it the DSM focuses on disorders which are common in the United States and is published by the American Psychiatric Association (APA) (Cooper, 2018). Thus, this diagnostic tool might not be applicable to South Africa due to the diversity in this country and how the cultural beliefs in ancestors or other spirits and witchcraft or sorcery is taken into consideration in the African perspective of mental illness and health (Shange & Ross, 2022). Hence, in South Africa, this means that the diagnosis of a mental illness is not only dependent on the symptoms presented by patients. In African

traditional healing, the diagnosis of mental illness also involves the throwing of bones, consultation with ancestors, observation of the patient's behaviour and seeking familial background (Shange & Ross, 2022).

1.7.2 Schizophrenia

Schizophrenia has a global prevalence of approximately 0.28% and is ranked 15th globally in the group of causes of years lived with disability (Dutta *et al.,* 2023). The onset of schizophrenia often develops during adolescence to the early 20s, and it is rare for symptoms to begin during childhood or late-life (Addington and Heinssen, 2012; Nowrouzi *et al.,* 2015). However, the late-onset of schizophrenia can also occur in the mid '50s (Jablensky, 2009; Addington and Heinssen, 2012; Stochl *et al.,* 2019). The incidence of men diagnosed with schizophrenia is slightly higher compared to women (Abel *et al.,* 2010), but it has been reported that women are most likely to be diagnosed with this illness later in life compared to men (Fischer and Buchanan, 2020). Literature shows that the first episode in males occurs about 4 to 5 years earlier than in females and the prognosis is worse in men than in women (Usall *et al.,* 2003; Grossman *et al.,* 2008; Purcell *et al.,* 2009).

In a recent study investigating the global burden of disease, schizophrenia was reported to have the highest functional burden among 235 physical and mental health cases (Langer, 2015). Its cruciality is further exacerbated by how current antipsychotic treatments are only effective in improving and managing the positive symptoms of the illness while their effectiveness against the negative symptoms and cognitive impairment is lacking (Kahn *et al.,* 2015). Furthermore, the current antipsychotic medication is associated with unfavourable side effects; such as, metabolic disturbances, motor impairments, complications relating to the cardiovascular system and abnormal levels of prolactin (Leucht *et al.,* 2013; Solmi *et al.,* 2017). These side effects add to the already existing morbidity and mortality rates related to schizophrenia (Olfson *et al.,* 2015; Hjorthoj *et al.,* 2017; Correll *et al.,* 2020).

1.7.2.1 Pathogenesis and Neuropsychology of Schizophrenia

Since the 1970s, the catecholamine neurotransmitter, norepinephrine has been noted to play a role in the pathogenesis of schizophrenia (Yamamoto and Hornykiewicz, 2004); however, the pathophysiology of schizophrenia still remains unclear. Dopaminergic neurotransmission also plays a crucial role in schizophrenia. The striatal D2 receptor mediated hyper-dopaminergic state of the brain is associated with the positive symptoms of schizophrenia, while negative symptoms are associated with frontal, cortical D1 and subcortical dopamine receptor mediated deficit (Abi-Dargham, 2003, 2004; Laruelle,

2014; Galderisi *et al.,* 2015; Collo *et al.,* 2020). An additional factor associated with the pathogenesis of schizophrenia and its negative and cognitive symptoms is glutamatergic dysfunction, which can also contribute to positive symptoms (Merritt *et al.,* 2013). Fischer and Carpenter (2009) suggested that this illness represents a syndrome made up of multiple diseases with similar symptoms and signs, thus resulting in the cause of schizophrenia being unclear and its treatment challenging. The hyper-activation of the dopaminergic system results in symptoms of schizophrenia such as, hallucination and delusion (positive), while on the other hand, the reduced activation of the D1 receptor and glutamate contributes to anhedonia and social withdrawal (negative), and memory deficit (cognitive). The glutamatergic system affects the signaling of the N-methyl-D-aspartate (NMDA) receptor which plays a significant role in synaptic plasticity and neurotransmission. The underactivity of the NMDA receptors results in morphological alterations which lead to structural modifications in the brain and also lead to chronic psychosis (Marsman *et al.,* 2013; Uno and Coyle, 2019).

It is also highly likely that schizophrenia may be triggered by a reaction between genetic material and the environment since environmental factors can also play a major role in influencing epigenetic processes in the same way as the genetic make-up of an individual can influence how one responds to environmental changes or stressors (Fischer and Buchanan, 2020). Modifications in DNA methylation are one of the first epigenetic alterations associated with schizophrenia (Richetto and Meyer, 2021). Obstetrical complications, infections, inflammation, cannabis use, cigarette smoking and immigration are the major environmental risk factors reported by various authors in schizophrenia (Cardno *et al.,* 1999; Kringlen, 2000; Youngmann *et al.,* 2009; Brown and Derkits, 2010; Benros *et al.,* 2011; Cannon, 2015). Under obstetrical complications, perinatal problems; such as, preterm labour, fetal hypoxia, hemorrhage, blood-group incompatibilities and maternal infection are reported to increase the risk of developing schizophrenia by twice the normal rate (Clarke *et al.,* 2006). The mechanism used by infectious agents to increase the risk of developing schizophrenia is not clear as there is minimal evidence suggesting damage to the central nervous system caused by infections (Fischer and Buchanan, 2020). However, the dopaminergic and glutamatergic hypotheses are still considered to be significant with regards to the neuropsychology (Stepnicki *et al.,* 2018).

1.7.2.2 Brain Anatomy in Relation to Schizophrenia

The human brain is one of the largest and multiplex organs in the body. The brain together with the spinal cord make up the central nervous system which is responsible for the analysis, integration and response of the body by means of sensory input and motor output (Hiatt, 2020). Alterations or abnormalities in the structure of the brain have been reported in patients with schizophrenia, and these include reduction of the total brain volume and grey matter, decrease in brain mass, reduction in the

volume of structures such as the hippocampus, amygdala, thalamus, anterior cingulate cortex, frontal gyri, frontal and temporal lobes, insula and the prefrontal region (Harrison *et al.,* 2003; Haijma *et al.,* 2013; Dabiri *et al.,* 2022). An enlargement of the ventricles of the brain (Figure 2) and change in volume of nuclei basales are also noted in patients with schizophrenia (Makara-Studzinska and Los, 2012; Koszla *et al.,* 2020).

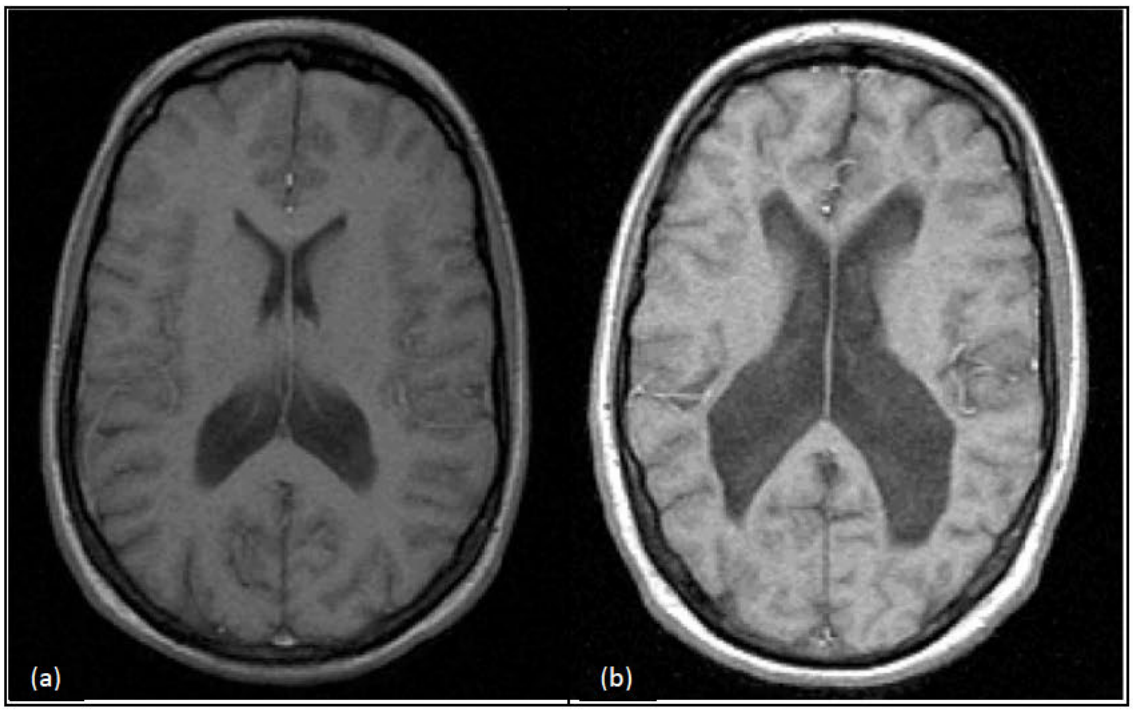


Figure 2: T2-weighted MRI scans of discordant monozygotic twins showing normal ventricles in a healthy twin (a) and ventriculomegaly in the second twin with schizophrenia (b). *(Adapted from Woolley and McGuire, 2005).*

When considering brain lateralisation, it has been demonstrated that asymmetries in the volumes of the aforementioned structures are also associated with schizophrenia (Okada *et al.,* 2016). According to Koszla *et al.* (2020), alterations and irregularities in the anatomy and physiology of the brain are often observed in the hippocampus in patients with schizophrenia. According to Chakos *et al.* (2005), a high reduction of the volume in the hippocampus is observed in chronic schizophrenia patients compared to patients who are at the early stages of the illness. Anatomical modifications of the hippocampus have also been reported to have a negative effect on the function or activity of this structure as schizophrenia patients have been reported to exhibit a lower number of synapses between the pyramidal neurons and the hippocampal mossy fibers (Kolomeets *et al.,* 2005; Donegan and Lodge, 2017). According to Donegan and Lodge, (2017), another part of the brain involved in the pathology of schizophrenia is the pre-frontal cortex. This structure is responsible for working memory which is also disturbed in

schizophrenia; thus, it has been concluded that interruption of the activity of the prefrontal cortex plays a major role to the cognitive dysfunction and negative symptoms of schizophrenia (Koszla *et al.,* 2020).

1.7.2.3 Symptoms of Schizophrenia

Schizophrenia is mainly classified into three types based on the symptoms that occur, viz. positive symptoms (Type I), negative symptoms (Type II) and cognitive impairment (Type III) (Koster *et al.,* 2014). To evaluate clinical severity or treatment response and to assess the symptoms of schizophrenia, the Positive and Negative Syndrome (or Severity) Scale abbreviated as PANSS is used (Haro *et al.,* 2003). The PANSS is a 30-item and 7-point rating instrument which is used to measure the three dimensions of schizophrenia; viz. positive symptoms, negative symptoms and general psychopathology (Wu *et al.,* 2018). Each PANSS item is rated on a scale of increasing psychopathology as follows: 1 = absent, 2 = minimal, 3 = mild, 4 = moderate, 5 = moderate to severe, 6 = severe and 7 = extreme. Ratings across all items are then summed up and the PANSS is scored; for the Positive and Negative Scales the score ranges from 7 to 49 and from 16 to 112 for the general psychopathology (Kay *et al.,* 1987). The Positive Scale measures delusions, conceptual disorganisation, hallucinations, excitement, grandiosity, suspiciousness or persecution, and hostility (Wu *et al.,* 2018), while the Negative Scale assesses blunted affect, emotional withdrawal, poor rapport, passive or apathetic social withdrawal, difficulty in abstract thinking, lack of spontaneity and conversation flow, and stereotyped thinking (Wu *et al.,* 2018). The General Psychopathology Scale of the PANSS consists of 16 items which assess disorganisation symptoms and severity of schizophrenia. These items are somatic concerns, anxiety, guilt feelings, tension, mannerisms and posturing, depression, motor retardation, un-cooperativeness, unusual thoughts, disorientation, poor attention, lack of judgment and insight, disturbance of volition, poor impulse control, preoccupation, and active social avoidance (Wu *et al.,* 2018).

The limitations of the 3 PANSS subscales (positive, negative and general psychopathology) include time-consumption as it takes approximately 30 to 45 minutes to administer and it has been reported to inadequately capture all aspects of the symptoms of schizophrenia (Chen *et al.* 2020). Therefore, interview-based assessments such as Cognitive Assessment Interview (CAI) or Schizophrenia Cognition Rating Scale (SCRS) are further used to evaluate the cognitive symptoms of schizophrenia in conjunction with a skills assessment, such as the UCSD Performance-based Skills Assessment in order to measure functional capacity (Targum and Keefe, 2008). Each assessment used for the evaluation of cognitive symptoms may also be approximately 30 to 45 minutes.

1.7.2.3.1 Positive Symptoms of Schizophrenia

These symptoms include disorganised speech and thoughts, delusions, hallucinations and disorganised behaviour (Koster *et al.,* 2014). Positive symptoms have been reported to be one of the causes of aggressive behaviour in patients diagnosed with schizophrenia (Wu *et al.,* 2018). These symptoms are more sensitive to agents or drugs which target the dopamine system (Leucht *et al.,* 2013).

1.7.2.3.2 Negative Symptoms of Schizophrenia

The negative symptoms of schizophrenia refer more to psychosocial impairment and are reported to be more persistent compared to positive symptoms (Tamminga *et al.,* 1998; Peralta *et al*., 2000; Savill *et al.,* 2015). These symptoms are regarded as the core component of schizophrenia and are less susceptible to treatment compared to the other symptoms of schizophrenia (Chue and Lalonde, 2014; Galderisi *et al.,* 2018). Negative symptoms include lack of drive and motivation marked by apathy or avolition, lack of enjoyment or pleasure (anhedonia) and social interactions (asociality), and lack of facial expression, gesturing and speech output such as alogia or aprosodia and blunting of affect (Choi and Medalia, 2010; Foussias and Remington, 2010; Blanchard *et al.,* 2011; Koster *et al.,* 2014; Galderisi *et al.,* 2018). According to Norman *et al.* (2000), these symptoms have been found to critically affect the quality of life and social functioning of an individual. Galderisi *et al.* (2018) describes them as having an effect in the "real-life" functioning of individuals diagnosed with schizophrenia. The incidence of these symptoms in first episode patients is reported to be between 41% and 54% of cases (Bobes *et al.,* 2009; Makinen *et al.,* 2010).

When working with negative symptoms, it is important to bear in mind that these symptoms may also be observed in other psychiatric illnesses, and that they are commonly seen in patients with depression (Ellenbroek and Cools, 2000). According to Galderisi *et al.* (2018), distinguishing between the negative symptoms of schizophrenia and depression may be a challenge without accurate clinical assessment. In a study conducted by Knights and Hirsch (1981), it was reported that in 80% of cases of patients with schizophrenia at least one depressive symptom occurred and three or more depressive symptoms were observed in 65% of cases. Studies conducted by Emerich and Sanberg (1991) and Hogan and Awad (1992) also reported that antipsychotic medication may give rise to symptoms such as dysphoria, akinesia, facial rigidity and lethargy which may be mistaken for negative symptoms of schizophrenia; thus, a distinction between primary and secondary negative symptoms was made.

1.7.2.3.2.1 Primary Negative Symptoms

Another term that is used for the negative and enduring symptoms of schizophrenia is deficit symptoms, and distinguishing between these symptoms and secondary negative symptoms plays a crucial role in their treatment as the deficit symptoms have been reported to have no standard treatment since they do not respond well to various medications (Chue and Lalonde, 2014; Galderisi *et al.,* 2018). When considering primary persistent negative symptoms with a specified minimum severity of symptoms, the incidence of these symptoms ranges from 23% to 26% of cases (Tek *et al.,* 2001; Bobes *et al.,* 2009; Makinen *et al.,* 2010; Chang *et al.,* 2011).

1.7.2.3.2.2 Secondary Negative Symptoms

Secondary negative symptoms are described as the symptoms which are induced by a contaminant illness such as reactive depression, or by certain antipsychotic medication (Carpenter *et al.,* 1988). Once the cause of these symptoms has been addressed, improvement is observed relatively quickly compared to primary negative symptoms which are reported to be more persistent (Moller, 2007).

1.7.2.3.3 Cognitive Symptoms

Cognitive symptoms include deficiency in grasp of social interactions and/or situations, lack of memory, attention and deficits in executive functioning (Koster *et al.,* 2014). These symptoms are said to reflect how well the brain of an individual learns, stores and utilises information.

1.7.2.5 Treatment of Schizophrenia

1.7.2.5.1 Biomedical Medicine

Commonly when attempting to treat schizophrenia, the major targets are D2 (dopamine), 5-HT_{2A} (5-hydoxytryptamine 2A: G protein-coupled receptor belonging to the serotonin receptor family) and α_1 receptors (Bantick *et al.,* 2001; Ogren *et al.,* 2008; Meltzer, 2012). Dopamine D2 blocking agents are the only approved and effective drug class for the treatment of this disorder and other related psychotic disorders since their discovery in the 1950s (Abi-Dargham, 2004). However, therapy concentrating only on the dopaminergic neurotransmitter system has been shown to not treat schizophrenia (Merritt *et al.,* 2013). Since negative symptoms of schizophrenia are the most persistent and resistant symptoms, clinical research currently focuses on biological systems such as glutamatergic neurotransmission in an attempt to target these symptoms (Chue and Lalonde, 2014). In recent studies, neurotransmitters such

as acetylcholine, serotonin, glutamate, γ-aminobutyric acid (GABA) and cytokines have been reported to also contribute to the pathophysiology of schizophrenia (Dutta *et al.,* 2023).

The recommendation according to the schizophrenia treatment guidelines from the American Psychiatric Association states that pharmacotherapy selection should be individualised based on the characteristics and preference of each patient; furthermore, patients should also be consulted when the choice of treatment is being selected (Lehman *et al.,* 2004; Maroney, 2020). For acute schizophrenia symptom management, second generation antipsychotic drugs are recommended as first-line treatment (Lehman *et al.,* 2004). However; according to the proposed updated treatment guidelines for schizophrenia, the use of antipsychotic drugs is the only single component of the treatment paradigm (Maroney, 2020). Patients respond differently to pharmacological therapy and approximately 10% to 30% of patients are reported to have a poor or partial response (Marder and Cannon, 2019). In addition, it has been reported that approximately 30% of patients with schizophrenia still have persistent residual or psychotic symptoms which affect their functioning even if they experience some improvement (Kane *et al.,* 2019). Gaps in pharmacological therapy for treatment of schizophrenia have been identified, which include: a need to improve treatment-resistant schizophrenia, a need for drugs that are capable for treating negative symptoms and improving cognition, agents that improve adherence and have improved adverse effect profiles (Fellner, 2017). New strategies; such as new dosages aiding in adherence, TAAR-1 agonists and σ-2 antagonists, and also modulation of serotonin and glutamate, which attempt to fill these gaps are still being researched and some have been recently approved (Maroney, 2020).

1.7.2.5.2 Traditional Medicine

According to Kaplan and Sadock, (1988), mental illness tends to be viewed as culture-free in western psychiatry as this entity does not assign causality to cultural beliefs (which include beliefs in spirits, gods etc.). However, cultural beliefs, values and concepts play a significant role in influencing health-seeking pathways, and THPs play a major role in managing disease in many cultures (Versola-Russo, 2005). In South Africa, some black African communities believe that ill health in their lives or in their families is brought upon them by culture-related phenomena (Monama and Basson, 2017). Mental health disorders are believed to be caused by bewitchment or sorcery. In some cases they may be interpreted as *ubizo* (an ancestral calling) *or ukuthwasa* (need to train as a healer) and these can only be treated or managed by THPs (Zabow, 2007). Other indigenous names that are used to refer to mental disorders are *amafufunyana* which is defined as the possession by evil spirits and *ukuphambana or ukuhlanya* which is defined as madness (Zabow, 2007).

In traditional medicine or African traditional healing, THPs use approaches that are based on cultural, religious and social systems to provide health care to the community, and these methods may include the use of plant, animal and/or mineral substances to treat different ailments (WHO, 1978). THPs may classify as *Isangoma* or a diviner who use divination to determine the cause of illness, *Inyanga* or herbalist which mainly prescribes traditional medical remedies for treatment of illness, and *Umthandazi* or faith healer who mainly use water and prayer, although sometimes ash and salt may be included in the healing process (Zuma *et al.,* 2016). In South Africa, the number of THPs practising full-time is approximately 68 000, while the total number of those practising full-time and part-time is approximately 300 000 (Street *et al.,* 2018). Traditional medicine by definition is the combination of knowledge, skills and practices based on the experiences, beliefs and theories indigenous to different cultures which are used for diagnosis, treatment and prevention of mental and physical illness as well as for overall maintenance of health (WHO, 2013).

In Sub-Saharan Africa, THPs are perceived to have the unique skill to manage various conditions which allopathic clinicians lack, and these conditions may involve or be caused by spells, spirits, sorcery or social misconduct (Kale, 1995; Pinkoane, 2005; Makundi *et al.,* 2006; Audet *et al.,* 2017). Other advantages of THPs compared to allopathic clinicians are that traditional healers speak languages typically preferred by patients, live closer to patients, and are often available to meet with patients without any difficulty in order to discuss causes of illness and diagnoses as well as the treatment or management of illnesses (Audet *et al.,* 2012). It has been reported that patients with various acute and chronic physical and mental conditions commonly consult traditional healers rather than seek help from allopathic services only, or sometimes use the two in conjunction due to the above-mentioned factors and other limitations linked with allopathic workforce and infrastructure (Hundt *et al.,* 2004; Babb *et al.,* 2007; Moshabela *et al.,* 2011; Audet *et al.,* 2013).

1.8 Brief Overview of General Methodology

1.8.1 Study Design

Biomedical treatment approaches for the treatment of schizophrenia, particularly for the negative symptoms, are considered lacking due to a number of factors which include the adverse side effects associated with them. Therefore, with the majority of the African population, particularly the Black South African population reported to have a preference for traditional medicine as a treatment option for both physical and mental aliments, this study aimed to investigate the role of the THPs and the effects of traditional medicine for the treatment of the negative symptoms of schizophrenia. This study was divided into two components (Figure 3). Component one comprised the administration of questionnaires to 100 THPs (data collected in November 2021) from the Harry Gwala District

Municipality (HGDM) and focus group discussions with 31 of the THPs (data collected in February 2022) who had indicated, on the questionnaires, to have previously diagnosed and treated mental illness. From the data collected from this component, the THPs indicated the use of herbal plants and the administration of a medicinal concoction for the treatment of mental disorders, particularly schizophrenia, to be the most common traditional healing method. Seven medicinal plants reported to treat mental illness by the THPs, were then sourced from the Berea and Dalton *muthi* markets. The phytochemical compounds were identified from these medicinal plants using Gas Chromatography Mass Spectrometry analysis and their cytotoxic activity was tested using the MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide] Assay in the first part of the second component. In the second part of this component, one of the aforementioned plants (*Salix mucronata*) was selected for testing in a pharmacological animal model and the antipsychotic potential of this plant was compared with the effects of a commonly used antipsychotic drug for the treatment of the negative symptoms of schizophrenia, risperidone. Behavioural tests conducted included the 3-chambered sociability test for asociality and the sucrose preference test for anhedonia. The Biochemical tests included dopamine (on VTA and PFC) and acetylcholine (on PFC and Hippocampus) Enzyme Linked Immunosorbent Assay (ELISA).

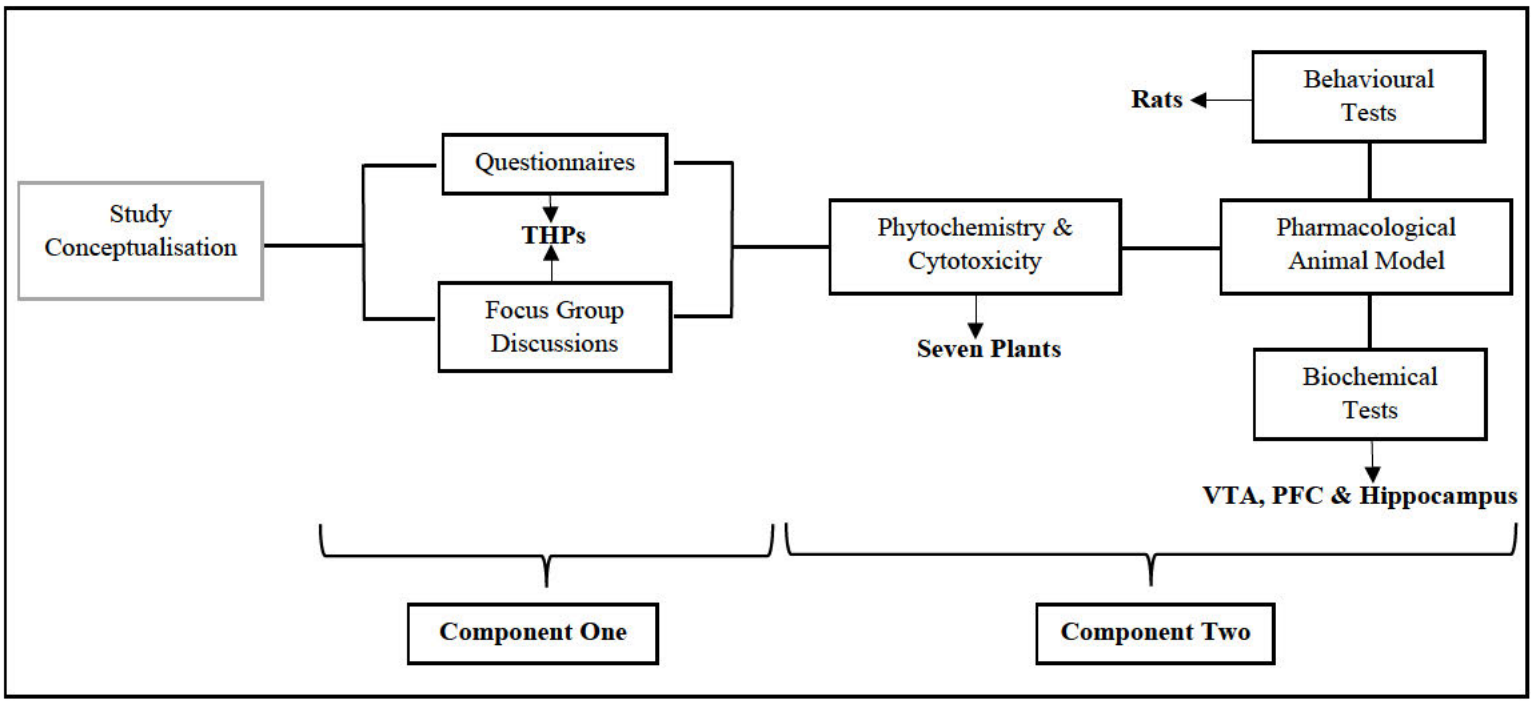


Figure 3: Schematic diagram of the Study Design

Detailed methodologies for each phase of the study are documented in the chapters and papers as presented in the thesis outline below.

1.8.2 Ethical Consideration

For the first component of the study, ethical approval was obtained from the Humanities and Social Sciences Research Ethics Committee of the University of KwaZulu-Natal (Ethics Clearance number: HSSREC/00002944/2021; Ethics Approval attached in Appendix 2) and an informed consent form was signed by all participants prior to participating in this study. The animal experimental component was approved by the Animal Research Ethics Committee of the University of KwaZulu-Natal (Ethics Clearance number: AREC/000/5188/2023; Ethics Approval attached in Appendix 3) and the Department of Agriculture, Land Reform and Rural Development of the Republic of South Africa (Permit number: SDAH-Epi-23021009580, Permit attached in Appendix 4).

1.9 REFERENCES

1. Abbo, C., 2011. Profiles and outcome of traditional healing practices for severe mental illnesses in two districts of Eastern Uganda. *Global Health Action*, 4(1), p.7117.
2. Abel, K.M., Drake, R. and Goldstein, J.M., 2010. Sex differences in schizophrenia. *International Review of Psychiatry*, 22(5), pp.417-428.
3. Abi-Dargham, A., 2003. Probing cortical dopamine function in schizophrenia: what can D1 receptors tell us?. *World Psychiatry*, 2(3), p.166-171.
4. Abi-Dargham, A., 2004. Do we still believe in the dopamine hypothesis? New data bring new evidence. *International Journal of Neuropsychopharmacology*, 7(1), pp.S1-S5.
5. Addington, J. and Heinssen, R., 2012. Prediction and prevention of psychosis in youth at clinical high risk. *Annual Review of Clinical Psychology*, 8, pp.269-289.
6. Ae-Ngibise, K., Cooper, S., Adiibokah, E., Akpalu, B., Lund, C., Doku, V. and Mhapp Research Programme Consortium, 2010. 'Whether you like it or not people with mental problems are going to go to them': a qualitative exploration into the widespread use of traditional and faith healers in the provision of mental health care in Ghana. *International Review of Psychiatry*, 22(6), pp.558-567.
7. Ahmed, M.N., Azam, K. and Nur, M., 2014. Traditional knowledge and formulations of medicinal plants used by the traditional medical practitioners of Bangladesh to treat schizophrenia like psychosis. *Schizophrenia Research and Treatment*, 2014, ID.679810, pp.1-10.
8. American Psychiatric Association. (2013). Diagnostic and statistical manual of mental disorders: DSM-5 (5th ed.). Washington, D.C: American Psychiatric Publishing.
9. Audet, C.M., Sidat, M., Blevins, M., Moon, T.D., Vergara, A. and Vermund, S.H., 2012. HIV knowledge and health-seeking behavior in Zambe zia Province, Mozambique. *SAHARA-J: Journal of Social Aspects of HIV/AIDS*, 9(1), pp.41-46.
10. Audet, C.M., Salato, J., Blevins, M., Amsalem, D., Vermund, S.H. and Gaspar, F., 2013. Educational intervention increased referrals to allopathic care by traditional healers in three high HIV-prevalence rural districts in Mozambique. *PloS One*, 8(8), e70326, pp.1-8.
11. Audet, C.M., Ngobeni, S., Graves, E. and Wagner, R.G., 2017. Mixed methods inquiry into traditional healers' treatment of mental, neurological and substance abuse disorders in rural South Africa. *PloS One*, 12(12), e0188433, pp.1-14.
12. Babb, D.A., Pemba, L., Seatlanyane, P., Charalambous, S., Churchyard, G.J. and Grant, A.D., 2007. Use of traditional medicine by HIV-infected individuals in South Africa in the era of antiretroviral therapy. *Psychology, Health and Medicine*, 12(3), pp.314-320.
13. Bantick, R.A., Deakin, J.F.W. and Grasby, P.M., 2001. The 5-HT1A receptor in schizophrenia: a promising target for novel atypical neuroleptics?. *Journal of Psychopharmacology*, 15(1), pp.37-46.

14. Barbato, A., WHO Nations for Mental Health Initiative and World Health Organization, 1997. Schizophrenia and public health (No. WHO/MSA/NAM/97.6). World Health Organization.
15. Beng, K.S., 2004. The last hours and days of life: a biopsychosocial-spiritual model of care. *Asia Pacific Family Medicine*, 4, pp.1-3.
16. Benjamin, F., Vickerman-Delport, S.A. and Roman, N.V., 2021. Mental health care services in rural South Africa: a human capabilities approach. *Social Work in Mental Health*, 19(5), pp.365-380.
17. Benros, M.E., Nielsen, P.R., Nordentoft, M., Eaton, W.W., Dalton, S.O. and Mortensen, P.B., 2011. Autoimmune diseases and severe infections as risk factors for schizophrenia: a 30-year population-based register study. *American Journal of Psychiatry*, 168(12), pp.1303-1310.
18. Bialon, M. and Wąsik, A., 2022. Advantages and limitations of animal schizophrenia models. *International Journal of Molecular Sciences*, 23(11), ID.5968, pp.1-32.
19. Bifftu, B.B., Takele, W.W., Guracho, Y.D. and Yehualashet, F.A., 2018. Depression and its help seeking behaviors: a systematic review and meta-analysis of community survey in Ethiopia. *Depression Research and Treatment*, 2018, ID.1592596, pp.1-11.
20. Biswal, R., Subudhi, C. and Acharya, S.K., 2017. Healers and healing practices of mental illness in India: The role of proposed eclectic healing model. *Journal of Health Research and Reviews (In Developing Countries)*, 4(3), pp.89-95.
21. Blanchard, J.J., Kring, A.M., Horan, W.P. and Gur, R., 2011. Toward the next generation of negative symptom assessments: the collaboration to advance negative symptom assessment in schizophrenia. *Schizophrenia Bulletin*, 37(2), pp.291-299.
22. Bobes, J., Arango, C., Garcia-Garcia, M. and Rejas, J., 2009. Prevalence of negative symptoms in outpatients with schizophrenia spectrum disorders treated with antipsychotics in routine clinical practice: findings from the CLAMORS study. *The Journal of Clinical Psychiatry*, 71(3), pp.280-286.
23. Brown, A.S. and Derkits, E.J., 2010. Prenatal infection and schizophrenia: a review of epidemiologic and translational studies. *American Journal of Psychiatry*, 167(3), pp.261-280.
24. Brown, J., Iacovelli, L., Di Cicco, G., Grayson, B., Rimmer, L., Fletcher, J., Neill, J.C., Wall, M.J., Ngomba, R.T. and Harte, M., 2022. The comparative effects of mGlu5 receptor positive allosteric modulators VU0409551 and VU0360172 on cognitive deficits and signalling in the sub-chronic PCP rat model for schizophrenia. *Neuropharmacology*, 208, ID.108982, pp.1-11.
25. Campos, M.T.F., Rodrigues, J.P., 2022. Influence of spirituality and religiosity on the use of alcohol and drugs: an integrative review. *SMAD Revista Electronica Salud Mental, Alcohol y Drogas*, 18(2), pp.1-11.
26. Carbon, M. and Correll, C.U., 2014. Thinking and acting beyond the positive: the role of the cognitive and negative symptoms in schizophrenia. *CNS Spectrums*, 19(1), pp.35-53.

27. Cannon, T.D., 2015. How schizophrenia develops: cognitive and brain mechanisms underlying onset of psychosis. *Trends in Cognitive Sciences*, 19(12), pp.744-756.
28. Carbon, M. and Correll, C.U., 2014. Thinking and acting beyond the positive: the role of the cognitive and negative symptoms in schizophrenia. *CNS Spectrums*, 19(1), pp.35-53.
29. Cardno, A.G., Marshall, E.J., Coid, B., Macdonald, A.M., Ribchester, T.R., Davies, N.J., Venturi, P., Jones, L.A., Lewis, S.W., Sham, P.C. and Gottesman, I.I., 1999. Heritability estimates for psychotic disorders: the Maudsley twin psychosis series. *Archives of General Psychiatry*, 56(2), pp.162-168.
30. Carpenter Jr, W.T., Heinrichs, D.W. and Wagman, A.M., 1988. Deficit and nondeficit forms of schizophrenia: the concept. *The American Journal of Psychiatry*, 145(5), pp.578-583.
31. Chakos, M.H., Schobel, S.A., Gu, H., Gerig, G., Bradford, D., Charles, C. and Lieberman, J.A., 2005. Duration of illness and treatment effects on hippocampal volume in male patients with schizophrenia. *The British Journal of Psychiatry*, 186(1), pp.26-31.
32. Chang, W.C., Hui, C.L., Tang, J.Y., Wong, G.H., Lam, M.M., Chan, S.K. and Chen, E.Y., 2011. Persistent negative symptoms in first-episode schizophrenia: a prospective three-year follow-up study. *Schizophrenia Research*, 133(1-3), pp.22-28.
33. Chen, J., Patil, K.R., Weis, S., Sim, K., Nickl-Jockschat, T., Zhou, J., Aleman, A., Sommer, I.E., Liemburg, E.J., Hoffstaedter, F. and Habel, U., 2020. Neurobiological divergence of the positive and negative schizophrenia subtypes identified on a new factor structure of psychopathology using non-negative factorization: an international machine learning study. *Biological Psychiatry*, 87(3), pp.282-293.
34. Choi, J. and Medalia, A., 2010. Intrinsic motivation and learning in a schizophrenia spectrum sample. *Schizophrenia Research*, 118(1-3), pp.12-19.
35. Chue, P. and Lalonde, J.K., 2014. Addressing the unmet needs of patients with persistent negative symptoms of schizophrenia: emerging pharmacological treatment options. *Neuropsychiatric Disease and Treatment*, 10, pp.777-789.
36. Clarke, M.C., Harley, M. and Cannon, M., 2006. The role of obstetric events in schizophrenia. *Schizophrenia Bulletin*, 32(1), pp.3-8.
37. Collo, G., Mucci, A., Giordano, G.M., Merlo Pich, E. and Galderisi, S., 2020. Negative symptoms of schizophrenia and dopaminergic transmission: translational models and perspectives opened by iPSC techniques. *Frontiers in Neuroscience*, 14, ID.632, pp.1-14.
38. Cooper, R., 2018. Diagnosing the diagnostic and statistical manual of mental disorders. Routledge.
39. Correll, C.U., Davis, R.E., Weingart, M., Saillard, J., O'Gorman, C., Kane, J.M., Lieberman, J.A., Tamminga, C.A., Mates, S. and Vanover, K.E., 2020. Efficacy and safety of lumateperone for treatment of schizophrenia: a randomized clinical trial. *JAMA Psychiatry*, 77(4), pp.349-358.
40. Crellin, N.E., Priebe, S., Morant, N., Lewis, G., Freemantle, N., Johnson, S., Horne, R., Pinfold, V., Kent, L., Smith, R. and Darton, K., 2022. An analysis of views about supported reduction or

discontinuation of antipsychotic treatment among people with schizophrenia and other psychotic disorders. *BMC Psychiatry*, 22(1), pp.1-11.

41. Cumella, E., 2002. Bio-psycho-social-spiritual: Completing the model. The Remuda Review: *The Christian Journal of Eating Disorders*, 1, pp.1-5.
42. Dabiri, M., Dehghani Firouzabadi, F., Yang, K., Barker, P.B., Lee, R.R. and Yousem, D.M., 2022. Neuroimaging in schizophrenia: A review article. *Frontiers in Neuroscience*, 16, ID.1042814, pp.1-22.
43. de Bartolomeis, A., Ciccarelli, M., De Simone, G., Mazza, B., Barone, A. and Vellucci, L., 2023. Canonical and non-canonical antipsychotics' dopamine-related mechanisms of present and next generation molecules: a systematic review on translational highlights for treatment response and treatment-resistant schizophrenia. *International Journal of Molecular Sciences*, 24(6), pp. 1-57.
44. Deng, H. and Xu, J., 2017. Wendan decoction (Traditional Chinese medicine) for schizophrenia. *Cochrane Database of Systematic Reviews*, 6, pp.1-92.
45. Docrat, S., Besada, D., Cleary, S., Daviaud, E. and Lund, C., 2019. Mental health system costs, resources and constraints in South Africa: a national survey. *Health Policy and Planning*, 34(9), pp.706-719.
46. Donegan, J.J. and Lodge, D.J., 2017. Cell-based therapies for the treatment of schizophrenia. *Brain Research*, 1655, pp.262-269.
47. Dutta, R.R., Picton, B., Brown, N.J., Yang, C., Lee, M., Sung, H., Lopez, A.M. and Paff, M., 2023. Schizophrenia and neurosurgery: systematic review and theories. *Neurosurgical Focus*, 54(2), e7, pp.1-11.
48. Dworkin, E.R., 2020. Risk for mental disorders associated with sexual assault: A meta-analysis. *Trauma, Violence, and Abuse*, 21(5), pp.1011-1028.
49. Edwards, S.D., 1986. Traditional and modern medicine in South Africa: A research study. *Social Science and Medicine*, 22(11), pp.1273-1276.
50. Ellenbroek, B.A. and Cools, A.R., 2000. Animal models for the negative symptoms of schizophrenia. *Behavioural Pharmacology*, 11(3 & 4), pp.223-233.
51. Emerich D.F. and Sanberg P.R., 1991. Neuroleptic dysphoria. *Biological Psychiatry*. 29(1991), pp.201-203.
52. Fellner, C., 2017. New schizophrenia treatments address unmet clinical needs. *Pharmacy and Therapeutics*, 42(2), p.130-134.
53. Fischer, B.A. and Buchanan, R.W., 2020. Schizophrenia in adults: Epidemiology and pathogenesis. *UpToDate, Rose, BD (Ed), UpToDate,* Waltham, MA.
54. Fischer, B.A. and Carpenter, W.T., 2009. Will the Kraepelinian dichotomy survive DSM-V?. *Neuropsychopharmacology*, 34(9), pp.2081-2087.
55. Foster, D.H., Freeman, M. and Pillay, Y. eds., 1997. Mental health policy issues for South Africa. Medical Association of South Africa, Multimedia Publications.

56. Foussias, G. and Remington, G., 2010. Negative symptoms in schizophrenia: avolition and Occam's razor. *Schizophrenia Bulletin*, 36(2), pp.359-369.
57. Freeman, M. and Motsei, M., 1992. Planning health care in South Africa—is there a role for traditional healers?. *Social Science and Medicine*, 34(11), pp.1183-1190.
58. Freeman, M., Lee, T. and Vivian, W., 1994. Evaluation of mental health services in the Orange Free State. Centre for Health Policy.
59. Galderisi, S., Merlotti, E. and Mucci, A., 2015. Neurobiological background of negative symptoms. *European archives of psychiatry and clinical neuroscience*, 265, pp.543-558.
60. Galderisi, S., Mucci, A., Buchanan, R.W. and Arango, C., 2018. Negative symptoms of schizophrenia: new developments and unanswered research questions. *The Lancet Psychiatry*, 5(8), pp.664-677.
61. GBD 2019 Mental Disorders Collaborators, 2022. Global, regional, and national burden of 12 mental disorders in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. *The Lancet Psychiatry*, 9(2), pp.137-150.
62. Gilbody, S., Bagnall, A.M., Duggan, L., Tuunainen, A. and Cochrane Schizophrenia Group, 1996. Risperidone versus other atypical antipsychotic medication for schizophrenia. *Cochrane Database of Systematic Reviews*, 2010(3), pp.1-44.
63. Glick, L.B., 1967. Medicine as an ethnographic category: the gimi of the New Guinea highlands. *Ethnology*, 6(1), pp.31-56.
64. Gopalkrishnan, N., 2018. Cultural diversity and mental health: Considerations for policy and practice. *Frontiers in Public Health*, 6, ID.179, pp.1-7.
65. Grant, M., Bhana, A., Kathree, T., Khuzwayo, N., van Rensburg, A.J., Mthethwa, L., Gigaba, S., Ntswe, E., Luvuno, Z. and Petersen, I., 2023. The feasibility of a Community Mental Health Education and Detection (CMED) tool in South Africa. *SSM-Mental Health*, 3, ID.100188, pp.1-10.
66. Green, E.C. and Makhubu, L., 1984. Traditional healers in Swaziland: toward improved cooperation between the traditional and modern health sectors. *Social Science and Medicine*, 18(12), pp.1071-1079.
67. Grossman, L.S., Harrow, M., Rosen, C., Faull, R. and Strauss, G.P., 2008. Sex differences in schizophrenia and other psychotic disorders: a 20-year longitudinal study of psychosis and recovery. *Comprehensive Psychiatry*, 49(6), pp.523-529.
68. Haijma, S.V., Van Haren, N., Cahn, W., Koolschijn, P.C.M., Hulshoff Pol, H.E. and Kahn, R.S., 2013. Brain volumes in schizophrenia: a meta-analysis in over 18 000 subjects. *Schizophrenia Bulletin*, 39(5), pp.1129-1138.
69. Halim, N., Beard, J., Mesic, A., Patel, A., Henderson, D. and Hibberd, P., 2018. Intimate partner violence during pregnancy and perinatal mental disorders in low and lower middle income

countries: A systematic review of literature, 1990–2017. *Clinical Psychology Review*, 66, pp.117-135.

70. Haro, J.M., Kamath, S.A., Ochoa, S.O., Novick, D., Rele, K., Fargas, A., Rodriguez, M.J., Rele, R., Orta, J., Kharbeng, A. and Araya, S., 2003. The Clinical Global Impression–Schizophrenia scale: a simple instrument to measure the diversity of symptoms present in schizophrenia. *Acta Psychiatrica Scandinavica*, 107, pp.16-23.
71. Harrison, P.J., Freemantle, N. and Geddes, J.R., 2003. Meta-analysis of brain weight in schizophrenia. *Schizophrenia Research*, 64(1), pp.25-34.
72. Hiatt, J.L., 2020. Textbook of head and neck anatomy. Jones and Bartlett Learning.
73. Hjorthoj, C., Stürup, A.E., McGrath, J.J. and Nordentoft, M., 2017. Years of potential life lost and life expectancy in schizophrenia: a systematic review and meta-analysis. *The Lancet Psychiatry*, 4(4), pp.295-301.
74. Hogan, T.P. and Awad, A.G., 1992. Subjective response to neuroleptics and outcome in schizophrenia: a re-examination comparing two measures. *Psychological Medicine*, 22(2), pp.347-352.
75. Hossain, M.M., Purohit, N., Sharma, R., Bhattacharya, S., McKyer, E.L.J. and Ma, P., 2020. Suicide of a farmer amid COVID-19 in India: Perspectives on social determinants of suicidal behavior and prevention strategies. SocArXiv ekam3, Center for Open Science.
76. Hundt, G.L., Stuttaford, M., Ngoma, B. and SASPI TEAM, 2004. The social diagnostics of stroke-like symptoms: healers, doctors and prophets in Agincourt, Limpopo Province, South Africa. *Journal of Biosocial Science*, 36(4), pp.433-443.
77. Incayawar, M., 2008. Efficacy of Quichua healers as psychiatric diagnosticians. *The British Journal of Psychiatry*, 192(5), pp.390-391.
78. Jablensky, A. Epidemiology of schizophrenia. In M. G. Gelder, N. C. Andreasen, J. J. L-I. Jr, & J. R. Geddes (Eds.), New Oxford Textbook of Psychiatry. Oxford University Press. 2009; 2(1): 540-553.
79. Kahn, R.S., Sommer, I.E., Murray, R.M., Meyer-Lindenberg, A., Weinberger, D.R., Cannon, T.D., O'Donovan, M., Correll, C.U., Kane, JM., van Os, J., Insel, T.R., 2015. Schizophrenia. N*ature Reviews Disease Primers,* 12(1), ID.15067, pp.1-23.
80. Kale, R., 1995. Traditional healers in South Africa: A parallel health care system. *British Medical Journal*, 310(6988), pp.1182-1186.
81. Kane, J.M., Agid, O., Baldwin, M.L., Howes, O., Lindenmayer, J.P., Marder, S., Olfson, M., Potkin, S.G. and Correll, C.U., 2019. Clinical guidance on the identification and management of treatment-resistant schizophrenia. *The Journal of Clinical Psychiatry*, 80(2), ID.2783, pp.1-9.
82. Kantrowitz, J.T., 2021. Trace amine-associated receptor 1 as a target for the development of new antipsychotics: current status of research and future directions. *CNS Drugs*, 35(11), pp.1153-1161.

83. Kapur, R.L., 1975. Mental health care in rural India: a study of existing patterns and their implications for future policy. *The British Journal of Psychiatry*, 127(3), pp.286-293.
84. Kaplan, H.I. and Sadock, B.J., 1988. Synopsis of psychiatry: Behavioral sciences clinical psychiatry. Williams & Wilkins Co.
85. Kay, S.R., Fiszbein, A. and Opler, L.A., 1987. The positive and negative syndrome scale (PANSS) for schizophrenia. *Schizophrenia Bulletin*, 13(2), pp.261-276.
86. Kleinman, A., Eisenberg, L. and Good, B., 1978. Culture, illness, and care: clinical lessons from anthropologic and cross-cultural research. *Annals of Internal Medicine*, 88(2), pp.251-258.
87. Kleinman, A. and Sung, L.H., 1979. Why do indigenous practitioners successfully heal?. *Social Science & Medicine. Part B: Medical Anthropology*, 13(1), pp.7-26.
88. Klinghardt, D., 2005. The five levels of healing. *Explore*, 14(4), pp.1-5.
89. Knights, A. and Hirsch, S.R., 1981. 'Revealed'depression and drug treatment for schizophrenia. *Archives of General Psychiatry*, 38(7), pp.806-811.
90. Koenig, H.G., 2008. Religion and mental health: what should psychiatrists do?. *Psychiatric Bulletin*, 32(6), pp.201-203.
91. Kolomeets, N.S., Orlovskaya, D.D., Rachmanova, V.I. and Uranova, N.A., 2005. Ultrastructural alterations in hippocampal mossy fiber synapses in schizophrenia: a postmortem morphometric study. *Synapse*, 57(1), pp.47-55.
92. Koster, L.S., Carbon, M. and Correll, C.U., 2014. Emerging drugs for schizophrenia: an update. *Expert Opinion on Emerging Drugs*, 19(4), pp.511-531.
93. Koszla, O., Targowska-Duda, K.M., Kędzierska, E. and Kaczor, A.A., 2020. In vitro and in vivo models for the investigation of potential drugs against schizophrenia. *Biomolecules*, 10(1), p.160.
94. Kringlen, E., 2000. Twin studies in schizophrenia with special emphasis on concordance figures. *American Journal of Medical Genetics*, 97(1), pp.4-11.
95. Langer, S.Z., 2015. α2-Adrenoceptors in the treatment of major neuropsychiatric disorders. *Trends in Pharmacological Sciences*, 36(4), pp.196-202.
96. Laruelle, M., 2014. Schizophrenia: from dopaminergic to glutamatergic interventions. *Current opinion in pharmacology*, 14, pp.97-102.
97. Leavey, G., 2010. The appreciation of the spiritual in mental illness: A qualitative study of beliefs among clergy in the UK. *Transcultural Psychiatry*, 47(4), pp.571-590.
98. Lehman, D.R., Chiu, C.Y. and Schaller, M., 2004. Psychology and culture. *Annual Review of Psychology*, 55, pp.689-714.
99. Leucht, S., Cipriani, A., Spineli, L., Mavridis, D., Örey, D., Richter, F., Samara, M., Barbui, C., Engel, R.R., Geddes, J.R. and Kissling, W., 2013. Comparative efficacy and tolerability of 15 antipsychotic drugs in schizophrenia: a multiple-treatments meta-analysis. *The Lancet*, 382(9896), pp.951-962.

100. Lowe, P., Krivoy, A., Porffy, L., Henriksdottir, E., Eromona, W. and Shergill, S.S., 2018. When the drugs don't work: treatment-resistant schizophrenia, serotonin and serendipity. *Therapeutic Advances in Psychopharmacology*, 8(1), pp.63-70.

101. Lucana, S. and Elfers, J., 2020. Sacred medicine: Indigenous healing and mental health. *The Qualitative Report*, 25(12), pp.4482-4495.

102. Luther, L., Jarvis, S.A., Spilka, M.J. and Strauss, G.P., 2023. Global reward processing deficits predict negative symptoms transdiagnostically and transphasically in a severe mental illness-spectrum sample. *European Archives of Psychiatry and Clinical Neuroscience*, 2023, pp.1-12.

103. Makara-Studzińska, M. and Łos, R., 2012. Neuroanatomical structural changes seen in patients with schizophrenia and their healthy siblings. *Polski Merkuriusz Lekarski: Organ Polskiego Towarzystwa Lekarskiego*, 33(193), pp.51-53.

104. Makinen, J., Miettunen, J., Jääskeläinen, E., Veijola, J., Isohanni, M. and Koponen, H., 2010. Negative symptoms and their predictors in schizophrenia within the Northern Finland 1966 Birth Cohort. *Psychiatry Research*, 178(1), pp.121-125.

105. Makundi, E.A., Malebo, H.M., Mhame, P., Kitua, A.Y. and Warsame, M., 2006. Role of traditional healers in the management of severe malaria among children below five years of age: the case of Kilosa and Handeni Districts, Tanzania. *Malaria Journal*, 5(1), pp.1-9.

106. Maphumulo, W.T. and Bhengu, B.R., 2019. Challenges of quality improvement in the healthcare of South Africa post-apartheid: A critical review. *Curationis*, 42(1), pp.1-9.

107. Marder, S.R. and Cannon, T.D., 2019. Schizophrenia. *New England Journal of Medicine*, 381(18), pp.1753-1761.

108. Marder, S.R. and Kirkpatrick, B., 2014. Defining and measuring negative symptoms of schizophrenia in clinical trials. *European Neuropsychopharmacology*, 24(5), pp.737-743.

109. Maroney, M., 2020. An update on current treatment strategies and emerging agents for the management of schizophrenia. *The American Journal of Managed Care*, 26(3), pp.S55-S61.

110. Marquand, A.F., Kia, S.M., Zabihi, M., Wolfers, T., Buitelaar, J.K. and Beckmann, C.F., 2019. Conceptualizing mental disorders as deviations from normative functioning. *Molecular Psychiatry*, 24(10), pp.1415-1424.

111. Marzukhi, M.A., Ghazali, N.M., Yaman, R. and Leh, O.L.H., 2023, July. Assessing the Effect of High-Density Housing on Mental Health of Urban Population. Case Study: Federal Territory of Kuala Lumpur. In IOP Conference Series: Earth and Environmental Science, 1217(1), p.012020. IOP Publishing.

112. Marsman, A., Van Den Heuvel, M.P., Klomp, D.W., Kahn, R.S., Luijten, P.R. and Hulshoff Pol, H.E., 2013. Glutamate in schizophrenia: a focused review and meta-analysis of 1H-MRS studies. *Schizophrenia Bulletin*, 39(1), pp.120-129.

113. Mbanga, N.I., Niehaus, D.J.H., Mzamo, N.C., Wessels, C.J., Allen, A., Emsley, R.A. and Stein, D.J., 2002. Attitudes towards and beliefs about schizophrenia in Xhosa families with affected probands. *Curationis*, 25(1), pp.69-73.

114. Mbendana, D., Mamabolo, K., Truter, M., Kritzinger, Q. and Ndhlala, A.R., 2019. Practices at herbal (muthi) markets in Gauteng, South Africa and their impact on the health of the consumers: A case study of KwaMai-Mai and Marabastad muthi markets. *South African Journal of Botany*, 126, pp.30-39.

115. Meltzer, H.Y., 2012. Serotonergic mechanisms as targets for existing and novel antipsychotics. *Current Antipsychotics*, pp.87-124.

116. Merritt, K., McGuire, P. and Egerton, A., 2013. Relationship between glutamate dysfunction and symptoms and cognitive function in psychosis. *Frontiers in Psychiatry*, 4, ID.151, pp.1-8.

117. Mindu, T., Mutero, I.T., Ngcobo, W.B., Musesengwa, R. and Chimbari, M.J., 2023. Digital mental health interventions for young people in rural South Africa: Prospects and challenges for implementation. *International Journal of Environmental Research and Public Health*, 20(2), ID.1453, pp.1-15.

118. Miola, A., Cattarinussi, G., Antiga, G., Caiolo, S., Solmi, M. and Sambataro, F., 2022. Difficulties in emotion regulation in bipolar disorder: a systematic review and meta-analysis. *Journal of Affective Disorders*, 302, pp.352-360.

119. Mkabile, S. and Swartz, L., 2022. Traditional healers' explanatory models of intellectual disability in Cape Town. *Transcultural Psychiatry*, 59(3), pp.263-273.

120. Mokgobi, M.G., 2014. Understanding traditional African healing. *African Journal for Physical Health Education, Recreation and Dance*, 20(sup-2), pp.24-34.

121. Moller, H.J., 2007. Clinical evaluation of negative symptoms in schizophrenia. *European Psychiatry*, 22(6), pp.380-386.

122. Monama, D. D. and Basson, W. J., 2017. Looking in the mirror: The cultural experiences of patients diagnosed with schizophrenia. *Pula: Botswana Journal of African Studies*, 31(2), 46-58.

123. Moshabela, M., Pronyk, P., Williams, N., Schneider, H. and Lurie, M., 2011. Patterns and implications of medical pluralism among HIV/AIDS patients in rural South Africa. *AIDS and Behavior*, 15, pp.842-852.

124. Moshabela, M., Zuma, T. and Gaede, B., 2016. Bridging the gap between biomedical and traditional health practitioners in South Africa. *South African Health Review*, 2016(1), pp.83-92.

125. Murambidzi, I., 2016. Conceptualisation of mental illness among Christian clergy in Harare, Zimbabwe (Master's thesis, University of Cape Town).

126. National Department of Health (NDoH). Strategic plan 2020/21 – 2024/25. Pretoria: National Department of Health, 2020. ISBN: 978-0-621-48280-5. Available: https://www.health.gov.za/wp-content/uploads/2020/11/depthealthstrategicplanfinal2020-21to2024-25-1.pdf (Accessed 9 October 2023)

127. Nattrass, N., 2005. Who consults sangomas in Khayelitsha? An exploratory quantitative analysis. *Social Dynamics*, 31(2), pp.161-182.

128. Nguse, S. and Wassenaar, D., 2021. Mental health and COVID-19 in South Africa. *South African Journal of Psychology*, 51(2), pp.304-313.

129. Norman, R.M., Malla, A.K., McLean, T., Voruganti, L.P.N., Cortese, L., McIntosh, E., Cheng, S. and Rickwood, A., 2000. The relationship of symptoms and level of functioning in schizophrenia to general wellbeing and the Quality of Life Scale. *Acta Psychiatrica Scandinavica*, 102(4), pp.303-309.

130. Nowrouzi, B., Kamhi, R., Hu, J., Kennedy, J.L., Matmari, M. and De Luca, V., 2015. Age at onset mixture analysis and systematic comparison in schizophrenia spectrum disorders: Is the onset heterogeneity dependent on heterogeneous diagnosis?. *Schizophrenia Research*, 164(1-3), pp.83-91.

131. Ogren, S.O., Eriksson, T.M., Elvander-Tottie, E., D'Addario, C., Ekström, J.C., Svenningsson, P., Meister, B., Kehr, J. and Stiedl, O., 2008. The role of 5-HT1A receptors in learning and memory. *Behavioural Brain Research*, 195(1), pp.54-77.

132. Okada, N., Fukunaga, M., Yamashita, F., Koshiyama, D., Yamamori, H., Ohi, K., Yasuda, Y., Fujimoto, M., Watanabe, Y., Yahata, N. and Nemoto, K., 2016. Abnormal asymmetries in subcortical brain volume in schizophrenia. *Molecular Psychiatry*, 21(10), pp.1460-1466.

133. Olfson, M., Gerhard, T., Huang, C., Crystal, S. and Stroup, T.S., 2015. Premature mortality among adults with schizophrenia in the United States. *JAMA Psychiatry*, 72(12), pp.1172-1181.

134. Oyebode, O., Kandala, N.B., Chilton, P.J. and Lilford, R.J., 2016. Use of traditional medicine in middle-income countries: a WHO-SAGE study. *Health Policy and Planning*, 31(8), pp.984-991.

135. Padmanabhanunni, A., Jackson, K., Noordien, Z., Pretorius, T.B. and Bouchard, J.P., 2022. Characterizing the nature of professional training and practice of psychologists in South Africa. In *Annales Médico-Psychologiques, Revue Psychiatrique*, 180(4), pp. 360-365). Elsevier Masson.

136. Patel, K.R., Cherian, J., Gohil, K. and Atkinson, D., 2014. Schizophrenia: overview and treatment options. *Pharmacy and Therapeutics*, 39(9), p.638-645.

137. Peralta, V., Cuesta, M.J., Martinez-Larrea, A. and Serrano, J.F., 2000. Differentiating primary from secondary negative symptoms in schizophrenia: a study of neuroleptic-naive patients before and after treatment. *American Journal of Psychiatry*, 157(9), pp.1461-1466.

138. Pinkoane, M.G., 2005. Incorporation of the traditional healers into the national health care delivery system (Doctoral thesis, North-West University).

139. Plana-Ripoll, O., Pedersen, C.B., Holtz, Y., Benros, M.E., Dalsgaard, S., De Jonge, P., Fan, C.C., Degenhardt, L., Ganna, A., Greve, A.N. and Gunn, J., 2019. Exploring comorbidity within mental disorders among a Danish national population. *JAMA Psychiatry*, 76(3), pp.259-270.

140. Prince, M., Patel, V., Saxena, S., Maj, M., Maselko, J., Phillips, M.R. and Rahman, A., 2007. No health without mental health. *The Lancet*, 370(9590), pp.859-877.

141. Puckree, T., Mkhize, M., Mgobhozi, Z. and Lin, J., 2002. African traditional healers: what health care professionals need to know. *International Journal of Rehabilitation Research*, 25(4), pp.247-251.

142. Purcell, S.M., Wray, N.R., Stone, J.L., Visscher, P.M., O'Donovan, M.C., Sullivan, P.F., Sklar, P., Ruderfer, D.M., McQuillin, A., Morris, D.W., International Schizophrenia Consortium., 2009. Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. *Nature*, 460(7256), pp.748-752.

143. Raphalalani, S., Becker, P.J., Böhmer, M.W. and Krüger, C., 2021. The role of Mental Health Care Act status in dignity-related complaints by psychiatric inpatients: A cross-sectional analytical study. *South African Journal of Psychiatry*, 27(1), ID.1602, pp.1-8.

144. Richetto, J. and Meyer, U., 2021. Epigenetic modifications in schizophrenia and related disorders: molecular scars of environmental exposures and source of phenotypic variability. *Biological Psychiatry*, 89(3), pp.215-226.

145. Ruffieux, Y., Wettstein, A., Maartens, G., Folb, N., Vieira, C.M., Didden, C., Tlali, M., Williams, C., Cornell, M., Schomaker, M. and Johnson, L.F., 2023. Life-years lost associated with mental illness: a cohort study of beneficiaries of a South African medical insurance scheme. *medRxiv*, [Preprint].

146. Rumbold, B.D., 2007. A review of spiritual assessment in health care practice. *Medical Journal of Australia*, 186, pp.S60-S62.

147. Sanavi, M.A., Ghazvini, H., Zargari, M., Ghalehnoei, H. and Hosseini-Khah, Z., 2022. Effects of clozapine and risperidone antipsychotic drugs on the expression of CACNA1C and behavioral changes in rat 'Ketamine model of schizophrenia. *Neuroscience Letters*, 770, ID.136354, pp.1-7.

148. Sanz-Fuentenebro, J., Taboada, D., Palomo, T., Aragües, M., Ovejero, S., Del Alamo, C. and Molina, V., 2013. Randomized trial of clozapine vs. risperidone in treatment-naïve first-episode schizophrenia: results after one year. *Schizophrenia Research*, 149(1-3), pp.156-161.

149. Saravanan, B., 2002. Explanatory models in psychiatry. *The British Journal of Psychiatry*, 181(4), pp.351-351.

150. Savill, M., Banks, C., Khanom, H. and Priebe, S., 2015. Do negative symptoms of schizophrenia change over time? A meta-analysis of longitudinal data. *Psychological Medicine*, 45(8), pp.1613-1627.

151. Schooler, N., Robinson, D., Marcy, P., Homan, P., Achtyes, E. and Kane, J.M., 2020. O9. 7. Technology Assisted Relapse Prevention Program Reduces Hospitalization In Schizophrenia Patients At High Risk For Relapse. *Schizophrenia Bulletin*, 46(1), pp.S23-S24.

152. Schulz, S.C. and Murray, A., 2016. Assessing cognitive impairment in patients with schizophrenia. *The Journal of Clinical Psychiatry*, 77(2), pp.3-7.

153. Shange, S. and Ross, E., 2022. “The Question Is Not How but Why Things Happen”: South African Traditional Healers’ Explanatory Model of Mental Illness, Its Diagnosis and Treatment. *Journal of Cross-Cultural Psychology*, 53(5), pp.503-521.

154. Shettima, F.B., Sheikh, T.L., Wakil, M.A., Abdulaziz, M., Rabebbe, I.B. and O Abdulmalik, J., 2023. Prevalence and Correlates of Extrapyramidal Side Effects Among Patients with Schizophrenia Spectrum Disorders on Typical and Atypical Antipsychotics. *Archives of Psychiatry Research: An International Journal of Psychiatry and Related Sciences*, 59(2), pp.225-240.

155. Solmi, M., Murru, A., Pacchiarotti, I., Undurraga, J., Veronese, N., Fornaro, M., Stubbs, B., Monaco, F., Vieta, E., Seeman, M.V. and Correll, C.U., 2017. Safety, tolerability, and risks associated with first-and second-generation antipsychotics: a state-of-the-art clinical review. *Therapeutics and Clinical Risk Management*, pp.757-777.

156. Solmi, M., Radua, J., Olivola, M., Croce, E., Soardo, L., Salazar de Pablo, G., Il Shin, J., Kirkbride, J.B., Jones, P., Kim, J.H. and Kim, J.Y., 2022. Age at onset of mental disorders worldwide: large-scale meta-analysis of 192 epidemiological studies. *Molecular Psychiatry*, 27(1), pp.281-295.

157. Sonnenschein, S.F., Gomes, F.V. and Grace, A.A., 2020. Dysregulation of midbrain dopamine system and the pathophysiology of schizophrenia. *Frontiers in psychiatry*, 11, ID.613, pp.1-11.

158. South African National Department of Health (DoH), 2013. National Mental Health Policy Framework and Strategic Plan 2013–2020. Pretoria: Republic of South Africa.

159. Spedding, M.F., Stein, D.J. and Sorsdahl, K., 2014. Task-shifting psychosocial interventions in public mental health: A review of the evidence in the South African context. *South African Health Review*, 2014(1), pp.73-87.

160. Statistics South Africa [StatsSA]. Mbalo Brief – October 2017. 2017.

161. Stein, D.J., 2014. A new mental health policy for South Africa. SAMJ: *South African Medical Journal*, 104(2), pp.115-116.

162. Stein, D.J., Benjet, C., Gureje, O., Lund, C., Scott, K.M., Poznyak, V. and Van Ommeren, M., 2019. Integrating mental health with other non-communicable diseases. *The BMJ*, 364.

163. Stepnicki, P., Kondej, M. and Kaczor, A.A., 2018. Current concepts and treatments of schizophrenia. *Molecules*, 23(8), ID.2087, pp.1-29.

164. Stochl, J., Whittier, A., Wagner, A.P., Veijola, J., Jääskeläinen, E., Miettunen, J., Khandaker, G.M. and Jones, P.B., 2019. Association between developmental milestones and age of schizophrenia onset: Results from the Northern Finland Birth Cohort 1966. *Schizophrenia Research*, 208, pp.228-234.

165. Street, R.A., Smith, M., Moshabela, M., Shezi, B., Webster, C. and Falkenberg, T., 2018. Traditional health practitioners and sustainable development: a case study in South Africa. *Public Health*, 165, pp.1-5.

166. Stroup, T.S., A. Lieberman, J., S. Swartz, M. and McEvoy, J.P., 2000. Comparative effectiveness of antipsychotic drugs in schizophrenia. *Dialogues in Clinical Neuroscience*, 2(4), pp.373-379.

167. Stroup, T.S. and Gray, N., 2018. Management of common adverse effects of antipsychotic medications. *World Psychiatry*, 17(3), pp.341-356.

168. Tamminga, C.A., Buchanan, R.W. and Gold, J.M., 1998. The role of negative symptoms and cognitive dysfunction in schizophrenia outcome. *International Clinical Psychopharmacology*, 13, pp.S21-S26.

169. Targum, S.D. and Keefe, R.S., 2008. Cognition and schizophrenia: is there a role for cognitive assessments in diagnosis and treatment?. *Psychiatry (Edgmont)*, 5(12), p.55-59.

170. Tek, C., Kirkpatrick, B. and Buchanan, R.W., 2001. A five-year followup study of deficit and nondeficit schizophrenia. *Schizophrenia Research*, 49(3), pp.253-260.

171. Tirodkar, M.A., Baker, D.W., Makoul, G.T., Khurana, N., Paracha, M.W. and Kandula, N.R., 2011. Explanatory models of health and disease among South Asian immigrants in Chicago. *Journal of Immigrant and Minority Health*, 13, pp.385-394.

172. Uno, Y. and Coyle, J.T., 2019. Glutamate hypothesis in schizophrenia. *Psychiatry and Clinical Neurosciences*, 73(5), pp.204-215.

173. Usall, J., Ochoa, S., Araya, S. and Márquez, M., 2003. Gender differences and outcome in schizophrenia: a 2-year follow-up study in a large community sample. *European Psychiatry*, 18(6), pp.282-284.

174. van den Heuvel, L.L., Smit, A.M., Stalder, T., Kirschbaum, C., Seedat, S. and Emsley, R., 2022. Hair cortisol levels in schizophrenia and metabolic syndrome. *Early Intervention in Psychiatry*, 16(8), pp.902-911.

175. Velligan, D.I. and Rao, S., 2023. Schizophrenia: salient symptoms and pathophysiology. *The Journal of clinical psychiatry*, 84(1), ID.45113, pp.1-2.

176. Versola-Russo, J.M., 2005. Cultural and demographic factors of schizophrenia. *International Journal of Psychosocial Rehabilitation*, 10(2), pp.89-102.

177. Weru, J.K. and Nafula, E.W., 2022. Psychosocial-Spiritual Healing. *Global Perspectives in Cancer Care: Religion, Spirituality, and Cultural Diversity in Health and Healing*, 28, p.295-301.

178. Whiteford, H.A., Degenhardt, L., Rehm, J., Baxter, A.J., Ferrari, A.J., Erskine, H.E., Charlson, F.J., Norman, R.E., Flaxman, A.D., Johns, N. and Burstein, R., 2013. Global burden of disease attributable to mental and substance use disorders: findings from the Global Burden of Disease Study 2010. *The Lancet*, 382(9904), pp.1575-1586.

179. Woolley, J. and McGuire, P., 2005. Neuroimaging in schizophrenia: what does it tell the clinician?. *Advances in Psychiatric Treatment*, 11(3), pp.195-202.

180. Wootton, O., King, A., Moy, K., Stein, D.J. and Susser, E.S., 2023. Disability benefits protect against lost income for South Africans living with Schizophrenia. *Social Psychiatry and Psychiatric Epidemiology*, 2023, pp.1-5.

181. World Health Organization. Depression Fact Sheet. https://www.who.int/news-room/fact-sheets/detail/depression. Accessed 09 October 2023.

182. World Health Organization. 1978. The promotion and development of traditional medicine: Report of a WHO meeting [held in Geneva from 28 November to 2 December 1977].

183. World Health Organization. Depression and other common mental disorders: global health estimates. No. WHO/MSD/MER/2017.2. World Health Organization, 2017.

184. World Health Organisation. WHO Policy Perspective on Medicines. Traditional Medicine: Growing Needs and Potential. Geneva, Switzerland. 2002.

185. World Health Organisation. Investing in Mental Health. Geneva, Switzerland. 2003.

186. World Health Organisation. Promoting Mental Health: Concepts, Emerging Evidence, Practise. Geneva, Switzerland. 2005.

187. World Health Organization. Mental health action plan 2013-2020. Geneva: Author; 2013.

188. World Health Organisation. 2022. Mental Disorders [Fact Sheet]. https://www.who.int/news-room/fact-sheets/detail/mental-disorders#:~:text=A%20mental%20disorder%20is%20characterized,different%20types%20of%20mental%20disorders. (Accessed 04 March 2023).

189. Wu, C., Wang, C. and Li, L., 2018. Speech-on-speech masking and psychotic symptoms in schizophrenia. *Schizophrenia Research: Cognition*, 12, pp.37-39.

190. Yamamoto, K. and Hornykiewicz, O., 2004. Proposal for a noradrenaline hypothesis of schizophrenia. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 28(5), pp.913-922.

191. Young, M., 2016. Private vs. public healthcare in South Africa (Honours thesis, Western Michigan University).

192. Youngmann, R., Pugachova, I. and Zilber D ès Sc, N., 2009. Patterns of psychiatric hospitalization among Ethiopian and former Soviet Union immigrants and persons born in Israel. *Psychiatric Services*, 60(12), pp.1656-1663.

193. Zabow, T., 2007. Traditional healers and mental health in South Africa. *International Psychiatry,* 4(4), pp.81-83.

194. Zuma, T., Wight, D., Rochat, T. and Moshabela, M., 2016. The role of traditional health practitioners in Rural KwaZulu-Natal, South Africa: generic or mode specific?. *BMC Complementary and Alternative Medicine*, 16(1), pp.1-13.

Prologue 1

The previous chapter (Chapter 1) provided an overview of mental health and mental disorders in a global and South African context. It also showed how Western and Traditional medicines differ in terms of their approach to the diagnosis and treatment of mental illnesses. Based on the literature reviewed in the previous chapter, a gap exists regarding the profiles of the THPs who treat mental illnesses and on the methods of diagnosis and treatment of such ailments. The following chapter, Chapter 2, is an extension of the literature review as it explores the different types of traditional medicine and herbal plants used by the global population from the different continents, regions and countries. This chapter investigates whether traditional methods for the treatment of schizophrenia, are similar or different, seeing that each community is reported to be influenced by their cultural and religious beliefs in their traditional treatment approaches. This literature is presented in the form of a systematic review paper.

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CHAPTER 2

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Global perspectives on the traditional approaches used in the treatment of schizophrenia: A systematic review

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ABSTRACT

Background: Traditional healing considers a holistic approach when diagnosing and treating patients for mental ailments, and is the preferred approach globally.
Objective: This review documented traditional healing approaches for treatment of schizophrenia used in different regions globally.
Methods: PICO framework was used to facilitate literature search from Google Scholar, PubMed, Medline, Cochrane, Scopus, APA PsycINFO, and Web search. Studies documenting methods of treatment from the perspective of traditional healers, patients and/or caregivers were included and also studies which investigated herbal plants used in traditional healing in vitro and in vivo were included. Review articles, magazine/newspaper articles, editorials, letters, comments/opinion articles, and articles with inaccessible full text were excluded. The risk of bias was assessed using MMAT and SYRCLE tools. University Capacity Development Programme funded this review.
Results: 74 articles were included, these documented traditional healing practices used in Africa, Asia, America, Europe, and Oceania. Common approach globally was herbal medicine. Other reported methods included faith-based healing, consultation with the ancestors, performing rituals, acupuncture, and music and yoga therapies. Inhumane approaches included starving, beating, cutting and confining patients. In some cases, traditional healing was used as adjunctive treatment. The overall risk of bias for studies in this review was low.
Conclusion: Traditional healing contributes in bridging the treatment gap for schizophrenia in developing countries. However, there is a lack of standardisation of the approaches employed in the different regions, and the safety and effectiveness of some of these approaches remain questionable.

1. Background

In low- and middle-income countries, access to formal psychiatric treatment remains a challenge for approximately 76–85 % of individuals diagnosed with serious mental disorders; thus, traditional healing approaches are considered a viable option for treatment (Nortje et al., 2016; WHO, 2021). Additionally, it has been established that over 80 % of the global population relies on traditional, alternative or complementary medicine for mental illnesses (Budzak and Brankovic, 2022). Traditional treatment interventions include herbal remedies, where their plant extracts or bioactive compounds may be considered for the production of neuroprotective drugs (Shaterzadeh-Yazdi et al., 2018). Neuroprotective drugs are significant in minimising brain ageing, neuropathological disorders as a result of neurodegeneration, and brain pathology, which are factors contributing 8 % to the total death rate, with an incidence of 2 in 1000 individuals (Kumar and Khanum, 2012). According to Kim and Na (2017), neuroprotection is the process which assists in homeostasis and restoration of the functional integrity of the brain in response to neurobiological stressors or neuropathology. When this process fails, the central nervous system may be compromised due to anatomical and functional impairments, which lead to the development of disorders such as schizophrenia (Kim and Na, 2017).

Schizophrenia is a neurodegenerative disorder affecting approximately 1 in 300 individuals or 1 % of the global population and ranks as

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one of the top ten causes of disability worldwide (WHO, 2022; Velligan and Rao, 2023). In 79 % of cases, heredity is estimated to contribute to the onset of schizophrenia. Other factors which may contribute to the cause of this disorder are environmental and social (Velligan and Rao, 2023). Schizophrenia symptoms include delusions, hallucinations and incoherence (positive symptoms); asociality, anhedonia, alogia and reduced energy (negative symptoms); and lack of attention and memory (cognitive dysfunction) (APA, 2013; Kahn et al., 2015). Schizophrenia and/or its symptoms may be associated with culture-bound conditions in different regions; for instance, in South Africa it is associated with a culture-bound syndrome called *ufufunyana/amafufunyana*. There is an overlap of symptoms (such as aggression, hallucination, alogia, unresponsiveness and lack of motivation) between schizophrenia and *ufufunyana* (in IsiZulu/IsiXhosa) which may also be described as the possession by the evil spirit caused by witchcraft (Mzimkulu and Simbayi, 2006, Molot, 2017). For this reason, *ufufunyana* is sometimes classified as schizophrenia.

In Western medicine, antipsychotic drugs have been the cornerstone treatment method for schizophrenia since their discovery in 1960; however, the response to these drugs is deemed unsatisfactory in approximately 30 % of patients (Ojagbemi and Gureje, 2020). The efficacy of antipsychotic medication is also compromised by the adverse side effects and how negative and cognitive symptoms of schizophrenia may persist with their use (Ojagbemi and Gureje, 2020; Crellin et al., 2022). Since schizophrenia may affect individuals from any community and geographical area worldwide, based on the cultural context, ancient traditional or natural therapies peculiar to that particular community may be used to treat this disorder since they have been reported to be effective for thousands of years (Ojagbemi and Gureje, 2020). In Africa, patients with psychiatric ailments (including schizophrenia) accessed traditional healers over other healthcare providers or access traditional healing prior to biomedical healthcare in approximately 73.5 % cases in Nigeria, 50.3 % in Ethiopia, 23.3 % in Ghana, and 22.7 % in Malawi, 11.5 %–38.5 % in South Africa, and 41 % in Sudan (James et al., 2018). Additionally, according Shi et al. (2021), for thousands of years, Chinese herbal medicine has been used for the treatment of schizophrenia. In Malatya (Turkey, Middle East), alternative medicine is used by approximately 51.6 % of patients for the treatment of schizophrenia (Yazici et al., 2016). Due to how they have more time with patients compared to the biomedical healthcare givers, traditional healers are commonly consulted first for the management of schizophrenia in different parts of the world (Mounkoro et al., 2020). With the evident use of alternative medicine for different psychiatric issues globally, the purpose of this review was to document the treatment approaches used in traditional healing practices in different regions globally for the treatment of schizophrenia. This review also addressed the following questions: (i) which plants are used for the treatment of schizophrenia where herbal remedies are an option?; (ii) how are these plants prepared and used?; (iii) what properties do these plants possess?; (iv) is there scientific evidence supporting the use of these plants?

2. Methodology

2.1. Search strategy

The Population, Intervention, Comparison and Outcomes (PICO) framework was used to breakdown the review question (Table 1).

The following six databases were used to complete a comprehensive search of the literature: Google Scholar, PubMed, Medline, Cochrane, Scopus, APA PsycINFO, and Web search through Chrome. Search terms, their synonyms and alternatives (Table 2) were used in alternation with the Boolean operators such as OR, AND, and "phrase" to compose different phrases. These search terms were used to obtain all included studies, and the different studies (i.e. studies including human sample, studies where herbal medicine was tested in-vitro and in-vivo, and ethnobotanical/ethno-zoological studies) were categorised during the review write-up phase. The literature search commenced on the 19th of August 2021 and was completed on the 4th of March 2022.

Table 1
Review question.

Full Review Question *Provide the full review question in sentence format, and then break up the question according to the PICO framework (or other frameworks as appropriate).*	
What are the treatment methods used in traditional medicine to treat and manage schizophrenia?	
Sample	Schizophrenia
Phenomena of interest	Traditional medicine
Intervention	Treatment or management
Comparison	Animal model/Patients (human sample)
Evaluation	Effectiveness

Table 2
Search terms used in all the selected databases.

Schizophrenia	**Treatment or management**	**Traditional medicine/ approaches/ interventions**	**Effectiveness**
Ufufunyana/ amafufunyana	Cure	Customary remedy	Efficacy
Insanity	Medication	Ancestral medicine/ remedy	Efficiency
Madness	Therapy	Medicinal plants	Validity
Lunacy	Healing	Complementary/ alternative medicine	
Nervous disorder			
Delusions			
Mania			

2.2. Study selection

Two investigators screened each identified article. However, a third screener was invited for a final decision if the inclusion was debatable. Google forms (which included the review's inclusion and exclusion criteria, and review questions) were used to take notes of the details of each study, after careful screening of the articles, each screener had to state if the article was going to be included in the review and also provide a reason for their choice. Where an article was said to fit for inclusion in the review by one screener and excluded by the other screener, the third screener was invited to make a decision. The flow chart for the selection of the included studies is presented in Fig. 1 below. In the initial search and after the removal of duplicates, a total of 2292 abstracts was obtained.

2.3. Eligibility criteria

2.3.1. Inclusion criteria

- Studies in English
- Studies on humans and animal models
- There were no restrictions based on life stage at exposure or assessment, sex, animal species or strain and mental disorder model
- Studies with titles profiling of herbal plants and their uses

2.3.2. Exclusion criteria

- Review papers
- Studies for which full text was not available or accessible
- Magazine and/or newspaper articles, editorials, letters, comments/ opinion articles
- Studies which reported on traditional treatment of mental illness in general and not specific to schizophrenia

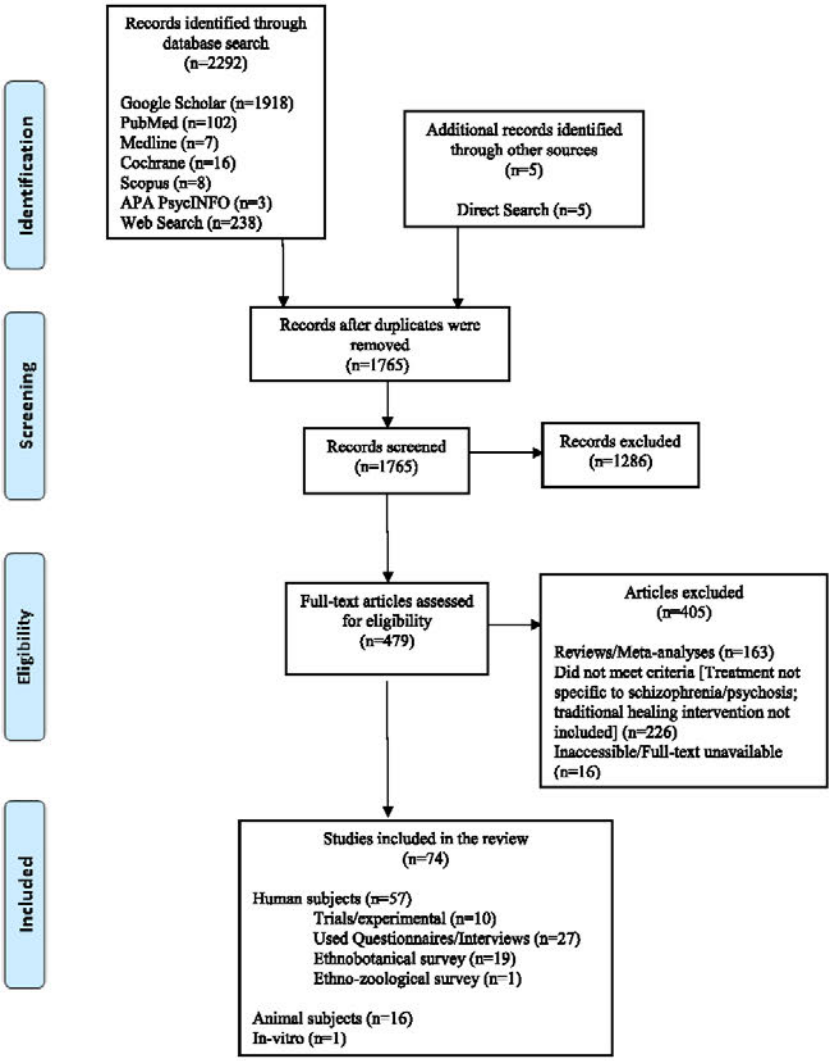


Fig. 1. : Literature review process using PRISMA flowchart.

2.4. Quality assessment

The Mixed Methods Appraisal Tool (MMAT) version 2018 (Hong et al., 2018) was used to assess the quality and risk of bias for human studies (Table A, Appendix), and the SYstematic Review Centre for Laboratory animal Experimentation (SYRCLE) risk of bias tool (Hooijmans et al., 2014) was used for animal studies (Table B, Appendix) included in this review.

3. Results/review

A total of 74 (69 from the systematic search and 5 from the additional direct search) articles were included in this review, with 28 from Africa, 31 from Asia, 7 from America, 5 from Europe, 2 from Oceania and one article from a collaboration between different regions from different continents (Fig. 2a–f). Of the 69 articles from the systematic search, 27 used quantitative and qualitative investigations involving a human sample (traditional healers, patients or caregivers), 16 reported on herbal plants tested on animal models, 19 reported on findings from ethnobotanical or ethno-medicinal surveys where in some of the articles traditional healers were involved as informants, 5 were control trials, one in-vitro, and one was an ethno-zoological survey. Of the 5 additional data from a direct search for articles on yoga therapy for the treatment of schizophrenia, which were clinical trials/experimental research on a human sample, 4 were from Asia (1 from Turkey and 3 from India), and 1 was from America (United States of America). The traditional interventions in the treatment of schizophrenia, psychosis, madness, insanity and herbal plants possessing antipsychotic properties are presented in tables below (Tables 3–4).

Clinical and methodological heterogeneity or diversity was noted in the studies included in this review. Variability was noted in the selection and number of participants, the intervention for the management schizophrenia or its symptoms, and in the design of each study. The participants who contributed to the information reported in the studies which included a human sample included in- and out-patients, (including patients with schizophrenia and psychosis), traditional healers, herb sellers, farmers, village elders, family heads and other members, housewives, and locals/inhabitants. The number of participants ranged from 2 to 1888. The type of studies included qualitative studies where interviews were conducted with the participants, some

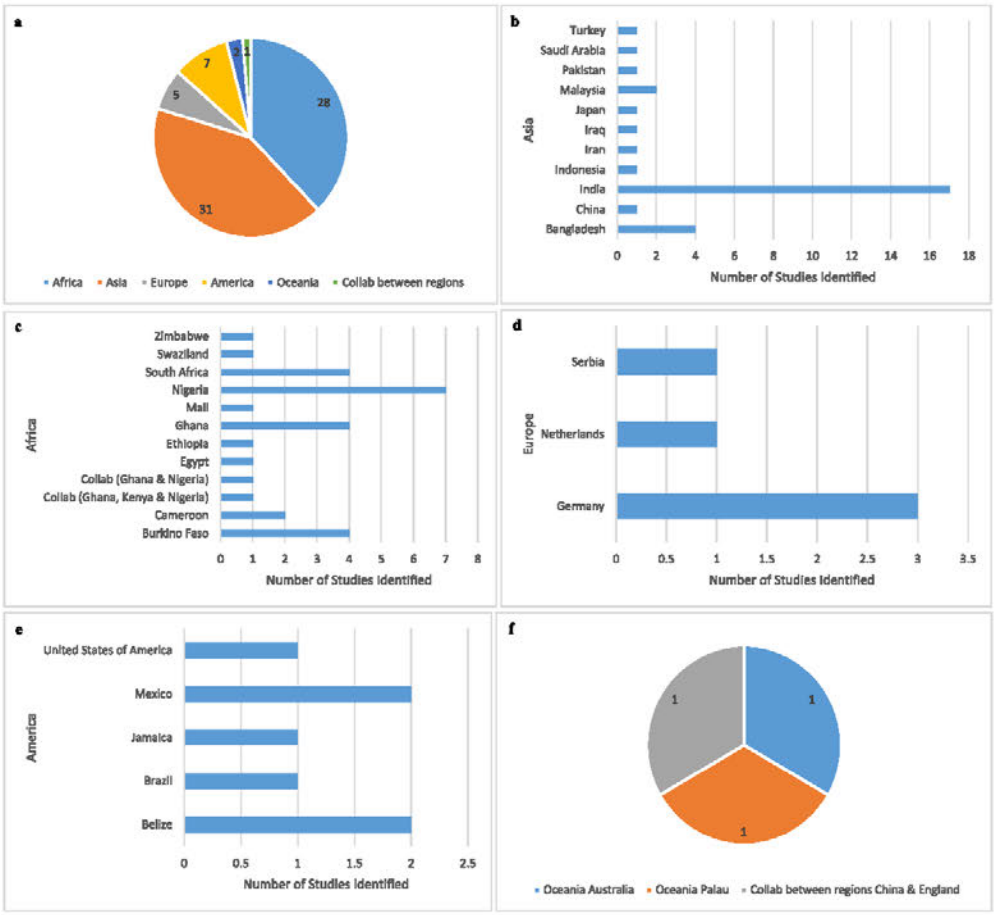


Fig. 2a-f. Distribution of studies identified in different regions.

studies used questionnaires for data collection, and in some of the studies the traditional interventions were tested in the participants for their efficacy. The traditional approaches used for the management of schizophrenia and/or its symptoms ranged from religious/faith healing (use of Holy water and ash, prayers, and fasting), spiritual (yoga therapy, worshipping Kung Yum goddess, Buddha and ancestors, performing rituals, cleansing, and exorcism), and psychosocial interventions (counselling). Other interventions included acupuncture, massages and the use of herbal and animal products for remedies. The efficacy and safety of most of these interventions are yet to be supported by scientific evidence, and some approaches are considered inhumane (such as, starvation, beating, confinement, and inappropriate bodily exposure). However, some of the medicinal plants used herbal remedies have been tested for their properties and effect in in-vitro and in-vivo studies.

The variability in the animal studies included in this review was also noted in sample type and number, and the methodology based on the properties that were tested in each study. The sample ranged from macrophage cells, Swiss albino mice, Imprint Control Region (ICR) mice, C57Bl/6 J mice, to Wistar rats. The duration of treatment with the plant extracts varied depending on the tests that were conducted. All tested plants demonstrated a positive antipsychotic potential and reversed the induced behavioural anomalies in the used animal models.

3.1. Treatment options and herbal plants used for the treatment of schizophrenia in different regions

table 3

4. Discussion

In traditional healing, the explanation of causal factors for physical, emotional and mental illnesses is associated with witchcraft, sorcery and spirits (gods, ancestors or evil spirits), while biomedical practitioners have been reported to lack the ability to treat conditions associated with supernatural factors (Audet et al., 2017). Furthermore, unlike biomedical psychiatric care, traditional healing or medicine has been reported by various authors to provide holistic care which considers a patient's cultural and spiritual beliefs, cosmology and world perspective (Ibrahim et al., 2020). Additionally, it is reported that on the onset of illness, psychiatric patients first seek healthcare from traditional healers (Razali and Yassin, 2008; Mounkoro et al., 2020). However, there exists a paucity of studies supporting the use of traditional approaches used by the traditional healers for the treatment of severe mental disorders; viz. bipolar and psychotic disorders (Nortje et al., 2016; Ibrahim *et al.*, 2020); hence, the purpose of this review was to document what has been investigated, with a particular interest in schizophrenia in order for knowledge gaps to be easily identified. Practices involved in traditional healing range from long-standing to evolving based on the diverse theories and beliefs associated with different cultures and communities

Table 3
Traditional treatment options and herbal plants used in different regions for the treatment of schizophrenia - Studies involving human participants.

Region	Country	Author	Year	Study Design	Treatment Intervention	Treatment Duration	Plant/Herb/ Remedy/Natural Product	Preparation	Ailment/s or Effects/ Activity	Subjects	Outcomes/ Comments	Bias (%)
AFRICA												
East Africa	**Ethiopia**	Baheretibeb et al.	2021	Case Study Qualitative	Collaboration between traditional medicine (faith healing) & biomedical treatment.	-	Holy water & prayers simultaneously with biomedical medication (collaboration)	-	Schizophrenia, substance use & mood disorders	1888 patients	Patients comfortable with using both traditional healing approaches & biomedical treatment	50
Northeast Africa	**Egypt**	Ibrahim et al.	2020	Quantitative	Herbal medicine, Prayers, Spiritual sessions for communicating with Jin, Hijama or surgical procedures	-	-	-	Schizophrenia	232 patients	Patients with schizophrenia prefer to consult traditional healers first	90
Southern Africa	**South Africa**	Sorsdahl et al.	2010	Qualitative	Herbal medicine	A few minutes for the *Amafufunyana* spirit to release	*Indonya & umdlebe*	Herbs (½ teaspoon of umdlebe), mixed with 1 teaspoon Methylated spirit, ½ teaspoon Benzene, a few pieces of Indonya, 1 teaspoon of vinegar	*Amafufunyana*	50 traditional healers	Modern ingredients incorporated by some of the traditional healers are potentially toxic	100
		Audet et al.	2017	Mixed-methods	Herbal medicine	-	-	Prepared as remedies to be delivered via drops to patients' ears & eyes	Nhlanyi (losing touch with reality, which may be translated as madness) & psychosis	160 traditional healers	Some healers indicated that they may not always be able to cure psychosis, but only minimise associated behaviours	40
		Mzimkulu & Simbaya	2006	Qualitative	Induced vomiting through the nose, induced vomiting through the mouth, steaming in herbs & nasal inhalation of smoke from herbs	-	-	-	Psychosis	4 traditional healers treating psychiatric patients admitted to a psychiatric hospital	Despite the differences that exist between traditional and biomedical healing, patients may seek treatment from both approaches simultaneously	80
		Ngobe et al.	2021	Qualitative	Herbal medication & nasal inhalation	-	-	Prepared mixture from materials such as roots obtained from numerous plants, & animal substances, is boiled and the resulting steam	Psychotic disturbance	10 traditional healers	Involvement of ancestors is essential in traditional healing for the management of psychotic disorders	90

(*continued on next page*)

Table 3 (*continued*)

Region	Country	Author	Year	Study Design	Treatment Intervention	Treatment Duration	Plant/Herb/ Remedy/Natural Product	Preparation	Ailment/s or Effects/ Activity	Subjects	Outcomes/ Comments	Bias (%)
								is inhaled by patients, & cool extracts are used to bath the body				
	Swaziland	Ngobe	2015	Qualitative	Herbal medicine, casting out evil, steaming, washing, induced vomiting, traditional incisions, performing rituals – all to cleanse patient of evil spirits	-	-	-	Mental illness (including psychotic illness)	10 traditional healers	Treatment option depends on what is believed to have caused the illness	80
	Zimbabwe	Kajawu et al.	2015	Qualitative	Herbs, animal products, counselling & healing rituals; also, ritual baths, sniffing (kufemba), scarifications (kutema nyora), invocations (kuchema kumidzimu) & vows (mhiko), where healers chant healing words or expel evil spirits from the patient	-	Python fat	"don't mix my medicine [*muti*] with Western medicine [*mushonga wechirungu*]; the healing spirit [*sekuru*] does not allow that, otherwise my herbs [*muti*] won't work"	Treatment intervention for mental disorders n general; python fat specific for psychosis	30 traditional healers	Pythons are considered as calm animals & it is believed that using python fat is perceived will calm aggressive behaviour Traditional healing will not be effective if combined with biomedical medicine	100
sub-Saharan African countries	**Ghana, Kenya & Nigeria**	Esan et al.	2019	Mixed-methods	Herbs & fetish practices, prayer & fasting, scarification, orthodox medications, rituals; other forms of non-humane treatment: starvation, beating, exposure to elements, overcrowding, cohabitation of sexes, sleeping on bare floor, inappropriate bodily exposure, confinement (to a room)	-	-	-	Epilepsy, puerperal psychosis, alcohol or drug use, madness, psychosis or insanity & depression	693 traditional healers (205 in Ghana, 406 in Kenya and 82 in Nigeria)	Some practices considered therapeutic by traditional healers are inhumane, & some of these practices may prevalent but done in secrecy or are hidden	60
	Ghana & Nigeria	Gureje et al. (*)	2020	Quantitative (cluster-randomized)	Collaboration of traditional & biomedical healing traditional healing: herbal medicine, rituals & psychosocial interventions	6 months	-	-	Psychosis	Adult patients with active psychotic symptoms admitted to traditional and faith healers facilities	Collaboration between traditional & biomedical healing was effective & cost-effective	80
West Africa	**Burkina Faso**	Kinda et al. (α)	2017	Mixed-methods	Herbal medicine	-	*Abrus precatorius, Acacia nilotica, Acacia sieberiana, Adansonia digitate,*	Maceration, decoction, calcination & trituration	Madness & mental disorders	53 traditional healers	Identified plants possess psychoactive properties	90

(*continued on next page*)

Table 3 (*continued*)

Region	Country	Author	Year	Study Design	Treatment Intervention	Treatment Duration	Plant/Herb/ Remedy/Natural Product	Preparation	Ailment/s or Effects/ Activity	Subjects	Outcomes/ Comments	Bias (%)
							Afzelia africana, Allium sativum, Annona senegalensis, Balanites aegyptiaca, Calotropis procera, Citrus aurantifolia, Cymbopogon giganteus, Dalbergia melanoxylon, Daniellia oliveri, Datura innoxia, Diospyros mespiliformis, Ficus iteophylla, Guiera senegalensis, Khaya senegalensis, Leptadenia hastate, Mitracarpus villosus, Mitragyna inermis, Moringa oleifera, Parkia biglobosa, Pericopsis laxiflora, Pseudocedrela kotschyi, Scoparia dulcis, Securidaca longepeduinculata, Tamarindus indica, Vitellaria paradoxa, Vitex doniana Sweet, Ximenia americana & Zanthoxylum zanthoxyloides					
		Ouoba et al. (β)	2020	Mixed-methods	Wild mammals in traditional medicine	-	*Civetticis civetta* *Loxodonta africana*	Anus Skin	Madness	18 respondents (farmers, pastoralists and traditional healers)	Wild mammals are essential in managing mental ailments in some traditional healing practices	90
		Rouamba et al.	2018	Mixed-methods	Herbal medicine	-	*Crotalaria retusa*	Whole plant powdered & burnt in the room	Psychotropic disorders (madness, hallucination & melancholy)	40 herbalists and traditional healers	*C. retusa* is reported to be a pyrrolizidine toxic plant, & herbalists & traditional healers are unaware of the potential toxicity of this plant	80
		Tibiri et al.	2015	Mixed-methods	Herbal medicine	-	*Lannea microcarpa* *Saba senegalensis*	Stem bark maceration used orally or for bathing Fruit decoction used orally or for bathing	Schizophrenia	16 traditional healers	This study provided database of Dozo traditional knowledge of herbals plants	70

(*continued on next page*)

Table 3 (*continued*)

Region	Country	Author	Year	Study Design	Treatment Intervention	Treatment Duration	Plant/Herb/ Remedy/Natural Product	Preparation	Ailment/s or Effects/ Activity	Subjects	Outcomes/ Comments	Bias (%)
	Ghana	Debrah et al.	2018	Qualitative	Spiritual interventions, herbal medicine, dietary supplements & music therapies	-	-	-	Schizophrenia	51 participants (family and clinical caregivers)	Patients with schizophrenia & bipolar disorders are likely to resort traditional and faith-based healing, and music therapy	80
	Mali	Mounkoro et al. (∝)	2020	Mixed-methods	Herbal medicine, sacrifice, incantations, physical restraint, Koranic verses, flagellation, fetish, conventional medicine & massage	-	*Allium sativum L., Sclerocarya birrea, Lannea acida, Annona senegalensis, Xylopia aethiopica, Leptadenia lancifolia, Carallluma dalzielii, Saba senegalensis, Glossonema boveanum, Commiphora africana, Boscia angustifolia, Guiera senegalensis, Combretum glutinosum, Combretum micranthum, Euphorbia balsamifera, Bauhinia reticulate, Daniellia oliveri, Cassia sieberiana, Senna italic, Faidherbia albida, Entada africana, Parkia biglobosa, Adansonia digitate, Sterculia setigera, Khaya senegalensis, Ficus platyphylla, Ficus abutilifolia, Ficus thonningii, Ficus cordata, Flueggea virosa, Cymbopogon giganteus, Chrysopogon nigritanus, Securidaca longepedunculata, Mitragyna inermis, Gardenia ternifolia, Vitellaria paradoxa, Aloe vera, Piper guineense, Zingiber officinale, Aframomum melegueta*	How each plant is prepared varied from being powdered, macerated, mixed with other herbs, etc.	Schizophrenia	20 traditional healers	Most plants are extinct &endangered	90

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Table 3 (*continued*)

Region	Country	Author	Year	Study Design	Treatment Intervention	Treatment Duration	Plant/Herb/ Remedy/Natural Product	Preparation	Ailment/s or Effects/ Activity	Subjects	Outcomes/ Comments	Bias (%)
	Nigeria	Sonibare et al. (α)	2005	Mixed-methods	Herbal medicine	-	*Abelmoschus esculentus, Acacia nilotica, Aframomum melegueta, Allium cepa, Allium sativum, Alternanthera sessilis, Amaranthus viridis, Arachis hypogea, Asparagus africanus, Blighia sapida, Capsicum frutescens, Cassytha filiformis, Celosia trigynia, Cissampelos owariensis, Citrus medica, Clerodendrum capitum, Costus afer, Crinum jagus, Cyrtosperma senegalensis, Dialium guineense, Dracaena arborea, Elaeis guineensis, Elytraria marginata, Enantia chlorantha, Erythrophleum ivorensis, Euphorbia kameronica, Garcinia kola, Globimetula braunii, Leptoderris micrantha, Lonchocarpus cyanenscens, Massularia acuminate, Milicia excels, Monodora myristica, Nauclea latifolia, Picarlima nitida, Piper guineensis, Pterocarpus osun, Pyreniacantha staudtii, Rauwolfia vomitoria, Spilanthes ficaulis, Terminalia ivorensis, Uraria picta & Xylopia aethiopica*	-	Anti-psychotic properties	Herbalists & herb sellers	Most plants with antipsychotic potential were from the *Amaranthaceae, Annonaceae & Liliaceae* families	90
ASIA												
East Asia	**China**	Zhang et al. (*)	2001	Quantitative (randomized)	Combination of herbal medicine & antipsychotic drug	12 weeks	*Ginkgo biloba*	Extract prepared as tablets	Antioxidant effect	82 schizophrenic patients	Extract of *Ginkgo biloba* may enhance the efficiency of antipsychotic haloperidol for	50

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Table 3 (*continued*)

Region	Country	Author	Year	Study Design	Treatment Intervention	Treatment Duration	Plant/Herb/ Remedy/Natural Product	Preparation	Ailment/s or Effects/ Activity	Subjects	Outcomes/ Comments	Bias (%)
											treatment of chronic schizophrenia	
	Japan	Miyaoka et al. (*)	2015	Quantitative (randomized)	Herbal medicine	4 weeks	Yokukansan (mixture of dried herbs containing rhizome of A. lancea (4 g), Poria (4 g), C. rhizome (3 g), Angelicae radix (Angelica acutiloba) (3 g), Bupleuti radix (2 g), Glycyrrhizae radix (1.5 g), Uncariae uncis cum ramulus (3 g)	-	Associated with 5-hydroxytryptamine (5-HT) receptor (potential target for the treatment of schizophrenia)	120 patients with treatment-resistant schizophrenia	Yokukansan demonstrated potential to improve excitement/ hostility symptoms in treatment-resistant schizophrenia	90
Middle East	**Iran**	Parvizi et al. (*)	2019	Quantitative (randomized)	Combination of herbal medicine & antipsychotic drug (risperidone)	8 weeks	*Cuscuta epithymum*	Prepared as capsules	Schizophrenia/ antioxidant and neuroprotective properties	30 admitted patients with schizophrenia	*Cuscuta epithymum* improved positive and negative symptoms, and cognitive impairment of patients with schizophrenia	80
	Iraq	Al-Jiboori	2007	Mixed-methods	Religious methods (writing of prayers & amulet, reading of the Quran (also in water), exorcism), herbal medicine (Soaout - inhalation of a herbal powder), physical punishment, cautery, strangulation & hitting the face of patients with shoes for the treatment of Bell's palsy	Ranged from less than 3 months to more than 2 years	-	-	Psychiatric disorders	750 patients	Traditional healing and supernatural belies of causation of psychiatric Disorders delayed psychiatric treatments	50
	Saudi Arabia	Sayed et al.	1999	Mixed-methods	Reading the Quran, herbal medicine (or a combination of the 2), cautery & rughia	-	-	-	Psychiatric disorders	227 patients	Frequency of attending traditional healers was associated with a diagnosis of schizophrenia, psychiatric hospitalization, elder age group, and common in females	70
	Turkey	Budak & Yilmaz (*)	2019	Quantitative (randomized)	Yoga therapy (adjunctive treatment to antipsychotic medication)	8 weeks	-	-	Schizophrenia	50 schizophrenic patients	Yoga showed effectiveness in improving medication adherence and clinical insight	90
South Asia	**Bangladesh**	Ahmed & Azam (α)	2014	Mixed-methods	Herbal medicine	-	*Piper retrofractum*, *Ficus hirta*, *Thevetia*	Some are taken orally (i.e. as a	Schizophrenia like psychosis	3 traditional healers	Plants used by traditional healers	70

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Table 3 (*continued*)

Region	Country	Author	Year	Study Design	Treatment Intervention	Treatment Duration	Plant/Herb/ Remedy/Natural Product	Preparation	Ailment/s or Effects/ Activity	Subjects	Outcomes/ Comments	Bias (%)
							peruviana, Datura metel L., Euphorbia neriifolia, Randia dumetorum, Aegle marmelos, Litsea polyantha, Coccinia grandis, Ocimum americanum, Abroma augusta, Vitex negundo, Cannabis sativa & Morinda citrifolia	vegetable with rice, prepared as a paste), some are prepared as tablets/pills, some are used to make vapour inhaled via the nose & used for oil to massage scalp			were validated by the search of relevant scientific literature	
		Rahmatullah et al. (α)	2010	Mixed-methods	Herbal medicine	-	*Aegle marmelos (L.) Corr.*	Leaves are crushed and applied to scalp	Insanity	2 traditional healers	Of the 49 reported plants, only one is used in the treatment of insanity	80
		Rahmatullah et al. (α)	2014	Mixed-methods	Herbal medicine	-	*Kaempferia rotunda L.*	-	Insanity	2 traditional healers	Non-herbal substances used by traditional healers for insanity were a mixture (termed Rosh shindip) of calcined mercury with powdered iron, silver, gold, copper, brass, mica, and coral	60
		Zohora et al. (α)	2016	Qualitative	Herbal medicine	-	*Phyllanthus emblica*	Fruits pulp	Insanity	9 traditional healers	One plant is used for various health issues	90
	India	Campion & Bhugra	1997	Qualitative	Religious healing (chanting mantra, use of rhayath, Ingestion of Holy water & ash, sprinkling of holy water, application of ash, use of dolls, there are objects given to patient for keeping at home, animal sacrifice & the use of lemon fruit	15 min to up to 24 h (duration of consultation with religious healer	-	-	Psychiatric disorders	198 psychiatric patients	Patients with schizophrenia and delusional disorders consulted traditional healers more than patients with other disorders	80
		Dey et al. (α)	2017	Qualitative	Herbal medicine	-	*Indigofera linifolia Phyllanthus emblica*	Roots worn around the neck, when pasted they are applied topically on head & forehead	Insanity/madness	65 traditional healers	Herbal plants combat restrictions of biomedical medication such as drug resistance and adverse side effects	90
		Duraiswamy et al. (*)	2007	Quantitative (randomized)	Yoga therapy (adjunctive treatment to antipsychotic medication)	4 months	-	-	Schizophrenia	61 schizophrenic patients	Patients in the Yoga therapy group showed better quality of life, and	70

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Table 3 (*continued*)

Region	Country	Author	Year	Study Design	Treatment Intervention	Treatment Duration	Plant/Herb/ Remedy/Natural Product	Preparation	Ailment/s or Effects/ Activity	Subjects	Outcomes/ Comments	Bias (%)
											social and occupational functions	
		Jalal et al. (α)	2008	Qualitative	Herbal medicine	-	*Epipactis helleborine*	-	Insanity	Unspecified number of local people & traditional healers	Orchids are close to being endangered	90
		Kumari et al. (α)	2019	Qualitative	Herbal medicine	-	*Rauvolfia serpentine*	-	Schizophrenia	60 informants (tribal chieftains, traditional healers and community elders)	The efficacy and success of traditional Medicine is trusted by the people of Chatra	90
		Ningsangrenla & Rao	2019	Qualitative	Psycho-spiritual approach, herbal medicine &/or manual approach	-	-	-	Schizophrenia	510 rural & 300 urban households	Traditional healing still remains the first point of contact for mental disorders	80
		Paikkatt et al.	2015	Quantitative	Yoga therapy (adjunctive treatment to antipsychotic medication)	1 month	-	-	Schizophrenia	30 chronic schizophrenic patients	Patients in the Yoga therapy group showed better quality of life, and psychopathology	80
		Ramu et al.	1992	Quantitative	Ayurvedic Treatment comprising shodhana (cleaning) & shamana (palliative) measures & satwawajayachikitsa (psychobehavioural therapy)	15 days	-	-	Schizophrenia	36 schizophrenic patients	Improvement in psychological functions were noted with Ayurvedic treatment	70
		Rao et al. (*)	2021	Quantitative (randomized)	Yoga therapy (adjunctive treatment to antipsychotic medication)	12 weeks	-	-	Schizophrenia	96 schizophrenic patients	Patients in the Yoga therapy group showed improvements in the negative symptoms	90
		Rout et al. (α)	2012	Qualitative	Herbal medicine	-	*Clerodendrum infortunatum*	Leaf juice is diluted in water	Insanity	217 respondents	Some plants are associated with superstitions and black magic	80
		Sankaranarayanan et al. (α)	2010	Qualitative	Herbal medicine	-	*Evolvulus alsinoides Linn.*	-	Insanity	275 inhabitants	Plant reported to contain evolvine, pentatriacontane, triacontane sistosterol, a glycoflavone, 4-methoxy vtexin and phenolic acid	90
		Singh et al. (α)	2002	Qualitative	Herbal medicine	-	*Evolvulus alsinoides*	Leaf paste	Insanity	Unspecified number of local informants & other elders	Different parts of the plant are prepare differently for different uses	80
		Singh et al. (α)	2020	Qualitative	Herbal medicine	-	*Ipomea obscura Convolvulace ae*	Leaf sap	Insanity	-	Different parts of the plant are prepare differently for different uses	90

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Table 3 (*continued*)

Region	Country	Author	Year	Study Design	Treatment Intervention	Treatment Duration	Plant/Herb/ Remedy/Natural Product	Preparation	Ailment/s or Effects/ Activity	Subjects	Outcomes/ Comments	Bias (%)
		Singhal et al. (α)	2016	Qualitative	Herbal medicine	-	*Catharanthus roseus*	-	Insanity	Unspecified number of knowledgeable people (such as family heads, housewives, elder persons, experienced farmers of the community)	Different parts of the plant have different uses	90
	Pakistan	Mushtaq et al. (α)	2012	Qualitative	Herbal medicine	-	*Aerva javanica*	Dry leaves are powdered & mixed with senna, wild mint, nigella seeds & gugal (1 tbsp of mixture twice a day)	Insanity	300 informants (including traditional healers, farmers, herb seller and elders)	Conservation of herbal plants saves them from extinction	100
							Pegarum hermala	Decoction of leaves				
Southeast Asia	**Malaysia**	Phang et al.	2010	Mixed-methods	Prayer, holy water, "do good-avoid evil" & dietary advices, massage & herbal medicine	-	-	-	Psychosis	50 in-patients (with first-episode psychosis)	Majority of the patients did not benefit nor get worse after receiving traditional medicine	70
		Razali & Yassin	2008	Qualitative	Holy water, herbal medicine, massage, talisman & others	Ranged from less than 2 weeks to up to 24 weeks	-	-	Psychosis & epilepsy	120 patients with schizophrenia, schizophreniform disorder & epilepsy	Consultation with traditional healers is higher in patients with psychotic illness	90
EUROPE												
Balkans	**Serbia**	Nikolic et al.	2018	Mixed-methods	Herbal medicine	-	*Matricaria chamomilla & Melissa officinalis*	Prepared as an infusion/tea	Psychotic symptoms & anxiety	106 outpatients in remission phase of psychotic mental disorders	Interaction between herbal remedies & biomedical medication still requires investigation	90
North-western Europe	**Netherlands**	Bosch et al.	2015a	Qualitative	Acupuncture according to traditional Chinese medicine principles	-	-	-	Depression & schizophrenia	60 patients with schizophrenia & depression	The use of acupuncture in treatment of patients with schizophrenia & depression showed positive effects	80
Western Europe	**Germany**	Bosch et al. (*)	2015b	Quantitative (Randomised)	Acupuncture (add on to Western Medication)	12 weeks	-	-	Schizophrenia, depression & sleep disorders	50 patients with schizophrenia, 50 patients with depression, 25 healthy control	Use of acupuncture was safe & practical in patients with schizophrenia, & had a positive effect in patients with depression	80
		Bosch et al.	2017	Case report Mixed-methods	Acupuncture (add on to Western Medication)	12 weeks	-	-	Schizophrenia & sleep disorder	Adult male outpatient with long-term	Acupuncture was effective in alleviating sleep	80

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Table 3 (*continued*)

Region	Country	Author	Year	Study Design	Treatment Intervention	Treatment Duration	Plant/Herb/ Remedy/Natural Product	Preparation	Ailment/s or Effects/ Activity	Subjects	Outcomes/ Comments	Bias (%)
										schizophrenia & sleep disorders	disorders & negative symptoms of schizophrenia	
AMERICA												
Central America	**Belize**	Bourbonnais-Spear et al. (α)	2005	Mixed-methods	Ritual of smoking, prayer & Herbal medicine	-	Families: *Acanthaceae, Adiantaceae, Araceae, Aristolochiaceae, Aspleniaceae, Asteraceae, Bixaceae, Buddlejaceae, Fabaceae (Caesalpnioideae), Fabaceae (Papilionoideae), Loranthaceae, Marantaceae, Phytolaccaceae, Piperaceae, Rubiaceae, Rutaceae, Schizaeaceae, Selaginellaceae, Solanaceae*	For ritual of smoking, elements from the cause or location of fright and/or plants are burnt letting the smoke rise around the patient	Madness	6 traditional healers	Madness is believed to be caused by witchcraft, jealousy & obsessive behaviour	80
		Choco	n.d.	Qualitative	Herbal medicine	-	*Anthurium gladiifolium Schott, Anthurium pentaphyllum,*	Leaves can be crushed in room temperature water just enough so that the individual can bathe the head	Madness	4 traditional healers	Young & fresh plants are preferred for mental disorders	80
							Souroubea sympetala	Leaves can be crushed in 2 gallons of room temperature water so that it can be drunk three times daily and can be used to constantly bathe the head				
							Mollinedia guatemalensis	Leaves are boiled in water enough to have for drink and to bathe the body with				
North America	**Mexico**	Zacharias	2006	Mixed-methods	Ethnotherapeutic treatment practices of *curanderos*	6 months	-	-	Psychiatric disorders (including schizophrenia, panic and	3 healers & their patients (8)	Mexican *Curanderismo* showed	90

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Table 3 (*continued*)

Region	Country	Author	Year	Study Design	Treatment Intervention	Treatment Duration	Plant/Herb/ Remedy/Natural Product	Preparation	Ailment/s or Effects/ Activity	Subjects	Outcomes/ Comments	Bias (%)
									dependence syndrome)		psychotherapeutic effect	
	United States of America	Visceglia & Lewis (*)	2011	Quantitative (randomized)	Yoga therapy (adjunctive treatment to antipsychotic medication)	8 weeks	-	-	Schizophrenia	18 schizophrenic patients	Patients in the Yoga therapy group showed better quality of life, and psychopathology	90
Southern North America	**Jamaica**	James & Peltzer	2012	Mixed-methods	Spiritual healing, herbal medicine, rituals, prayer, use of olive oil, lay hands and take patients for a bath	-	-	-	Psychiatric disorders (including schizophrenia)	60 psychiatric patients & 30 western trained mental health practitioners	Biomedical practitioners do not perceive traditional medicine as advantageous among patients with psychiatric illness	90
South America	**Brazil**	Rodrigues & Carlini (α)	2006	Qualitative	Herbal medicine & rituals	-	*Anacardium giganteum W. Hancock ex Engl., Chondrodendron tomentosum Ruiz & Pav, Clitoria guianensis (Aubl.) Benth., Dischorisandra affinis Mart, Myrcia multiflora (Lam.) DC., Pterocarpus michelii Britton, Spondias lutea L., Tachigalia paniculata Aubl.*	-	Craziness/madness (symptoms similar to schizophrenic & psychotic disorders)	Informal interviews with 200 adult village inhabitants (7 were traditional healers & selected to participate in this study)	Mentioned plants reported to have psychoactive properties	90
OCEANIA												
-	**Australia**	Turner et al. (*)	2019	Quantitative (Randomized)	Adjunctive herbal medicine	24 weeks	*Garcinia mangostana Linn. (Mangosteen) Pericarp*	Prepared as 500 mg capsules (2 capsules = 1000 mg/day)	Schizophrenia	150 patients with schizophrenia or schizoaffective disorder	Plant showed positive effects as an adjunctive treatment	80
-	**Palau**	Sullivan et al.	2000	Mixed-methods	Herbal medicine	-	*Areca catechu*	-	Schizophrenia	70 outpatients with schizophrenia	Betel chewing was linked with milder symptomatology & minimized use of recreational drugs	80
Collaboration between Countries from Different Regions												
-	England & China	Furnham & Wong	2007	Quantitative	Acupuncture, Buddhist prayers, exorcism of evil spirits, herbal medicine, seeking help from God, psychological treatments, worshipping Kung Yum goddess & the Buddha, balancing your Yin & Yang, counselling, community treatment programmes, ancestral worship	-	-	-	Schizophrenia	200 participants (British & Chinese)	Chinese believed that causation & treatment is influenced by superstitious & religious factors	80

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Table 3 (*continued*)

Region	Country	Author	Year	Study Design	Treatment Intervention	Treatment Duration	Plant/Herb/ Remedy/Natural Product	Preparation	Ailment/s or Effects/ Activity	Subjects	Outcomes/ Comments	Bias (%)
					(burning candles & joss sticks), Freudian therapy (psychoanalysis), massage with aromatic oils, reading the Holy Bible & Taoism							

Trial (*); Ethnobotanical Survey (α); Ethno-zoological Survey (β)

(Bodeker and Burford, 2006; Ningsangrenla and Rao, 2019). The broadness and diversity of traditional medicine has also led to the evolution of varying forms of indigenous healing methods; such as, African, Chinese and Indian traditional medicines (Abdullahi, 2011). Based on the literature reviewed in this paper, the diversity of traditional medicine has been reflected as different regions having different methods to manage mental illnesses. The prevalent traditional healing method across all regions globally in the current review was the use of herbal remedies; however, the plants used and their method of preparation vary considerably. The use of herbal medicine (also referred to as phytotherapy or herbalism) dates back to ancient civilization and is currently reported to be common in approximately 80 % of the global population (Balkrishna et al., 2024), and this validates the findings of this review. The danger with the rising demand of herbal products is the increase in fraudulent practices which include the use of synthetic compounds in substitution or addition to herbal material; this then necessitates quality control and standardization of herbal medicine (Balkrishna et al., 2024). Medicinal plants contain a number of bioactive compounds which act in alleviating, curing and preventing various diseases (Ahmed and Azam, 2014). The positive properties (i.e. antioxidant, antipsychotic, anti-inflammatory, etc.) of the medicinal plants and their constituents are commonly reported, while details on side effects and cytotoxicity of these remain lacking. Other issues that need to be considered with the use of medicinal plants include how foreign contaminants such as microbiological agent and pesticide residues or heavy metals can also trigger adverse effects (Balkrishna et al., 2024).

In Africa, the use of herbal medicine was the prevalent traditional approach across all regions and was used in a number of countries, including Ethiopia, Kenya, Burkina Faso, Ghana and Nigeria in West Africa, Cameroon in Central Africa, South Africa, Swaziland and Zimbabwe in Southern Africa, and Egypt in Northeast Africa. In South Africa, the methods of administering the identified herbs included steaming, burning the herbs, nasal inhalation, enema, induced vomiting and traditional incisions (Mzimkulu and Simbayi, 2006; Ngobe et al., 2021). In Asia, the use of herbal medicine for the treatment of schizophrenia and other mental disorders is also prevalent in countries like Bangladesh, India, Pakistan, Indonesia, Malaysia, China, Japan, Iran, Iraq and Saudi Arabia. Modes of administering the herbal remedies include applying the powdered plant onto the scalp of the patient, as noted in Bangladesh (Rahmatullah et al., 2010). In South America (Brazil), herbal medicine and rituals were used to treat craziness or madness with symptoms similar to schizophrenic and psychotic disorders (Rodrigues and Carlini, 2006). In Central America (Belize), herbal medicine, rituals of smoking and prayer were used in the treatment of madness (Choco, 2024; Bourbonnais-Spear et al., 2005). The current review has noted an underreporting of how herbal remedies are prepared, details on the dosage, the duration of treatment and in the efficacy of the herbal plants used for the treatment of psychiatric disorders. Only a few clinical trials prove herbal medicine and other traditional healing approaches to be effective, even so, in most cases these are use as adjunctive treatment to biomedical healing approaches. Additional, in some cases, plants used in herbal remedies are not disclosed due to how they are considered sacred information by the healers, and this is consistent with the reports by Sorsdahl et al. (2010). Also, in some cases, the traditional healers are not aware of the toxicity of the plants they use (Rouamba et al., 2018).

Other traditional healing approaches used in Ghana involved consultation with the gods, dietary supplements and music therapies (Debrah et al., 2018). According to Debrah et al. (2018), when patients listen to their favourite music, they feel better as their mood is regulated, and this makes them cope better with their conditions. This is in agreeance with the reports from Jackson (2015), who also added that the effectiveness of music therapies is due to how they incorporate positive psychology interventions. Dietary supplements have been reported to be used as adjunctive treatment with antipsychotic drugs, and the amino acids these contain assist in the biosynthesis of

Table 4
Studies where the herbal plants used in different regions for the treatment of schizophrenia have been tested in-vitro and in-vivo.

Region	Country	Author	Year	Study Design	Plant/Herb/ Remedy/ Natural Product	Preparation	Subjects/Cells	Treatment Duration	Ailment/s or Effects/ Activity	Outcome	Bias
AFRICA Central Africa	Cameroon	Ngoungoure et al.	2019	Quantitative In-vitro	*Voacanga africana; Cola acuminata (P. Beauv.) Schott & Endl.*	Dried & powdered different parts of plants were extracted with methylene chloride/ methanol	LPS-stimulated RAW 264.7 macrophage cells	15 min (anti-inflammatory activities), 4 h (cytotoxicity)	Schizophrenia	All investigated plants showed AChE inhibitory, anti-inflammatory & antioxidant properties	-
		Taiwe et al.	2012	Quantitative In-vivo	*Crassocephalum bauchiense (Hutch.) Milne-Redh (Asteraceae)*	Dried & powdered leaves prepared as aqueous extract	Male & female Swiss mice (24–26 g).	24 h	Antipsychotic properties	Antipsychotic properties of *Crassocephalum bauchiense* are potential due to blockade of dopamine D-2 receptors	50
West Africa	Ghana	Amoateng et al.	2017a	Quantitative In-vivo	*Synedrella nodiflora*	Dried & powdered plant prepared as hydro-ethanolic extract	Female Imprint Control Region (ICR) mice (20–30 g)	Animals observed after15, 30, 60, 120 & 180 min, & 24 h	Schizophrenia	Plant showed acetylcholine-like & serotonin-like effects, & can also potentiate effects of chlorpromazine & haloperidol.	55
		Amoateng et al.	2017b	Quantitative In-vivo	*Albizia zygia*	Dried & powdered leaves were prepared as ethanolic extract	Female ICR mice (20–30 g)	Animals observed after15, 30, 60, 120 & 180 min, & 24 h	Antipsychotic-like effects	Plant extract decreased the frequency & duration of apomorphine-induced climbing activities in mice	80
		Kumbol et al	2018	Quantitative In-vivo	*Albizia zygia*	Roots were powdered & macerated in 70 % ethanol	Male ICR mice (25–30 g)	60 min (for apomorphine cage climbing test, ketamine-induced hyperlocomotion & Ketamine-impaired social interaction), 7 days (for ketamine-enhanced immobility	Schizophrenia	Plant extract showed potential to alleviate all (viz. positive, negative & cognitive) symptoms of schizophrenia	80
	Nigeria	Akinpelu et al.	2018	Quantitative In-vivo	*Milicia excelsa (welw.) C.C. Berg*	Powdered leaves extracted in 70 % ethanol	Adult male albino mice (18–25 g)	1 h	Insanity	Plant extract inhibited the hypermotility induced by ketamine, inhibited climbing behaviour in apomorphine-induced climbing, & reduced number & duration of grooming. Thus indicating antipsychotic properties	80
		Ior et al.	2020	Quantitative In-vivo	*Terminalia macroptera*	Powdered leaves macerated in 70 % methanol	Male & female Swiss albino mice (25–30 g)	1 h (for apomorphine climbing test, Spontaneous motor activity), 7 days (for chronic ketamine administration)	Psychosis	Plant extract showed neuroprotective potential, & alleviated ketamine-induced behavioural abnormalities	65
		Ior et al.	2021	Quantitative In-vivo				1 h (for apomorphine induced stereotypic climbing), 7 days (for open-field, Y-maze & forced swim tests after ketamine administration, & for catalepsy test)	Schizophrenia	Plant extract showed antipsychotic activity through alleviating ketamine-induced hyperactivity, immobility, & memory deficit & reducing stereotypic climbing behavior due to apomorphine.	65

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Table 4 (*continued*)

Region	Country	Author	Year	Study Design	Plant/Herb/ Remedy/ Natural Product	Preparation	Subjects/Cells	Treatment Duration	Ailment/s or Effects/ Activity	Outcome	Bias
		Magaji et al.	2014	Quantitative In-vivo	*Securinega virosa*	Powdered leaves macerated in methanol	Swiss Albino mice of either sex (20±2 g)	24 h (for acute toxicity study), 30 min for swim-induced grooming test)	Psychiatric illness	Plant extract indicated antipsychotic & sedative properties	60
		Maiha et al.	2013	Quantitative In-vivo	*Ficus thonningii*	Powdered leaves extracted in 70 % methanol	Swiss albino mice of either sex (24 ± 4 g)	30 min	Antipsychotic potential	Plant extract showed potential D1 dopaminergic antagonistic effect	55
		Oyepeju & Sunday	2018	Quantitative In-vivo	*Securidacalonge pedunculata*	Dried & powdered plant was macerated in 70 % ethanol	Albino mice of either sex (18–30 g)	30–60 min	Anti-psychotic properties	Plant extract reduced stereotypic behaviour induced by apomorphine	80
ASIA											
South Asia	**India**	Ingale & Kasture	2012	Quantitative In-vivo	n-butanol extract of *Passiflora incarnata* leaves & flowers	Dried & powdered leaves & flowers prepared as an ethanol extract	Swiss albino mice of either sex (25–30 g)	30–60 min	Antipsychotic/ neuroleptic potential	Flavonoids contribute to the activity of plant's leaves & flowers extracts	65
		Mishra et al.	2018	Quantitative In-vivo	*Bacopa monnieri*	Mixture of powdered 38 herbal plants	Swiss albino mice	7 days	Seizures associated with schizophrenia	Plant extract showed anti-schizophrenia, anticonvulsant & memory-enhancing effects	65
		Venkataramaiah et al.	2021	Quantitative In-vivo	3-(3, 4-dimethoxy phenyl)-1–4(methoxy phenyl) prop-2-en-1-one (from methanol extract of *Celastrus paniculatus*)	Isolated bioactive compound	Male adult Wistar rats (150 ± 25 g)	7 days	Schizophrenia	Bioactive compound showed anti-schizophrenic & neuroprotective properties	70
Southeast Asia	**Indonesia**	Hidayat et al.	2021	Quantitative In-vivo	Cinnamon Extract (*Cinnamomum burmannii*)	Maceration in 96 % ethanol	Male Wistar rats (200 ± 20 g)	14 days	Anti-psychotic activity	Extract improved psychotic symptoms	65
EUROPE											
Western Europe	**Germany**	Coors et al.	2019	Quantitative In-vivo	*Rhodiola rosea*	Finely chopped root was extracted using 80 % aqueous ethanol at room temperature	Male Wistar rats & C57Bl/ 6 J mice	15 min	Antipsychotic-like effects	Apomorphine & Dizocilpine-induced prepulse inhibition deficits were restored by pre-treatment with extract	65
AMERICA											
North America	**Mexico**	Santillan-Urquiza et al.	2019	Quantitative In-vivo	*Galphimia glauca*	Dried plant macerated in n-hexane & methanol	Male ICR mice (28–31 g)	3 days (dopaminergic p/w), 19 days (glutamatergic p/w)	Schizophrenia	Plant extract counteracted stereotyped & grooming behaviours induced with apomorphine	70

neurotransmitters which then play a role in alleviating mental disorders (Debrah et al., 2018). Traditional healing approaches in Nigeria involved *Bori* (seeking consent for permission to treat patients from spirits), and in Zimbabwe vows (mhiko) are used, where healers chant healing words or expel evil spirits from the patient (Kajawu et al., 2015). Other methods used in Africa included psycho-spiritual approaches, for example the use of Holy water and prayers in Ethiopia (Baheretibeb et al., 2021), faith-based and spiritual interventions (confessions, prayers and fasting) in Ghana (Debrah et al., 2018), and prayers and spiritual sessions for communicating with *Jin* and *Hijama* used in Egypt (Ibrahim et al., 2020). In Ethiopia, faith healers who use Holy water as a treatment method are believed to be inspired by the apostle, St. Paul who was a physician, and they believe that Holy water is part of the sacrament which symbolises purity (Baheretibeb et al., 2021). Baheretibeb et al. (2021) also added that the use Holy water as a form of intervention is an Orthodox Christian approach which is believed to possess a healing power through the spirit of Jesus Christ which assists in the exorcism of evil spirits believed to cause mental illness (Baheretibeb et al., 2021). Prayer, Holy water, "do good-avoid evil" and dietary advice were also reported in Malaysia (Razali and Yassin, 2008; Phang et al., 2010), while the writing of prayers, the wearing of an amulet, reading of the Quran (also in water) and exorcism were reported in Iraq (Al-Jiboori, 2007) and Saudi Arabia (Sayed et al., 1999). Psycho-spiritual and religious healing practices in Asia which include chanting *mantra*, use of *rhayath*, ingestion of Holy water and ash and sprinkling of holy water were reported also in India (Campion and Bhugra, 1997). Othrt methods used in India included animal sacrifice and the use of lemon fruit (Campion and Bhugra, 1997), and Ayurvedic treatment comprising mechanical, manual, *shodhana* (cleaning) and *shamana* (palliative) measures and *satwawajayachikitsa* (psychobehavioural therapy) (Mukundan *et al.*, 1992; Ningsangrenla and Rao, 2019).

Acupuncture in addition to biomedical approaches was common in Europe, especially in the Netherlands and Germany (Bosch et al., 2015; 2017), and is used in conjunction with herbal medicine in Germany (Coors et al., 2019) and Serbia (Nikolic et al., 2018). In North America (Mexico), ethnotherapeutic treatment practices of *curanderos* (traditional native healers) (Zacharias, 2006) and herbal medicine (Santillán-Urquiza et al., 2019) were used to treat psychiatric disorders, including schizophrenia. In Jamaica, spiritual healing, herbal medicine, rituals, prayer, use of olive oil, laying hands and taking patients for a bath were used to treat psychiatric disorders, including schizophrenia (James and Peltzer, 2012). In Australia, adjunctive herbal medicine was used for the treatment of schizophrenia (Turner et al., 2019). In a collaborative study between China and England, acupuncture, Buddhist prayers and meditation, exorcism of evil spirits, herbal medicine, seeking help from God, psychological treatments which included worshipping the goddess Kung Yum and the Buddha, balancing your Yin and Yang, counselling, community treatment programmes, antipsychotic drugs, ancestor worship (burning candles and joss sticks), apologising for breaches of taboos and wrongs against your ancestors, Freudian therapy (psychoanalysis), going to self-help groups, massage with aromatic oils, producing a more comfortable and less stressful environment, reading the Holy Bible and Taoism were all reported as having been used to treat schizophrenia. Another method used in Asia (India and Turkey) and America (USA) was yoga therapy. Studies reporting on the role yoga therapy in alleviating mental illness were identified in an additional direct search. Yoga, originating from India, is a form of mind-body exercise (improves both physical and mental health) which is classified as a form of Complementary and Alternative Medicine by the National Institutes of Health (Woodyard, 2011, Rao et al., 2021). Yoga has proved to be useful as adjunctive treatment for psychiatric disorders, including schizophrenia, even though its mechanism in these disorders in yet to be full understood (Duraiswamy et al., 2007, Paikkatt et al., 2015). Yoga therapy has also been reported to increasing medication adherence and clinical insight in schizophrenic patients, and this also supports the use of this therapy as adjunctive treatment (Budak and Yilmaz, 2019).

Inhumane methods are also used in some of the African countries, such as starvation (or restriction of food), chaining and beating the patient, exposure to the elements, overcrowding, cohabitation of sexes, sleeping on a bare floor, inappropriate bodily exposure, confinement (or isolation in dark room) and restriction of visitors which are interventions reported in Kenya, Ghana and Nigeria (Esan et al., 2019). *Soaout*, which is the inhalation of herbal powder, physical punishment, cautery, strangulation and hitting the face of patients with shoes for the treatment of Bell's palsy in Iraq was also documented (Al-Jiboori, 2007). These unorthodox interventions and the lack of evidence of the efficacy and safety of other interventions such as herbal medicine, then compromise the human rights and safety of the patients or users of these interventions. In general, traditional healing practices lack clear structure, standardization and proper guidelines for their use (Ningsangrenla and Rao, 2019). This could be a contributing factor to why collaborations between traditional and biomedical healing sometimes seem impractical.

5. Conclusion, limitations and recommendations

As evidenced in this systematic review, traditional healing plays a significant role in communities where it is the accessible and preferred option in bridging the treatment gap for mental disorders in developing or low- and middle-income countries. Various traditional healing approaches are used and administered in different ways. A consistent method is the administration of different herbal plants based on the region and the traditional healing cultural or religious beliefs. However, the safety and effectiveness of these approaches still require scrutiny since there is limited knowledge about the phytochemical constitution or bioactive compounds and cytotoxic effects of the various plants used. Part of the question that the current review aimed to address was the efficacy of traditional healing approaches in the treatment of schizophrenia, however, only a few studies report on the effectiveness of the traditional approaches reported to be used in various regions. This then adds as a limitation of this review as the effectiveness of some of the herbal plants used was only reported after testing in animal models. With that being said, only a few of the wide range of herbal plants used in traditional medicine are tested for their properties, efficacy and cytotoxicity. There is also scarcity of well-conducted studies methodologically, which could address all parts of the current review's question; hence, studies using different methodologies and mixed-methods are included in this review. Additionally, the unverified and unproven inhumane methods used in some countries also require scrutiny as these might do more harm than good and thus might arouse contention for this alternative treatment approach. The strength that can be reported from the findings of this review is how gaps regarding the accurate dosage, treatment duration and efficacy of traditional medicine have been highlighted. It is then recommended that future research consider addressing these gaps, and addressing these gaps may also contribute in increasing the trustworthiness of traditional medicine.

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CRediT authorship contribution statement

Ntombifuthi Ngubane: Writing – original draft, Visualization, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Musa Mabandla:** Supervision. **Brenda De Gama:** Writing – review & editing, Supervision, Conceptualization.

Declaration of Competing Interest

None.

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Appendix

Table A: Assessment of risk of bias of studies involving a human sample

Author	Year	Screening Questions																				Quality Score (%)
		Qualitative					Quantitative (randomised)					Quantitative (descriptive)					Mixed methods					
		1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	
Human Studies																						
Baheretibeb et al.	2021	✓	×	✓	-	×																50
Ibrahim et al.	2020											✓	✓	✓	-	✓						90
Sorsdahl et al.	2010	✓	✓	✓	✓	✓																100
Audet et al.	2017																×	×	✓	-	-	40
Mzimkulu & Simbaya	2006	✓	✓	-	-	✓																80
Ngobe et al.	2021	✓	✓	✓	-	✓																90
Ngobe	2015	✓	✓	-	-	✓																80
Kajawu et al.	2015	✓	✓	✓	✓	✓																100
Esan et al.	2019																×	-	✓	-	✓	60
Gureje et al.	2020						✓	✓	✓	-	-											80
Kinda et al.	2017																✓	✓	✓	-	✓	90
Ouoba et al.	2020																✓	✓	✓	-	✓	90
Rouamba et al.	2018																✓	✓	✓	-	-	80
Tibiti et al.	2015																✓	✓	-	-	-	70
Debrah et al.	2018	✓	-	✓	✓	-																80
Mounkoro et al.	2020																✓	✓	✓	-	✓	90
Sonibare et al.	2005																✓	✓	✓	-	✓	90
Zhang et al.	2001						-	✓	×	-	-											50
Miyaoka et al.	2015						✓	✓	✓	-	✓											90
Parvizi et al.	2019						✓	✓	✓	-	-											80
Al-Jiboori	2007																×	✓	-	-	-	50
Sayed et al.	1999																×	✓	✓	-	✓	70
Budak & Yilmaz	2019						✓	✓	✓	-	✓											90
Ahmed & Azam	2014																×	✓	✓	-	✓	70
Rahmatullah et al.	2010																✓	✓	-	-	✓	80
Rahmatullah et al.	2014																×	✓	-	-	✓	60
Zohora et al.	2016	✓	✓	✓	-	✓																90
Campion & Bhugra	1997	✓	✓	-	-	✓																80
Dey et al.	2017	✓	-	✓	✓	✓																90
Duraiswamy et al.	2007						-	✓	-	-	✓											70
Jalal et al.	2008	✓	✓	-	✓	✓																90
Kumari et al.	2019	✓	✓	-	-	✓																90
Ningsangrenla & Rao	2019	✓	-	✓	✓	-																80
Paikkatt et al.	2015						✓	✓	-	-	✓											80
Ramu et al.	1992											✓	-	-	-	✓						70
Rao et al.	2021						✓	✓	✓	-	✓											90
Rout et al.	2012	✓	-	✓	✓	-																80
Sankaranarayanan et al.	2010	✓	✓	-	✓	✓																90
Singh et al.	2002	✓	✓	-	-	✓																80
Singh et al.	2020	✓	✓	✓	-	✓																90
Singhal et al.	2016	✓	-	✓	✓	✓																90
Mushtaq et al.	2012	✓	✓	✓	✓	✓																100
Phang et al.	2010																✓	✓	-	-	-	70
Razali & Yassin	2008	✓	✓	-	✓	✓																90
Nikolic et al.	2018																✓	✓	✓	-	✓	90
Bosch et al.	2015a	✓	✓	-	-	✓																80
Bosch et al.	2015b						✓	✓	✓	-	-											80
Bosch et al.	2017																✓	✓	-	-	✓	80
Bourbonnais-Spear et al.	2005																✓	✓	-	-	✓	80
Choco	n.d.	✓	✓	-	-	✓																80
Zacharias	2006																✓	✓	✓	-	✓	90
Visceglia & Lewis	2011						✓	✓	✓	-	✓											90
James & Peltzer	2012																✓	✓	✓	-	✓	90
Rodrigues & Carlini	2006	✓	✓	-	✓	✓																90
Turner et al.	2019						-	✓	✓	-	✓											80
Sullivan et al.	2000																✓	✓	-	-	✓	80
Furnham & Wong	2007											✓	✓	-	-	✓						80

Table B: Assessment of risk of bias of studies involving a human sample

Author	Year	Screening/Signaling Questions										Quality Score (%)
		1	2	3	4	5	6	7	8	9	10	
Animal Studies												
Taiwe et al.	2012	×	-	×	-	×	-	-	✓	✓	✓	50
Amoateng et al.	2017a	×	✓	×	-	×	-	-	✓	✓	✓	55
Amoateng et al.	2017b	-	✓	✓	✓	×	-	✓	✓	✓	✓	80
Kumbol et al	2018	-	✓	✓	✓	×	-	✓	✓	✓	✓	80
Akinpelu et al.	2018	-	✓	✓	✓	×	-	✓	✓	✓	✓	80
Ior et al.	2020	×	✓	-	-	×	-	✓	✓	✓	✓	65
Ior et al.	2021	×	✓	-	-	×	-	✓	✓	✓	✓	65
Magaji et al.	2014	×	✓	-	-	×	×	✓	✓	✓	✓	60
Maiha et al.	2013	×	✓	-	-	×	×	-	✓	✓	✓	55
Oyepeju & Sunday	2018	-	✓	✓	✓	×	-	✓	✓	✓	✓	80
Ingale & Kasture	2012	×	✓	-	-	×	-	✓	✓	✓	✓	65
Mishra et al.	2018	×	✓	-	-	×	-	✓	✓	✓	✓	65
Venkataramaiah et al.	2021	-	✓	-	-	×	-	✓	✓	✓	✓	70
Hidayat et al.	2021	×	✓	-	-	×	-	✓	✓	✓	✓	65
Coors et al.	2019	×	✓	-	-	×	-	✓	✓	✓	✓	65
Coors et al.	2019	-	✓	-	-	×	-	✓	✓	✓	✓	70

References

Abdullahi, A.A., 2011. Trends and challenges of traditional medicine in Africa. Afr. J. Tradit. Complement. Altern. Med. 8 (5S), 115–123.

Ahmed, N., Azam, N.K., M., 2014. Traditional knowledge and formulations of medicinal plants used by the traditional medical practitioners of Bangladesh to treat schizophrenia like psychosis. Schizophr. Res. Treat. 1–10.

Akinpelu, L., Akanmu, M., Obuotor, E., 2018. Antipsychotic effects of ethanol leaf extract and fractions of Milicia excelsa (Moraceae) in mice. J. Pharm. Res. Int. 22 (6), 1–10.

Al-Jibouri, S.D., 2007. The frequency of psychiatric patients using traditional and spiritual medicine. Tikrit Med. J. 13 (2), 9–15.

American Psychiatric Association. (2013). Diagnostic and statistical manual of mental disorders: DSM-5 (5th ed). Washington, D.C. American Psychiatric Publishing.

Amoateng, P., Adjei, S., Osei-Safo, D., Kukuia, K.K., Bekoe, E.O., Karikari, T.K., Kombian, S.B., 2017a. Extract of Synedrella nodiflora (L) Gaertn exhibits antipsychotic properties in murine models of psychosis. BMC Complement. Altern. Med. 17 (1), 14.

Amoateng, P., Osei-Safo, D., Kukuia, K.K.E., Adjei, S., Akure, O.A., Agbemelo-Tsomafo, C., Adu-Poku, S.N., Agyeman-Budu, K.Y., 2017b. Psychotropic effects of an alcoholic extract from the leaves of Albizia zygia (Leguminosae-Mimosoideae). Evid. Based Complement. Altern. Med. 2017, 1–13. ID.9297808,.

Audet, C.M., Ngobeni, S., Graves, E., Wagner, R.G., 2017. Mixed methods inquiry into traditional healers' treatment of mental, neurological and substance abuse disorders in rural South Africa. PloS One 12 (12), e0188433.

Baheretibeb, Y., Wondimagegn, D., Law, S., 2021. Holy water and biomedicine: a descriptive study of active collaboration between religious traditional healers and biomedical psychiatry in Ethiopia. Br. J. Psychiatry Open 7 (3), 1–5 e92.

Balkrishna, A., Sharma, N., Srivastava, D., Kukreti, A., Srivastava, S., Arya, V., 2024. Exploring the safety, efficacy, and bioactivity of herbal medicines: bridging traditional wisdom and modern science in healthcare. Future Integr. Med. 3 (1), 35–49.

Bodeker, G., Burford, G. and Kronenberg, F. eds., 2006. Traditional, complementary and alternative medicine: policy and public health perspectives. World Scientific.

Bosch, P., de Rover, P., Staudte, H., Lim, S., van den Noort, M., 2015a. Schizophrenia, depression, and sleep disorders: their traditional oriental medicine equivalents. J. Acupunct. Meridian Stud. 8 (1), 17–22.

Bosch, P., Van Den Noort, M., Yeo, S., Lim, S., Coenen, A., Van Luijtelaar, G., 2015b. The effect of acupuncture on mood and working memory in patients with depression and schizophrenia. J. Integr. Med. 13 (6), 380–390.

Bosch, P., Staudte, H., Yeo, S., Lee, S.H., Lim, S., van den Noort, M., 2017. Acupuncture treatment of a male patient suffering from long term schizophrenia and sleep disorders. J. Tradit. Chin. Med. 37 (6), 862–867.

Boutbonnais-Spear, N., Awad, R., Maquin, P., Cal, V. and Arnason, J.T., 2005. Ethnobotany of Q'eqchi'Maya medicinal plants used for the treatment of neurological and mental health disorders in Southern Belize. Arnason J T. Catling PM, Small E, et al. Biodiversity & Health: Focusing Research to Policy. *NRC Research Press*, Ottawa, Ontario, pp.61-65.

Budak, F.K., Yilmaz, E., 2019. The effect of yoga on clinical insight and medication adherence in patients with schizophrenia-a randomized controlled trial. Eur. J. Integr. Med. 30, 1–6.

Budzak, A., Branković, M., 2022. Alternative ways to mental health: exploring psychological determinants of preference for CAM treatments. Stud. Psychol. 64 (1), 118–135.

Campion, J., Bhugra, D., 1997. Experiences of religious healing in psychiatric patients in South India. Soc. Psychiatry Psychiatr. Epidemiol. 32, 215–221.

Choco, M.P., n.d. A Preliminary Study of Ethnobotanical Use of Plant Species by the Q'eqchi'Maya Community in Toledo District, (Thesis, University of Belize).2024.

Coors, A., Brosch, M., Kahl, E., Khalil, R., Michels, B., Laub, A., Franke, K., Gerber, B., Fendt, M., 2019. Rhodiola rosea root extract has antipsychotic-like effects in rodent models of sensorimotor gating. J. Ethnopharmacol. 235, 320–328.

Crellin, N.E., Priebe, S., Morant, N., Lewis, G., Freemantle, N., Johnson, S., Horne, R., Pinfold, V., Kent, L., Smith, R., Darton, K., 2022. An analysis of views about supported reduction or discontinuation of antipsychotic treatment among people with schizophrenia and other psychotic disorders. BMC Psychiatry 22 (1), 1–11.

Debrah, A.B., Buabeng, K.O., Donnir, G., Akwo Kretchy, I., 2018. A caregiver perspective of complementary and alternative medicine use among patients with schizophrenia and bipolar disorders. Int. J. Ment. Health 47 (4), 298–310.

Dey, A., Gorai, P., Mukherjee, A., Dhan, R., Modak, B.K., 2017. Ethnobiological treatments of neurological conditions in the Chota Nagpur Plateau, India. J. Ethnopharmacol. 198, 33–44.

Duraiswamy, G., Thirthalli, J., Nagendra, H.R., Gangadhar, B.N., 2007. Yoga therapy as an add-on treatment in the management of patients with schizophrenia–a randomized controlled trial. Acta Psychiatr. Scand. 116 (3), 226–232.

Esan, O., Appiah-Poku, J., Othieno, C., Kola, L., Harris, B., Nortje, G., Makanjuola, V., Oladeji, B., Price, L., Seedat, S., Gureje, O., 2019. A survey of traditional and faith healers providing mental health care in three sub-Saharan African countries. Soc. Psychiatry Psychiatr. Epidemiol. 54, 395–403.

Furnham, A., Wong, L., 2007. A cross-cultural comparison of British and Chinese beliefs about the causes, behaviour manifestations and treatment of schizophrenia. Psychiatry Res. 151 (1-2), 123–138.

Gureje, O., Appiah-Poku, J., Bello, T., Kola, L., Araya, R., Chisholm, D., Esan, O., Harris, B., Makanjuola, V., Othieno, C., Price, L., 2020. Effect of collaborative care between traditional and faith healers and primary health-care workers on psychosis outcomes in Nigeria and Ghana (COSIMPO): a cluster randomised controlled trial. Lancet 396 (10251), 612–622.

Hidayat, R., Wulandari, P., Marchira, C.R., Pratiti, B., 2021. Efficacof cinnamon extract (Cinnamomum burmannii) as supplementation in lit-psychotic-induced rats through oxidative stress regulation in neuronal cells. Open Access Maced. J. Med. Sci. 9 (A), 177–182.

Hong, Q.N., Pluye, P., Fàbregues, S., Bartlett, G., Boardman, F., Cargo, M., Dagenais, P., Gagnon, M.-P., Griffiths, F., Nicolau, B., O'Cathain, A., Rousseau, M.-C., Vedel, I., 2018. Mixed Methods Appraisal Tool (MMAT), version. Registration of Copyright (#1148552). Canadian Intellectual Property Office, Industry Canada.

Hooijmans, C.R., Rovers, M.M., De Vries, R.B., Leenaars, M., Ritskes-Hoitinga, M., Langendam, M.W., 2014. SYRCLE's risk of bias tool for animal studies. BMC Med. Res. Methodol. 14, 1–9.

Ibrahim Awaad, M., Mohsen Ibrahim, N., Nagnib, R.M., Khalil, S.A., Elhabiby, M., Khaled Mohamed, Z., 2020. Role of traditional healers in the pathway to care of patients with schizophrenia in Egypt. Int. J. Soc. Psychiatry 66 (4), 382–388.

Ingale, S.P., Kasture, S.B., 2012. Psychopharmacological profile of Passiflora incarnata Linn in mice. Int. J. Phytopharm. 3 (3), 263–268.

Ior, L.D., Otimenyin, S.O., Gushit, J.S., 2020. Neuroprotective effects of methanol extract of Terminalia macroptera leaf in mice. Afr. J. Pharm. Pharmacol. 14 (8), 300–307.

Ior, L.D., Otimenyin, S.O., Okwuasaba, F.K., 2021. Antipsychotic-like effect of ethyl acetate fraction of Terminalia macroptera leaf in mice. IBRO Neurosci. Rep. 10, 83–89.

Jackson, N.A., 2015. Music therapy and chronic mental illness: overcoming the silent symptoms. Music Ther. Perspect. 33 (2), 90–96.
Jalal, J.S., Kumar, P., Pangtey, Y.P.S., 2008. Ethnomedicinal orchids of Uttarakhand, western Himalaya. Ethnobot. Leafl. 2008 (1), 1227–1230.
James, C.C., Peltzer, K., 2012. Traditional and alternative therapy for mental illness in Jamaica: patients' conceptions and practitioners' attitudes. Afr. J. Tradit. Complement. Altern. Med. 9 (1), 94–104.
James, P.B., Wardle, J., Steel, A., Adams, J., 2018. Traditional, complementary and alternative medicine use in Sub-Saharan Africa: a systematic review. BMJ Glob. Health 3 (5), 1–18.
Kahn, R.S., Sommer, I.E., Murray, R.M., Meyer-Lindenberg, A., Weinberger, D.R., Cannon, T.D., O'Donovan, M., Correll, C.U., Kane, J.M., van Os, J., Insel, T.R., 2015. Schizophrenia. Nat. Rev. Dis. Prim. 12 (1), 1–23. ID.15067.
Kajawu, L., Chingarande, S.D., Jack, H., Ward, C., Taylor, T., 2015. What do African traditional medical practitioners do in the treatment of mental disorders in Zimbabwe? Int. J. Cult. Ment. Health 9 (1), 44–55.
Kim, Y.K., Na, K.S., 2017. Neuroprotection in schizophrenia and its therapeutic implications. Psychiatry Investig. 14 (4), 383–391.
Kinda, P.T., Zerbo, P., Guenné, S., Compaoré, M., Ciobica, A., Kiendrebeogo, M., 2017. Medicinal plants used for neuropsychiatric disorders treatment in the Hauts Bassins region of Burkina Faso. Medicines 4 (2), 1–21 e32.
Kumar, G.P., Khanum, F., 2012. Neuroprotective potential of phytochemicals. Pharmacogn. Rev. 6 (12), 81–90.
Kumari, R., Kumar, A., Kumar, B., 2019. Ethnobotanical investigation of medicinal plants used by rural communities of district Chatra, Jharkhand, India. J. Biotechnol. Biochem. 5 (6), 34–49.
Kumbol, V.W.A., Abotsi, W.K.M., Ekuadzi, E., Woode, E., 2018. Albizia zygia root extract exhibits antipsychotic like properties in murine models of schizophrenia. Biomed. Pharmacother. 106, 831–841.
Magaji, M.G., Yakubu, Y., Magaji, R.A., Musa, A.M., Yaro, A.H., Hussaini, I.M., 2014. Psychopharmacological potentials of methanol leaf extract of Securinega virosa Roxb (Ex Willd) Baill. in mice. Pak. J. Biol. Sci.: PJBS 17 (6), 855–859.
Maiha, B.B., Mohammed, B., Magaji, M.G., 2013. Psychopharmacological potential of methanol leaf extract of Ficus thonningii (Blume) in mice. Niger. J. Pharm. Sci. 12 (2), 30–34.
Mishra, A., Mishra, A.K., Jha, S., 2018. Effect of traditional medicine brahmi vati and bacoside A-rich fraction of Bacopa monnieri on acute pentylenetetrzole-induced seizures, amphetamine-induced model of schizophrenia, and scopolamine-induced memory loss in laboratory animals. Epilepsy Behav. 80, 144–151.
Miyaoka, T., Furuya, M., Horiguchi, J., Wake, R., Hashioka, S., Tohyama, M., Mori, N., Minabe, Y., Iyo, M., Ueno, S., Ezoe, S., 2015. Efficacy and safety of yokukansan in treatment-resistant schizophrenia: a randomized, double-blind, placebo-controlled trial (a Positive and Negative Syndrome Scale, five-factor analysis). Psychopharmacology 232, 155–164.
Molot, M., 2017. Discourses of Psychiatry and Culture: The Interface Between Western and Traditional Medicine in the Treatment of Mental Illness. Independent Study Project (ISP) Collection. 2582.
Mounkoro, P.P., Togola, A., de Jong, J., Diallo, D., Paulsen, B.S., van't Klooster, C., 2020. Ethnobotanical survey of plants used by traditional health practitioners for treatment of schizophrenia spectrum disorders in Bandiagara, Mali, West Africa. J. Herb. Med. 24, 1–13. ID.100402.
Mushtaq, A., Muhammad, Z., Ajab, K.M., Shazia, S., Mujtaba, S.G., Jan, G., 2012. Ethnomedicinal investigation of phytomedicines among the local communities of arid areas of Pakistan. Indian J. Tradit. Knowl. 1 (3), 436–446.
Mzimkulu, K.G., Simbayi, L.C., 2006. Perspectives and practices of Xhosa-speaking African traditional healers when managing psychosis. Int. J. Disabil. Dev. Educ. 53 (4), 417–431.
Ngobe, A.J., 2015. Swati traditional healers' conceptualisation of causes and treatment of mental illness (Masters Dissertation, University of Limpopo).
Ngobe, A., Semenya, S., Sodi, T., 2021. Psychological ailments and their treatment protocols: a case study of Swati traditional healers in Mpumalanga Province, South Africa. Afr. Health Sci. 21 (2), 884–895.
Ngoungoure, V.L., Mfotie Njoya, E., Ngamli Fewou, S., Ella, A.F., McGaw, L.J., Moundipa, P.F., 2019. Acetylcholinesterase inhibitory, anti-inflammatory and antioxidant properties of some Cameroonian medicinal plants used to treat some neurological disorders. Investig. Med. Chem. Pharmacol. 2 (2), 1–13.
Nikolic, G., Stojanović, N.M., Randjelović, P.J., Manojlović, S., Radulović, N.S., 2018. An epidemiological study on herbal product self-medication practice among psychotic outpatients from Serbia: a cross-sectional study. Saudi Pharm. J. 26 (3), 335–341.
Ningsangrenla, L., Rao, P.S.S., 2019. Traditional healing practices and perspectives of mental health in Nagaland. J. North East India Stud. 9 (2), 33–56.
Nortje, G., Oladeji, B., Gureje, O., Seedat, S., 2016. Effectiveness of traditional healers in treating mental disorders: a systematic review. Lancet Psychiatry 3 (2), 154–170.
Ojagbemi, A., Gureje, O., 2020. The potential role of traditional medicine in the management of schizophrenia. Curr. Psychiatry Rep. 22, 1–7.
Ouoba, D., Dibloni, O.T., Mano, K., Ouoba, Y., Kabre, B.G., 2020. Role of wild mammals in traditional medicine and mystic practices in the province of Oubritenga, Burkina Faso. Int. J. Biol. Chem. Sci. 14 (4), 1322–1340.
Oyepeju, O., Sunday, O.O., 2018. Neuropharmacological evaluation of the ethanol roots extract of Securidacalonge pedunculata (Polygalaceae) in Mice. Int. J. Pharm. Pharm. Res.: Hum. J. 11 (4), 195–205.
Parvizi, M., Fadai, F., Khodaei-Ardakani, M.R., Amin, G., Abdi, L., Noroozi, M., Ansari, I., 2019. Effect of Cuscuta epithymum acquainted with risperidone on the improvement of clinical symptoms and cognitive impairment in patients with schizophrenia: a triple-blind randomized placebo-controlled trial. Galen. Med. J. 8, 1–13 e1334.
Paikkatt, B., Singh, A.R., Singh, P.K., Jahan, M., Ranjan, J.K., 2015. Efficacy of yoga therapy for the management of psychopathology of patients having chronic schizophrenia. Indian J. Psychiatry 57 (4), 355–360.
Phang, C.K., Marhani, M., Salina, A.A., 2010. Prevalence & experience of contact with traditional healers among patients with first-episode psychosis in Hospital Kuala Lumpur. Malays. J. Psychiatry 19 (2), 59–67.
Rahmatullah, M., Azam, M.N.K., Mollik, M.A.H., Hasan, M.M., Hassan, A.I., Jahan, R., Jamal, F., Nasrin, D., Ahmed, R., Rahman, M.M., Khatun, M.A., 2010. Medicinal plants used by the Kavirajes of Daulatdia Ghat, Kushtia district, Bangladesh. Am.-Eurasia J. Sustain. Agric. 4 pp.219-29.
Rahmatullah, M., Haque, M.E., Mondol, M.R.K., Hasan, M., Aziz, T., Jahan, R., Seraj, S., 2014. Medicinal formulations of the Kuch tribe of Bangladesh. J. Altern. Complement. Med. 20 (6), 428–440.
Ramu, M.G., Venkataram, B.S., Mukundan, H., Shankara, M.R., Leelavathy, S., Janakiramaiah, N., 1992. A controlled study of Ayurvedic treatment in the acutely ill patients with Schizophrenia (Unmada): rationale and results. Nimhans J. 10 (1), 1–16.
Rao, N.P., Ramachandran, P., Jacob, A., Joseph, A., Thonse, U., Nagendra, B., Chako, D. M., Shiri, S., Hassan, H., Sreenivas, V., Maran, S., 2021. Add on yoga treatment for negative symptoms of schizophrenia: a multi-centric, randomized controlled trial. Schizophr. Res. 231, 90–97.
Razali, S.M., Yassin, A.M., 2008. Complementary treatment of psychotic and epileptic patients in Malaysia. Transcult. Psychiatry 45 (3), 455–469.
Rodrigues, E., Carlini, E.A., 2006. Plants with possible psychoactive effects used by the Krahô Indians, Brazil. Braz. J. Psychiatry 28, 277–282.
Rouamba, A., Ouédraogo, V., Karama, I., Compaoré, M., Kiendrebeogo, M., 2018. Ethnomedicinal use of Crotalaria retusa L.(Fabaceae), a pyrrolizidine alkaloid toxic plant. Int. J. Biochem. Res. Rev. 23 (2), 1–6.
Rout, J., Sajem, A.L., Nath, M., 2012. Medicinal plants of North Cachar Hills district of Assam used by the Dimasa tribe. Indian J. Tradit. Knowl. 11 (3), 520–527.
Sankaranarayanan, S., Bama, P., Ramachandran, J., Kalaichelvan, P.T., Deecaraman, M., Vijayalakshimi, M., Dhamotharan, R., Dananjeyan, B., Sathya Bama, S., 2010. Ethnobotanical study of medicinal plants used by traditional users in Villupuram district of Tamil Nadu, India. J. Med. Plants Res. 4 (12), 1089–1101.
Santillan-Urquiza, M.A., Herrera-Ruiz, M., Zamilpa, A., Jiménez-Ferrer, E., Román-Ramos, R., Alegría-Herrera, E.Y., Tortoriello, J., 2019. Galphimia glauca and natural galphimines block schizophrenia like symptoms induced with apomorphine and MK-801 in mice. Evid. Based Complement. Altern. Med. 2019, 1–11. ID 8404258.
Sayed, M., Abosinaina, B., Rahim, S.I., 1999. Traditional healing of psychiatric patients in Saudi Arabia. Curr. Psychiatry 6 (1), 11–23.
Shaterzadeh-Yazdi, H., Samarghandian, S., Farkhondeh, T., 2018. Effects of crocins in the management of neurodegenerative pathologies: a review. Neurophysiology 50, 302–308.
Shi, X.J., Fan, F.C., Liu, H., Ai, Y.W., Liu, Q.S., Jiao, Y.G., Cheng, Y., 2021. Traditional Chinese medicine decoction combined with antipsychotic for chronic schizophrenia treatment: a systematic review and Meta-analysis. Front. Pharmacol. 11, 1–10.
Singh, A.K., Raghubanshi, A.S., Singh, J.S., 2002. Medical ethnobotany of the tribals of Sonaghati of Sonbhadra district, Uttar Pradesh, India. J. Ethnopharmacol. 81 (1), 31–41.
Singh, R., Upadhyay, S.K., Rani, A., Kumar, P., Sharma, P., Sharma, I., Singh, C., Chauhan, N., Kumar, M., 2020. Ethnobotanical study of weed flora at District Ambala, Haryana, India: comprehensive medicinal and pharmacological aspects of plant resources. Int. J. Pharm. Res. 2020 (1), 1941–1956.
Singhal, P., Raina, P., Dobhal, P., 2016. Studies on the Ethnomedicinal Plants of Suddhowala region of Doon valley. Int. J. Pharm. Life Sci. 7 (12), 5375–5383.
Sonibare, M.A., Soladoye, M.O., Subuloye, T.O., 2005. Ethnobotanical survey of anti-psychotic plants in Lagos and Ogun States of Nigeria. Eur. J. Sci. Res. 19 (4), 634–644.
Sorsdahl, K.R., Fisher, A.J., Wilson, Z., Stein, D.J., 2010. Explanatory models of mental disorders and treatment practices among traditional healers in Mpumulanga, South Africa. Afr. J. Psychiatry 13 (4), 284–290.
Sullivan, R.J., Allen, J.S., Otto, C., Tiobech, J., Nero, K., 2000. Effects of chewing betel nut (Areca catechu) on the symptoms of people with schizophrenia in Palau, Micronesia. Br. J. Psychiatry 177 (2), 174–178.
Taïwe, G.S., Bum, E.N., Talla, E., Dawe, A., Moto, F.C.O., Ngoupaye, G.T., Sidiki, N., Dabole, B., Dzeufiet, P.D.D., Dimo, T., De Waard, M., 2012. Antipsychotic and sedative effects of the leaf extract of Crassocephalum bauchiense (Hutch.) Milne-Redh (Asteraceae) in rodents. J. Ethnopharmacol. 143 (1), 213–220.
Tibiri, A., Sawadogo, W.R., Dao, A., Elkington, B.G., Ouedraogo, N., Guissou, I.P., 2015. Indigenous knowledge of medicinal plants among Dozo hunters: An ethnobotanical survey in Niamberla village, Burkina Faso. J. Altern. Complement. Med. 21 (5), 294–303.
Turner, A., McGrath, J.J., Dean, O.M., Dodd, S., Baker, A., Cotton, S.M., Scott, J.G., Kavanagh, B.E., Ashton, M.M., Walker, A.J., Brown, E., 2019. Protocol and rationale: a 24-week double-blind, randomized, placebo controlled trial of the efficacy of adjunctive Garcinia mangostana Linn.(mangosteen) pericarp for schizophrenia. Clin. Psychopharmacol. Neurosci. 17 (2), 297–307.
Velligan, D.I., Rao, S., 2023. Schizophrenia: salient symptoms and pathophysiology. J. Clin. Psychiatry 84 (1), 45113.
Venkataramaiah, C., Lakshmi Priya, B., Rajendra, W., 2021. Perturbations in the catecholamine metabolism and protective effect of "3 (3, 4-dimethoxy phenyl)-1-4 (methoxy phenyl) prop-2-en-1-one" during ketamine-induced schizophrenia: an in vivo and in silico studies. J. Biomol. Struct. Dyn. 39 (10), 3523–3532.
Visceglia, E., Lewis, S., 2011. Yoga therapy as an adjunctive treatment for schizophrenia: a randomized, controlled pilot study. J. Altern. Complement. Med. 17 (7), 601–607.

Woodyard, C., 2011. Exploring the therapeutic effects of yoga and its ability to increase quality of life. Int. J. Yoga 4 (2), 49–54.
World Health Organisation. (2021). Comprehensive mental health action plan 2013–2030. Geneva: World Health Organization. Licence: CC BY-NC-SA 3.0 IGO.
World Health Organisation. 2022. Mental Disorders [Fact Sheet]. https://www.who.int/news-room/fact-sheets/detail/mental-disorders#:~:text=A%20mental%20disorder%20is%20characterized,different%20types%20of%20mental%20disorders. (Accessed 04 March 2023).
Yazici, E., Yazici, A.B., Ince, M., Erol, A., Erdoğan, A., Ikiz, H.S., Kirpinar, I., 2016. The search for traditional religious treatment amongst schizophrenic patients: the current situation. Anatol. J. Psychiatry/Anadolu Psikiyatr. Derg. 17 (3), 174–180.
Zachatias, S., 2006. Mexican curanderismo as ethnopsychotherapy: a qualitative study on treatment practices, effectiveness, and mechanisms of change. Int. J. Disabil. Dev. Educ. 53 (4), 381–400.
Zhang, X.Y., Zhou, D.F., Su, J.M., Zhang, P.Y., 2001. The effect of extract of Ginkgo biloba added to haloperidol on superoxide dismutase in inpatients with chronic schizophrenia. J. Clin. Psychopharmacol. 21 (1), 85–88.
Zohora, F.T., Mahmud, S.N., Kar, A., Mahmud, S., Shibly, A.Z., 2016. A survey on medicinal plant usage by folk medicinal practitioners in different villages of Haluaghat Upazila, Mymensingh District, Bangladesh. Indian J. Res. Pharm. Biotechnol. 4 (5), 200–208.

Prologue 2

The findings from the previous chapter indicated herbal medicine as the common traditional treatment approach globally. However, the medicinal plants used for the treatment of schizophrenia and other mental disorders vary widely. This could be influenced by how different plants are found in different parts of the world depending on climate and other varying factors. The following chapter, Chapter 3, quantitatively and qualitatively investigates the profiles of THPs involved in the diagnosis and treatment of mental illnesses, particularly schizophrenia in KwaZulu-Natal, South Africa. It also investigates the traditional methods used for diagnosis and treatment of these disorders. The quantitative findings are documented in Paper 2 while the qualitative findings are documented in Paper 3.

Paper 2

Paper Title	**:** A quantitative evaluation of traditional health practitioners' perspectives on mental disorders in KwaZulu-Natal: knowledge, diagnosis, and treatment practices
Status	**:** Published
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Paper 3

Paper Title	**:** The Influence of Culture on the Cause, Diagnosis and Treatment of Serious Mental Illness (*Ufufunyana*): Perspectives of Traditional Health Practitioners in the Harry Gwala District, KwaZulu-Natal
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CHAPTER 3

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A quantitative evaluation of traditional health practitioners' perspectives on mental disorders in KwaZulu-Natal: knowledge, diagnosis, and treatment practices

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ABSTRACT

Traditional healing practices are prominent in recent studies. Particularly, there is focused attention on holistic healing in treatment interventions for various illnesses, including mental disorders. This paper quantitatively evaluates the perspectives of traditional health practitioners (THPs) regarding their knowledge, diagnosis, and treatment practices of mental disorders, especially schizophrenia. Special attention was paid to symptoms of schizophrenia as they seem to overlap with the symptoms of bewitchment and an ancestral calling. Self-developed questionnaires were administered to 100 THPs from Harry Gwala District, KwaZulu-Natal. Black, middle-aged female THPs classified as diviners were prevalent. They reflected knowledge of mental disorders and played a role in their diagnosis and treatment. Medicinal concoction was the commonly prescribed treatment option.

KEYWORDS

Traditional healers; Traditional healing; mental health; mental illness; schizophrenia

Introduction

Mental disorders affect approximately one in every eight people globally, with approximately 24 million or 1 in 300 people affected by schizophrenia (WHO, 2022). Schizophrenia is the least common of the mental disorders, affecting approximately 1% of the global population; however, it ranks as one of the top 10 illnesses which contribute to the global burden of disease and leading causes of disability (Velligan & Rao, 2023). The management and/or treatment of schizophrenia commonly emanates from the biopsychosocial model which does not consider religious/spiritual beliefs (Grover, Davuluri, & Chakrabarti, 2014), and antipsychotic drugs, psychological and social interventions, psychotherapy, and/or electroconvulsive therapy are prescribed for patients in Western Medicine (Deng & Xu, 2017; Patel, Cherian, Gohil, & Atkinson, 2014). However, antipsychotic medication is not always effective and its side effects can be severe and unbearable; thus, patients are said to withdraw from this type of treatment (Crellin et al., 2022). The inconsistent effectiveness of the current antipsychotic drugs in the treatment of negative symptoms of

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schizophrenia is high; thus, they are reported to be the most challenging with a prevalence of approximately 23–26% (Hajiaghaee & Akhondzadeh, 2018). Therefore, there is a need to consider alternative approaches such as indigenous medical approaches to comprehensively effect treatment especially in contexts where African traditional beliefs influence the choice of treatment sought for various illnesses including mental disorders.

In many African regions, it is common for people to have a belief in an omnipresent God and ancestral spirits who are believed to be mediators between God and the living (Mokgobi, 2014). Generational relationships through ancestral reverence are believed to influence the balance, health, and protection of an individual (Edwards, Makunga, Thwala, & Mbele, 2009). Therefore, people in African regions have a common belief that ill health may be influenced by these culture-related reasons (Monama & Basson, 2017) and mental disorders may possibly be caused by sorcery or may be interpreted as an ancestral calling (*ubizo* in IsiZulu) to become a traditional health practitioner (THP) (Gutema & Mengstie, 2022). These occurrences or events are also suggested to be mainly treated or managed by THPs (Zabow, 2007). THPs are individuals who provide health care to the community with the aid of approaches that are based on cultural, religious, and social systems and these methods may include the use of plant, animal, and/or mineral substances to treat different ailments (WHO, 1978). There are three main classifications of the THPs, namely, *Isangoma* (also referred to as the diviner – use divination to determine the cause of illness), *Inyanga* (also referred to as the herbalist – mainly prescribes traditional medical remedies for treatment of illness), and *Umthandazi* (also referred to as the faith healer – mainly uses water and prayer, sometimes ash and salt may be included in the healing process) (Zuma, Wight, Rochat, & Moshabela, 2016).

According to Kaplan and Sadock (1988), mental illness may tend to be viewed as free of cultural beliefs by western psychiatry, whereas cultural beliefs, values, and concepts play a significant role in influencing health-seeking behaviors in traditional healing. According to Arshinoff, Roldan, and Balboni (2023), the provision of spiritual care, which follows a generalist-specialist model, is a fundamental component in holistic medical care for patients diagnosed with nervous disorders. Other spiritually integrated models for both physical and mental illness include body-mind-spirit interventions and the pluralistic approaches which take into consideration whatever that is considered sacred by the patient (Captari, Sandage, Vandiver, Jankowski, & Hook, 2023). A lack of consideration of these models and sociocultural factors when dealing with mental illness may limit the effectiveness of treatment interventions (van Der Zeijst et al., 2022).

Indigenous names that are often used to refer to mental illness are *ukuphambana* or *ukuhlanya* (considered as madness) and the symptoms in patients with schizophrenia are explained as a culture-bound syndrome used

"to explain aberrant behavioral and psychological phenomena" termed *amafufunyana* in IsiZulu and IsiXhosa (Niehaus et al., 2004; Volkan, 2021), which in the traditional beliefs or healing setting, is defined as the possession by evil spirits (Zabow, 2007). Schizophrenia presents with symptoms that overlap with symptoms of conditions considered to be a result of sorcery and an ancestral calling in the African or traditional healing setting. According to van der Zeijst et al. (2021), hallucinations, anxiety, confusion, sleeping issues, social withdrawal, loss of feeling, hope, and self-esteem and loss of capacity for enjoyment are observed in individuals with an ancestral calling. Schizophrenia in the Western Medicine setting is also characterized by symptoms which include delusions and hallucinations (positive symptoms), asociality, alogia, and anhedonia (negative symptoms) and lack of attention and memory (cognitive impairment) (APA, 2013; Deng & Xu, 2017; Koster, Carbon, & Correll, 2014). In an African or traditional healing setting, conditions relating to sorcery and ancestral calling are mainly treated by THPs where the cultural background (i.e. cultural values and beliefs) plays a significant role in their diagnosis and treatment approaches (Zabow, 2007). This paper quantitatively evaluates the perspectives of THPs in their knowledge and diagnosis of mental disorders, particularly schizophrenia. The treatment approaches or practices of the THPs, particularly for schizophrenia, were also investigated. It hypothesizes that perceptions of mental illness and its diagnosis and treatment from the THPs differed from Western Medicine.

Materials and methods

Study location and sampling

The current study was conducted in the Harry Gwala District Municipality (HGDM) located southwest of KwaZulu-Natal. Self-developed questionnaires were administered to 100 THPs from the four local municipalities, namely, the Greater Kokstad, uMzimkhulu, Ubuhlebezwe, and Dr Nkosazana Dlamini Zuma (Figure 1). Black African individuals from South Africa constitute 96.88% in HGDM (Cogta, 2020). IsiZulu language is spoken by 62.70% of the population while 28.60% speak IsiXhosa language (StatsSA, 2011) and approximately 52.65% and 47.35% of the population are females and males, respectively (Cogta, 2020).

Participants (i.e. the THPs) were randomly selected via convenience sampling. The district's database of THPs was used by the THPs' district chairperson to select the THPs who participated in the study and the selected THPs were invited to either a community hall or local municipality office to complete the questionnaires. Each questionnaire was enclosed in a plastic pocket, a new pen was provided to each participant, and sanitizers were used constantly to minimize the spread of Covid-19. The questionnaires were self-



Figure 1. Local municipalities of the harry gwala district municipality (adapted from municipalities. Co.za; accessed 10 march 2022).

administered and filled by the participants; however, assistance was provided by the investigators to the participants when clarity was required. Pilot testing of the questionnaire was conducted prior to its administration in order to examine the validity of each question before data collection in November 2021.

Inclusion and exclusion criteria

Registered THPs in the district's database were included in the study, while unregistered THPs were excluded from this study.

Ethical considerations

Prior to data collection, ethical approval was obtained from the Humanities and Social Sciences Research Ethics Committee of the University of KwaZulu-Natal (HSSREC/00002944/2021) and written informed consent was signed by all participants before participating in this study.

Confidentiality

Participants were assigned participation numbers to maintain confidentiality. Completed questionnaires were stored in a locked area and only the investigators had access.

Data analysis

Data analysis was conducted using the R Statistical Computing Software of the R Core Team, 2020, version 3.6.3. All variables were categorical; hence, the results were described as counts and percentage frequencies, and presented in the form of descriptive statistics. Pie, simple, component, and multiple bar charts were also included. Dependent variables in this study were knowledge, and methods of diagnosis and treatment of mental disorders, especially schizophrenia, while independent variables were age, sex, classification, period of practice, and locality. To determine the association between these variables, a Chi-Square Test was used and where the distribution of the cross tabulations contained an expected value of less than five, a Fisher's exact test was applied. For two groups' stratification, and in the case of a significant difference between the Chi-Square or Fisher's exact tests, a row-wise paired z-test was used as a post hoc analysis tool following the omnibus tests (Chi-Square or Fisher's exact test). A p-value $\leq .05$ was considered statistically significant in this study.

Results

Socio-demographic profiles

A 100% response rate was obtained, and 35% of the participants were from the Buhlebezwe local municipality, with 26%, 21%, and 18% of the THPs from Dr Nkosazana Zuma, uMzimkhulu, and Greater Kokstad municipalities, respectively. The female THPs were prevalent (74%) among the participants, and males were 25%, one participant identified as other. The prevalent age group was the middle age group (60%) with participants between the ages of 35 and 59 years, while 24% of the THPs were between 15 and 34 years and 16% were over 60 years. The THPs self-classified as diviner (*Isangoma* in IsiZulu language) in 63%, faith healer (*Umthandazi* in IsiZulu language) in 10%, herbalist (*Inyanga* IsiZulu language) in 3% of cases, and a combination of these classifications in 23% of cases. One participant was an initiate. The level of experience was at Entry level (0–5 years) in 37% of cases, 34% were at the Mid-level (6–15 years), 24% at Senior level (16–30 years), and 5% were at Expert level with over 30 years of experience.

A total of 71% of participants did not respond on their area of specialization. Of the participants who responded, 41.4% specialized in head-related

illnesses classified as Group 1 in this study, 37.9% were more of general practitioners (Group 2), those who specialized in aches, wounds, and related ailments (Group 3) were 10.3%, and the other remaining 10.3% specialized in abdomen and maternity-related ailments (Group 4) (Table 1).

Mental disorders in general

Knowledge of mental disorders

THPs (83%) declared knowledge of mental disorders. In particular, 31.3% maintained that mental disorders are conditions whereby one loses their mind, 16.9% offered that they are conditions that affect one's thinking, mood, and behavior, and 50.6% did not know if mental disorders could be defined as any of these.

Diagnosis of mental disorders

Mental disorders were diagnosed by 60% of the participants (Table 2). Most of the THPs who diagnosed mental disorders were from Greater Kokstad municipality ($p = .043$). Schizophrenia was the commonly diagnosed mental disorder with 42% of the THPs having diagnosed this disorder (Table 2). Some of the participants (18%) had diagnosed more than one of the stipulated mental disorders, and 20% had diagnosed other mental disorders apart from these. Only 3% of the participants specified the other diagnosed mental disorders and these were specified as aches (headaches), stroke (cerebro-vascular disease), and epilepsy. The methods used to diagnose mental disorders included questioning patients about their background (3%), consulting with the ancestors (28%), reading patients for signs (12%), and using patients' symptoms for guidance (6%). More than one of these methods were used by 37% of the THPs and 14% of THPs indicated use of other methods (Table 2).

Schizophrenia

Knowledge of schizophrenia

Although only 42% of the THPs had indicated that they had diagnosed schizophrenia, 65% knew what schizophrenia was and 67.7% of these THPs detailed that this disorder can be defined as losing touch with reality. Some of the participants (4.6%) offered that it is madness/craziness and 24.6% maintained that it is the combination of these. Other participants (3.1%) identified schizophrenia as something else other than those listed. When questioned if delusions, hallucinations, hostility, suspiciousness, grandiosity, and conceptual disorganization are symptoms of schizophrenia, 58% answered "yes" (Table 3). When questioned if the lack of motivation, lack of enjoyment and social interactions, asociality, anhedonia, and alogia are symptoms of schizophrenia, 50% responded "yes" (Table 3).

Table 1. Area of specialization in the field of traditional medicine.

Age		15-34 yrs (*N*=24)	35-59 yrs (*N*=60)	60+yrs (*N*=16)	p-value	Overall (*N*=100)
Specialization area					0.108	
Group 1 (Head, Mental Illnesses, Stroke, and Epilepsy)		3 (37.5%)	8 (44.4%)	1 (33.3%)	Fisher's	12 (41.4%)
Group 2 (General, Ukuhlola, Performs Rituals, Removes Spells and Initiation)		5 (62.5%)	6 (33.3%)	0 (0.0%)		11 (37.9%)
Group 3 (Aches, Wounds, Shingles, Umeqo, and Feet)		0 (0.0%)	3 (16.7%)	0 (0.0%)		3 (10.3%)
Group 4 (Abdomen- and maternity-related issues)		0 (0.0%)	1 (5.6%)	2 (66.7%)		3 (10.3%)
Local Municipality	uMzimkhulu (*N*=21)	Greater Kokstad (*N*=18)	Buhlebezwe (*N*=35)	Dr. Nkosazana Zuma (*N*=26)	p-value	Overall (*N*=100)
Specialization area					0.243	
Group 1 (Head, Mental Illnesses, Stroke, and Epilepsy)	2 (50.0%)	0 (0.0%)	9 (52.9%)	1 (14.3%)	Fisher's	12 (41.4%)
Group 2 (General, Ukuhlola, Performs Rituals, Removes Spells and Initiation)	1 (25.0%)	1 (100.0%)	4 (23.5%)	5 (71.4%)		11 (37.9%)
Group 3 (Aches, Wounds, Shingles, Umeqo, and Feet)	0 (0.0%)	0 (0.0%)	3 (17.6%)	0 (0.0%)		3 (10.3%)
Group 4 (Abdomen- and maternity-related issues)	1 (25.0%)	0 (0.0%)	1 (5.9%)	1 (14.3%)		3 (10.3%)
Sex		Female (*N*=74)	Male (*N*=25)	p-value		Overall (*N*=99)
Specialization area				Fisher's, $p = .256$		
Group 1 (Head, Mental Illnesses, Stroke, and Epilepsy)		8 (47.1%)	4 (33.3%)			12 (41.4%)
Group 2 (General, Ukuhlola, Performs Rituals, Removes Spells and Initiation)		4 (23.5%)	7 (58.3%)			11 (37.9%)
Group 3 (Aches, Wounds, Shingles, Umeqo, and Feet)		3 (17.6%)	0 (0.0%)			3 (10.3%)
Group 4 (Abdomen- and maternity-related issues)		2 (11.8%)	1 (8.3%)			3 (10.3%)
Self-classification	Combinations of all classifications (*N*=23)	Inyanga (*N*=3)	Isangoma (*N*=63)	Umthandazi (*N*=10)	p-value	Overall (*N*=99)
Specialization area					0.556	
Group 1 (Head, Mental Illnesses, Stroke, and Epilepsy)	2 (66.7%)	1 (50.0%)	7 (31.8%)	2 (100.0%)	Fisher's	12 (41.4%)
Group 2 (General, Ukuhlola, Performs Rituals, Removes Spells and Initiation)	1 (33.3%)	0 (0.0%)	10 (45.5%)	0 (0.0%)		11 (37.9%)
Group 3 (Aches, Wounds, Shingles, Umeqo, and Feet)	0 (0.0%)	0 (0.0%)	3 (13.6%)	0 (0.0%)		3 (10.3%)
Group 4 (Abdomen- and maternity-related issues)	0 (0.0%)	1 (50.0%)	2 (9.1%)	0 (0.0%)		3 (10.3%)
Practice period	Entry level (*N*=37)	Mid-level (*N*=34)	Senior level (*N*=24)	Expert level (*N*=5)	p-value	Overall (*N*=100)
Specialization area					0.820	
Group 1 (Head, Mental Illnesses, Stroke, and Epilepsy)	4 (44.4%)	4 (33.3%)	3 (50.0%)	1 (50.0%)	Fisher's	12 (41.4%)
Group 2 (General, Ukuhlola, Performs Rituals, Removes Spells and Initiation)	3 (33.3%)	4 (33.3%)	3 (50.0%)	1 (50.0%)		11 (37.9%)

(*Continued*)

Table 2. Diagnosis of mental disorders.

Local Municipality	uMzimkhulu (*N*=21)	Greater Kokstad (*N*=18)	Buhlebezwe (*N*=35)	Dr. Nkosazana Zuma (*N*=26)	p-value	Overall (*N*=100)
Once diagnosed mental disorder					0.043	
No	11 (52.4%)	2 (11.1%)	16 (45.7%)	11 (42.3%)	Chisq.	40 (40.0%)
Yes	10 (47.6%)	16 (88.9%)	19 (54.3%)	15 (57.7%)		60 (60.0%)
Mental disorders diagnosed					0.127	
Anxiety disorder	2 (9.5%)	1 (5.6%)	3 (8.6%)	6 (23.1%)	Fisher's	12 (12.0%)
Major depressive disorder	1 (4.8%)	1 (5.6%)	3 (8.6%)	2 (7.7%)		7 (7.0%)
More than one of these disorders	4 (19.0%)	2 (11.1%)	7 (20.0%)	5 (19.2%)		18 (18.0%)
Other	6 (28.6%)	0 (0.0%)	7 (20.0%)	7 (26.9%)		20 (20.0%)
Schizophrenia	8 (38.1%)	14 (77.8%)	14 (40.0%)	6 (23.1%)		42 (42.0%)
Substance use disorder	0 (0.0%)	0 (0.0%)	1 (2.9%)	0 (0.0%)		1 (1.0%)
Other mental disorders diagnosed					0.922	
Specified (Aches, Stroke and Epilepsy)	0 (0.0%)	0 (0.0%)	2 (5.7%)	1 (3.8%)		3 (3.0%)
How mental disorders diagnosed					0.010	
Ask patient about their background for guidance	2 (9.5%)	0 (0.0%)	1 (2.9%)	0 (0.0%)	Fisher's	3 (3.0%)
Consult with ancestors for guidance	1 (4.8%)	11 (61.1%)	7 (20.0%)	9 (34.6%)		28 (28.0%)
More than one of these methods	12 (57.1%)	3 (16.7%)	11 (31.4%)	11 (42.3%)		37 (37.0%)
Other	4 (19.0%)	1 (5.6%)	5 (14.3%)	4 (15.4%)		14 (14.0%)
Read patient for signs and guidance	1 (4.8%)	2 (11.1%)	8 (22.9%)	1 (3.8%)		12 (12.0%)
Use patient's symptoms for guidance	1 (4.8%)	1 (5.6%)	3 (8.6%)	1 (3.8%)		6 (6.0%)

Treatment of schizophrenia

Schizophrenia was treated by 49% of the participants and 77.8% were from Greater Kokstad (p = .020) (Table 4). From those who treated schizophrenia, 33.3%, 52.4%, 30%, and 47.8% classified as Herbalists, Diviners, Faith Healers, and a combination of these, respectively. When considering the treatment of schizophrenia in relation to the period of practice of the THPs, 32.4%, 64.7%, 54.2%, and 40% who were at the entry level, mid-level, senior level, and expert level treated schizophrenia, respectively. There was a statistical significance between the participants who treated schizophrenia and the period of practice as THPs (p = .041) (Table 4). Of the participants who mentioned treating schizophrenia, 59.2% used a medicinal concoction, 2% used a cleansing bath for patients, 2% performed rituals on patients, 32.7% used a combination of

Table 3. Knowledge on the symptoms of schizophrenia.

Local Municipality	uMzimkhulu (*N*=21)	Greater Kokstad (*N*=18)	Buhlebezwe (*N*=35)	Dr. Nkosazana Zuma (*N*=26)	p-value	Overall (*N*=100)
Know of positive symptoms of schizophrenia					0.289	
I don't know	8 (38.1%)	2 (11.1%)	9 (25.7%)	10 (38.5%)	Fisher's	29 (29.0%)
No	2 (9.5%)	2 (11.1%)	4 (11.4%)	5 (19.2%)		13 (13.0%)
Yes	11 (52.4%)	14 (77.8%)	22 (62.9%)	11 (42.3%)		58 (58.0%)
Know of negative symptoms of schizophrenia					0.110	
I don't know	12 (57.1%)	4 (22.2%)	9 (25.7%)	11 (42.3%)	Fisher's	36 (36.0%)
No	3 (14.3%)	1 (5.6%)	6 (17.1%)	4 (15.4%)		14 (14.0%)
Yes	6 (28.6%)	13 (72.2%)	20 (57.1%)	11 (42.3%)		50 (50.0%)

Table 4. Participants who treat schizophrenia.

Local Municipality	uMzimkhulu (*N*=21)	Greater Kokstad (*N*=18)	Buhlebezwe (*N*=35)	Dr. Nkosazana Zuma (*N*=26)	p-value	Overall (*N*=100)
Treat schizophrenia					0.020	
No	12 (57.1%)	4 (22.2%)	17 (48.6%)	18 (69.2%)	Chisq.	51 (51.0%)
Yes	9 (42.9%)	14 (77.8%)	18 (51.4%)	8 (30.8%)		49 (49.0%)
Self-classification	Combinations of these classifications (*N*=23)	Inyanga (*N*=3)	Isangoma (*N*=63)	Umthandazi (*N*=10)	p-value	Overall (*N*=99)
Treat schizophrenia					0.576	
No	12 (52.2%)	2 (66.7%)	30 (47.6%)	7 (70.0%)	Fisher's	51 (51.5%)
Yes	11 (47.8%)	1 (33.3%)	33 (52.4%)	3 (30.0%)		48 (48.5%)
Practice period	Entry level (*N*=37)	Mid-level (*N*=34)	Senior level (*N*=24)	Expert level (*N*=5)	p-value	Overall (*N*=100)
Treat schizophrenia					0.041	
No	25 (67.6%)	12 (35.3%)	11 (45.8%)	3 (60.0%)	Fisher's	51 (51.0%)
Yes	12 (32.4%)	22 (64.7%)	13 (54.2%)	2 (40.0%)		49 (49.0%)

these methods, and 6.1% use other methods. Of those who did not treat schizophrenia, 29.4% maintained that they would use a medicinal concoction, 2% would take patients for a cleansing bath, 7.8% would use a combination of these and 58.8% would use other methods to treat this mental disorder.

Traditional medicine and treatment methods for other (general) ailments

On the treatment of general ailments, the participants reported that they provided a traditional medicinal concoction to their patients in 37% of cases, prayed for them in 7% of the cases, referred them to pharmacy or clinic in 4% of cases, took them for a cleansing bath in 8% of cases, used a combination of these in 38% of cases, and used other methods in 6% of cases (Table 5). The majority (89%) of the participants maintained that their treatment approaches are effective, 28% of the THPs based this response on their level of confidence, while 32% conducted a follow-up with the patients, 33% used a combination of the two and 7% declared other reasons. Most (85%) of the participants believed that traditional medicine is a medicinal concoction from indigenous plants, 2% mentioned that these are similar to pharmacological medicine and 13% were of the opinion that traditional medicine is something else (Table 5).

Table 5. General traditional treatment methods and the effectiveness of traditional medicine.

Local Municipality	uMzimkhulu (*N*=21)	Greater Kokstad (*N*=18)	Buhlebezwe (*N*=35)	Dr. Nkosazana Zuma (*N*=26)	p-value	Overall (*N*=100)
General treatment methods					<0.001	
A combination of the above mentioned	11 (52.4%)	4 (22.2%)	10 (28.6%)	13 (50.0%)	Fisher's	38 (38.0%)
Give them a medicinal concoction	4 (19.0%)	5 (27.8%)	17 (48.6%)	11 (42.3%)		37 (37.0%)
Other	1 (4.8%)	0 (0.0%)	3 (8.6%)	2 (7.7%)		6 (6.0%)
Pray for them	4 (19.0%)	0 (0.0%)	3 (8.6%)	0 (0.0%)		7 (7.0%)
Refer them to a pharmacy/clinic	1 (4.8%)	2 (11.1%)	1 (2.9%)	0 (0.0%)		4 (4.0%)
Take them for a cleansing bath	0 (0.0%)	7 (38.9%)	1 (2.9%)	0 (0.0%)		8 (8.0%)
Treatment option effective					0.052	
I don't know	5 (23.8%)	0 (0.0%)	2 (5.7%)	4 (15.4%)	Fisher's	11 (11.0%)
Yes	16 (76.2%)	18 (100.0%)	33 (94.3%)	22 (84.6%)		89 (89.0%)
Treatment option effectiveness assessment					0.149	
Do follow-up; Confident in my treatment option	8 (38.1%)	2 (11.1%)	12 (34.3%)	11 (42.3%)	Fisher's	33 (33.0%)
Confident in my treatment option	7 (33.3%)	8 (44.4%)	7 (20.0%)	6 (23.1%)		28 (28.0%)
Do follow-up	3 (14.3%)	8 (44.4%)	13 (37.1%)	8 (30.8%)		32 (32.0%)
Other	3 (14.3%)	0 (0.0%)	3 (8.6%)	1 (3.8%)		7 (7.0%)
What is traditional medicine					0.488	
A medicinal concoction made from indigenous plants	20 (95.2%)	17 (94.4%)	28 (80.0%)	20 (76.9%)	Fisher's	85 (85.0%)
Other	1 (4.8%)	1 (5.6%)	6 (17.1%)	5 (19.2%)		13 (13.0%)
Similar to pharmacological medicine	0 (0.0%)	0 (0.0%)	1 (2.9%)	1 (3.8%)		2 (2.0%)

Discussion

Socio-demographic profiles

The predominance of female THPs in this study differed from previous authors (Moeng & Potgieter, 2011; Semenya & Potgieter, 2014) who reported that traditional healing is a male dominated practice. According to Moeng and Potgieter (2011), female healers may not be able to handle the strenuous process of collecting medicinal plants. Moreover, Semenya and Potgieter (2014) expanded that males dominate this practice as they are breadwinners for their families in the rural areas. However, the results of this study reflect a different notion which is similar to De Wet, Nzama, and Van Vuuren (2012) and Mathibela, Egan, Du Plessis, and Potgieter (2015) who also reported female healers dominating this practice. This shows that the prevalent gender among THPs is context-specific and could be due to various factors or reasons. For instance, according to Ogana and Ojong (2015), the higher number of female diviners (*Izangoma*) could be due to males who have an ancestral calling rejecting the possession by the spirits. In some cases, the dominating sex in this practice may depend on the area of specialization, for example, Kacholi and Amir (2022) reported a dominance of male THPs which was concluded to be due to how males were comfortable discussing matters relating to sex and sexually transmitted diseases compared to females.

The prevalent age groups of THPs reported in this study were similar to those identified by other authors (Kacholi & Amir, 2022; Ndawonde, 2006; Semenya & Potgieter, 2014). This age group is hypothesized to be dominant because these individuals are the working group that supports their families (Ndawonde, 2006). A small number of THPs in this study had an expert-level experience, this could be due to the previous prohibition of openly practicing traditional healing in South Africa until this healing practice was formally recognized after the promulgation of the THPs Act no. 22 of 2007 (Mendu & Ross, 2019). Even if the THPs practiced in secret prior to this Act, it can be hypothesized that they only considered the years of practice after the Act was declared as their formal years of experience as healers.

The majority of the THPs in the HGDM classified as *Isangoma* (Diviner), followed by the healers who classified themselves as a combination of the three classifications. The possible reason for this finding is reflected by Du Toit (1971) who stated that when the expertise of a healer exceeds treating with herbs and the healer is a doctor for divining, then that healer is classified as *Isangoma*. This finding of THPs identifying as more than one of these classifications also corroborates the report by Appelbaum Besisle *et al.* (2015) which states that most of the interviewed THPs in their study identified with more than one of the traditional healing classifications. A majority of the THPs in this study did not have specific areas of specialization, this could be due to the

regular practice of treating patients as they arrive and the dependence on the guidance from the ancestors regarding the different types of ailments.

Mental disorders in general

Knowledge of mental disorders

The majority of the THPs were knowledgeable about the existence of mental disorders in this study, although a few had what may be regarded as "an accurate knowledge" of mental disorders in relation to Western Medicine and their definitions varied from definitions in Western Medicine as well. These findings are similar to how Atilola (2015) described psychiatric terms as unknown or poorly understood in sub-Saharan Africa. This could be due to how THPs perceive illness in a holistic manner and most of them have a belief that these disorders are influenced by the spiritual world (Marques, Freeman, & Carter, 2022).

Diagnosis of mental disorders

The methods used by the THPs to diagnose mental disorders included questioning patients about their background, consulting with the ancestors, reading patients for signs, and using patients' symptoms for guidance. Most THPs used a combination of these methods for mental disorder diagnosis. Understanding the background/familial situation of a patient before treatment was recommended as previously highlighted in a study by Al-Krenawi and Graham (1997). In allopathic medicine, one of the commonly used diagnostic tools to diagnose mental disorders is the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) which only focuses on the symptoms presented by the patients, unlike in traditional healing where a diagnosis is dependent on various factors, namely, biological, socio-cultural, and spiritual factors.

Most of the THPs (who were prevalent in Greater Kokstad) stated that they have been diagnosed with mental disorders in their years as healers, this agrees with Simonds and Christopher (2013) who documented that people first seek medical care related to mental health from THPs in 20–40% of cases in Africa. Schizophrenia was the commonly diagnosed mental disorder within HGDM, and this is similar to the study by Mbwayo, Ndetei, Mutiso, and Khasakhala (2013) in Kenya. Other diagnosed disorders in the region included anxiety disorder, major depressive disorder, substance abuse, and epilepsy. These findings differed slightly from previous studies where the common diagnosed disorders through traditional healing in Pakistan were major depression, anxiety disorder, and psychosis (Saeed, Gater, Hussain, & Mubbashar, 2000). In the African context, psychotic disorders (i.e. psychotic depression, mania, mood disorder, and schizophrenia) were the prevalent mental disorders diagnosed in Uganda (Abbo, Ekblad, Waako, Okello, & Musisi, 2009) while depression and anxiety disorder were the commonly diagnosed disorders in

Tanzania and Kenya (Mbwayo, Ndetei, Mutiso, & Khasakhala, 2013; Ngoma, Prince, & Mann, 2003). A few of the participants listed headaches and stroke as some of the mental disorders they have diagnosed, and this reflects how the distinction between neurologic and psychiatric disorders seems arbitrary (Martin, 2002). It can also be hypothesized that the THPs identified stroke as it may be associated with some of the mental disorders since there is one in three stroke patients who are also diagnosed with clinical depression (Ojagbemi et al., 2017).

Schizophrenia

Knowledge of schizophrenia

Prior to the discovery of antipsychotic drugs in the 1960s, traditional healing approaches had been in use for thousands of years for the treatment of schizophrenia (Ojagbemi & Gureje, 2020). As reflected by the findings of this study, the majority of THPs in the HGDM possess knowledge and understanding of schizophrenia. Delusions, hallucinations, hostility, suspiciousness, grandiosity, and conceptual disorganization were recognized by most of the THPs as symptoms of schizophrenia. Lack of motivation, lack of enjoyment and social interactions, asociality, anhedonia, and alogia were also recognized by half of the THPs as symptoms of schizophrenia. In some communities, symptoms of schizophrenia may be associated with spiritual explanations such as the influence of the devil, demons, and/or other spirits, witchcraft, and an inadequate relationship with the ancestral spirits (Mohr & Huguelet, 2004; Verginer & Juen, 2019).

Treatment of schizophrenia

It was the THPs who had mid-level experience and who classified as *Isangoma* who commonly treated schizophrenia, followed by those who had senior-level experience and those who classify as the combination of *Inyanga*, *Isangoma*, and/or *Umthandazi*. The dominating local municipality in the treatment of schizophrenia was Greater Kokstad followed by Buhlebezwe. The commonly used treatment method for schizophrenia was the administration of a medicinal concoction. Sometimes patients may be taken for a cleansing bath and/or have rituals performed on or for them in order to treat this mental disorder. These findings concur with Dein (2020) in that the choice of treatment approach may be influenced by the classification of the THPs and what is believed to have caused the illness. Faith healing has been reported to lack effectiveness in treating mental illness; however, spiritual treatment methods (prayer and fasting) were reported to be effective in the treatment of these illnesses (Appelbaum Belisle et al., 2015). Herbs, medicinal concoction, sweat lodges, sacred bundles, drumming, smudging, and smoking tobacco have also been reported by other authors to be effective in managing mental illness

(Musyimi, Mutiso, Nandoya, & Ndetei, 2016). Despite the global presence and contribution of traditional medicine in the treatment of mental disorders, the standard of care and the evidence from which to assess the effectiveness of traditional healing are still lacking. However, since there is a significant number of people who use THPs for treatment and management of mental disorders, these healers and their treatment approaches cannot be disregarded (Abbo, Odokonyero, & Ovuga, 2019).

When it comes to Western medicine, the cornerstone in the treatment of schizophrenia are the antipsychotic drugs; however, the treatment of this mental disorder is still a challenge despite the numerous pharmacological advances that have been made till date (Wada et al., 2022). Some of the factors that contribute to this are the adverse side effects from the antipsychotic drugs as these side effects may lead to poor adherence, change and discontinuation in antipsychotic medication, and partial or non-response to treatment (Brodeur et al., 2022). Non-pharmacological therapy that includes psychotherapeutic approaches (viz. individual, group, and cognitive behavioral) may also be used to manage schizophrenia (Crismon et al., 2014). According to Lindenmayer et al. (2009), non-pharmacological approaches can assist in ensuring that patients adhere to their medication and also fill in the gaps found in pharmacological treatment approaches.

In South Africa, the number of THPs practicing is approximately 368 000 (Street et al., 2018). There are approximately only 1.5 psychiatrists and 2.5 psychologists per 100 000 people in South Africa and this is a clear indication of the lack of allopathic mental health care providers, particularly in the public sector (Padmanabhanunni, Jackson, Noordien, Pretorius, & Bouchard, 2022; WHO, 2019). Communities from sub-Saharan Africa offer that THPs possess the ability to diagnose and manage physical and mental ailments that allopathic specialists are unable to treat as these illnesses are specific to Black African people (Schierenbeck, Johansson, Andersson, Krantz, & Ntaganira, 2018). Other reasons why traditional healing is still the preferred option for diagnosis and treatment of various ailments in Africa are cost-effectiveness, dissatisfaction with Western health care and the conformity of traditional medicine with spiritual and cultural beliefs and practices (Mpinga et al., 2013). Poverty has been reported to be the major factor that contributes to why in low-income and middle-income countries traditional medicine remains the commonly used mode of treatment for different ailments. In these countries most of the population resides in rural areas while facilities that provide Western medicine are located in urban areas and this is one of the reasons why traditional medicine is more accessible (Gureje et al., 2015). Language barrier may also be a contributing factor to why people may choose to consult with THPs as in a study conducted in the Eastern Cape, South Africa, where it was reported that most medical doctors were English-speaking and this led to ineffective communication between doctors and patients who were not English-speakers

(Hussey, 2012). It is then uncertain if these patients understood the nature of their diagnosis and the choice of treatment communicated by their doctors.

Despite the evident contrasting differences between traditional healing and western medicine, there are calls for THPs to be integrated into formal or mainstream health services and calls for a collaboration between Western and non-Western treatment approaches (WHO, 2013). According to Gureje et al. (2020), this collaboration needs to follow a cautious program due to the concerns that treatment approaches used by the THPs might be harmful since they include the use of untested or unknown concoctions, shackling, and prolonged fasting periods which are forced, and also a concern that these might also infringe human rights of patients diagnosed with serious mental disorders. When a trial of this collaboration was done in Nigeria and Ghana for the treatment of mental disorders, it provided improved patient outcomes at a reduced overall care cost (Gureje et al., 2020).

Traditional medicine and treatment methods other (general) ailments

The approach used by the THPs for the treatment of physical ailments is the same as for treating mental disorders, whereby a medicinal concoction is prescribed on its own or with the addition of prayer and a cleansing bath. This alignment of treatment methods can be explained by the holistic approach used by THPs when diagnosing an illness as they take into consideration the biological, socio-cultural, and spiritual factors. It is rare for the THPs to refer their patients to a pharmacy or clinic since the majority of the THPs are confident in the effectiveness of their treatment. Some THPs conducted a follow-up with their patients in order to confirm the effectiveness of their treatment. The effectiveness of traditional medicine may also be influenced by how patients accurately and openly describe their symptoms to THPs as compared to medical doctors (this could be influenced by language barrier); hence, THPs are likely to have a thorough and clear description of the symptoms presented by the patients and assists in selecting the best treatment option (van der Watt et al., 2018). In cases where traditional medicine is ineffective as a treatment option, THPs then refer their patients to biomedical health care facilities. This then broadens the possibilities for a collaboration between traditional healing and biomedicine, although some THPs might still not be convinced of the value of allopathic medicine in illnesses where supernatural factors are said to be the cause of illness.

THPs in most African cultures are considered to be divinely connected and obtain their power from God (Mokgobi, 2014). In addition to God, in most African communities, people also culturally believe that their lives and well-being are influenced by ancestors (Mokgobi, 2014). Also, in African traditions, the explanation as to why one suffers from a particular mental illness at a particular time is associated with gods or ancestors and witchcraft or sorcery (Nwagbo &

Tingir, 2022). In other cases, ill health, emotional and spiritual breakdowns may be associated with an ancestral calling; thus, mental illness may be caused by the refusal to accede to this call which is said to lead to the ancestors being angry with the person who is refusing the calling (Shange & Ross, 2022). Most THPs maintain that they are called by their ancestors to become healers in order to assist and provide health care to the community with the aid of medicinal plants and wisdom from the ancestors (Zuma, Wight, Rochat, & Moshabela, 2016). Hence, the use of medicinal plants or herbal remedies for different ailments as this is the eminent treatment option in South Africa (Rasethe *et al.*, 2022). According to Zuma, Wight, Rochat, and Moshabela (2016), approximately 70% of Black South Africans rely on traditional medicine for treatment and approximately 60% of this population consult with THPs. It is also likely that patients will consult with THPs before seeking allopathic health care (Dein, 2020).

Conclusion and recommendations

In conclusion, mental disorders are known, diagnosed, and treated by THPs at HGDM in KwaZulu-Natal. Diagnosis and treatment approaches used by THPs involved consulting ancestors. The commonly used treatment option in traditional healing is a medicinal concoction; however, it is recommended that further studies be conducted on the individual medicinal plants used for the concoctions to evaluate their effectiveness and safety so that this type of healing can be scientifically proven and to ensure that none or minimal side effects are associated with this treatment approach. Another area that requires exploration would be the relationship between THPs and biomedical practitioners; as to how can they work together to provide health care to the community without one system misunderstanding the other?

Limitations

This study focused on only 1 of 11 municipalities in the KwaZulu-Natal province; hence, the findings cannot be generalized to the other municipalities. The sample of THPs represented in this study was only 25% of the total registered THPs in the HGDM. Additionally, the findings of this study are quantitative with the sole use of descriptive statistics.

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References

Abbo, C., Ekblad, S., Waako, P., Okello, E., & Musisi, S. (2009). The prevalence and severity of mental illnesses handled by traditional healers in two districts in Uganda. *African Health Sciences, 9*(1), S16–22.

Abbo, C., Odokonyero, R., & Ovuga, E. (2019). A narrative analysis of the link between modern medicine and traditional medicine in Africa: A case of mental health in Uganda. *Brain Research Bulletin, 145*, 109–116. doi:10.1016/j.brainresbull.2018.07.018

Al-Krenawi, A., & Graham, J. R. (1997). Spirit possession and exorcism in the treatment of a Bedouin psychiatric patient. *Clinical Social Work Journal, 25*(2), 211–222. doi:10.1023/A:1025714626136

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders: DSM-5* (5th ed.). Washington, D.C: American Psychiatric Publishing.

Appelbaum Belisle, H., Hennink, M., Ordóñez, C. E., John, S., Ngubane-Joye, E., Hampton, J. . . . Marconi, V. C. (2015). Concurrent use of traditional medicine and ART: Perspectives of patients, providers and traditional healers in Durban, South Africa. *Global Public Health, 10*(1), 71–87. doi:10.1080/17441692.2014.967709

Arshinoff, R., Roldan, C., & Balboni, T. (2023). Spirituality and spiritual distress in neurologic illness [Abstract]. In *Handbook of clinical neurology* (Vol. 191, pp. 221–234). Elsevier. doi:10.1016/B978-0-12-824535-4.00004-5.

Atilola, O. (2015). Level of community mental health literacy in sub-saharan Africa: Current studies are limited in number, scope, spread, and cognizance of cultural nuances. *Nordic Journal of Psychiatry, 69*(2), 93–101. doi:10.3109/08039488.2014.947319

Brodeur, S., Vanasse, A., Courteau, J., Courteau, M., Stip, E., Fleury, M. J. . . . Roy, M. A. (2022). Antipsychotic utilization trajectories three years after initiating or reinitiating treatment of schizophrenia: A state sequence analysis approach. *Acta Psychiatrica Scandinavica, 145*(5), 469–480. doi:10.1111/acps.13411

Captari, L. E., Sandage, S. J., Vandiver, R. A., Jankowski, P. J., & Hook, J. N. (2023). Integrating Positive Psychology, Religion/Spirituality, and a Virtue Focus Within Culturally Responsive Mental Healthcare. In E. B. Davis, E. L. Worthington Jr, S. A. Schnitker (Eds.), *Handbook of Positive Psychology, Religion, and Spirituality*. Cham: Springer. doi:10.1007/978-3-031-10274-5_26

Cooperative Governance and Traditional Affairs (Cogta). (2020). *Harry gwala district municipality, kzn. profile and analysis: District development model*. Pretoria, South Africa: Harry Gwala District Municipality (cogta.gov.za).

Crellin, N. E., Priebe, S., Morant, N., Lewis, G., Freemantle, N., Johnson, S. . . . Moncrieff, J. (2022). An analysis of views about supported reduction or discontinuation of antipsychotic treatment among people with schizophrenia and other psychotic disorders. *BMC Psychiatry*, *22*(1), 185. doi:https://doi.org/10.1186/s12888-022-03822-5

Crismon, L., Argo, T. R., & Buckley, P. F. (2014). Schizophrenia. In J. T. DiPiro, R. L. Talbert, G. C. Yee, G. R. Matzke, B.G. Wells, & L. Posey (Eds.), *Pharmacotherapy: A pathophysiologic approach* (9th ed., pp. 1019–1046). New York, New York: McGraw-Hill.

Dein, S. (2020). Religious healing and mental health. *Mental Health, Religion & Culture*, *23*(8), 657–665. doi:10.1080/13674676.2020.1834220

Deng, H., & Xu, J. (2017). Wendan decoction (traditional Chinese medicine) for schizophrenia. *Cochrane Database System Review*, *6*(6), CD012217. doi:10.1002/14651858.CD012217.pub2

De Wet, H., Nzama, V. N., & Van Vuuren, S. F. (2012). Medicinal plants used for the treatment of sexually transmitted infections by lay people in northern maputaland, KwaZulu–Natal province, South Africa. *South African Journal of Botany*, *78*, 12–20. doi:https://doi.org/10.1016/j.sajb.2011.04.002

Du Toit, B. M. (1971). Isangoma: An adaptive agent among urban Zulu. *Anthropological Quarterly*, *1*(2), 51–65. doi:10.2307/3316770

Edwards, S., Makunga, N., Thwala, J., & Mbele, B. (2009). The role of the ancestors in healing: Indigenous African healing practices. *Indilinga African Journal of Indigenous Knowledge Systems*, *8*(1), 1–11. doi:10.4314/indilinga.v8i1.48234

Grover, S., Davuluri, T., & Chakrabarti, S. (2014). Religion, spirituality, and schizophrenia: A review. *Indian Journal of Psychological Medicine*, *36*(2), 119–124. doi:10.4103/0253-7176.130962

Gureje, O., Appiah-Poku, J., Bello, T., Kola, L., Araya, R., Chisholm, D. . . . Seedat, S. (2020). Effect of collaborative care between traditional and faith healers and primary health-care workers on psychosis outcomes in Nigeria and Ghana (COSIMPO): A cluster randomised controlled trial. *The Lancet*, *396*(10251), 612–622. doi:10.1016/S0140-6736(20)30634-6

Gureje, O., Nortje, G., Makanjuola, V., Oladeji, B., Seedat, S., & Jenkins, R. (2015). The role of global traditional and complementary systems of medicine in treating mental health problems. *The Lancet Psychiatry*, *2*(2), 168–177. doi:10.1016/S2215-0366(15)00013-9

Gutema, B. T., & Mengstie, M. M. (2022). Perceived causes, diagnosis methods and treatment practices of mental illness among traditional healers of the Berta community in Assosa, Ethiopia. *The International Journal of Social Psychiatry*. doi:10.1177/00207640221128163

Hajiaghaee, R., & Akhondzadeh, S. (2018). Herbal medicine in the treatment of schizophrenia: A review. *Journal of Medicinal Plants*, *17*(67), 1–5.

Hussey, N. (2012). The language barrier: The overlooked challenge to equitable health care. *South African Health Review*, *1*, 189–195.

Kacholi, D. S., & Amir, H. M. (2022). Ethnobotanical survey of medicinal plants used by traditional healers in managing Gonorrhoea and Syphilis in Urambo District, Tabora Region, Tanzania. *Journal of Herbs, Spices & Medicinal Plants*, *28*(2), 179–192. doi:10.1080/10496475.2022.2035476

Kaplan, H. I., & Sadock, B. J. (1988). *Synopsis of psychiatry: Behavioral sciences clinical psychiatry*. Philadelphia, United States: Williams & Wilkins Co.

Koster, L. S., Carbon, M., & Correll, C. U. (2014). Emerging drugs for schizophrenia: An update. *Expert Opinion Emerging Drugs*, *19*(4), 1–21. doi:10.1517/14728214.2014.958148

Lindenmayer, J. P., Liu-Seifert, H., Kulkarni, P. M., Kinon, B. J., Stauffer, V., Edwards, S. E. . . . Volavka, J. (2009). Medication non-adherence and treatment outcomes in patients with

schizophrenia or schizoaffective disorder with suboptimal prior response. *The Journal of Clinical Psychiatry*, *70*(7), 990–996. doi:10.4088/JCP.08m04221

Marques, B., Freeman, C., & Carter, L. (2022). Adapting traditional healing values and beliefs into therapeutic cultural environments for health and well-being. *International Journal of Environmental Research and Public Health*, 426. doi:10.3390/ijerph19010426

Martin, J. B. (2002). The integration of neurology, psychiatry, and neuroscience in the 21st century. *The American Journal of Psychiatry*, *159*(5), 695–704.

Mathibela, M. K., Egan, B. A., Du Plessis, H. J., & Potgieter, M. J. (2015). Socio-cultural profile of bapedi traditional healers as indigenous knowledge custodians and conservation partners in the Blouberg area, Limpopo province, South Africa. *Journal of Ethnobiology Ethnomedicine*, *11*(1), 49. doi:10.1186/s13002-015-0025-3

Mbwayo, A. W., Ndetei, D. M., Mutiso, V., & Khasakhala, L. I. (2013). Traditional healers and provision of mental health services in cosmopolitan informal settlements in Nairobi, Kenya. *African Journal of Psychiatry*, *16*(2), 134–140. doi:10.4314/ajpsy.v16i2.17

Mendu, E., & Ross, E. (2019). Biomedical healthcare and African traditional healing in the management of HIV and AIDS: Complimentary or competing cosmologies? *African Journal of AIDS Research*, *18*(2), 104–114. doi:10.2989/16085906.2019.1619600

Moeng, E. T., & Potgieter, M. J. (2011). The trade of medicinal plants by muthi shops and street vendors in the Limpopo province, South Africa. *Journal of Medicinal Plants Research*, *5*, 558–564.

Mohr, S., & Huguelet, P. (2004). The relationship between schizophrenia and religion and its implications for care. *Swiss Medical Weekly*, *134*(25–26), 369–376. doi:10.4414/smw.2004.10322

Mokgobi, M. G. (2014). Understanding traditional African healing. *African Journal of Physics Health Education Recreation Dance*, *20*(2), 24–34.

Monama, D. D., & Basson, W. J. (2017). Looking in the mirror: The cultural experiences of patients diagnosed with schizophrenia. *Pula: Botswana Journal of African Studies*, *31*(2), 46–58.

Mpinga, E. K., Kandolo, T., Verloo, H., Bukonda, N. K., Kandala, N. B., & Chastonay, P. (2013). Traditional/Alternative medicines and the right to health: Key elements for a convention on global health. *Health and Human Rights*, *15*(1), E44–57.

Musyimi, W., Mutiso, V. N., Nandoya, E. S., & Ndetei, D. M. (2016). Forming a joint dialogue among faith healers, traditional healers and formal health workers in mental health in a Kenyan setting: Towards common grounds. *Journal of Ethnobiology and Ethnomedicine*, *12*(1), 4. doi:10.1186/s13002-015-0075-6

Ndawonde, B. G. (2006). Medicinal Plant Sales: A Case Study in Northern Zululand M.Sc. Dissertation. KwaDlangezwa: University of Zululand.

Ngoma, M. C., Prince, M., & Mann, A. (2003). Common mental disorders among those attending primary health clinics and traditional healers in urban Tanzania. *The British Journal of Psychiatry: The Journal of Mental Science*, *183*(4), 349–355. doi:10.1192/bjp.183.4.349

Niehaus, D. J., Oosthuizen, P., Lochner, C., Emsley, R. A., Jordaan, E., Mbanga, N. I. ... Stein, D. J. (2004). A culture-bound syndrome 'amafufunyana'and a culture-specific event 'ukuthwasa': Differentiated by a family history of schizophrenia and other psychiatric disorders. *Psychopathology*, *37*(2), 59–63. doi:10.1159/000077579

Nwagbo, C. A., & Tingir, M. (2022). The effectiveness of traditional healing in the treatment of mental illness in Africa: A critical review. *International Journal for Psychotherapy in Africa*, *7*(1), 120–135. doi:10.1016/S2215-0366(15)00515-5

Ogana, W., & Ojong, V. B. (2015). A study of literature on the essence of ubungoma (divination) and conceptions of gender among izangoma (diviners). *Journal for the Study of Religion*, *28*(1), 52–80.

Ojagbemi, A., & Gureje, O. (2020). The potential role of traditional medicine in the management of schizophrenia. *Current Psychiatry Reports*, *22*(12), 1–7. doi:10.1007/s11920-020-01196-7

Ojagbemi, A., Owolabi, M., Akinyemi, R., Arulogun, O., Akinyemi, J., Akpa, O. … Ovbiagele, B. (2017). Prevalence and predictors of anxiety in an African sample of recent stroke survivors. *Acta Neurologica Scandinavica*, *136*(6), 617–623. doi:10.1111/ane.12766

Padmanabhanunni, A., Jackson, K., Noordien, Z., Pretorius, T. B., & Bouchard, J. P. (2022). Characterizing the nature of professional training and practice of psychologists in South Africa. *Annales Médico-psychologiques, revue psychiatrique*, *180*(4), 360–365. doi:10.1016/j.amp.2022.02.012

Patel, K. R., Cherian, J., Gohil, K., & Atkinson, D. (2014). Schizophrenia: Overview and treatment options. *P & T*, *39*(9), 638–645.

Rasethe, M., Potgieter, M., & Pfab, M. (2022). The utilization of selected Threatened or Protected plant species; a case of Limpopo Province, South Africa. *Sustainability*, *14*(3), 1073.

Saeed, K., Gater, R., Hussain, A., & Mubbashar, M. (2000). The prevalence, classification and treatment of mental disorders among attenders of native faith healers in rural Pakistan. *Social Psychiatry and Psychiatric Epidemiology*, *35*(10), 480–485. doi:10.1007/s001270050267

Schierenbeck, I., Johansson, P., Andersson, L. M., Krantz, G., & Ntaganira, J. (2018). Collaboration or renunciation? The role of traditional medicine in mental health care in Rwanda and Eastern cape province, South Africa. *Global Public Health*, *13*(2), 159–172. doi:10.1080/17441692.2016.1239269

Semenya, S. S., & Potgieter, M. J. (2014). Bapedi traditional healers in the Limpopo province, South Africa: Their socio-cultural profile and traditional healing practice. *Journal of Ethnobiology Ethnomedicine*, *10*(1), 4. doi:10.1186/1746-4269-10-4

Shange, S., & Ross, E. (2022). "The question is not how but why things happen": South African traditional healers' explanatory model of mental illness, its diagnosis and treatment. *Journal of Cross-Cultural Psychology*, *53*(5), 503–521. doi:10.1177/00220221221077361

Simonds, V. W., & Christopher, S. (2013). Adapting Western research methods to indigenous ways of knowing. *American Journal of Public Health*, *103*(12), 2185–2192. doi:10.2105/AJPH.2012.301157

Statistics South Africa. (2011). *Statistics by place*. Pretoria, South Africa: Statistiocs South Africa.

Street, R. A., Smith, M., Moshabela, M., Shezi, B., Webster, C., & Falkenberg, T. (2018). Traditional health practitioners and sustainable development: A case study in South Africa. *Public Health*, *165*, 1–5. doi:10.1016/j.puhe.2018.07.021

van der Watt, A. S. J., van de Water, T., Nortje, G., Oladeji, B. D., Seedat, S., & Gureje, O. (2018). & partnership for mental health development in sub-saharan Africa (PaM-D) research team. *Social Psychiatry and Psychiatric Epidemiology*, *53*(6), 555–566. doi:10.1007/s00127-018-1519-9

van Der Zeijst, M. C., Veling, W., Makhathini, E. M., Mbatha, N. D., Shabalala, S. S., Van Hoeken, D. … Hoek, H. W. (2022). Course of psychotic experiences and disorders among apprentice traditional health practitioners in rural South Africa: 3-year follow-up study. *Frontiers in Psychiatry*, *13*, 956003. doi:10.3389/fpsyt.2022.956003

van der Zeijst, M. C., Veling, W., Makhathini, E. M., Mtshemla, S., Mbatha, N. D., Shabalala, S. S. … Hoek, H. W. (2021). Psychopathology among apprentice traditional health practitioners: A quantitative study from rural KwaZulu-Natal, South Africa. *Transcultural Psychiatry*, *58*(4), 486–498. doi:10.1177/1363461520949672

Velligan, D. I., & Rao, S. (2023). The epidemiology and global burden of schizophrenia. *The Journal of Clinical Psychiatry*, *84*(1), 45094. doi:10.4088/JCP.MS21078COM5

Verginer, L., & Juen, B. H. (2019). Spiritual explanatory models of mental illness in West Nile, Uganda. *Journal of Cross-Cultural Psychology*, *50*(2), 233–253. doi:10.1177/0022022118813652

Volkan, K. (2021). Schizophrenia, culture, and culture-bound syndromes. *Psychology Research and Applications*, *3*(1), 1–13. doi:10.22606/pra.2021.31001

Wada, M., Noda, Y., Iwata, Y., Tsugawa, S., Yoshida, K., Tani, H. ... Nakajima, S. (2022). Dopaminergic dysfunction and excitatory/inhibitory imbalance in treatment-resistant schizophrenia and novel neuromodulatory treatment. *Molecular Psychiatry*, *27*(7), 2950–2967. doi:10.1038/s41380-022-01572-0

World Health Organisation. (2022). Mental Disorders [Fact Sheet]. (Retrieved 4 March 2023). https://www.who.int/news-room/fact-sheets/detail/mental-disorders#:~:text=A%20mental%20disorder%20is%20characterized,different%20types%20of%20mental%20disorders.

World Health Organization. (1978). The promotion and development of traditional medicine: Report of a WHO meeting [held in Geneva from 28 November to 2 December 1977].

World Health Organization. (2013). *WHO traditional medicine strategy: 2014-2023*. World Health Organization. https://apps.who.int/iris/handle/10665/92455

World Health Organization. (2019). Global Health Observatory Data Repository. Human Resources Data by Country: Psychiatrists Working in Mental Health Sector per 100,000 Population. Retrieved 11 July 2022. http://apps.who.int/gho/data/node.main.MHHR?lang=en .

Zabow, T. (2007). Traditional healers and mental health in South Africa. *International Psychiatry: Bulletin of the Board of International Affairs of the Royal College of Psychiatrists*, *4*(4), 81–83. doi:10.1192/S174936760000521X

Zuma, T., Wight, D., Rochat, T., & Moshabela, M. (2016). The role of traditional health practitioners in Rural KwaZulu-Natal, South Africa: Generic or mode specific? *BMC Complementary and Alternative Medicine*, *16*(1), 304. doi:10.1186/s12906-016-1293-8

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ORIGINAL ARTICLE

The Influence of Culture on the Cause, Diagnosis and Treatment of Serious Mental Illness (*Ufufunyana*): Perspectives of Traditional Health Practitioners in the Harry Gwala District, KwaZulu-Natal

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Abstract

Cultural beliefs influence the perceived cause, methods of diagnosis and treatment of mental illnesses. A qualitative study was conducted among traditional health practitioners (THPs) in the Harry Gwala District Municipality to further explore this influence. Purposive sampling assisted in the recruitment of 31 participants (9 males and 22 females). The four key themes this study investigated in relation to mental illness included its causes, methods of diagnosis, common symptoms observed and treatment approaches used by THPs, and the system of patient management. Culturally, mental illness was reported to be caused by witchcraft and an ancestral calling in this study. Mental illness was predominantly diagnosed by spiritual intervention which included divination through consultation with the ancestors, familial background, burning of incense which can also be part of communicating with the ancestors and through examining the patient. The common symptoms included aggression, hallucination and unresponsiveness. Prevalent modes of treatment included the use of a medicinal concoction and performing cultural rituals where ancestors and other spirits were assumed influential. The duration of the treatment process was dependent on guidance from the ancestors. Most causal aspects of mental illness from diagnosis to treatment seemed to be influenced by cultural beliefs and ancestors.

Keywords Ancestors · Mental illness · Traditional health practitioners · Cultural beliefs · Schizophrenia

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Introduction

The use of traditional medicine also referred to as complementary and alternative medicine (CAM) dates back several decades and has been noted in different communities globally. This type of medicine is often referred to as an informal folk treatment which represents practices that are outside of conventional medicine (Budzak & Brankovic, 2022; Helha & Wang, 2022). According to the World Health Organisation (2019b), the term "complementary" describes approaches that may be used as augmentation to biomedical medicine, these include massage and meditation; whereas the term "alternative" describes methods such as herbal remedies which are used instead of conventional medicine. Despite traditional medicine lacking sufficient empirical investigation, it is estimated that approximately 80% of the global population uses this type of medicine for the treatment and management of ailments associated with mental, emotional, spiritual, physical and functional aspects (WHO, 2019b; Budzak & Brankovic, 2022; NCCIH, 2022). Traditional healing practices used by traditional health practitioners (THPs) play a significant role in the delivery of mental healthcare since receiving formal psychiatric treatment in low and middle-income countries remains a challenge for approximately 76-85% of individuals with serious mental illnesses (Nortje et al., 2016; WHO, 2021). The common factors that contribute to the inaccessibility to formal psychiatric healthcare and to the widespread use of traditional medicine in developing countries include, cost-effectiveness, dissatisfaction with biomedical, healthcare and the scarcity of mental healthcare professionals (Mpinga et al., 2013). Particularly, it has been reported in South Africa that there are approximately 1.5 psychiatrists and 2.5 psychologists per 100 000 individuals (WHO, 2019a; Padmanabhanunni et al., 2022). Traditional medicine then becomes the next available option due to its emphasis on holistic healthcare and conformity with spiritual and cultural beliefs and practices (Helha & Wang, 2022; Mpinga et al., 2013).

South Africa is a diverse country with its largest proportion constituted by 81.4% of the Black African population group which is divided into different tribes which are characterised by distinctive cultural beliefs and practices (StatsSA, 2022; Makhetha et al., 2024). Of the 11 official languages spoken by the different ethnic groups in South Africa, isiZulu (24.4%) and isiXhosa (16.3%) are the most commonly spoken languages with AmaZulu also being the largest of the ethnic groups (StatsSA, 2022). IsiZulu is dominant in the province of KwaZulu-Natal (KZN) and in some parts of Mpumalanga, Gauteng and Free State provinces, and isiXhosa is dominant in the Eastern Cape Province and the southwestern part of KZN (StatsSA, 2022). The effectiveness of traditional healing, as reported by its patients, is influenced by language as the THPs speak the languages native to their patients; thus, patients are able to accurately describe their symptoms to them compared to biomedical doctors (Ngubane & De Gama, 2023; Van der Watt et al., 2018). In many African communities, God (*uMvelinqangi* in Nguni languages) and the ancestors (*amadlozi* and *iinyanya* in isiZulu and isiXhosa, respectively) are the center of healing and are closely connected, with the

ancestral spirits perceived as mediators between God and the living (Mokgobi, 2014; Zuma et al., 2016). The ancestral spirits are also reported to provide guidance to the THPs in diagnosing and treating different ailments, including mental illnesses (Zuma et al., 2016).

Culture is a significant factor that influences how various communities, particularly African communities, explain and perceive mental illness (Kleinman, 1977; Monama & Basson, 2017). Cultural background including values and belief systems, also play a pivotal role in the diagnosis and treatment of mental illnesses in the traditional healing community. Additionally, supernatural causal explanations which include bewitchment or witchcraft, sorcery, evil spirits and an ancestral calling (a gift or a calling to become a THP) are believed to be the cause of mental illness, particularly psychosis, in this community in Africa (Zabow, 2007; Al-Krenawi, 2019; van der Zeijst et al., 2021a, 2021b, 2022). However, in some instances, the pathological potential of the ancestral calling in mental illness may be argued as cultural beliefs vary between different communities (van der Zeijst et al., 2021a, 2021b). Nonetheless, psychotic and mood-related symptoms have been reported to be common in individuals with an ancestral calling (van der Zeijst et al., 2022). Usually the spirits of the ancestors, particularly if they have been neglected or are not remembered and properly treated, can become spirit agents that cause mental illness, which is commonly associated with "bad spirits" that could originate from rocks, lakes and rivers (Verginer & Juen, 2019). Additionally, in the traditional healing context, schizophrenic symptoms are described as *ufufunyana/amafufunyana* which is also defined as a culture-bound syndrome and is further used in explaining "aberrant behavioral and psychological phenomena" (Molot, 2017; Volkan, 2021). *Ufufunyana* is reported to be caused by witchcraft leading to evil spirit possession (Molot, 2017). This results in different views on the cause of mental illness between biomedical and traditional healing. There also exists the notion in biomedical healing that the cause of mental illness is biologically-oriented while African traditional healing emphasizes supernatural or religious-magical views (Teferra & Shibre, 2012).

In biomedical healing, mental illness is defined as a health problem that affects the feelings, thoughts and behaviour of an individual which may result in the person's quality of life being reduced (Ngobe et al., 2021). According to the World Health Organisation (2022), mental disorders are depicted by the disturbance of behaviour, cognition and emotional regulation of an individual. Numerous factors that contribute to mental health problems in South Africa include use substances, unemployment, poverty, diseases such as HIV/AIDS, political violence and transgenerational trauma as a result of European colonialisation (Madzhie et al., 2014; Gone & Kirmayer, 2020; Shange & Ross, 2022). The Diagnostic and Statistical Manual of Mental Disorders (DSM-5) manual is used in biomedical healthcare to diagnose mental illness based on the defining symptoms of each disorder. Also, treatment options are dependent on the type of illness and include; medications (such as antipsychotic drugs), psychotherapy, brain-stimulation and substance misuse treatments (Mayo clinic, n.d.). Traditional healing differs from biomedical healing as there is emphasis on the consultation with ancestors and the prescription of a medicinal concoction as common methods used by THPs for the diagnosis and treatment or management of mental illness (Shange & Ross, 2022; Sorsdahl et al., 2010). In cases of an ancestral

calling, *ukuthwasa* (training to become a THP) may be the recommended form of treatment intervention (van der Zeijst et al., 2021a, 2021b).

This paper aimed to qualitatively obtain insights from the THPs' perspective into how culture influences the explanation of mental disorders; viz. their cause, methods of diagnoses and treatment approaches used in the traditional healing practice. It also aimed to identify the common symptoms observed by the THPs in mentally ill patients and document the system used by the THPs to manage their patients. Further, this study had a specific focus on schizophrenia as symptoms of this disorder are said to be caused by a calling to become a THP (Sorsdahl et al., 2010) and are associated with conditions involving ancestors and/or other spirits and sorcery or bewitchment in the African healing setting.

Material and Methods

Research Design

The larger study included quantitative (Ngubane & De Gama, 2023) and qualitative components; however, the current article focuses only on the qualitative dimension of the research. With the use of focus group discussions, the phenomenology theoretical perspective was adopted in order to investigate the dynamics of the collective beliefs and perspectives of the THPs on mental illness. With this theory, data was analysed by allowing meanings to emanate from the in-depth semi-structured group interviews that were conducted. The thematic analysis method was adopted to extract and synthesize key information among the THPs using codes and themes.

Recruitment

This study was conducted in the Greater Kokstad (GK), uMzimkhulu (Mz), uBuhlebezwe (Bu) and Dr. Nkosazana Dlamini Zuma municipalities (NDZ) which are local municipalities of the Harry Gwala District Municipality (HGDM) located southwest of the KwaZulu-Natal Province (Fig. 1).

There were four focus groups as per the local municipalities with participants ranging from 5-10 per group. A group of 8 THPs were interviewed in Greater Kokstad Municipality, 8 THPs in uMzimkhulu Municipality, 10 in uBuhlebezwe Municipality and 5 were interviewed in Dr. Nkosazana Dlamini Zuma Municipality. All focus group discussions were conducted in February 2022. Purposive Sampling was used as the participants were selected from the THPs who had participated in the quantitative component of the research project and had indicated possession of knowledge on methods of diagnosis and treatment of mental disorders. In the previous study by Ngubane and De Gama (2023), 100 questionnaires were administered to randomly selected THPs from the HGDM as part of the quantitative investigation which aimed to add on the knowledge of the socio-demographic profiles of the THPs in this district, their knowledge and methods of diagnosis and treatment of mental disorders.



Fig. 1 Local Municipalities of the Harry Gwala district municipality (Adapted from https://municipalities.co.za)

The Participants

A total of 31 THPs (71% female; 29% males) participated in this study. The age range of the participants was between 30 and 67 years with a mean age of 47 years. A total of 52% THPs classified as a diviner (*Isangoma* in IsiZulu), 6% as an herbalist (*Inyanga* in IsiZulu), 6% as a faith healer (*Umthandazi* in IsiZulu) and 35% classified as a combination of these classifications. The years of experience of the THPs in the traditional healing practice ranged between 3 and 33 years with a mean range of 12 years (Table 1). The sample consisted of THPs from the Zulu and Xhosa ethnic groups of South Africa, who communicated in both IsiZulu and IsiXhosa languages. The THPs who participated in this study were registered under the Harry Gwala District Traditional Health Practitioners organization.

Focus Groups

The focus group discussions were guided by semi-structured questions from a self-developed interview sheet on the cause of mental illness, symptoms and the traditional methods of diagnosis and treatment of the different kinds of mental disorders or illnesses (Appendix). Each session was conducted between 40 and 90 min. A pre-test of the interview schedule was conducted with two THPs (which were excluded from the study) prior to its use in the actual focus group discussions in order to ensure reliability and validity of the questions. The group discussions, were co-facilitated by both authors and each participant was provided with an equal chance to contribute to the discussion. THPs participating in the group discussions were not asked or pressured to confirm contributions from one THP. All sessions were recorded and were supplemented by handwritten notes.

Table 1 Demographics of the participants as per focus group discussions in each local municipality

Local municipality	Participant No.	Age (years)	Gender	Classification	Experience (years)
Greater Kokstad	1	40	Female	Combination	17
	2	54	Female	Combination	15
	3	43	Female	Combination	4
	4	47	Female	Isangoma	10
	5	45	Female	Combination	20
	6	34	Female	Combination	8
	7	47	Female	Combination	12
	8	30	Male	Isangoma	7
Buhlebezwe	1	57	Female	Isangoma	14
	2	40	Male	Combination	18
	3	53	Female	Isangoma	10
	4	42	Female	Isangoma	7
	5	36	Female	Isangoma	18
	6	49	Female	Isangoma	8
	7	62	Male	Combination	33
	8	47	Male	Umthandazi	7
	9	64	Female	Umthandazi	24
	10	30	Female	Isangoma	4
uMzimkhulu	1	51	Female	Isangoma	16
	2	30	Female	Isangoma	4
	3	38	Male	Isangoma	7
	4	56	Female	Isangoma	8
	5	59	Female	Isangoma	13
	6	46	Female	Combination	10
	7	58	Male	Inyanga	16
	8	63	Female	Combination	3
Dr. Nkosazana Zuma	1	32	Male	Isangoma	14
	2	54	Female	Isangoma	4
	3	59	Female	Combination	29
	4	67	Male	Inyanga	5
	5	31	Male	Isangoma	9

Inclusion and Exclusion Criteria

Registered THPs in the Harry Gwala District Municipality who were invited to participate in this study based on their responses from the questionnaire administered prior to commencement of the qualitative component of the study were included. THPs who had not participated in the quantitative component of the study were excluded from the focus group discussions.

Data Analysis

All focus group sessions were recorded and later transcribed verbatim in the language that they were conducted in. Key information from the transcriptions was translated into English by both authors independently in order to ensure consistency and accuracy. The six phases of thematic analysis as described by Braun and Clarke (2006) were used in order to identify meanings and patterns in the accounts of the participants. The authors independently coded each transcript manually. The patterns and trends which emerged from the data were then classified into themes, and areas of inconsistency and disagreement were discussed by both authors until consensus on the appropriate coding was reached. Both authors also reviewed and refined these classifications for proper definition of the themes. The refined themes which emerged are presented in the results section. The narratives of the participants are anonymously reported.

Ethical Consideration

Ethical approval for this study was obtained from the Human Social Sciences Research Ethics Committee of the University of KwaZulu-Natal (HSS-REC/00002944/2021) prior to data collection. All participants were fully informed about the aim and significance of the study prior to its commencement and an informed consent form was signed prior to participation.

Results

Of the 31 THPs that participated in this study, the majority were from the uBuhlebezwe Municipality [32%; n = 10]. The majority of the THPs were females [71%; n = 22], and 52% (n = 16) of the 31 THPs classified as *Isangoma* (Table 1). The age range of the participants was 30–67 years and their years of experience ranged from 3 to 33 years (Table 1).

The findings are presented as per the key themes of this study; viz. (1) cause of mental illness, (2) traditional methods of diagnosis, (3) identification of common symptoms and treatment methods of mental illness among patients, and (4) the system of patient management. The sub-themes that emanated under the cause of mental illness included witchcraft and ancestral calling. Divination through consultation with the ancestors, family background, burning of incense and examining the patient were the sub-themes that emerged under the methods of diagnosis. Aggression, hallucination, inability to speak and unresponsiveness emanated under the common symptoms observed and the sub-themes that emerged under the methods of treatment included the use of medicinal preparations, guidance from ancestors on treatment approach to use and performing of cultural rituals. Record keeping and referral to biomedical healthcare facilities were the sub-themes that emerged under the system of patient management. A total of 31 THPs participated in the focus group

discussions and individual responses were voluntarily offered with no pressure for other THPs to confirm this narrative. The comments offered by the THPs in each key theme are quoted below. Narratives from the THPs have been reported as n = x, and the following abbreviations were used for responses from each local municipality: Greater Kokstad (GK), uMzimkhulu (Mz), uBuhlebezwe (Bu) and Dr. Nkosazana Dlamini Zuma (NDZ) to offer information of how widely a specific response was mentioned. The total number of responses received for each narrative was presented as n^{total}.

Cause of Mental Illness

The prevalent causes of mental illness, particularly *ufufunyana* (which may be interpreted as schizophrenia and/or the possession by evil spirits), across all four local municipalities included an ancestral calling (or issues relating to ancestors) [n^{total} = 14: n = 4 (GK), n = 5 (Mz), n = 3 (Bu), n = 2 (NDZ)], witchcraft (which in some cases included bad spirits, i.e. *isilwane* in IsiZulu) [n^{total} = 13: n = 5 (GK), n = 3 (Mz), n = 5 (Bu)] and the use of substances [n^{total} = 7: n = 4 (GK), n = 2 (Bu), n = 1 (NDZ)]. In one case [n = 1 (GK)] *ufufunyana* was not classified as a mental disorder but the cause of mental illness.

> My patients were suffering from ufufunyana. The first one (of the aforementioned patients) ubehlushwa abantu abadala (had an ancestral calling), this started when they were young and it was ignored in their family until the ancestors took away their sanity. (P6, Greater Kokstad Municipality)
> Most of the patients I have treated had ufufunyana which was inflicted. The cause of mental illness in 2 of the patients was isilwane sihamba nalo ufufunyana (witchcraft inflicted ufufunyana). (P2, Greater Kokstad Municipality)
> Of the 4 patients that I have treated, drugs were the cause of mental illness in 2. (P1, Greater Kokstad Municipality)
> A person becomes mentally ill because of imimoya yokuthi uthakathiwe (bad spirits inflicted by witchcraft/bewitchment), or they are just crazy and some mental illness has to do with ancestors. In some cases, a person can have ancestors fighting for them, maybe that person is using a surname that is not theirs and the ancestors from their true surname those from the current surname end up fighting over them. (P3, uBuhlebezwe Municipality)

Other causes that were reported were confusion [n^{total} = 3: n = 1 (GK), n = 1 (Bu), n = 1 (NDZ)] and heredity [n = 1 (NDZ)].

> In some cases, it is just confusion, in some it is heredity and in some it is related to ancestors. (P2, Dr. Nkosazana Dlamini Zuma Municipality)

Defaulting from HIV treatment [n = 4 (Mz)] and stress [n^{total} = 4: n = 2 (Mz), n = 2 (NDZ)] were also reported as causes of mental illness.

> I have diagnosed ufufunyana caused by not accepting the spiritual gifts that your ancestors give to you. In other patients, mental illness is caused by too much stress which then leads to depression. Others default from their HIV treatment

and then say they have an ancestral calling when that is not the case. (P3, uMzimkhulu Municipality)
I have diagnosed ufufunyana and as I was questioning the patient's parents I discovered that the patient defaulted from their HIV treatment. It happens that when you default from HIV treatment you become mentally ill. Sometimes mental illness is due to umsamo ongalunganga (when the sacred place or alter where there is interconnectedness with the ancestors is not in a good state), sometimes ufufunyana is due to izinto zabantu (bewitchment) and ancestors. (P5, uMzimkhulu Municipality)

Traditional Methods of Diagnosis

The common method of diagnosis across all 4 local municipalities was divination through consultation with the ancestors [n^{total} = 10: n = 3 (GK), n = 1 (Mz), n = 3 (Bu), n = 3 (NDZ)].

You first consult with the ancestors and that is where you will get the answers and how you need to help your patient. (P3, Greater Kokstad Municipality)
I consult with ancestors as the patient comes in and they advise on what is wrong with the patient and how I should treat them. (P2, uMzimkhulu Municipality)

Seeking information from the family members or from the individuals accompanying the patient [n^{total} = 3: n = 1 (GK), n = 2 (Mz)] was also reported as a method of diagnosis.

You first find out from the people who bring in the patient what is wrong with the patient. You then consult with the ancestors and they will give you the diagnosis. (P2, Greater Kokstad Municipality)

Other methods used in uBuhlebezwe included burning of incense (*impepho* in IsiZulu) [n = 1 (Bu)].

The first thing we do as THPs when a patient is brought in is burn incense and then consult with the ancestors. (P2, uBuhlebezwe Municipality)

A method that can only be done by an experienced THP was deducing and confirming the diagnosis from just examining the patient [n = 1(NDZ)]. This examination was not elaborated further by the THP.

There are ways to examine the patient to better understand the diagnosis. (P1, Dr. Nkosazana Dlamini Zuma Municipality – 14 years of experience)

Identification of Common Symptoms and Treatment Methods of Mental Illness Among Patients

Common Symptoms

Aggression [n^{total} = 11: n = 5 (GK), n = 4 (Mz), n = 2 (Bu)], hallucination [n^{total} = 8: n = 4 (GK), n = 1 (Mz), n = 3 (NDZ)], inability to speak [n^{total} = 5:

n = 1 (GK), n = 3 (Bu), n = 1 (NDZ)], being incoherent [n^{total} = 4: n = 3 (Bu), n = 1 (NDZ)] and unresponsiveness [n = 2 (Mz)] were commonly observed in patients with mental illness. Other symptoms included not eating [n^{total} = 3: n = 1 (Mz), n = 2 (Bu)], inability to walk [n = 1(GK)] and lack of sleep [n = 1 (Mz)].

> One who had ufufunyana was chasing and killing chickens and also said they were seeing some people. Another one who had ufufunyana saw people who were chasing them. (P3, Greater Kokstad Municipality)
> One child could not walk and speak due to the illness. (P4, Greater Kokstad Municipality)
> The patient was tied up and in pain. They were said to be aggressive, they tore their clothes, destroyed everything in the house, they were not eating or sleeping. (P5, uMzimkhulu Municipality)

Other symptoms identified included hysterical screams [n^{total} = 6: n = 1 (GK), n = 1 (Mz), n = 3 (Bu), n = 1 (NDZ)], seizures [n^{total} = 6: n = 4 (GK), n = 2 (Bu)], being fearful [n = 2 (Bu)] and forgetfulness [n = 1 (Bu)].

> In some cases, it can happen that a person speaks things that do not make sense and they are forgetful. Also, it can happen that when a patient has bad spirits they will have fear and this may cause them to scream. (P2, uBuhlebezwe Municipality)
> A person with ufufunyana may just scream for no reason and does not talk a lot. And then there is a person who is just crazy and talks a lot, even things that do not make sense. (P1, Dr. Nkosazana Dlamini Zuma Municipality)

Treatment Methods

Mental illnesses were reported to be commonly treated using *umuthi* or a medicinal concoction [n^{total} = 16: n = 3 (GK), n = 5 (Mz), n = 5 (Bu), n = 3 (NDZ)]. The predominant modes of administration were inhalation [n^{total} = 7: n = 2 (GK), n = 4 (Bu), n = 1 (NDZ)], drinking [n^{total} = 7: n = 3 (GK), n = 2 (Mz), n = 2 (Bu)], putting or squeezing into the nose [n^{total} = 4: n = 1 (GK), n = 1 (Mz), n = 2 (Bu)], *ukuchatha* (enema) [n^{total} = 7: n = 1 (GK), n = 5 (Bu), n = 1 (NDZ)], *ukuphalazisa* (induced vomiting) [n^{total} = 4: n = 1 (Mz), n = 2 (Bu), n = 1 (NDZ)] and steaming [n^{total} = 3: n = 2 (Bu), n = 1 (NDZ)].

> When it comes to ufufunyana we use traditional medicines which we get from the forest. (P2, Greater Kokstad Municipality)
> Medicinal concoctions are used to treat mental illness and these are administered through inhalation, ukuphalaza (induced vomiting) and enema. (P1, uBuhlebezwe Municipality)
> Usually for mentally ill patients, medication is administered through inhalation to try and calm the patient, steaming, induced vomiting and enema are also used. (P5, Dr. Nkosazana Dlamini Zuma Municipality)

Cleansing the patient was reported to be the first step to treatment in some cases [$n^{total} = 3$: n = 1 (GK), n = 2(Bu)] and a patient could be treated by performing their required cultural rituals in other cases [$n^{total} = 4$: n = 2 (GK), n = 2 (Bu)].

> You first need to cleanse the patient before you can start with treatment. (P1, Greater Kokstad Municipality)
> When the illness has to do with ancestors, appropriate rituals must be done. (P6, Greater Kokstad Municipality)

Guidance from the ancestors on the best and most appropriate method of treatment was required by some of the THPs [$n^{total} = 6$: n = 2 (GK), n = 3 (Bu), n = 1 (NDZ)] prior to treating their patients.

> Usually we are guided by the ancestors we work for. But usually you have to give the patient with mental illness something for inhalation to ease the illness. (P5, Dr. Nkosazana Dlamini Zuma Municipality)

In some cases [n = 3 (Mz)], the treatment method was dependent on the symptoms presented by the patients.

> As there are different symptoms, the modes of treatment are also different. In some cases, a mentally ill person does not require to be treated with pills but with traditional medicines (ubulawu) when the illness has to do with ancestors. A person with umdlenyane (form of ufufunyana) is treated using a medicinal concoction. (P7, uMzimkhulu Municipality)

The System of Patient Management

Keeping patient records [$n^{total} = 15$: n = 5 (GK), n = 7 (Mz), n = 2 (Bu), n = 1 (NDZ)] and conducting follow-ups [$n^{total} = 6$: n = 2 (GK), n = 2 (Mz), n = 1 (Bu), n = 1 (NDZ)] was reported across all 4 local municipalities. Records included patient's personal details and history which included information on prior illnesses and treatment, which also included the history of any cultural practice or rituals that have been conducted before to or for the patient.

> It is important to keep records of your patients and to have their medical history. (P1, Greater Kokstad Municipality)
> We also need to know of traditional rituals that have been performed for or to the patient. (P6, Greater Kokstad Municipality)
> We do have books where we keep all records of our patients (their names, surnames, contact details and addresses). We also keep medical and treatment history of the patients. We also do follow-up on our patients and find out how the treatment process is going. (P2, uMzimkhulu Municipality)
> It is important that when a patient comes to you, their name, surname and contact details are recorded. (P5, Dr. Nkosazana Dlamini Zuma Municipality)

Record keeping was reported to ensure ease of tracking the patient's illness and medical history or records also assisted in providing clues for diagnosing and treating the current presented illness.

> We do have books where we keep all records of our patients. We also keep medical and treatment history of the patients. We also do follow-up on our patients and find out how the treatment process is going. (P1, uMzimkhulu Municipality)

Record keeping was stated to be mandatory as per regulations by the Department of Health.

> According to the regulations from the department of health, we have to have registers where we record details of patients that come to see us for each day. (P1, uBuhlebezwe Municipality)

THPs also reflected that they referred patients to biomedical institutions [$n^{total} = 9$: n = 2 (GK), n = 5 (Mz), n = 1 (Bu), n = 1 (NDZ)]. The common reason for referring patients to biomedical institutions across the 4 local municipalities was when traditional medicine approaches were ineffective and the THPs were unable to assist the patient. In Kokstad, pre-existing medical conditions such as diabetes and HIV were reported to be some of the illnesses which required patients to also use allopathic medicine prior to receiving traditional medicines.

> A patient with diabetes cannot stop taking their treatment when they are being treated with traditional medicines, same goes for a person with HIV. So, these ailments have to be attended to with the aid of western medicine prior to treatment with traditional medicines because umuthi is strong. (P2, Greater Kokstad Municipality)

THPs maintained that their treatment approaches were strong and required that their patients be in good health prior to receiving treatment from them; hence, it was preferable that patients first conduct a medical checkup and if they default from their western medication or if the THPs cannot treat them due to various reasons they are referred to biomedical facilities.

> When a person defaults from their HIV treatment you need to send or refer them to a clinic where they will advise what needs to be done. We also refer a person with ufufunyana to the clinic, especially if they do not eat, they need drips for energy because we cannot use umuthi on a weak person. Sometimes we take our patients to clinics to have them checked for underlying illnesses before we can use umuthi on them. (P1, uMzimkhulu Municipality)
>
> If I cannot treat the person I refer them to the clinic where they get treated. (P2, uMzimkhulu Municipality)
>
> I once had a mentally ill patient who would have seizures that were unstoppable and no matter what I did or gave to him I just could not help. So, what helped was rushing him to the nearest hospital or clinic. (P2, uBuhlebezwe Municipality)

> In this day and age there are ailments that we as THPs cannot treat. So even if we try to help a patient whichever way possible, there also has to be an intervention from western medicine for the patient to be treated. (P5, Dr. Nkosazana Dlamini Zuma Municipality).

Discussion

The psychiatric fraternity has been faced by culture-bound syndromes since past centuries, and these conditions are perceived to not fit accurately to the standard psychiatric classifications even though they are also said to be similar to psychiatric disorders in biomedical psychiatry (Wessels, 1985). One of these culture-bound syndromes is *ufufunyana/amafufunyana* which is defined as a possession by evil spirits through sorcery, with its sufferers being described as having hysterical psychoses (Wessel, 1985; Zabow, 2007; Molot, 2017). In IsiZulu/IsiXhosa, schizophrenia may be referred to as *amafufunyana* due to the alignment of symptoms between the two conditions, resulting in a complexity as they possess both a supernatural causation and biomedical-defined explanations (Molot, 2017). Discrepancies exist in the description of symptoms observed in patients diagnosed with *ufufunyana*; as its causation is usually reported differently depending on how respondents from different studies understand this condition (Parle, 2003). In the current study, *ufufunyana* was used by the THPs as a translation for schizophrenia or schizophrenic symptoms which is influenced by the specific cultural beliefs on ancestral calling and witchcraft thus affirming *ufufunyana* as a culture bound syndrome. However, *ufufunyana* was also indicated as one of the causes of mental illness in this study, agreeing with Fabrega (1991) in reporting u*fufunyana* to cause psychiatric illness. Zabow (2007) and Molot (2017) further attested to this in their definition of *ufufunyana* as a possession by evil spirits and the possession by these spirits which resulted in mental disturbance.

Culture-bound syndromes are described as a broad rubric that encompasses certain behavioral, affective and cognitive manifestations seen in specific cultures (Balhara, 2011). Although Balhara (2011) advocates for the relabeling of some of the culture-bound syndromes to functional somatic syndromes, the authors still adopt that the interpretation of *ufufunyana* as a culture-bound syndrome is accurate as per the findings of the current study. Additionally, even though there is an overlap of symptoms between *ufufunyana* and schizophrenia, due to the completely different views between African healing and biomedical healthcare on the causation of the two conditions, *ufufunyana* cannot be fully interpreted as a psychiatric condition such as schizophrenia. With the varying causal factors between these two conditions, the methods of diagnosis and treatment are inevitably unique in each fraternity, with all methods in African healing encompassed by cultural beliefs. For this reason, it seems more accurate for *ufufunyana* to be framed as a culture-bound syndrome since the manifestations associated with this condition are specific to the Zulu and Xhosa cultures.

The prevalent causes of mental illness in this study included the use of substances, witchcraft and an ancestral calling. The possession by evil or bad spirits,

bewitchment or witchcraft, having an ancestral calling and use of substances as the cause of mental illness were also reported by other authors from Ethiopia and Uganda in Africa (Teferra & Shibre, 2012; Verginer & Juen, 2019) and from Mpumalanga, Gauteng and KwaZulu-Natal provinces in South Africa (Kometsi et al., 2020; Ngobe et al., 2021; Shange & Ross, 2022; Sorsdahl et al., 2010). Other causes in this study were stress, trauma, confusion, heredity and defaulting from HIV treatment, of which stress and trauma were consistent with reports by Teferra and Shibre (2012) and Kometsi et al. (2020). Mental illness inherited from parents by children was also reported by Teferra and Shibre (2012) and Shange and Ross (2022) corroborating perspectives from the NDZ municipality in this study. The involvement of HIV in the etiology of mental illness was also reported by Kometsi et al. (2020); however, it was the positive status of this illness that led to depression and schizophrenia in their study, though defaulting from HIV treatment causing mental illness was not included.

The prevalent methods of diagnosis included divination through consultation with ancestors (spiritual intervention), seeking information from the family members or the individuals accompanying the patient, burning of incense and examining the patient which were consistent with the prevalent methods of diagnosis reported by Kpobi et al. (2019) and Shange and Ross (2022), but consultation with ancestors was accompanied by throwing of bones in the study by Shange and Ross (2022). Burning of incense (*impepho*) can be used in the communication with ancestors and can be also used to calm patients or the evil spirits possessing the patients (Belani et al., 2022; Ntshangase, 2012). Some of the THPs in the study by Sorsdahl et al. (2010) also maintained that consulting with ancestors aided in the diagnosis of mental illness. Aggression, hallucination, unresponsiveness and also being incoherent were prevalent symptoms in mentally ill patients in this study. Other symptoms included the inability to walk, inability to talk, visual hallucinations, lack of sleep, not eating, stress, calling out names of people unseen by others, auditory hallucinations, being fearful, screams, seizures, forgetfulness and alogia. Hallucinations and delusions were also reported by Ngobe et al. (2021). Hallucinations were reported by van der Zeijst et al. (2020) as the prevalent symptom of the psychotic symptoms among apprentice THPs, with depressive symptoms prevalent under mood-related symptoms. In some cases, the evil spirits could switch the presented symptoms in a patient in order to confuse the healer and keep them from understanding the cause of the illness, this then delayed the treatment process (Verginer & Juen, 2019).

The prevalent method for treatment of mental illness in this study was the use of a medicinal concoction which is prepared using herbs or herbal plants. The details on the medicinal plants used in the concoction were not shared as this knowledge was regarded as sacred by the THPs since it was gifted to them by their ancestors, which is consistent with the findings by Sorsdahl et al. (2010). The THPs recommended that the authors visit the *muthi* market (which may also refer to the Zulu pharmacy) in order to obtain more information on the medicinal plants used for mental illness. The commonly used modes of administration of these herbal remedies were inhalation, drinking, steaming, enema (*ukuchatha*) and induced vomiting (*ukuphalazisa*).

Giving patients a medicinal concoction for drinking and bathing was also reported by Sorsdahl et al. (2010). The THPs in this study maintained that cleansing the patient prior to treatment is significant and that sometimes a patient can be treated from their illness when cultural rituals have been kept for them. When considering conditions associated to an ancestral calling and other ancestral related issues, the THPs maintained that a patient can only be treated once they have accepted their calling and once all cultural rituals have been done for them. This was consistent with reports by Ngobe et al. (2021). It was also reported in this study that the treatment method is dependent on the symptoms presented by the patients and that guidance from the ancestors also plays a significant role towards determining how patients should be treated. Western medicine, particularly the pharmacological interventions only manage the symptoms of mental illness and do not attend to the root cause of the illness (Verginer & Juen, 2019), which is one of the factors contributing to the dissatisfaction with biomedical medicine. The THPs in this study maintained that the duration of treatment was dependent on the improvement of the symptoms presented by the patient and mainly on the guidance from the ancestors, hence the treatment process could last for days, months or years depending on what is communicated by the ancestors to the THP about the patient. The THPs reported that they follow-up on the patient's progress if the patient is not staying with them for the duration of the treatment process and the records of each patient are maintained as per the regulations by the Department of Health. If the THPs fail to treat the patient using all their traditional strategies, they refer them to biomedical facilities such as clinics and hospitals thereafter.

Based on the findings from this study, there is some possibility of a fully functional collaboration between the African healing and biomedical medicine in psychiatry. Aspiration for this is drawn from the apparent collaboration noted when the THPs refer their patients to biomedical facilities for pre-existing illnesses such as diabetes and HIV prior to the commencement of their treatment methods. However, a question remains, if the THPs acknowledge the role of biomedical medicine for the management of other ailments, why is this not the case for mental illness? Perhaps the education and training of THPs regarding mental disorders, their diagnosis and treatment methods used in Western psychiatry could pave a way for a collaboration in psychiatry. For example, in a pilot study by Mbeh et al. (2010), THPs were trained to provide education and appropriate healthcare to diabetic patients in Cameroon. The findings of their study suggested that through this initiative, the THPs were enthusiastic about a collaboration with biomedical healthcare for the management of diabetes. This current study also noted that in other cases the THPs only refer their patients for biomedical healthcare after their treatment attempts have failed. Considering that the duration of their treatment is not clear, this might increase the risk of the illness worsening before the patient receives biomedical healthcare. Thus, further questions arise as to whether the referred patients were correctly diagnosed and treated? Furthermore, concerns also arise on whether the guidance from ancestors regarding the methods of diagnosis and treatment could be misleading in some instances.

Conclusion, limitations and recommendations

It is evident from the study that the traditional healing practice is significantly influenced by ancestral spirits or the belief in these spirits. Ancestors and cultural beliefs also influence the beliefs regarding the etiology of mental illness and the methods used by the THPs to diagnose and treat mental illness. In order to further explore the extent of the influence of culture and ancestors in mental illness, it is recommended that future studies should also explore the perspectives of the patients and their caregivers. Further engagement with biomedical practitioners on their views of traditional healing practices for mental illness should be considered to offer a holistic understanding of healing within the mental health space in South Africa. Apart from referrals, it was evident that there was a working relationship or collaboration between traditional and biomedical medicine treatment approaches. This was validated by the THPs when they recommend patients to seek a medical check-up at the biomedical facilities prior to seeking traditional treatment. In addition, they also refer patients who have other ailments or pre-existing co-morbidities to biomedical facilities. However, this form of collaboration seemed one-sided as it was the THPs who referred their patients to biomedical facilities and no reports of referred patients from biomedical healthcare providers to the traditional healing practice were documented.

The major limitation of this study was the inability to obtain extensive details on the methods of diagnosis and treatment used by the THPs as almost all aspects of these were associated with the guidance from the ancestors and in other instances some information was described as sensitive and sacred, thus could not be shared with the investigators. It then becomes a challenge to attempt to find common ground between psychiatry between traditional healing and biomedical medicine. Perhaps the symptoms identified by the THPs could be a starting point in attempting to bridge the gap in the knowledge, diagnostic and management approaches between traditional and biomedical medicines. Although in most cases the causal factors for mental illness in African healing are linked with supernatural explanations, perhaps if the two healthcare entities allow themselves to learn from each other, a collaboration would be possible without one entity undermining the other based on the existing discrepancies.

Appendix: The Role of Indigenous Healers and the Effect of Indigenous Medicine in the Treatment of the Negative Symptoms of Schizophrenia in KwaZulu Natal

DATE: _____ **TIME:** _____ **PARTICIPANT NO:** _____

VENUE: _____

QUESTIONNAIRE PARTICIPANT NO: _____

Interview Questions

History of Practice in Mental Health Disorders

1. (a) Since the start of your practice, how many patients have you diagnosed with mental health disorders?
   (b) How often do you diagnose individuals suffering from mental disorders?
   (c) From your experience, what is the cause of these mental health disorders?
   (d) How many patients are presently being treated?

   i. What is the common age group?
   ii. What is the more common gender profile of these patients?
   iii. Do you verify if the patient has accessed treatment before?
Yes ☐ No ☐
   iv. What kind of treatment do patients usually access? (Western or Traditional)
   v. Do you keep a record of the treatment the patient has been exposed to previously?
Yes ☐ No ☐
   vi. What other patient history do you require to be shared with you?

Nature of disorders

2. What were the common symptoms noted?
3. Methods of diagnosis:

(a) Is there a way you categorize mental health disorders based on the symptoms?
Yes ☐ No ☐
(b) If so, what are the different categories? (Identify by name)

Indigenous Treatment:

(a) What is the proposed treatment for the different mental health disorders?
(b) If certain herbs are used, what are they?
(c) Where are they obtained?
(d) How are they used?
(e) What are they intended to do to make the patient better?
(f) Typically, how long is the patient on treatment with herbs, etc.?

4. (a) How do you determine when a patient has been bewitched or has a calling?
   (b) Are there different treatment approaches for these? If so, identify these modalities of treatment.

5. (a) Do you follow up on patients? If so, how often?

(b) What are the outcomes?

Treatment for Schizophrenia

6. (a) Are you familiar with the mental health disorder termed schizophrenia? If yes, how do you diagnose it and what is the management plan?
   (b) When a patient has the following symptoms, what would your diagnosis be?

i. Delusions
ii. Conceptual disorganization
iii. Hallucinations
iv. Grandiosity
v. Suspiciousness or persecution
vi. Hostility

7. When a patient has the following symptoms, what would your diagnosis be?

i. Asociality
ii. Lack of drive and motivation outlined by apathy or avolition
iii. Lack of enjoyment and social interactions
iv. Anticipatory anhedonia and lack of facial expression
v. Gesturing and speech output such as alogia or aprosody and blunting of affect

8. How do you treat or manage each symptom (negative symptoms of schizophrenia) mentioned in (c)?
9. If there is a medicinal concoction that is used to treat the symptoms mentioned in (c), what is the dosage and how is it measured?
10. What is the cost per treatment?

7. (a) Have you end up referred institutional care for a patient? If so how often?
   (b) What is your opinion on western medicine in managing mental health disorders?
   (c) Do you think traditional and western medicine can be used together? If so how?
   (d) How would you like to see traditional medicine used in communities more effectively?

General

Any further comments?

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Declarations

Conflict of interest The authors do not have any conflict of interest to disclose.

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References

Al-Krenawi, A. (2019). The impact of cultural beliefs on mental health diagnosis and treatment. In M. Zangeneh & A. Al-Krenawi (Eds.), *Culture, diversity and mental health—Enhancing clinical practice. Advances in mental health and addiction.* Cham: Springer.

Balhara, Y. P. S. (2011). Culture-bound syndrome: Has it found its right niche? *Indian Journal of Psychological Medicine, 33*(2), 210–215. https://doi.org/10.4103/0253-7176.92055

Belani, A., Oladimeji, K. E., Hyera, F. L., Matubatuba, C., & Juzang, I. J. (2022). Can the use of impepho leaves extend beyond traditional to modern medicine? An opinion. *Africa Journal of Nursing and Midwifery, 24*(2), 1–4. https://doi.org/10.25159/2520-5293/11872

Braun, V., & Clarke, V. (2006). Using thematic analysis in psychology. *Qualitative Research in Psychology, 3*(2), 77–101. https://doi.org/10.1191/1478088706qp063oa

Budzak, A., & Brankovic, M. (2022). Alternative ways to mental health: Exploring psychological determinants of preference for CAM treatments. *Studia Psychologica, 64*(1), 118–135. https://doi.org/10.31577/sp.2022.01.843

Fabrega, H., Jr. (1991). Psychiatric stigma in non-Western societies. *Comprehensive Psychiatry, 32*(6), 534–551. https://doi.org/10.1016/0010-440X(91)90033-9

Gone, J. P., & Kirmayer, L. J. (2020). Advancing indigenous mental health research: Ethical, conceptual and methodological challenges. *Transcultural Psychiatry, 57*(2), 235–249. https://doi.org/10.1177/1363461520923151

Helha, F. N., & Wang, Y. P. (2022). Trends in complementary and alternative medicine for the treatment of common mental disorders: A bibliometric analysis of two decades. *Complement Therapies in Clinical Practice, 46*(101531), 1–9. https://doi.org/10.1016/j.ctcp.2021.101531

Kleinman, A. (1977). Depression, somatisation and the new 'crosscultural psychiatry. *Social Science and Medicine, 11*, 269–276.

Kometsi, M. J., Mkhize, N. J., & Pillay, A. L. (2020). Mental health literacy: Conceptions of mental illness among African residents of Sisonke District in KwaZulu-Natal, South Africa. *South African Journal of Psychology, 50*(3), 347–358. https://doi.org/10.1177/0081246319891635

Kpobi, L. N. A., Swartz, L., & Omenyo, C. N. (2019). Traditional herbalists' methods of treating mental disorders in Ghana. *Transcultural Psychiatry, 56*(1), 250–266. https://doi.org/10.1177/1363461518802981

KwaZulu Natal Municipalities. https://municipalities.co.za/provinces/view/4/kwazulu-natal. Retrieved August 18, 2022

Madzhie, M., Mashamba, T. M., & Takalani, F. J. (2014). African traditional healers' perception and diagnosis of mental illness. *African Journal for Physical Health Education Recreation and Dance, 1*(2), 319–328.

Makhetha, M., Chabilal, N., & Aldous, C. (2024). Recent trends in hereditary breast cancer incidence by race and age in KwaZulu-Natal, South Africa: An 11-year single-centre retrospective study (2011–2021). *International Journal of Cancer, 154*(1), 65–70. https://doi.org/10.1002/ijc.34687

Mayo Clinic. (n.d.). Mental Illness. https://www.mayoclinic.org/diseases-conditions/mental-illness/diagnosis-treatment/drc-20374974#:~:text=Your%20treatment%20team&text=Physician%20assistant,psychologist%20or%20a%20licensed%20counselor. Retrieved January 19, 2023

Mbeh, G. N., Edwards, R., Ngufor, G., Assah, F., Fezeu, L., & Mbanya, J. C. (2010). Traditional healers and diabetes: Results from a pilot project to train traditional healers to provide health education and appropriate health care practices for diabetes patients in Cameroon. *Global Health Promotion, 17*(2), 17–26. https://doi.org/10.1177/1757975910363925

Mokgobi, M. G. (2014). Understanding traditional African healing. *African Journal for Physical Health Education, Recreation and Dance, 20*(2), 24–34.

Molot, M. (2017). Discourses of psychiatry and culture: The interface between western and traditional medicine in the treatment of mental illness. Independent Study Project (ISP) Collection. 2582. https://digitalcollections.sit.edu/isp_collection/2582

Monama, D. D., & Basson, W. J. (2017). Looking in the mirror: The cultural experiences of patients diagnosed with schizophrenia. *PULA: Botswana Journal of African Studies, 31*(2), 46–58.

Mpinga, E. K., Kandolo, T., Verloo, H., Bukonda, N. K., Kandala, N. B., & Chastonay, P. (2013). Traditional/alternative medicines and the right to health: Key elements for a convention on global health. *Health Human Rights, 15*(1), 44–57.

National Institutes of Health, National Center for Complementary and Integrative Health (NCCIH), complementary, alternative, or integrative health: What's in a name? https://nccih.nih.gov/. Retrieved August 19, 2022

Ngobe, A., Semenya, S., & Sodi, T. (2021). Psychological ailments and their treatment protocols: A case study of Swati traditional healers in Mpumalanga Province, South Africa. *African Health Sciences, 21*(2), 884–895. https://doi.org/10.4314/ahs.v21i2.50

Ngubane, N. P., & De Gama, B. Z. (2023). A quantitative evaluation of traditional health practitioners' perspectives on mental disorders in KwaZulu-Natal: Knowledge, diagnosis, and treatment practices. *Journal of Spirituality in Mental Health, 2023*, 1–21. https://doi.org/10.1080/19349637.2023.2194561

Nortje, G., Oladeji, B., Gureje, O., & Seedat, S. (2016). Effectiveness of traditional healers in treating mental disorders: A systematic review. *Lancet Psychiatry, 3*(2), 154–170. https://doi.org/10.1016/S2215-0366(15)00515-5

Ntshangase, M.C. (2012). Some gendered African ritual practices: The case of impepho (an indigenous African plant) (Master's dissertation, University of KwaZulu-Natal)

Padmanabhanunni, A., Jackson, K., Noordien, Z., Pretorius, T. B., & Bouchard, J. P. (2022, April). Characterizing the nature of professional training and practice of psychologists in South Africa. In *Annales Médico-psychologiques, revue psychiatrique* (180(4), 360-365). Elsevier Masson.

Parle, J. (2003). Witchcraft or Madness? The Amandiki of Zululand, 1894–1914. *Journal of Southern African Studies, 29*(1), 105–132. https://doi.org/10.1080/0305707032000060502

Shange, S., & Ross, E. (2022). "The Question Is Not How but Why Things Happen": South African Traditional Healers' Explanatory Model of Mental Illness, Its Diagnosis and Treatment. *Journal of Cross-Cultural Psychology, 53*(5), 503–521. https://doi.org/10.1177/00220221221077361

Sorsdahl, K., Flisher, A. J., Wilson, Z., & Stein, D. J. (2010). Explanatory models of mental disorders and treatment practices among traditional healers in Mpumulanga, South Africa. *African Journal Psychiatry, 13*(4), 284–290. https://doi.org/10.4314/ajpsy.v13i4.61878

Statistics South Africa. (2022). Census 2022 Results. https://census.statssa.gov.za/assets/documents/2022/Census_2022_SG_Presentation_10102023.pdf. Retrieved January 28, 2024

Teferra, S., & Shibre, T. (2012). Perceived causes of severe mental disturbance and preferred interventions by the Borana semi-nomadic population in southern Ethiopia: A qualitative study. *BMC Psychiatry, 12*(79), 1–9.

Van der Watt, A. S. J., van de Water, T., Nortje, G., Oladeji, B. D., Seedat, S., & Gureje, O. (2018). Partnership for Mental Health Development in Sub-Saharan Africa (PaM-D) research team. The perceived effectiveness of traditional and faith healing in the treatment of mental illness: A systematic

review of qualitative studies. *Social Psychiatry and Psychiatric Epidemiology, 53*(6), 555–566. https://doi.org/10.1007/s00127-018-1519-9

van der Zeijst, M. C., Veling, W., Makhathini, E. M., Mtshemla, S., Mbatha, N. D., Shabalala, S. S., Susser, I., Burns, J. K., Susser, E., & Hoek, H. W. (2021a). Psychopathology among apprentice traditional health practitioners: A quantitative study from rural KwaZulu-Natal. *South Africa. Transcultural Psychiatry, 58*(4), 486–498.

van der Zeijst, M. C., Veling, W., Makhathini, E. M., Mbatha, N. D., Shabalala, S. S., van Hoeken, D., Susser, E., Burns, J. K., & Hoek, H. W. (2022). Course of psychotic experiences and disorders among apprentice traditional health practitioners in rural South Africa: 3-Year follow-up study. *Frontiers in Psychiatry, 13*(956003), 1–17. https://doi.org/10.3389/fpsyt.2022.956003

van der Zeijst, M., Veling, W., Makhathini, E. M., Susser, E., Burns, J. K., Hoek, H. W., & Susser, I. (2021b). Ancestral calling, traditional health practitioner training and mental illness: An ethnographic study from rural KwaZulu-Natal. *South Africa. Transcultural Psychiatry, 58*(4), 471–485.

Verginer, L., & Juen, B. H. (2019). Spiritual Explanatory Models of Mental Illness in West Nile. *Uganda. Journal of Cross-Cultural Psychology, 50*(2), 233–253. https://doi.org/10.1177/0022022118813652

Volkan, K. (2021). Schizophrenia, culture, and culture-bound syndromes. *Psychology Research and Applications, 3*(1), 1–13. https://doi.org/10.22606/pra.2021.31001

Wessels, W. H. (1985). Understanding culture-specific syndromes in South Africa-the Western dilemma. *Modern Medicine, 10*(9), 51–63.

World Health Organization. (2019a). Global Health Observatory Data Repository. Human resources data by country: Psychiatrists working in mental health sector per100,000 population. http://apps.who.int/gho/data/node.main.MHHR?lang=en. Retrieved July 11, 2022

World Health Organisation. (2019b). *Global report on traditional and complementary medicine*. World Health Organization.

World Health Organisation. (2021). Comprehensive mental health action plan 2013–2030. World Health Organization. Licence: CC BY-NC-SA 3.0 IGO.

World Health Organisation. (2022). Mental disorders [Fact Sheet]. https://www.who.int/news-room/fact-sheets/detail/mental-disorders#:~:text=A%20mental%20disorder%20is%20characterized,different%20types%20of%20mental%20disorders. Retrieved January 19, 2023

Zabow, T. (2007). Traditional healers and mental health in South Africa. *International Psychiatry, 4*(4), 81–83.

Zuma, T., Wight, D., Rochat, T., & Moshabela, M. (2016). The role of traditional health practitioners in Rural KwaZulu-Natal, South Africa: generic or mode specific?. *BMC complementary and alternative medicine, 16*, 1–13.

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Prologue 3

The administration of a medicinal concoction was indicated as the common mode of treatment in the previous chapter. Seven medicinal plants (viz. *Dioscorea dregeana*, *Boophone disticha*, *Afroaster hispida*, *Salix mucronata*, *Artabotrys monteiroae Oliv.*, *Trimeria grandifolia* and *Clivia miniata*) were offered by the THPs at the Berea and Dalton markets (in Durban, KwaZulu-Natal) for the treatment of schizophrenia and other mental disorders. The phytochemical compounds and the cytotoxic effect of these plants are investigated in the following chapter, Chapter 4. In addition, the findings from this chapter are document in Manuscript 4 which was prepared in the style for the journal it was submitted to.

Manuscript Title	**:** Phytochemical Screening and Cytotoxic Effect of Herbal Plants Used by Traditional Health Practitioners in KwaZulu-Natal for the Treatment of Schizophrenia
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CHAPTER 4

MANUSCRIPT[4]

Title : Phytochemical Screening and Cytotoxic Effects of Herbal Plants Used by Traditional Health Practitioners in KwaZulu-Natal for the Treatment of Schizophrenia

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ABTRACT

Introduction: Treatment of schizophrenia remains a challenge due to non-adherence and limited accessibility to antipsychotic medication. This is aggravated by costly medication, limited access to mental healthcare services, adverse side effects associated with medication and preference for alternative treatments. This prompts the exploration of the benefits of using traditional medicine (TM) as an alternative treatment method. TM involves holistic intervention by managing both physical and mental ill-health through various ways that include the use of herbal plants. However, limited literature exists on the phytochemical constitution and cytotoxicity of these herbal plants. Hence, this study evaluated the phytochemistry and cytotoxicity of seven herbal plants (viz. *D. dregeana*, *B. disticha*, *A. hispida*, *S. mucronata*, *A. monteiroae Oliv.*, *T. grandifolia* and *C. miniata*) used by traditional health practitioners (THPs) in KwaZulu-Natal for treatment of schizophrenia. **Methods:** Gas Chromatography Mass Spectrometry (GC-MS) analysis was used to identify bioactive compounds in these herbal plants, while cytotoxic activity was investigated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) Assay in cultured liver cell lines. **Results:** The plant extracts yielded phytochemicals ranging from 20 to 75 in number, which were independent of the extraction solvent. Cell viability test showed no cytotoxicity at 50µg/ml and 100µg/ml for the methanol, ethanol and DCM extractions. Cell proliferation was promoted at 100µg/ml in methanol and DCM extracts, and at 50µg/ml in ethanol extracts. **Discussion/Conclusions:** The overall findings of this study suggest that herbal plants used by THPs for treatment of schizophrenia have numerous bioactive compounds and are safe for use in alternative treatment methods.

Keywords: Schizophrenia; Treatment; Herbal Plants; Phytochemistry; Cytotoxicity; Traditional Medicine

1. INTRODUCTION

Schizophrenia is a neurodegenerative psychiatric disorder which affects approximately 24 million individuals globally and is rated the third leading cause of disability in South Africa (Spedding *et al.,* 2014; WHO, 2022a). It is characterized by positive symptoms (delusions and hallucinations), negative symptoms (asociality, anhedonia and alogia) and cognitive impairment (lack of memory and attention) (APA, 2013; Koster *et al.,* 2014; Deng & Xu, 2017; Correll & Schooler, 2020). Since the pathophysiology of schizophrenia remains unclear; the dopamine and glutamate hypotheses remain commonly supported (Rabiee *et al.,* 2023). Thus, the prescription of dopamine (D2) partial receptor agonists and antagonists is understandable to manage the positive symptoms of this disorder; however, the management of negative and cognitive symptoms remains a challenge despite the development of first- and second-generation antipsychotic drugs (Kahn *et al.,* 2015; Ojagbemi & Gureje, 2020). Currently, antipsychotic and antidepressant drugs, which leave most patients with persistent adverse side effects are the approved and commonly prescribed medications for the management of the negative symptoms (Kantrowitz, 2021). Additionally, in low and middle-income countries, diagnostic, treatment and support resources for individuals affected by mental health illnesses remain limited (WHO, 2022c). The deficit of coherent and effective pharmacological medications for treatment of the negative symptoms of schizophrenia globally also presents a gravely unmet necessity, which leads to the exploration of alternative approaches such as the use of indigenous medical approaches.

Traditional medicines (TM) are recognised as established natural remedies which have contributed to the development of 40% of the approved pharmaceutical products (WHO, 2022b). According to the World Health Organisation (WHO) estimations, approximately 80% of the global population uses TM such as herbal remedies for the treatment of various ailments including mental illness (WHO, 2022b). Moreover, herbal concoctions were extensively used in the past by ancestors in most African communities for the treatment of mental illness (Okaiyeto & Oguntibeju, 2021). These methods for mental healthcare were dependent on the knowledge, skill and practices based on the experiences, beliefs and theories indigenous to different cultures (Ojagbemi & Gureje, 2020). However, TM and Traditional Health Practitioners (THPs) employ a holistic approach to health and illness with a deep connection between the spirit, mind, and the body, which makes their treatment approaches effective; with

patients rating themselves as cured post-treatment (Kapur 1975; Kleinman & Sung, 1979; Incayawar, 2008; Lucana & Elfers, 2020). However, contention exists on the magical and mystical explanations related to this type of healing (Green & Makhubu, 1984; Freeman & Motsei, 1992). In addition, the safety of the TM remains controversial as knowledge on the chemical components of the plants used in herbal medication is lacking. However, previous studies reported that the use of these concoctions may lead to organ damage and sometimes even death (Okaiyeto & Oguntibeju, 2021). Therefore, investigating the phytochemical constitution and cytotoxic activity of the herbal plants used in traditional concoctions for the treatment of different illnesses is significant. Thus, the aim of this study was to screen the phytochemistry and cytotoxicity of seven medicinal plants that may be used individually or in one concoction by THPs in KwaZulu-Natal for the treatment of mental illnesses, particularly schizophrenia, in cultured liver cell lines.

2. MATERIALS AND METHODS

2.1. Plant Samples

The investigators sourced seven herbal plants from the Berea and Dalton Muthi Markets, KwaZulu Natal, South Africa which were recommended by different THPs for the treatment of schizophrenia. The resident THPs from the *muthi* market offered only the particular parts of the seven plants that they commonly prescribe for the treatment of schizophrenia (Table 1), and these were used in this study.

Table 1: Herbal plants used for the treatment of schizophrenia

Plant Collected	Part used	*Muthi* Market	Voucher no.
udakwa (*Dioscorea dregeana (Kunth) T. Durand*),	Tuber	Dalton	NU0092561
incotho (*Boophone disticha*)	Bulb	Dalton	NU0092562
udlutshana (*Afroaster hispida (Aster bakerianus)*)	Roots	Dalton	NU0092563
umyezane (*Salix mucronata (Thunb)*)	Bark	Dalton	NU0092564
umazwenda (*Artabotrys monteiroae Oliv.*)	Roots	Berea	NU0092565
ilukuluku (*Trimeria grandifolia (Hochst) Warb. subsp. grandifolia*)	Roots	Berea	NU0092566
umayime (*Clivia miniata (Lindl.)*)	Roots	Berea	NU0092567

2.2. Plants Sample Preparation

Each collected sample was washed twice with distilled water and cut into small pieces. Thereafter, the sample was left to dry at room temperature under shade or away from direct sunlight. Once dry, the small pieces of each sample were powdered through using a laboratory blender and stored in containers for future use. These containers were stored at room temperature and away from direct sunlight.

2.3. Plants Sample Extraction

The method of orbital shaker assisted maceration was used. The blended plant material for each sample was prepared using the following solvents; methanol (MeOH), ethanol (EtOH) and Dichloromethane (DCM). The plant samples (10g) were suspended in 100ml of solvent (1:10/w/v) and shaken in an orbital shaker for 24h at 250rpm as per the method protocol by Kaur *et al.* (2020). The mixtures were filtered using no.1 filter papers. Solvents were evaporated with the aid of a heating water bath as per their boiling points until 20% was left in the crude extracts. The crude extracts were then dried under a stream of cold air and stored in a -20°C freezer for future use thereafter.

2.4. Phytochemistry

Gas chromatography-mass spectrometry (GC-MS) analysis (base on its availability) was used to determine the phytochemical compounds of the medicinal plants. The screening of the plant extracts was done using the GCMS-QP2010 Ultra with a column oven temperature of 50°C and hold time of 2 minutes. The samples were injected in a split mode (split ration of 100) with injection temperature at 280 °C. The pressure of the carrier gas was set at 40.6 kPa with a total flow of 93.9 mL/min, column flow of 0.90 mL/min, purge flow of 3.0 mL/min and linear velocity of 35.6 cm/sec. The compounds were then identified by comparing their spectra to those of the National Institute Standard and Technology (NIST) 11 library.

2.5. Cell culture

Cell lines were selected to avoid the use of live animals for toxicological testing and liver cell lines were used due to their similarity in genotypic and phenotypic characteristics with normal

liver cells (Jain *et al.,* 2018). Liver (Chang) cell lines were purchased from Merck Life Science ((Pty) Ltd. South Africa). A well-established cell culture protocol by Czifra *et al.* (2006) was used. Briefly, Eagle's minimum essential medium (EMEM) for culturing liver cell lines was supplemented with 10% fetal calf serum (FCS), pen/strep (1%) and L-glutamine (1%). Frozen liver cell lines were reconstituted in respective media and transferred into 25cm^3 flasks which were incubated at 37 ˚C in the presence of CO_2 (5%) in a humidified (89%) incubator. The cells were then allowed to grow, attach and become confluent. The attached confluent cells were trypsinized with trypsin (1 mL) after being washed three times with phosphate buffer saline solution (PBS). The cells were then sub-cultured in new flasks and incubated. Thereafter, the cell lines were plated in 96 well plates for experiments.

2.6. Cell viability

Cell viability was conducted on liver cell lines to examine the cytotoxic effects of the seven herbal plants (7 MeOH, 7 EtOH and 7 DCM extracts). Cell viability was assessed using the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cells were trypsinised and seeded into 96-well plates at a seeding density of 1.8 x 10^4 cells/well and incubated for 24 h to permit attachment and growth of cells to semi-confluency. Thereafter, the medium (0.5 mL) was replaced and plant extract doses (50 and 100 mmol/L) were freshly prepared in DMSO (0.1%) and added to the wells. The cells were then incubated at 37ºC for 24hrs and 48hrs, respectively. After each incubation period, the medium was removed and the MTT solution (5 mg/mL in phosphate buffered saline, 200 µL) was added to each of the wells. The cells were then incubated for 4hrs to allow for the formation of blue formazan crystals. The MTT solution was then replaced with DMSO (200 µL/well). Viable cells have active mitochondrial dehydrogenase enzymes, which reduce MTT dye by cleaving to the tetrazolium rings of the pale-yellow MTT to form dark blue formazan crystals (Bernas and Dobrucki, 2002). These crystals are largely impermeable to cell membranes and accumulate within the healthy cells. The number of surviving cells is directly proportional to the level of the purple formazan product (Bernas and Dobrucki, 2002). This colored solution was quantified by measuring the absorbance at 570 nm in a UV-visible spectrophotometer (Thermo Scientific Biomate, Cambridge, UK). The percentage cell viability was then calculated as follows:

$$\text{Cell viability (\%)} = \frac{[\text{A490 treated cells} - \text{background}]}{[A490\ control\ cells - background]} \text{ x } 100.$$

2.7. Statistical Analysis

All data were expressed as mean ± standard error of mean (S.E.M.) and the GraphPad Prism Software (version 7) was used to conduct data analysis. The differences between control and experimental groups were analysed using One-way Analysis of Variance (ANOVA) followed by the Tukey-Kramer post hoc test. Values of $p<0.05$ were considered statistically significant.

3. RESULTS

3.1. Phytochemistry

3.1.1. Methanol Extracts

GC-MS analysis of the methanol extract of *D. dregeana* led to the identification and quantification of a total of 66 compounds, with the major compounds being 9,12-octadecadienoic acid (22.53%), n-hexadecanoic acid (6.13%) and eicosanoic acid (4.66%). The analysis of *B. disticha* led to the identification and quantification of a total of 30 compounds, with the major compounds being 9,12-Octadecadienoic acid (2.41%), apocynin ethanone (1.15%) and phthalic acid, bis(7-methyloctyl) ester (1.09%). The analysis of *A. hispida* led to the identification and quantification of a total of 37 compounds, with the major compound being phthalic acid, bis(7-methyloctyl) ester (2.00%, 1.94%, 1.65% and 1.57%). The analysis of *S. mucronata* yielded a total of 25 compounds, and the major compounds were 1H-cycloprop[e]azulen-4-ol (15.63%), .beta.-sitosterol (15.15%), D:A-friedooleanan-3-ol (8.20%) and octadecanoic acid (5.53%). The analysis of *A. monteiroae Oliv.* yielded a total of 55 compounds, with the major compounds being erucic acid (11.86%), n-hexadecanoic acid (4.79%) and phthalic acid, bis(7-methyloctyl) ester (2.40%). The analysis of *T. grandifolia* yielded a total of 46 compounds, with the major compounds being n-hexadecanoic acid (3.06%), 1-hexacosanol/n-hexacosanol (2.18%) and phthalic acid, bis(7-methyloctyl) ester (1.65%). The analysis of *C. miniata* yielded a total of 45 compounds, with the major compounds being 9,12-octadecadienoic acid (53.69%), n-hexadecanoic acid (5.70%) and 9,12-octadecadienoyl chloride (1.78%). The GC-MS chromatograms of methanol extracts are presented in Figure 1a-g and details on the major bioactive compounds identified from each plant are presented in Table 2.

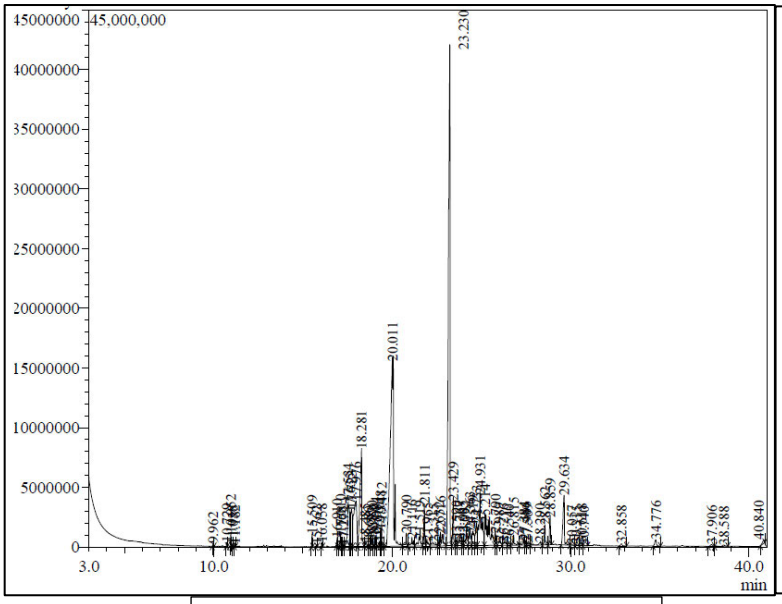


Figure 1a: *D. dregeana*

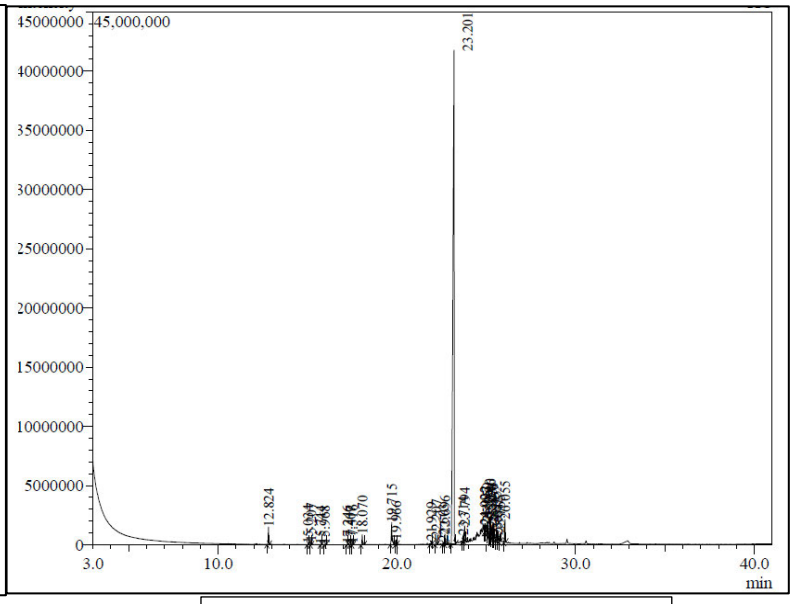


Figure 1b: *B. disticha*

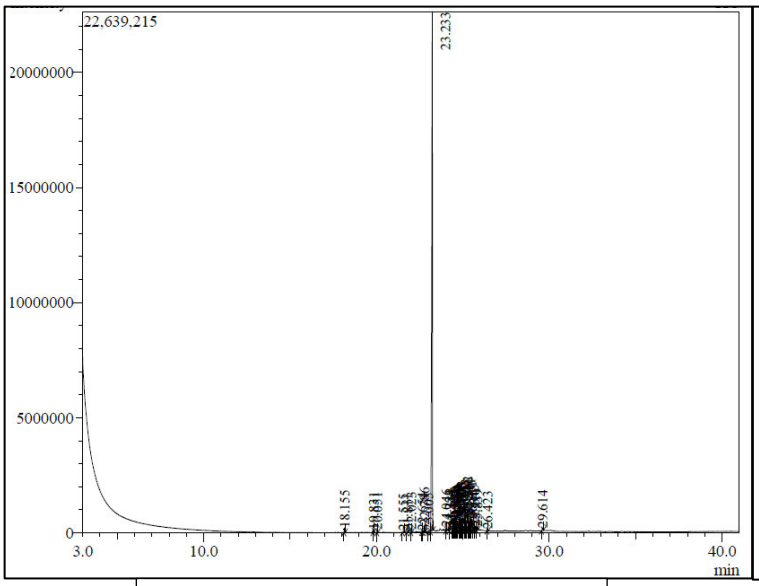


Figure 1c: *A. hispida*

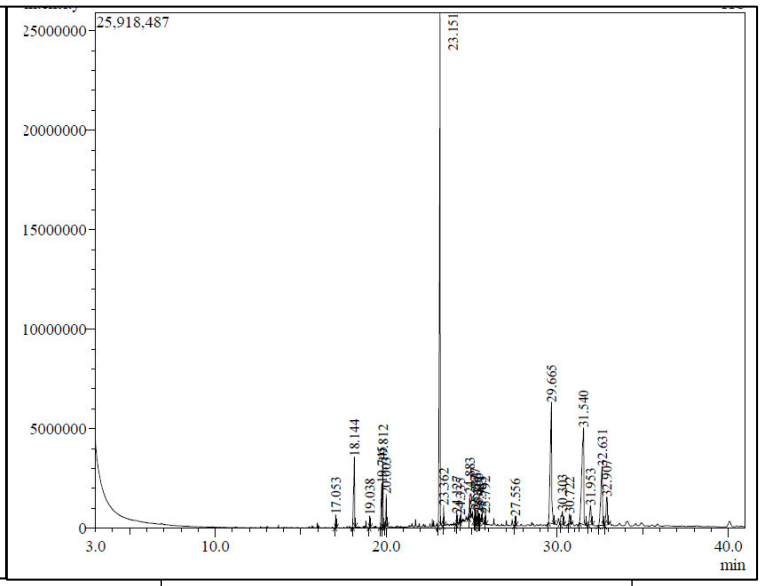


Figure 1d: *S. mucronata*

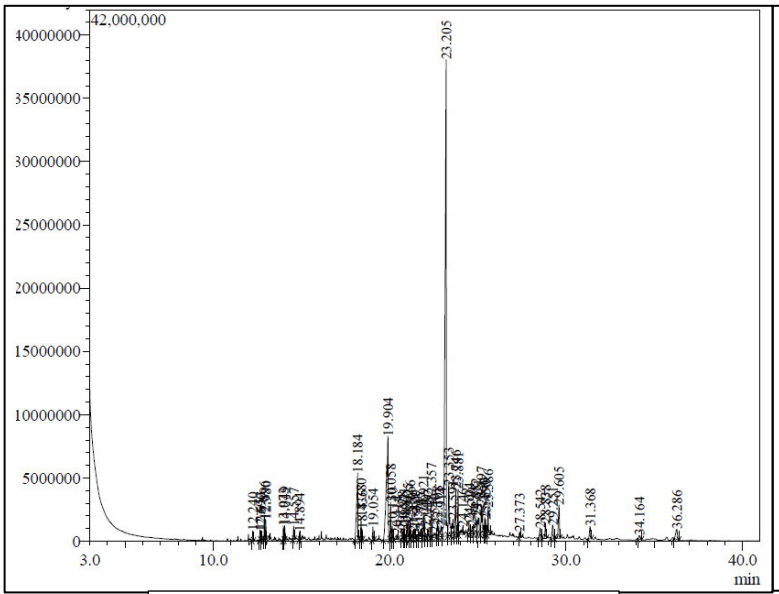


Figure 1e: *A. monteiroae Oliv.*

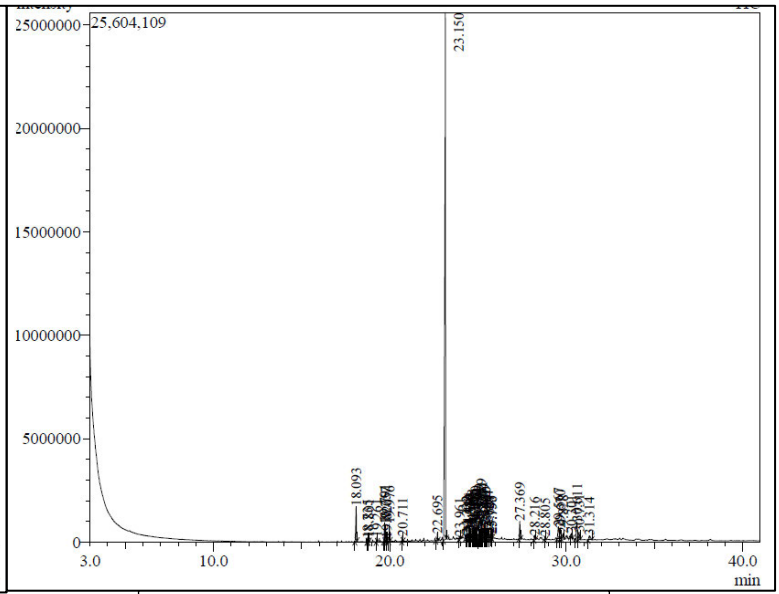


Figure 1f: *T. grandifolia*

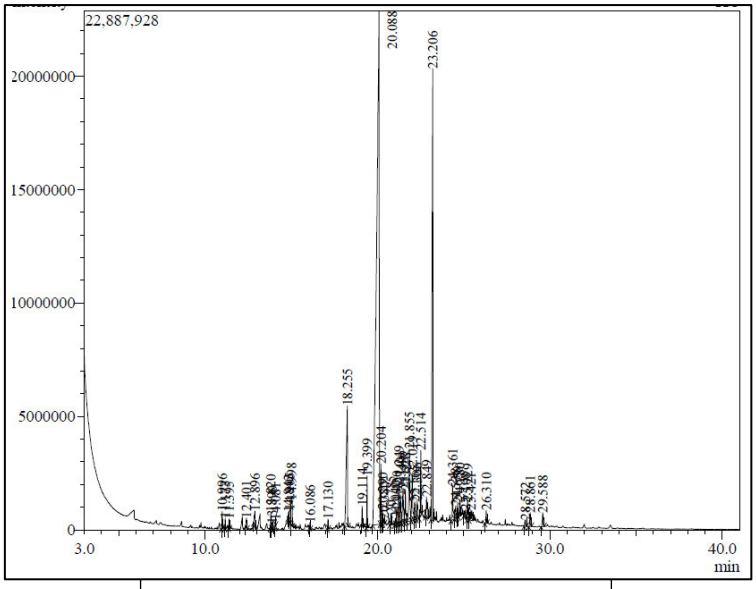
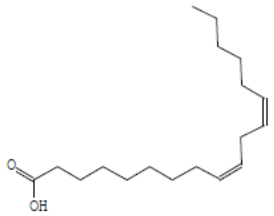
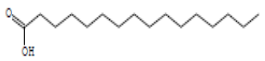
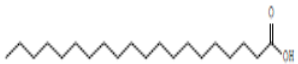
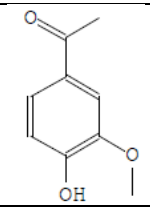
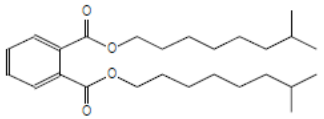
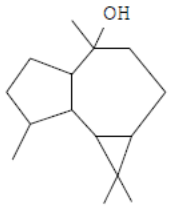
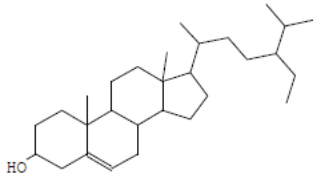
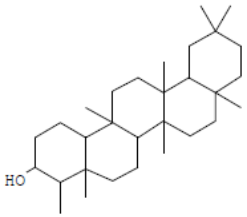
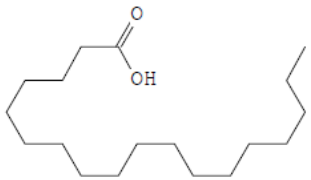


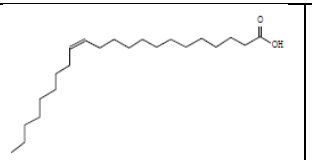
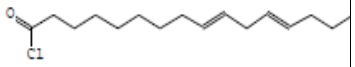
Figure 1g: *C. miniata*

Figure 1a-g: GC-MS chromatograms of methanol extracts of the 7 medicinal plants.

Table 2: Phytochemical compounds identified in methanol extracts

Plant/s (RT-min)	Compound Name/s	Molecular Weight	Formula	Structure	Previously Reported Pharmacological Properties/Activity
D. dregeana (20.11); *B. disticha* (19.715) & *C. miniata* (20.088)	9,12-Octadecadienoic acid (Z,Z)-cis-9,cis-12-Octadecadienoic acid, cis, cis-Linoleic acid, Grape seed oil	280	$C_{18}H_{32}O_2$		Anti-inflammatory, antimicrobial, cancer preventive, hypocholesterolemic, hepatoprotective, anti-arthritic, nematicide, insectifuge(cide), antihistaminic, anti-eczemic, anti-acne, 5-α reductase inhibitor, anti-androgenic & anti-coronary activity (Adeoye-Isijola *et al.,* 2018; Fagbemi *et al.,* 2021)
D. dregeana (18.280); *A. monteiroae Oliv.* (18.184); *T. grandifolia* (18.093) & *C. miniata* (18.255)	n-Hexadecanoic acid, Hexadecanoic acid n-Hexadecoic acid, Palmitic acid, Pentadecane-carboxylic acid	256	$C_{16}H_{32}O_2$		Antibacterial activity (Yff *et al.,* 2002); antinociceptive effect (Shafri, M. & Abdul Manan, 2012; Maroyi, 2018); antioxidant & antiandrogenic effect (Sudha & Padmini, 2023)
D. dregeana (24.930)	Eicosanoic acid, Arachic acid, Arachidic acid, Icosanoic acid, n-Eicosanoic acid, Arachidic acid (synthetic)	312	$C_{20}H_{40}O_2$		Aantiurease, antioxidant & antimicrobial activity (Zekeya *et al.,* 2022)
B. disticha (12.825)	Apocynin Ethanone, 1-(4-hydroxy-3-methoxyphenyl)-Acetophenone, 4'-hydroxy-3'-methoxy-Acetoguaiacon	166	$C_9H_{10}O_3$		Anti-inflammatory effect (Lafeber *et al.,* 1999); used in neurodegeneration, diabetic complications, cardiovascular disorders, pheochromocytoma, in lung, hepatocellular & pancreatic cancer (Salva *et al.,* 2021)

B. disticha (25.380); *A. hispida* (25.070, 25.130, 25.260 & 25.315); *A. monteiroae Oliv.* (25.197) & *T. grandifolia* (24.994)	Phthalic acid, bis(7-methyloctyl) ester, 1,2-Benzenedicarboxylic acid, bis(7-methyloctyl) ester, Bis(7-methyloctyl) phthalate	418	$C_{26}H_{42}O_4$		Antitumour activity (Sivasubramanian & Brindha, 2013)
S. mucronata (31.540)	1H-Cycloprop[e]azulen-4-ol, decahydro-1,1,4,7-tetramethyl-, [1aR-(1a.alpha.,4.beta.,4a.beta.,7.alpha.,7a.beta.,7b.alpha.)]-Viridiflorol	222	$C_{15}H_{26}O$		Anti-mycobacterial, anti-inflammatory & antioxidant activity (Trevizan *et al.,* 2016)
S. mucronata (29.665)	.beta.-Sitosterol, Stigmast-5-en-3-ol, (3.beta.)-Stigmast-5-en-3.beta.-ol, .alpha.-Dihydrofucosterol, .beta.-Sitosterin	414	$C_{29}H_{50}O$		Antioxidant, antimicrobial, angiogenic, immunomodulatory, antidiabetic, anti-inflammatory, anticancer & anti-nociceptive activity (Rashed, 2020); reduces LDL & prostate hyperplasia, also used in skin ointments (Sudha & Padmini, 2023)
S. mucronata (32.630)	D:A-Friedooleanan-3-ol, (3.alpha.)-, D:A-Friedooleanan-3.alpha.-ol, Friedelan-3.alpha.-ol, Friedelanol	428	$C_{30}H_{52}O$		Stomachic, carminative, diuretic, antidiarrhoeal, hypoglycaemic, antibacterial, antidysentric activity (Garg *et al.,* 2013)
S. mucronata (18.145)	Octadecanoic acid, Stearic acid, n-Octadecanoic acid, Humko Industrene R, Hydrofol Acid 150, Hystrene S-97	284	$C_{18}H_{36}O_2$		Antimicrobial & antifungal activity (Maroyi, 2018); treatment of schizophrenia (Sushmitha *et al.,* 2018)

A. monteiroae Oliv. (19.905)	Erucic acid 13-Docosenoic acid, (Z)- .delta.13-cis-Docosenoic acid cis-13-Docosenoic acid (Z)-13-Docosenoic acid	338	$C_{22}H_{42}O_2$		Protective & regulatory effects on nervous system (Gao *et al.,* 2022)
T. grandifolia (29.565)	1-Hexacosanol, n-Hexacosanol, Ceryl alcohol, Cerylic alcohol, Hexacosyl alcohol	382	$C_{26}H_{54}O$		Lowers LDL-Cholesterol (Pratama *et al.,* 2019)
C. miniata (22.020)	9,12-Octadecadienoyl chloride, (Z,Z)-, Linoleoyl chloride, Lineoleoyl chloride, Linoleic acid chloride	298	$C_{18}H_{31}ClO$		Antisecretory, choleretic, contraceptive, antispermatogenic & antitubercular properties (Eleazu, 2016);

3.1.2. Ethanol Extracts

GC-MS analysis of the ethanol extract of *D. dregeana* led to the identification and quantification of a total of 20 compounds which included 3-hydroxy-2,2,4-trimethyl-3-pentenoic acid (21.17%), erucic acid (10.87%), 9,12-octadecadienoic acid (7.35%) and .gamma.-sitosterol (5.99%). The analysis of *B. disticha* yielded a total of 28 compounds which included apocynin (ethanone) (26.05%) and 9,12-octadecadienoic acid (6.19%). The analysis of *A. hispida* yielded 75 compounds which included caryophyllene oxide (21.49%), beta.-sitosterol (18.33%), D:A-friedooleanan-3-ol (8.70%) and 1H-cycloprop[e]azulen-4-ol (4.18%). The analysis of *S. mucronata* led to the identification of a total of 70 compounds which included 1H-cycloprop[e]azulen-4-ol (15.10%), .gamma.-sitosterol (12.41%), n-hexadecanoic acid (10.83%), D:A-friedooleanan-3-ol (6.07%) and erucic acid (5.33%). The analysis of *A. monteiroae Oliv.* yielded a total of 58 compounds which included 9,12-octadecadienoic acid (13.20%), erucic acid (12.14%), cyclohexane (3.49%) and ephedrine (3.49%). The analysis of *T. grandifolia* led to the identification of a total of 51 compounds which included .beta.-amyrin (8.40%), cyclopentanecarboxylic acid (3.93%) and 1,3,5-triazine (3.88%). The analysis of *C. miniata* yielded a total of 26 compounds which included 9,12-octadecadienoic acid (54.27% and 10.51%) and n-hexadecanoic acid (7.11%). The GC-MS chromatograms of ethanol extracts are presented in Figure 2a-g and the major compounds (with high area% and height%) for each plant are documented in Table 3.

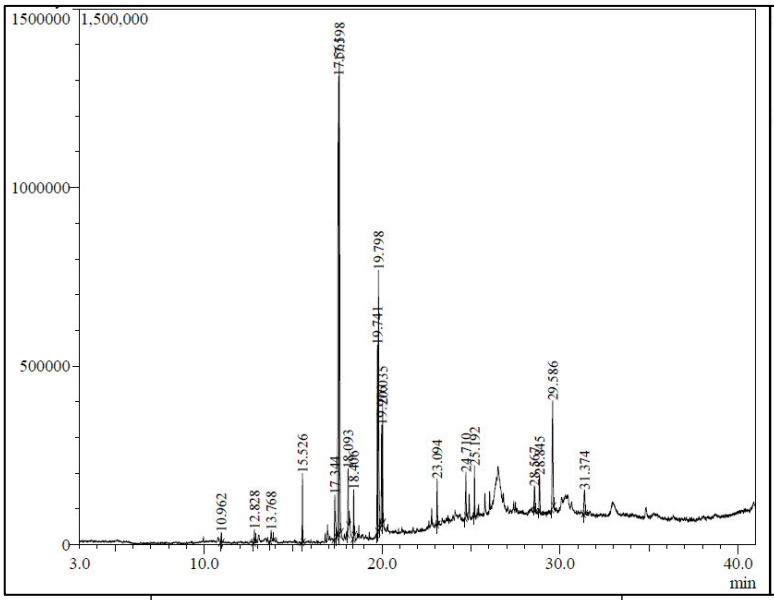


Figure 2a: *D. dregeana*

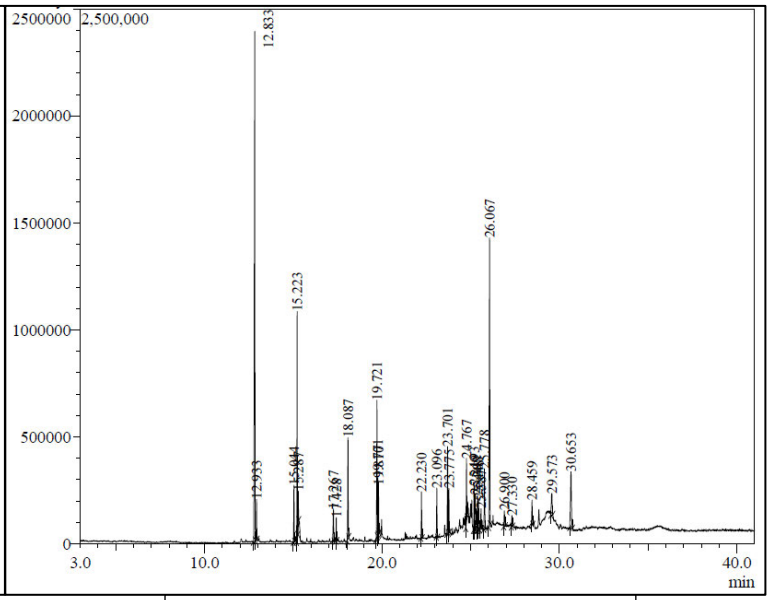


Figure 2b: *B. disticha*

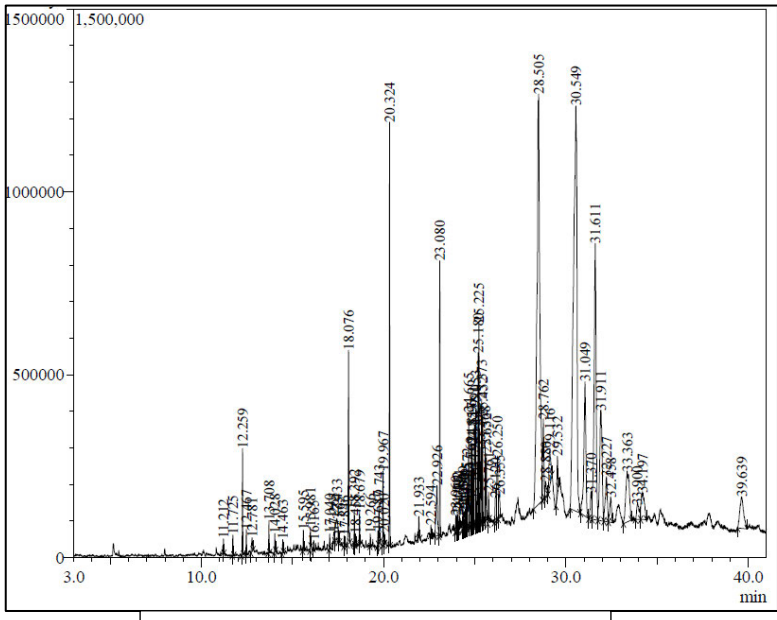


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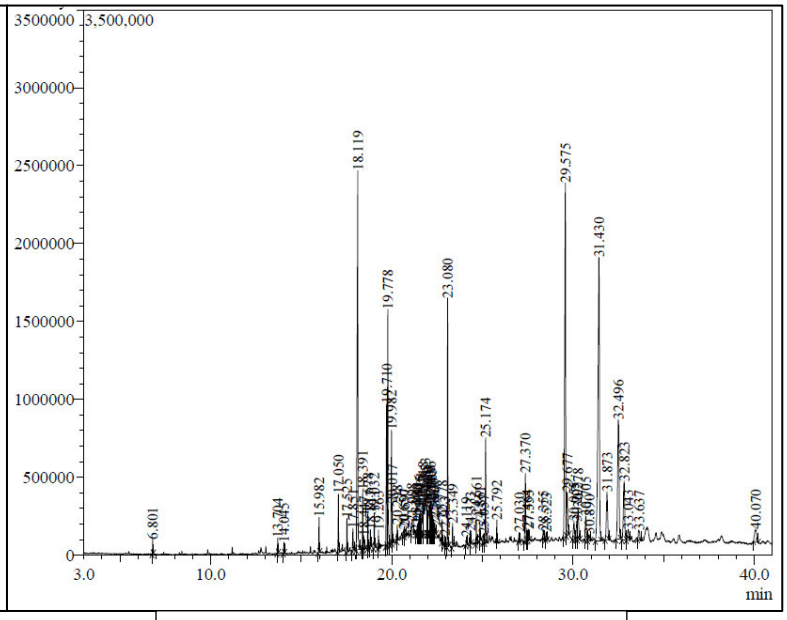


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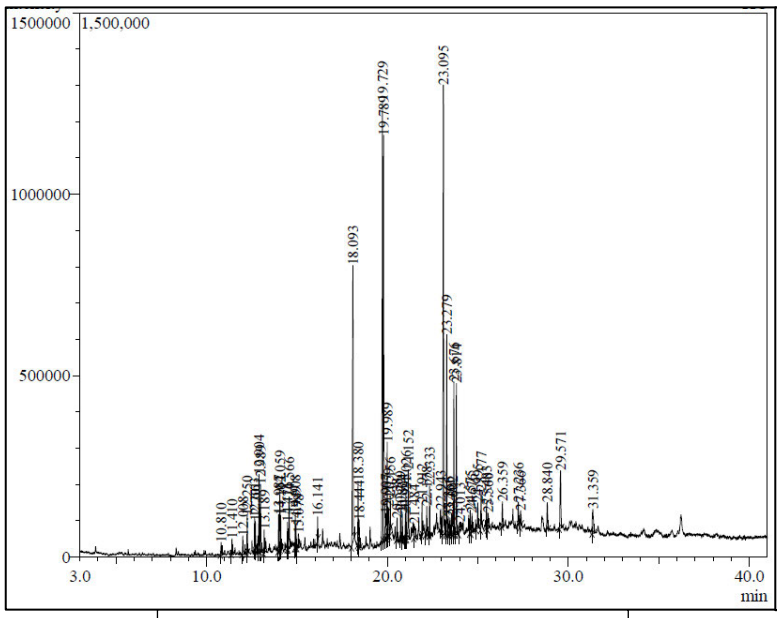


Figure 2e: *A. monteiroae Oliv.*

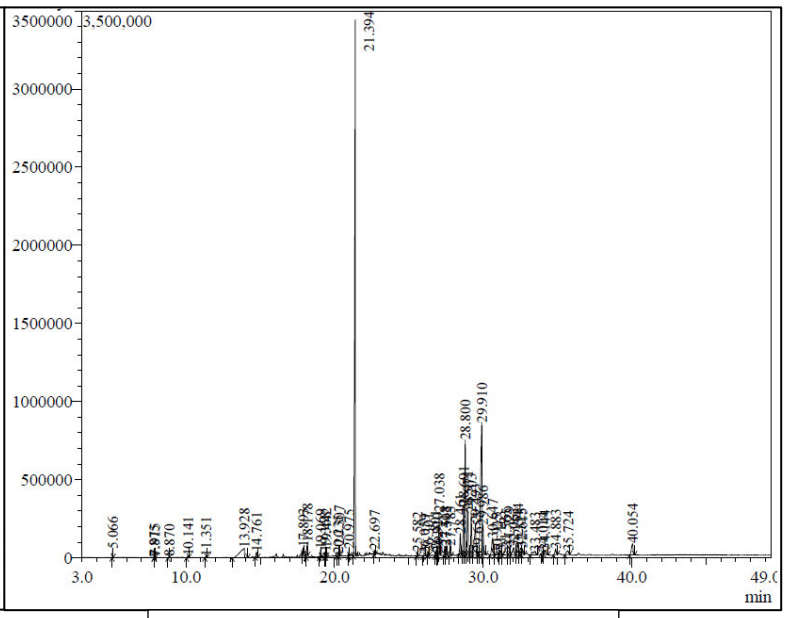


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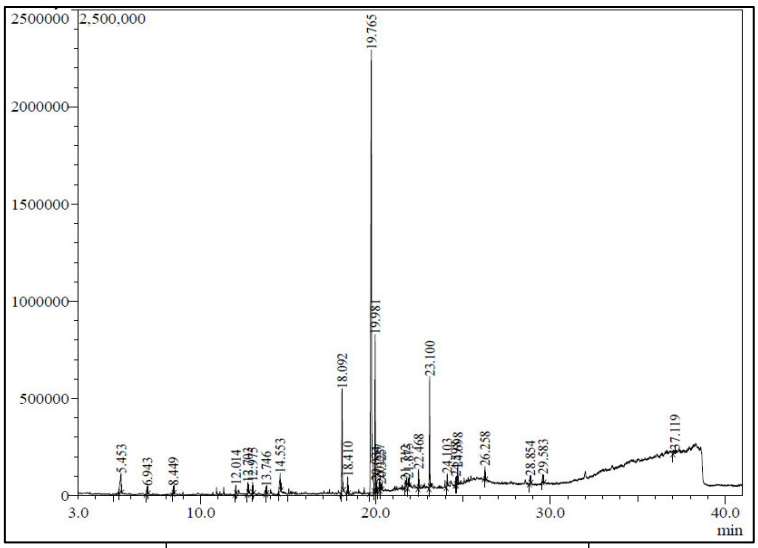
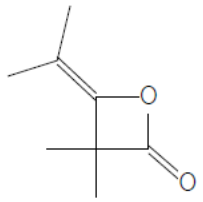
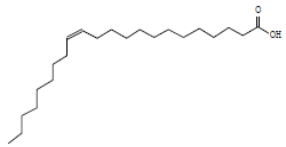
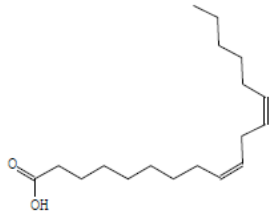
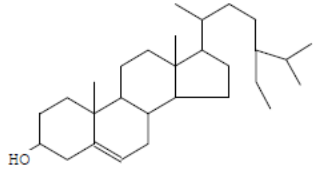
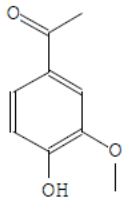
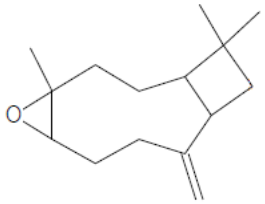
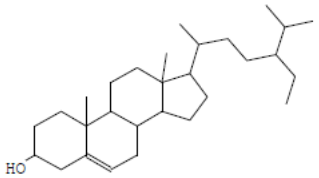
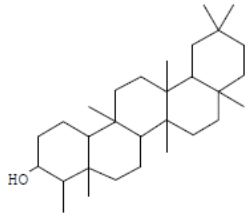
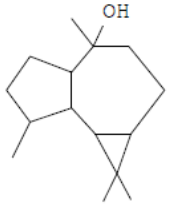


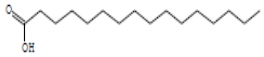
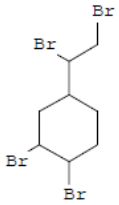
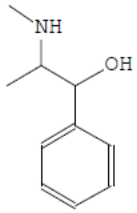
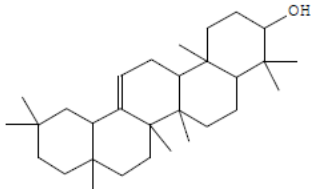
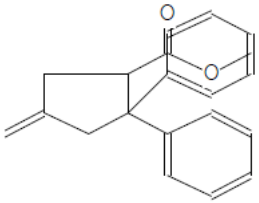
Figure 2g: *C. miniata*

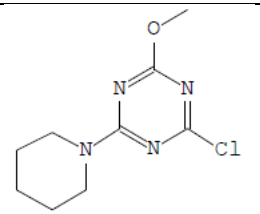
Figure 2a-g: GC-MS chromatograms of ethanol extracts of the 7 medicinal plants

Table 3: Phytochemical compounds identified in ethanol extracts

Plant/s (RT-min)	Compound Name	Molecular Weight	Formula	Structure	Previously Reported Pharmacological Properties/Activity
D. dregeana (17.600)	3-Hydroxy-2,2,4-trimethyl-3-pentenoic acid .beta.-lactone, Dimethylketene-.beta.-lactone dimer	140	$C_8H_{12}O_2$		Inhibit activity of lipases secreted by gastrointestinal tract (Fenteany *et al.,* 1994); enzymatic inhibition (Bai *et al.,* 2014)
D. dregeana (19.800); *S. mucronata* (19.780) & *A. monteiroae Oliv.* (19.790)	Erucic acid 13-Docosenoic acid, (Z)- .delta.13-cis-Docosenoic acid cis-13-Docosenoic acid (Z)-13-Docosenoic acid	338	$C_{22}H_{42}O_2$		Protective & regulatory effects on nervous system (Gao *et al.,* 2022)
D. dregeana (19.741); *B. disticha* (19.721); *A. monteiroae Oliv.* (19.730) & *C. miniata* (19.765 & 19.980)	9,12-Octadecadienoic acid (Z,Z)-cis-9,cis-12-Octadecadienoic acid, cis, cis-Linoleic acid, Grape seed oil	280	$C_{18}H_{32}O_2$		Anti-inflammatory, antimicrobial, cancer preventive, hypocholesterolemic, hepatoprotective, anti-arthritic, nematicide, insectifuge(cide), antihistaminic, anti-eczemic, anti-acne, 5-α reductase inhibitor, anti-androgenic & anti-coronary activity (Adeoye-Isijola *et al.,* 2018; Fagbemi *et al.,* 2021)
D. dregeana (29.585) & *S. mucronata* (29.575)	.gamma.-Sitosterol, Stigmast-5-en-3-ol, (3.beta.,24S)-, Stigmast-5-en-3.beta.-ol, (24S)-, Clionasterol, Fucosterol	414	$C_{29}H_{50}O$		Antidiabetic & anticancerous properties (Bano & Deora, 2019); antiviral action (de Sousa Ferrão & Janeque, 2023); anti-ulcer, antimicrobial, growth hormone of animals & plants, antipyretic & hypolipidemic activity (Sudha & Padmini, 2023)

B. disticha (12.835)	Apocynin, Ethanone, 1-(4-hydroxy-3-methoxyphenyl)-, Acetophenone, 4'-hydroxy-3'-methoxy-, Acetoguaiacon	166	$C_9H_{10}O_3$		Anti-inflammatory effect (Lafeber *et al.,* 1999); used in neurodegeneration, diabetic complications, cardiovascular disorders, pheochromocytoma, in lung, hepatocellular & pancreatic cancer (Salva *et al.,* 2021)
A. hispida (30.550)	Caryophyllene oxide, 5-Oxatricyclo[8.2.0.0(4,6)-]dodecane, 4,12,12-trimethyl-9-methylene-,	220	$C_{15}H_{24}O$		Potential anti-parasitic agent, used for colitis, inhibits multiplication of various cancer cells (Gyrdymova & Rubtsova, 2021)
A. hispida (28.505)	beta.-Sitosterol, Stigmast-5-en-3-ol, (3.beta.)- Stigmast-5-en-3.beta.-ol, .alpha.-Dihydrofucosterol, .beta.-Sitosterin	414	$C_{29}H_{50}O$		Antioxidant, antimicrobial, angiogenic, immunomodulatory, antidiabetic, anti-inflammatory, anticancer & anti-nociceptive activity (Rashed, 2020)
A. hispida (31.610) & *S. mucronata* (32.495)	D:A-Friedooleanan-3-ol, (3.alpha.)-, D:A-Friedooleanan-3.alpha.-ol, Friedelan-3.alpha.-ol, Friedelanol	428	$C_{30}H_{52}O$		Stomachic, carminative, diuretic, antidiarrhoeal, hypoglycaemic, antibacterial, antidysentric activity (Garg *et al.,* 2013)
A. hispida (31.050) & *S. mucronata* (31.050)	1H-Cycloprop[e]azulen-4-ol, decahydro-1,1,4,7-tetramethyl-, [1aR-(1a.alpha.,4.beta.,4a.beta.,7.alpha.,7a.beta.,7b.alpha.)]-Viridiflorol	222	$C_{15}H_{26}O$		Anti-mycobacterial, anti-inflammatory & antioxidant activity (Trevizan *et al.,* 2016)

S. mucronata (18.120) & *C. miniata* (18.092)	n-Hexadecanoic acid, Hexadecanoic acid n-Hexadecoic acid, Palmitic acid, Pentadecane-carboxylic acid	256	$C_{16}H_{32}O_2$		Antibacterial activity (Yff *et al.,* 2002); antinociceptive effect (Shafri & Abdul Manan, 2012; Maroyi, 2018); antioxidant & antiandrogenic activity (Sudha & Padmini, 2023)
A. monteiroae Oliv. (23.675)	Cyclohexane, 1,2-dibromo-4-(1,2-dibromoethyl)-, Citex BCL 462, 1,2-Dibromo-4-(1,2-dibromoethyl)cyclohexane	424	$C_8H_{12}Br_4$		Endocrine disruptor & activates androgen receptor (Asnake *et al.,* 2014)
A. monteiroae Oliv. (23.815)	Ephedrine, (-)-Ephedrine, L-Ephedrine, Efedrin, Ephedrin, Benzenemethanol, .alpha.-[1-(methylamino)ethyl]-,	165	$C_{10}H_{15}NO$		Bronchodilator & decongestant (adrenergic stimulant); treatment of asthma, colds, cough, fever, headache & nasal congestion (Limberger *et al.,* 2013)
T. grandifolia (28.800)	.beta.-Amyrin, Olean-12-en-3-ol, (3.beta.)-, Olean-12-en-3.beta.-ol, .beta.-Amyrenol, Olean-12-en-3-ol	426	$C_{30}H_{50}O$		Anti-nociceptive, anti-inflammatory, anxiolytic, antidepressant, anticonvulsant, gastroprotection, hepatoprotection, antihyperglycemic & hypolipidemic activity & weight gain reducer (Nogueira *et al.,* 2019)
T. grandifolia (29.490)	Cyclopentanecarboxylic acid, 4-methylene-2,2-diphenyl-, methyl ester, Methyl 4-methylene-2,2-diphenylcyclopentanecarboxylate	292	$C_{20}H_{20}O_2$		Anticonvulsant (Liu & Pollack, 1994)

T. grandifolia (28.690)	1,3,5-Triazine, 2-chloro-4-methoxy-6-(1-piperidyl)-, 2-Chloro-4-methoxy-6-(1-piperidinyl)-1,3,5-triazine	228	$C_9H_{13}C_lN_4O$		Antibacterial, fungicidal, antimalarial, anticancer, antiviral, antimicrobial, anti-inflammatory, antiamoebic, & antitubercular activity (Singh *et al.,* 2021)

3.1.3. DCM Extracts

GC-MS analysis of the DCM extract of *D. dregeana* led to the identification of a total of 21 compounds and the major phytochemicals included 3-hydroxy-2,2,4-trimethyl-3-pentenoic acid (16.89%), methylphenidate concerta (3.98%) and .beta.-sitosterol (3.06%). The analysis of *B. disticha* yielded a total of 48 compounds with the major bioactive compound being .beta.-carboline (9.77%). The analysis of *A. hispida* yielded 23 compounds while *S. mucronata* yielded 30 compounds with the major bioactive compound on both extracts being sebacic acid 12.02% and 7.25%, respectively. The analysis of *A. monteiroae Oliv.* yielded 26 compounds and the major bioactive compounds included alpha.-guaiene (5.64%) and .beta.-sitosterol (5.62%). The analysis of *T. grandifolia* yielded 56 compounds which included 4,4,6a,6b,8a,11,11,14b-octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2H-picen-3-one (12.58%), germanicol (9.51%) and sebacic acid (8.14%). The analysis of *C. miniata* yielded 45 compounds which included 9,12-octadecadienoic acid (59.01%), n-hexadecanoic acid (7.07%), oleic acid (2.19%) and cis-9-hexadecenal (1.90%). The GC-MS chromatograms of DCM extracts are presented in Figure 3a-g and the major compounds (with high area% and height%) for each plant are documented in Table 4.

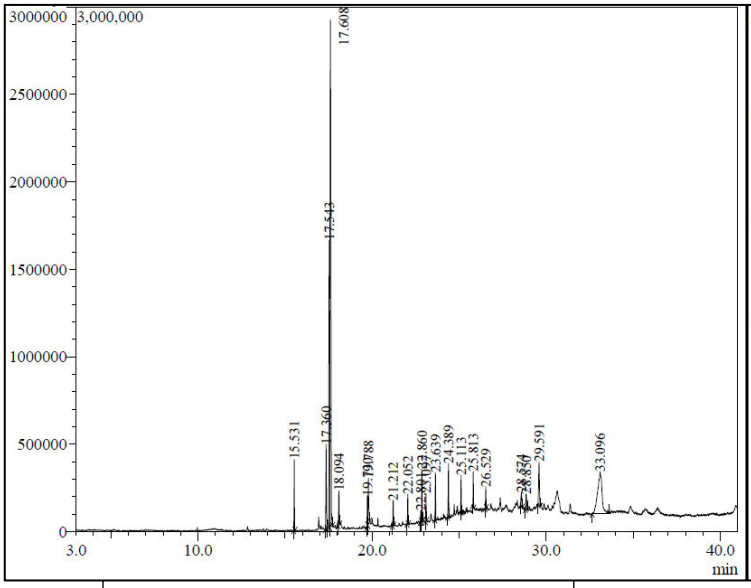


Figure 3a: *D. dregeana*

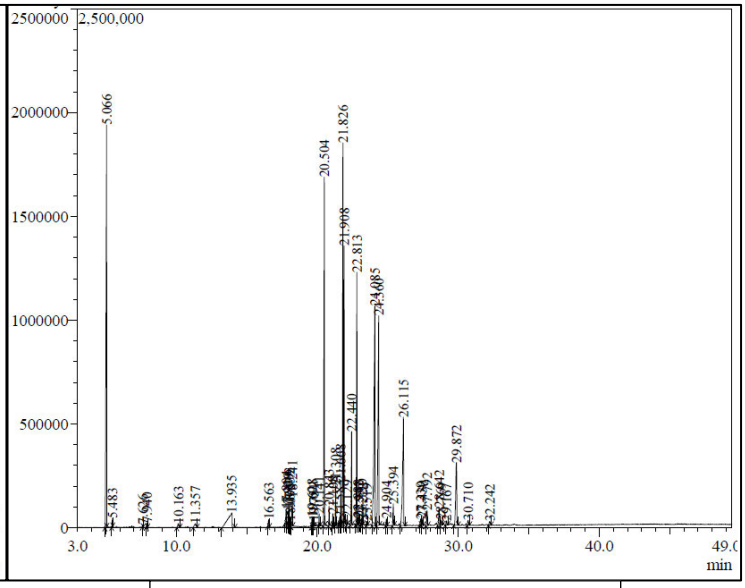


Figure 3b: *B. disticha*

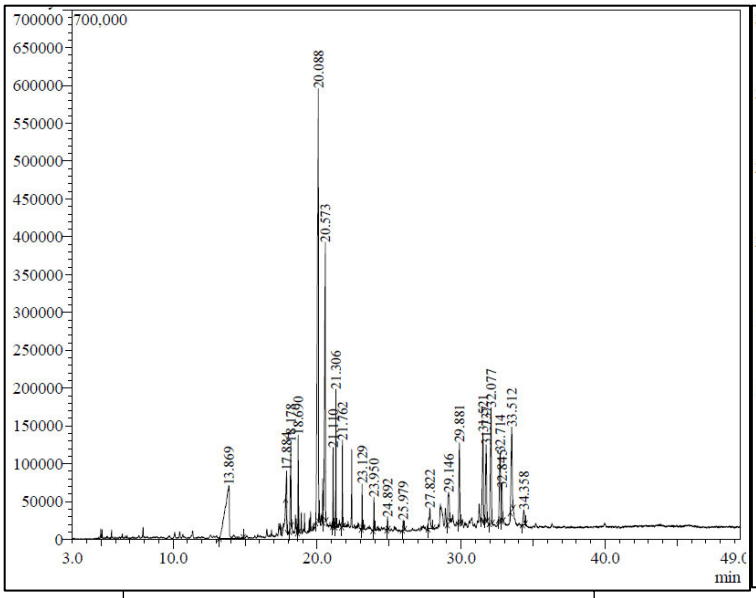


Figure 3c: *A. hispida*

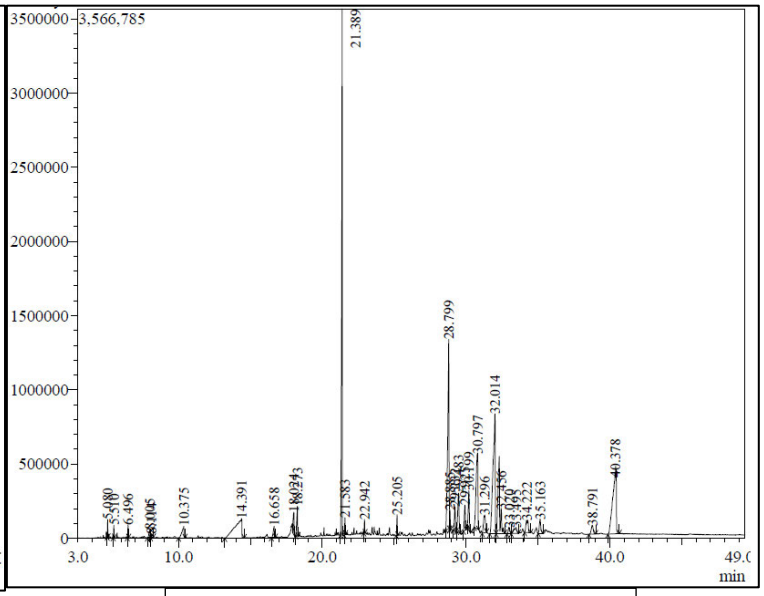


Figure 3d: *S. mucronata*

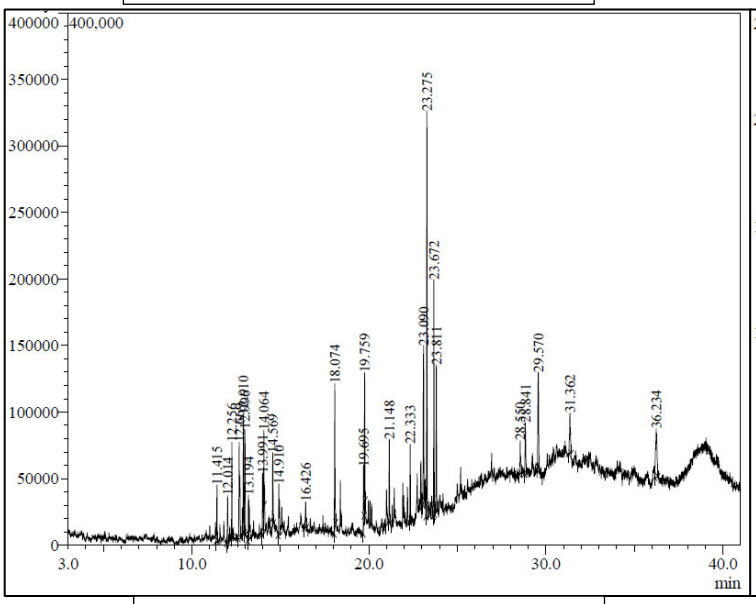


Figure 3e: *A. monteiroae Oliv.*

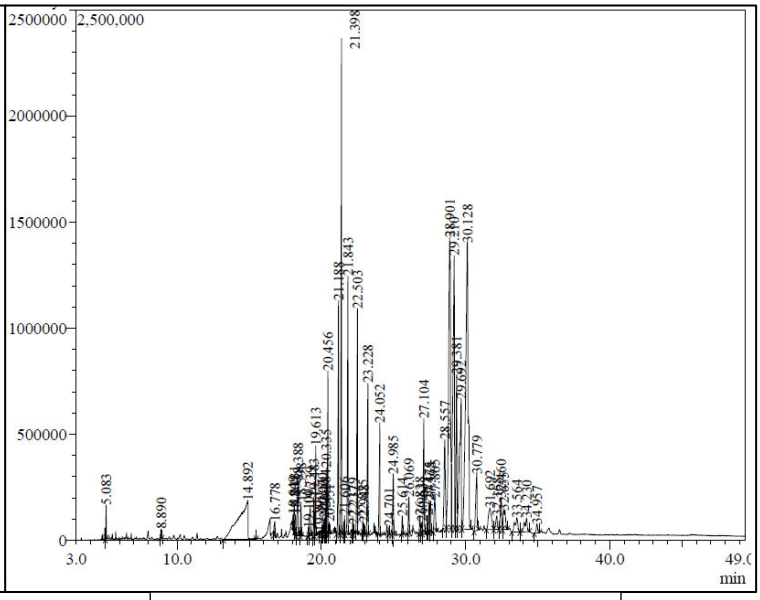


Figure 3f: *T. grandifolia*

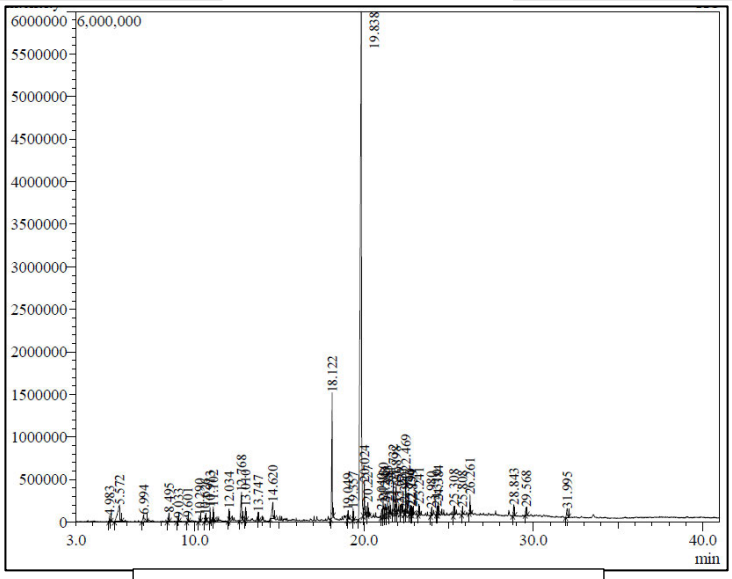
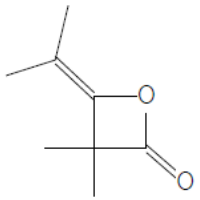
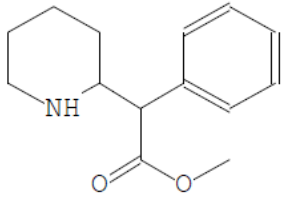
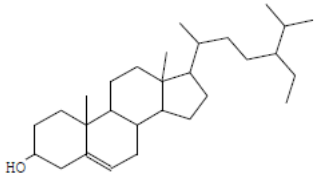
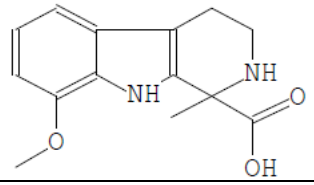
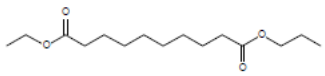
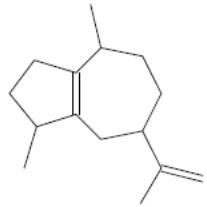
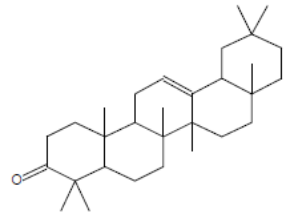
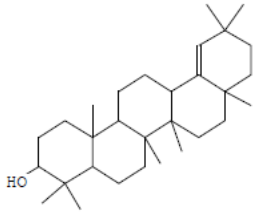


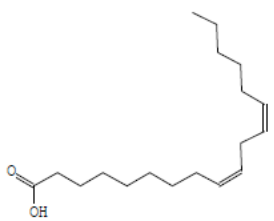
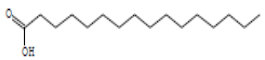
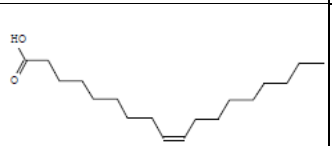
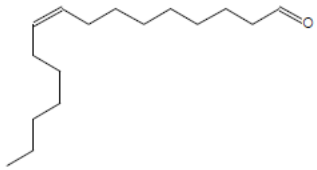
Figure 3g: *C. miniata*

Figure 3a-g: GC-MS chromatograms of DCM extracts of the 7 medicinal plants

Table 4: Phytochemical compounds identified in DCM extracts

Plant (RT-min)	Compound Name/s	Molecular Weight	Formula	Structure	Previously Reported Pharmacological Properties/Activity
D. dregeana (17.545)	3-Hydroxy-2,2,4-trimethyl-3-pentenoic acid .beta.-lactone, Dimethylketene-.beta.-lactone dimer	140	$C_8H_{12}O_2$		Inhibits activity of lipases secreted by gastrointestinal tract (Fenteany *et al.,* 1994); enzymatic inhibition (Bai *et al.,* 2014)
D. dregeana (17.360)	Methylphenidate Concerta 2-Piperidineacetic acid, .alpha.-phenyl-, methyl ester, .alpha.-Phenyl-2-piperidineacetic acid	233	$C_{14}H_{19}NO_2$		Treatment of attention-deficit/hyperactivity disorder (Katzman & Sternat, 2014)
D. dregeana (29.591) & *A. monteiroae Oliv.* (29.670)	.beta.-Sitosterol, Stigmast-5-en-3-ol, (3.beta.)-Stigmast-5-en-3.beta.-ol, .alpha.-Dihydrofucosterol, .beta.-Sitosterin	414	$C_{29}H_{50}O$		Antioxidant, antimicrobial, angiogenic, immunomodulatory, antidiabetic, anti-inflammatory, anticancer & anti-nociceptive activity (Rashed, 2020); reduces LDL & prostate hyperplasia, also used in skin ointments (Sudha & Padmini, 2023)
B. disticha (20.505)	.beta.-Carboline, 1,2,3,4-tetrahydro-1-carboxy-8-methoxy-1-methyl-, 8-Methoxy-1-methyl-2,3,4,9-tetrahydro-1H-beta-carboline-1-carboxylic acid	260	$C_{14}H_{16}N_2O_3$		Antioxidant, neuroprotective & anti-inflammatory activity (Piechowska *et al.,* 2019); sedative, hypnotic, anxiolytic, anticonvulsant, antiviral, antiparasitic/antimicrobial & antitumor effect (Szabo *et al.,* 2021)

A. hispida (13.870); *S. mucronata* (14.390) & *T. grandifolia* (14.892)	Sebacic acid, ethyl propyl ester	272	$C_{15}H_{28}O_4$		Improves glycaemic control, reduces hepatic gluconeogenesis & glucose output (Mingrone *et al.,* 2013)
A. monteiroae Oliv. (12.670)	alpha.-Guaiene, Azulene, 1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1.alpha.,4.alpha.,7.alpha.)]-	204	$C_{15}H_{24}$		Weak inhibitor of HERG (human ether-a-go-go-related gene) (Karimi *et al.,* 2021)
T. grandifolia (28.900)	4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2H-picen-3-one	424	C30H48O		Antioxidant, antibacterial, antitumour & cancer preventive activity (Helal *et al.,* 2019; Sudha & Padmini, 2023)
T. grandifolia (29.210)	Germanicol, Olean-18-en-3-ol	426	$C_{30}H_{50}O$		Anticancer & apoptotic effect (Hu *et al.,* 2016); anti-inflammatory effect (Al-Ramamneh *et al.,* 2022)

C. miniata (19.840)	9,12-Octadecadienoic acid (Z,Z)-cis-9,cis-12-Octadecadienoic acid, cis, cis-Linoleic acid, Grape seed oil	280	$C_{18}H_{32}O_2$		Anti-inflammatory, antimicrobial, cancer preventive, hypocholesterolemic, hepatoprotective, anti-arthritic, nematicide, insectifuge(cide), antihistaminic, anti-eczemic, anti-acne, 5-α reductase inhibitor, anti-androgenic & anti-coronary activity (Adeoye-Isijola *et al.,* 2018; Fagbemi *et al.,* 2021)
C. miniata (18.120)	n-Hexadecanoic acid, Hexadecanoic acid n-Hexadecoic acid, Palmitic acid, Pentadecane-carboxylic acid	256	$C_{16}H_{32}O_2$		Antibacterial activity (Yff *et al.,* 2002); antinociceptive effect (Shafri & Abdul Manan, 2012; Maroyi, 2018); antioxidant & antiandrogenic (Sudha & Padmini, 2023)
C. miniata (12.770)	Oleic Acid, 9-Octadecenoic acid (Z)-, .DELTA.9-cis-Oleic acid, cis-Oleic Acid, cis-9-Octadecenoic Acid, Emersol	282	$C_{18}H_{34}O_2$		Antimicrobial & antifungal activities (Maroyi, 2018)
C. miniata (21.730)	cis-9-Hexadecenal, 9-Hexadecenal, (Z)-, (Z)-9-Hexadecenal, Z-9-Hexadecenal, (9Z)-9-Hexadecenal	238	$C_{16}H_{30}O$		Antifungal activity (Hoda *et al.,* 2019); anti-inflammatory potential (Alves *et al.,* 2021)

3.2. Cytotoxicity

The cell viability of the methanol ethanol and DCM extracts of *D. dregeana*, *B. disticha*, *A. hispida*, *S. mucronata*, *A. monteiroae Oliv.*, *T. grandifolia* and *C. miniata* showed no cytotoxicity at the dosage of 50µg/ml and 100µg/ml concentrations after 24h treatment (Figure 4, 5 and 6). The methanol extracts of *S. mucronata*, *A. monteiroae Oliv*. and *T. grandifolia* showed increased cell viability at the highest dosage of 100µg/ml concentration (Figure 4). The ethanol extract of *D. dregeana* showed increased cell viability at the dosage of 50µg/ml and 100µg/ml concentrations, while the ethanol extracts of *A. monteiroae Oliv.* and *T. grandifolia* showed increased cell viability at the lowest dosage of 50µg/ml concentration (Figure 5). The DCM extracts of *A. hispida*, *S. mucronata*, *A. monteiroae Oliv.* and *C. miniata* showed increased cell viability at the highest dosage of 100µg/ml concentration (Figure 6).

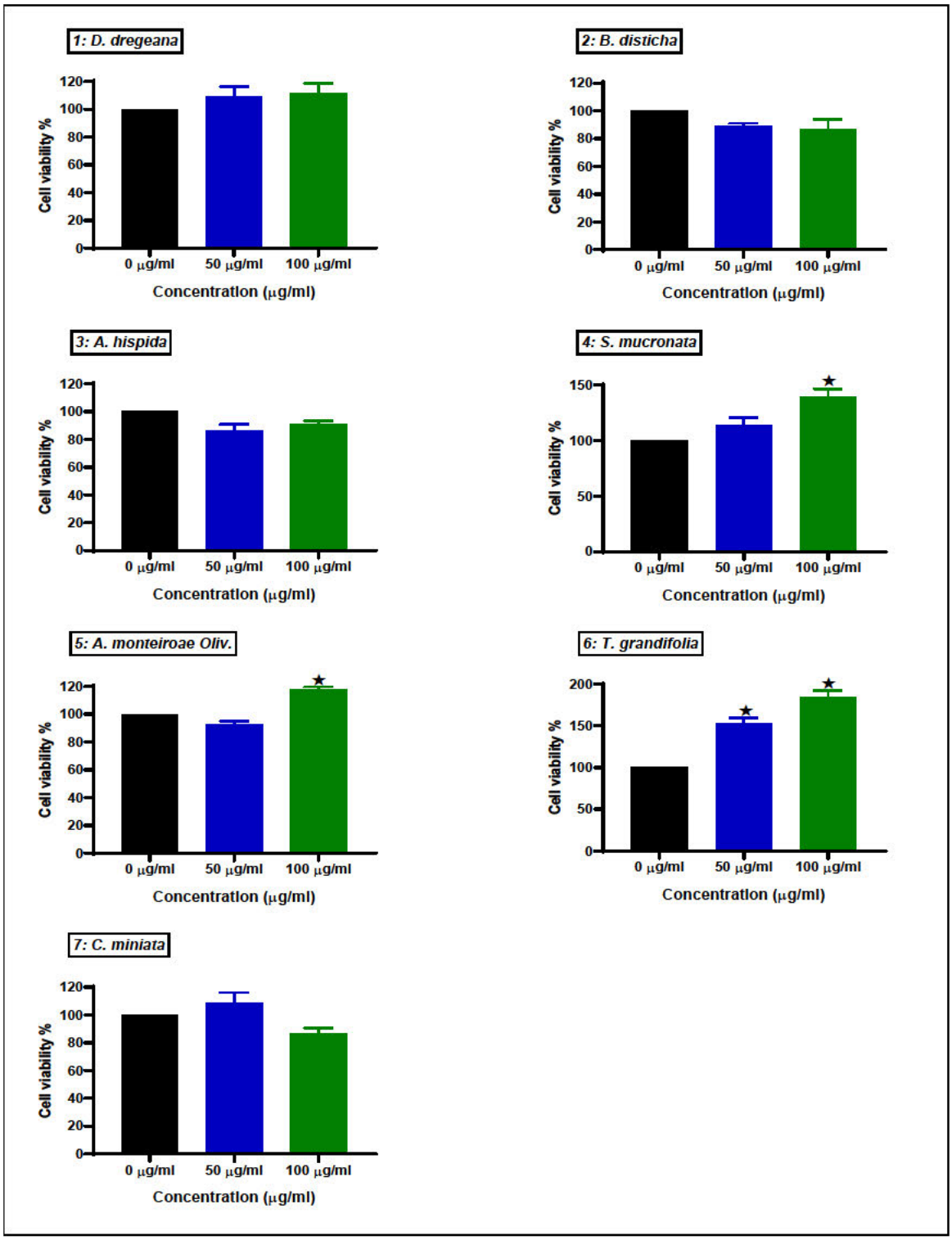


Figure 4: Cell viability of methanol extracts of the 7 medicinal plants after 24h treatment. Values are presented as means ± SEM (n = 6) in each group. * $p < 0.05$ compared to control group.

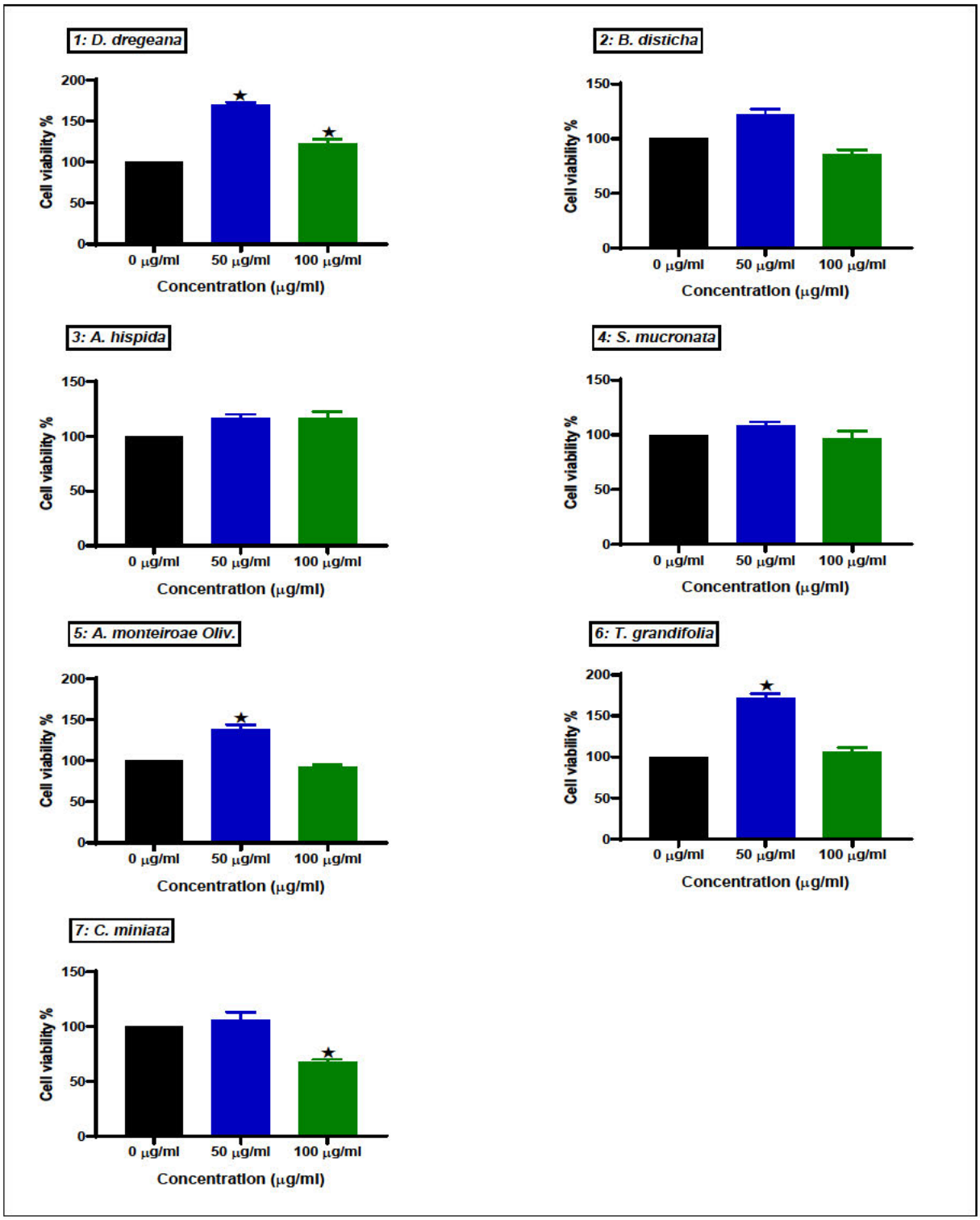


Figure 5: Cell viability of ethanol extracts of the 7 medicinal plants after 24 h treatment. Values are presented as means ± SEM (n = 6) in each group. * $p < 0.05$ compared to control group.

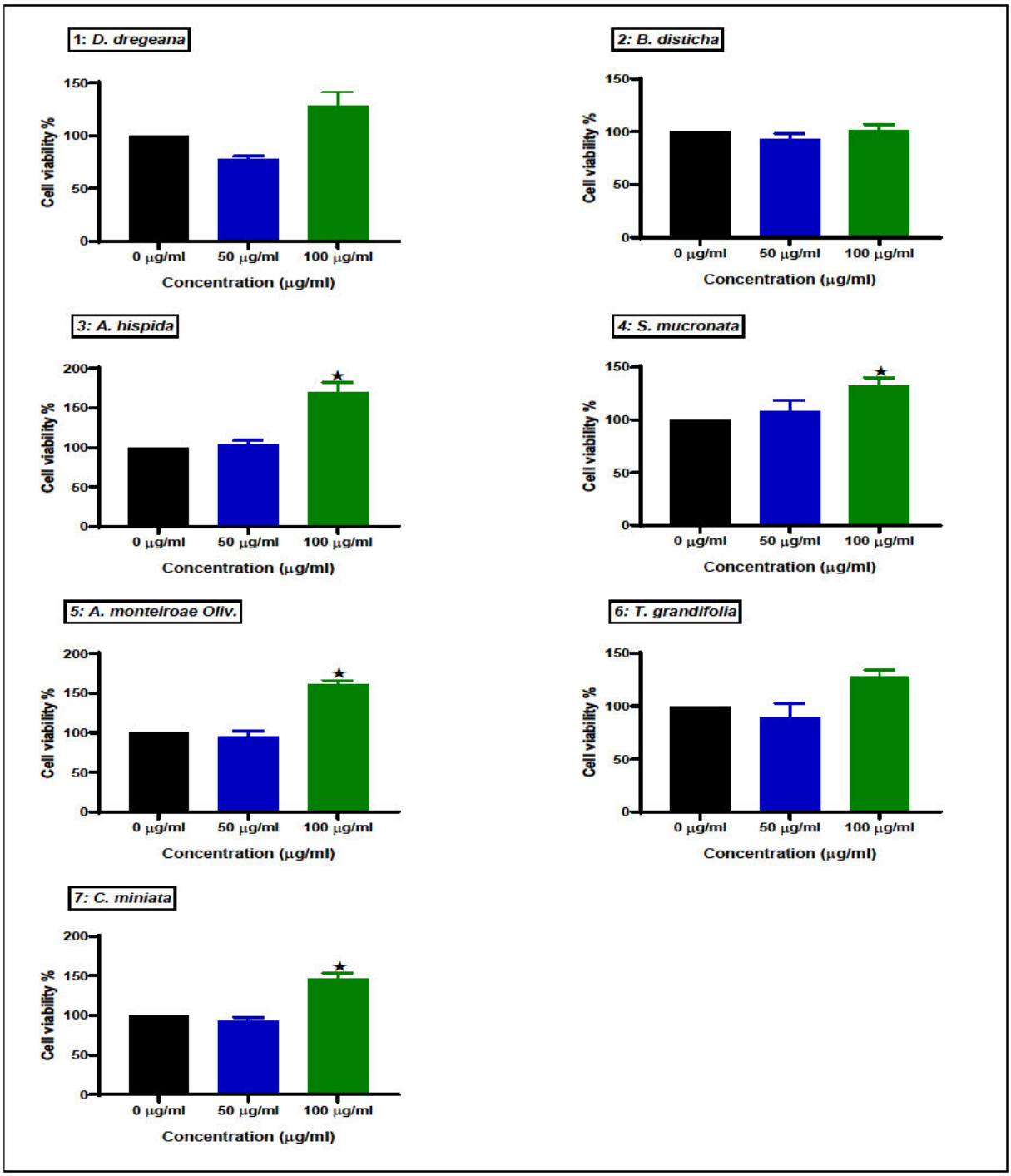


Figure 6: Cell viability of DCM extracts of the 7 medicinal plants after 24 h treatment. Values are presented as means ± SEM (n = 6) in each group. * $p < 0.05$ compared to control group.

4. DISCUSSION

4.1. Phytochemistry

Medicinal plants consist of bioactive compounds, which are mostly unknown; hence, their identification plays a significant role in the characterization of the chemical, biological and physical properties of these plants (de Souza-Melo *et al.,* 2021). Compounds with anti-inflammatory and antioxidant activity (i.e. 9,12-Octadecadienoic acid, n-hexadecanoic acid, eicosanoic acid, and apocynin ethanone were found in the methanol extracts of *D. dregeana, B. disticha*, *A. hispida*, *A. monteiroae Oliv., T. grandifolia*, and *C. miniata*, while the methanol extract of *S. mucronata* possessed compounds such as 1H-cycloprop[e]azulen-4-ol and .beta.-sitosterol which also have reported to have anti-inflammatory and antioxidant effects, and also octadecanoic acid which has been previously reported to treat schizophrenia (Table 2). In addition to the previously mentioned compounds, the ethanol extracts of *D. dregeana* (19.800); *S. mucronata* (19.780) & *A. monteiroae Oliv.* possessed erucic acid which has been reported to have protective and regulatory effects on nervous system (Table 3). A number of different compounds from the other extracts were yielded by the DCM extracts, in particular the presence of methylphenidate in D. dregeana was noted as this compound has been reported one of the most common mental disorders affecting children, attention-deficit/hyperactivity disorder (Table 4).The tuber of *D. dregeana* was reported as a remedy for the treatment of epilepsy, hysteria and convulsions, while the bulb of *B. disticha* has been used to sedate aggressive, psychotic patients (Stafford *et al.,* 2007). The use of *D. dregeana* in the treatment of neurological conditions such as epilepsy is supported by the presence of erucic acid in the ethanol extract of this plant in the current study which was reported by Gao *et al.* (2022) to have regulatory and protective effects on the nervous system. This study also identified the presence of erucic acid in the ethanol extracts of *S. mucronata* and *A. monteiroae Oliv*. One of the major compounds yielded by the methanol extract of *B. disticha* in the current study was apocynin ethanone, which could be a contributor to the plant's effect in psychosis due to its role in alleviating neurodegeneration as reported by Salva *et al.* (2021). Although psychosis is not classified as a neurodegenerative disorder, psychotic symptoms may manifest in the presence of underlying neurological disorders (Seritan, 2023). *B. disticha* has also been reported to induce hallucinations while also treating mental disorders in a study by Bonokwane *et al.* (2022). The reported contents of this plant in the aforementioned study included buphanamine, buphanadrine, buphanisine and distichamine which were not identified in this study. The use of *B. disticha* as a sedative agent can be linked to the presence of .beta.-carboline

in the DCM extract of this plant. This compound has been reported to possess sedative, hypnotic, anxiolytic and anticonvulsant effects (Szabo *et al.,* 2021) and also neuroprotective and anti-inflammatory activity (Piechowska *et al.,* 2109).

Maroyi (2022) reported the presence of sitosterol, stigmasterol, dodecanosyl 3-[4′-hydroxy-3′-methoxyphenyl] propenoate, 3,4′,5-trihydroxybibenzyl, crinamine and dioscorine in the tuber of *D. dregeana*. These compounds were also reported to have activity in psychosis and insanity which are also used to describe the symptoms of schizophrenia. In the current study, sitosterol (beta- and gamma-) was identified in the methanol extract of *S. mucronata*, ethanol extracts of *D. dregeana*, *S. mucronata* and *A. hispida*, and in the DCM extracts of *D. dregeana* and *A. monteiroae Oliv*. Caryophyllene, a terpenoid, was also identified in the ethanol extract of *A. hispida* in the current study which may contribute to the management of psychiatric symptoms that exist in schizophrenia, confirming the report by Kose *et al.* (2015). Terpenoids are present in the family Salicaceae (El-Sawi *et al.,* 2020); however, we did not identify these in the current study in *S. mucronata* which belongs to this family. The compound that supports the use of *S. mucronata* in the treatment of schizophrenia is octadecanoic acid identified in the current study and reported to be used to treat this disorder by Sushmitha *et al.* (2018).

N-methoxyoxoaporphine alkaloid artamonteirine and polycarpol were identified from *A. monteiroae Oliv.* in a study by Nyandoro *et al.* (2013), however, these compounds were not identified in current study. This could be due to the investigation of different parts of the plant in the two studies, where this study used the root of the plant while Nyandoro *et al.* (2013) used the stem bark. Thus supporting Rabizadeh *et al.* (2022) who reported that some phytochemicals may be highly concentrated on the outer layers of plants. *A. monteiroae Oliv.* has been reported to alleviate nervous system disorders such as hysteria (Mhlongo & Van Wyk, 2019), while erucic acid which was identified from the methanol and ethanol extracts in the current study was reported to have regulatory and protective effects on the nervous system (Gao *et al.,* 2022). Cyclohexane identified from the ethanol extract of *A. monteiroae Oliv.* was previously reported to have negative properties due its classification as an endocrine disruptor and androgen receptor activator (Asnake *et al.,* 2014). The leaves of *T. grandifolia* were reported to be used for mental disorders by Masondo *et al.* (2019); however, they did not specify the responsible compound for this activity. In the current study, *T. grandifolia* was linked with the alleviation

of mental disorders via .beta.-Amyrin which was identified from the ethanol extract of this plant. This compound was reported to have antidepressant effects by Nogueira *et al.* (2019). The roots of *C. miniata* were reported to contain linoleic acid and 5-hydroxymethyl-2-furancarboxaldehyde by Musara *et al.* (2021), cis-linoleic acid (also referred to 9,12-octadecadienoic acid) was also identified in the current study from the methanol, ethanol and DCM extracts of this plant. This compound has been previously reported to possess anti-inflammatory properties (Fagbemi *et al.,* 2021) which may alleviate neuropsychiatric disorders as these disorders may be caused by an inappropriately triggered inflammatory response (da Silva *et al.,* 2021).

4.2. Cytotoxicity

The increased cell viability shown by the different extracts at varying concentrations indicated a positive effect on cell proliferation. Certain bioactive compounds might have contributed to the non-cytotoxic effects, the promotion of cell proliferation and survival by the aforementioned plant extracts. The presence of n-hexadecanoic acid was indicated by the methanol extracts of *D. dregeana*, *A. monteiroae Oliv.*, *T. grandifolia and C.* miniata, ethanol extracts of *S. mucronata* and *C. miniata*, and the DCM extract of *C. miniata.* This compound was reported to have antioxidant activity by Sudha and Padmini (2023). Antioxidants reduce oxidative stress, thus promoting regeneration, proliferation and survival of cells (Shafi *et al.,* 2019). Antioxidant properties were also reported in .beta.-sitosterol by Rashed (2020), and this compound was present in the methanol extract of *S. mucronata*, the ethanol extract of *A. hispida* and the DCM extracts of *D. dregeana* and *A. monteiroae Oliv.* in this study. The presence of 9,12-octadecadienoic acid was indicated by the methanol extracts of *D. dregeana*, *B. disticha* and *C. miniata*, the ethanol extracts of *D. dregeana*, *B. disticha*, *A. monteiroae Oliv.* and *C. miniata*, and DCM extract of *C. miniata* in this study. This compound was reported to have hepato-protective activity by Fagbemi *et al.* (2021) which could have been a contributing factor to the promotion of cell proliferation and survival of the liver cells used in this study. The presence of .beta.-amyrin was indicated by the ethanol extract of *T. grandifolia*; this compound has also been reported to offer hepato-protection by Nogueira *et al.* (2019).

5. CONCLUSIONS

Based on the findings from the GC-MS analysis of the seven medicinal plants, it is evident that different phytochemicals can be identified from one plant and the types of bioactive compounds identified may depend on the solvent used for extraction; however, the total number of yielded compounds is independent of the solvent used. Thus, there may be more compounds present in the investigated herbal plants, which may have not been identified due to the selected solvents in this study. Furthermore, different parts of plants have varying concentrations of different phytochemicals and only specific parts, as offered by the THPs for treatment of schizophrenia, were investigated in this study. Nonetheless, little is known about the anti-psychotic effects of the identified bioactive compounds in these herbal plants; thus, further studies are recommended in order to better understand their mechanistic effects in the treatment of schizophrenia. The increased cell viability was dose-dependent with the highest dose (100 µg/mL) promoting cell proliferation in methanol and DCM extracts. An increased dose of the extracts might have contributed to a higher concentration of the identified compounds with hepato-protective, antioxidant or other related activity, thus promoting cell proliferation. Further studies on the cytotoxicity of each compound are recommended to better understand which phytochemicals contribute to the cytotoxic activity of some of the extracts of these herbal plants. Furthermore, a concoction made with one of the plants would have a mixture of various phytochemicals, and the complexity of the phytochemical contents would be increased in concoctions with a mixture or all of the investigated plants. As noted in the pharmacological uses of the different compounds, different bioactive compounds effect different organ systems and not all compounds have a positive effect on the nervous system or any other functional system in the body. Thus, isolation, characterization and optimization of the bioactive compounds is recommended for future research in order to determine a clear mechanism of action of these compounds which in future would assist in the production of new, possibly antipsychotic, drugs. Lastly, it is recommended that the non-cytotoxic extracts be tested on various animal models to understand their mechanistic effects in the treatment of schizophrenia, and or other mental disorders.

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Declaration of Interest

The authors have no potential conflict of interest to report.

6. REFERENCES

Adeoye-Isijola, M.O., Olajuyigbe, O.O., Jonathan, S.G. and Coopoosamy, R.M., 2018. Bioactive compounds in ethanol extract of Lentinus squarrosulus Mont-a Nigerian medicinal macrofungus. *African Journal of Traditional, Complementary and alternative medicines*, 15(2), pp.42-50. http://doi.org/10.21010/ajtcamv15i2.6

Al-Ramamneh, E.A.D.M., Ghrair, A.M., Shakya, A.K., Alsharafa, K.Y., Al-Ismail, K., Al-Qaraleh, S.Y., Mojski, J. and Naik, R.R., 2022. Efficacy of Sterculia diversifolia leaf extracts: Volatile compounds, antioxidant and anti-Inflammatory activity, and green synthesis of potential antibacterial silver nanoparticles. *Plants*, 11(19), p.2492, pp.1-17. https://doi.org/10.3390/plants11192492

Alves, J., Gaspar, H., Silva, J., Alves, C., Martins, A., Teodoro, F., Susano, P., Pinteus, S. and Pedrosa, R., 2021. Unravelling the anti-inflammatory and antioxidant potential of the Marine Sponge Cliona celata from the Portuguese coastline. *Marine Drugs*, 19(11), p.632, pp.1-17. https://doi.org/10.3390/md19110632

American Psychiatric Association. 2013. Diagnostic and statistical manual of mental disorders (5th ed.). https://doi.org/10.1176/appi.books.9780890425596

Asnake, S., Pradhan, A., Banjop-Kharlyngdoh, J., Modig, C. and Olsson, P.E., 2014. 1, 2-dibromo-4-(1, 2 dibromoethyl) cyclohexane (TBECH)–mediated steroid hormone receptor activation and gene regulation in chicken LMH cells. *Environmental Toxicology and Chemistry*, 33(4), pp.891-899. https://doi.org/10.1002/etc.2509

Bai, T., Zhang, D., Lin, S., Long, Q., Wang, Y., Ou, H., Kang, Q., Deng, Z., Liu, W. and Tao, M., 2014. Operon for biosynthesis of lipstatin, the beta-lactone inhibitor of human pancreatic lipase. *Applied and Environmental Microbiology*, 80(24), pp.7473-7483. https://doi.org/10.1128/AEM.01765-14

Bano, I. and Deora, G.S., 2019. Preliminary phytochemical screening and GC-MS analysis of methanolic leaf extract of Abutilon pannosum (Forst. F.) Schlect. from Indian Thar desert. *Journal of Pharmacognosy and Phytochemistry*, 8(1), pp.894-899.

Bernas, T. and Dobrucki, J., 2002. Mitochondrial and nonmitochondrial reduction of MTT: Interaction of MTT with TMRE, JC-1, and NAO mitochondrial fluorescent probes.

Cytometry: *The journal of the International Society for Analytical Cytology*, 47(4), pp.236-242. https://doi.org/10.1002/cyto.10080

Bonokwane, M.B., Lekhooa, M., Struwig, M. and Aremu, A.O., 2022. Antidepressant effects of South African plants: an appraisal of ethnobotanical surveys, ethnopharmacological and phytochemical studies. *Frontiers in Pharmacology*, 13, p.895286, pp.1-38. https://doi.org/10.3389/fphar.2022.895286

Correll, C.U. and Schooler, N.R., 2020. Negative symptoms in schizophrenia: a review and clinical guide for recognition, assessment, and treatment. *Neuropsychiatric Disease and Treatment*, 16, pp.519-534. https://doi.org/10.2147/NDT.S225643

Czifra, G., Tóth, I.B., Marincsák, R., Juhász, I., Kovács, I., Ács, P., Kovács, L., Blumberg, P.M. and Bíró, T., 2006. Insulin-like growth factor-I-coupled mitogenic signaling in primary cultured human skeletal muscle cells and in C2C12 myoblasts. A central role of protein kinase Cδ. *Cellular Signalling*, 18(9), pp.1461-1472. https://doi.org/10.1016/j.cellsig.2005.11.007

da Silva, M.G., Daros, G.C. and de Bitencourt, R.M., 2021. Anti-inflammatory activity of ayahuasca: Therapeutical implications in neurological and psychiatric diseases. *Behavioural Brain Research*, 400, p.113003, pp.1-8. https://doi.org/10.1016/j.bbr.2020.113003

de Sousa Ferrão, E.J.E. and Janeque, E.A.D.G., 2023. Anti-viral compounds from Jatropha curcas seed extract with anti-HIV-1 and anti-SARS-CoV-2 action. *African Journal of Pharmacy and Pharmacology*, 17(1), pp.1-9. http://doi.org/10.5897/AJPP2022.5328

de Souza-Melo, W.O., Figueiredo-Júnior, E.C., Freire, J.C.P., Costa, B.P., Lira, A.B., Freires, I.A., Cavalcanti, Y.W., Lopes, W.S., Tavares, J.F., Pessôa, H.D.L.F. and Pereira, J.V., 2021. Phytochemistry, antifungal and antioxidant activity, and cytotoxicity of Byrsonima gardneriana (A. Juss) extract. *Archives of Oral Biology*, 123, p.104994, pp.1-10. https://doi.org/10.1016/j.archoralbio.2020.104994

Deng, H. and Xu, J., 2017. Wendan decoction (Traditional Chinese medicine) for schizophrenia. *Cochrane Database of Systematic Reviews*, (6). https://doi.org/10.1002/14651858.CD012217.pub2

El-Sawi, S.A., Maamoun, A.A., Salama, A.H., Maamoun, M.A. and Farghaly, A.A., 2020. Application of micro-emulsion formulation in improving the antiproliferative performance of Salix mucronata (Thunb) leaves with chemical investigation of the active extract. *Acta Ecologica Sinica*, 40(4), pp.339-346. https://doi.org/10.1016/j.chnaes.2020.04.004

Eleazu, C.O., 2016. Characterization of the natural products in cocoyam (Colocasia esculenta) using GC–MS. *Pharmaceutical Biology*, 54(12), pp.2880-2885. https://doi.org/10.1080/13880209.2016.1190383

Fagbemi, K.O., Aina, D.A. and Olajuyigbe, O.O., 2021. Soxhlet extraction versus hydrodistillation using the clevenger apparatus: A comparative study on the extraction of a volatile compound from Tamarindus indica seeds. *The Scientific World Journal*, 2021, pp.1-8. https://doi.org/10.1155/2021/5961586

Fenteany, G., Standaert, R.F., Reichard, G.A., Corey, E.J. and Schreiber, S.L., 1994. A beta-lactone related to lactacystin induces neurite outgrowth in a neuroblastoma cell line and inhibits cell cycle progression in an osteosarcoma cell line. *Proceedings of the National Academy of Sciences*, 91(8), pp.3358-3362. https://doi.org/10.1073/pnas.91.8.3358

Freeman, M. and Motsei, M., 1992. Planning health care in South Africa—is there a role for traditional healers?. *Social Science and Medicine*, 34(11), pp.1183-1190. https://doi.org/10.1016/0277-9536(92)90311-D

Gao, L., Li, H., Li, B., Shao, H., Yu, X., Miao, Z., Zhang, L., Zhu, L. and Sheng, H., 2022. Traditional uses, phytochemistry, transformation of ingredients and pharmacology of the dried seeds of Raphanus sativus L.(Raphani Semen), A comprehensive review. *Journal of Ethnopharmacology*, 294, p.115387, pp.1-21. https://doi.org/10.1016/j.jep.2022.115387

Garg, R., Mohana, D.C. and Manjunath, K., 2013. In vitro antibacterial activity and phytochemical analysis of some traditional herbs. *Journal of Pharma and Bio Sciences*, 4(1), pp.994-1003.

Green, E.C. and Makhubu, L., 1984. Traditional healers in Swaziland: toward improved cooperation between the traditional and modern health sectors. *Social Science and Medicine*, 18(12), pp.1071-1079. https://doi.org/10.1016/0277-9536(84)90167-9

Gyrdymova, Y.V. and Rubtsova, S.A., 2021. Caryophyllene and caryophyllene oxide: A variety of chemical transformations and biological activities. *Chemical Papers*, 76, pp.1-39. https://doi.org/10.1007/s11696-021-01865-8

Helal, N.M., Ibrahim, N. and Khattab, H., 2019. Phytochemical analysis and antifungal bioactivity of Pulicaria undulata (L.) methanolic extract and essential oil. *Egyptian Journal of Botany*, 59(3), pp.827-844. https://doi.org/10.21608/EJBO.2019.12259.1308

Hoda, S., Gupta, L., Agarwal, H., Raj, G., Vermani, M. and Vijayaraghavan, P., 2019. Inhibition of Aspergillus fumigatus Biofilm and Cytotoxicity Study of Natural Compound Cis-9-Hexadecenal. *Journal of Pure and Applied Microbiology*, 13(2), pp.1207-1216. https://dx.doi.org/10.22207/JPAM.13.2.61

Hu, Y.L., Wang, X.B., Chen, D.D., Guo, X.J., Yang, Q.J., Dong, L.H. and Cheng L., 2016. Germanicol induces selective growth inhibitory effects in human colon HCT-116 and HT29 cancer cells through induction of apoptosis, cell cycle arrest and inhibition of cell migration. *Journal of BUON*; 21(3), pp.626-32.

Incayawar, M., 2008. Efficacy of Quichua healers as psychiatric diagnosticians. *The British Journal of Psychiatry*, 192(5), pp.390-391. https://doi.org/10.1192/bjp.bp.107.046938

Jain, A.K., Singh, D., Dubey, K., Maurya, R., Mittal, S. and Pandey, A.K., 2018. Models and methods for in vitro toxicity. *In vitro Toxicology*, 3, pp.45-65. Academic Press. https://doi.org/10.1016/B978-0-12-804667-8.00003-1

Kahn, R.S., Sommer, I.E., Murray, R.M., Meyer-Lindenberg, A., Weinberger, D.R., Cannon, T.D., O'Donovan, M., Correll, C.U., Kane, J.M., van Os, J. and Insel, T.R., 2015. Schizophrenia (Primer). *Nature Reviews: Disease Primers*, 12(1), ID.15067, pp.1-23. https://doi.org/10.1038/nrdp.2015.67

Kantrowitz, J.T., 2021. How do we address treating the negative symptoms of schizophrenia pharmacologically?. *Expert Opinion on Pharmacotherapy*, 22(14), pp.1811-1813. https://doi.org/10.1080/14656566.2021.1939677

Kapur, R.L., 1975. Mental health care in rural India: a study of existing patterns and their implications for future policy. *The British Journal of Psychiatry*, 127(3), pp.286-293. https://doi.org/10.1192/bjp.127.3.286

Karimi, I., Yousofvand, N. and Hussein, B.A., 2021. In vitro cholinesterase inhibitory action of Cannabis sativa L. Cannabaceae and in silico study of its selected phytocompounds. *In Silico Pharmacology*, 9, pp.1-15. https://doi.org/10.1007/s40203-021-00075-0

Katzman, M.A. and Sternat, T., 2014. A review of OROS methylphenidate (Concerta®) in the treatment of attention-deficit/hyperactivity disorder. *CNS Drugs*, 28(11), pp.1005-1033. https://doi.org/10.1007/s40263-014-0175-1

Kaur, R., Manchanda, P. and Sidhu, G. S., 2020. Phenolic compounds from peel and callus extracts of sweet lime (Citrus medica). *The Indian Journal of Agricultural Sciences. Delhi, India*, 90(6), pp. 1205-1208. https://doi.org/10.56093/ijas.v90i6.104803

Kleinman, A. and Sung, L.H., 1979. Why do indigenous practitioners successfully heal?. *Social Science and Medicine. Part B: Medical Anthropology*, 13(1), pp.7-26. https://doi.org/10.1016/0160-7987(79)90014-0

Kose, L.S., Moteetee, A. and Van Vuuren, S., 2015. Ethnobotanical survey of medicinal plants used in the Maseru district of Lesotho. *Journal of Ethnopharmacology*, 170, pp.184-200. https://doi.org/10.1016/j.jep.2015.04.047

Koster, L.S., Carbon, M. and Correll, C.U., 2014. Emerging drugs for schizophrenia: an update. *Expert Opinion on Emerging Drugs*, 19(4), pp.511-531. https://doi.org/10.1517/14728214.2014.958148

Lafeber, F.P.J.G., Beukelman, C.J., Van Den Worm, E., Van Roy, J.L.A.M., Vianen, M.E., Van Roon, J.A.G., Van Dijk, H. and Bijlsma, J.W.J., 1999. Apocynin, a plant-derived, cartilage-saving drug, might be useful in the treatment of rheumatoid arthritis. *Rheumatology*, 38(11), pp.1088-1093. https://doi.org/10.1093/rheumatology/38.11.1088

Limberger, R.P., Jacques, A.L.B., Schmitt, G.C. and Arbo, M.D., 2013. Pharmacological Effects of Ephedrine. *Natural Products*, 38, pp.1217-1237. Springer-VerlagBerlinHeidelberg https://doi.org/10.1007/978-3-642-22144-6_41

Liu, M.J. and Pollack, G.M., 1994. Pharmacokinetics and Pharmacodynamics of Valproate Analogues in Rats. IV. Anticonvulsant Action and Neurotoxocity of Octanoic Acid, Cyclohexanecarboxylic Acid, and 1-Methyl-1-Cyclohexanecarboxylic Acid. *Epilepsia*, 35(1), pp.234-243. https://doi.org/10.1111/j.1528-1157.1994.tb02939.x

Lucana, S. and Elfers, J., 2020. Sacred medicine: Indigenous healing and mental health. *The Qualitative Report*, 25(12), pp.4482-4495.

Maroyi, A., 2018. Albizia adianthifolia: botany, medicinal uses, phytochemistry, and pharmacological properties. *The Scientific World Journal*, 2018, ID. 7463584, pp.1-18. https://doi.org/10.1155/2018/7463584

Maroyi, A., 2022. Medicinal uses, phytochemistry and pharmacological properties of Dioscorea dregeana (Kunth) T. Durand & Schinz. *Medicinal Plants-International Journal of Phytomedicines and Related Industries*, 14(1), pp.57-63. https://doi.org/10.5958/0975-6892.2022.00006.5

Masondo, N.A., Stafford, G.I., Aremu, A.O. and Makunga, N.P., 2019. Acetylcholinesterase inhibitors from southern African plants: An overview of ethnobotanical, pharmacological potential and phytochemical research including and beyond Alzheimer's disease treatment. *South African Journal of Botany*, 120, pp.39-64. https://doi.org/10.1016/j.sajb.2018.09.011

Matinlinna, J.P., Lassila, L.V., Özcan, M., Yli-Urpo, A. and Vallittu, P.K., 2004. An introduction to silanes and their clinical applications in dentistry. *International Journal of Prosthodontics*, 17(2), pp.155-164.

Mhlongo, L.S. and Van Wyk, B.E., 2019. Zulu medicinal ethnobotany: New records from the Amandawe area of KwaZulu-Natal, South Africa. *South African Journal of Botany*, 122, pp.266-290. https://doi.org/10.1016/j.sajb.2019.02.012

Mingrone, G., Castagneto-Gissey, L. and Macé, K., 2013. Use of dicarboxylic acids in type 2 diabetes. *British Journal of Clinical Pharmacology*, 75(3), pp.671-676. https://doi.org/10.1111/j.1365-2125.2012.04177.x

Musara, C., Aladejana, E.B. and Aladejana, A.E., 2021. Clivia miniata (Lindl.) Bosse,(Amaryllidaceae): Botany, medicinal uses, phytochemistry and pharmacological properties. *Journal of Applied Pharmaceutical Science*, 11(2), pp.012-018. https://doi.org/10.7324/JAPS.2021.110202

Nogueira, A.O., Oliveira, Y.I.S., Adjafre, B.L., de Moraes, M.E.A. and Aragao, G.F., 2019. Pharmacological effects of the isomeric mixture of alpha and beta amyrin from Protium

heptaphyllum: a literature review. *Fundamental and clinical pharmacology*, 33(1), pp.4-12. https://doi.org/10.1111/fcp.12402

Nyandoro, S.S., Joseph, C.C., Nkunya, M.H. and Hosea, K.M., 2013. New antimicrobial, mosquito larvicidal and other metabolites from two Artabotrys species. *Natural Product Research*, 27(16), pp.1450-1458. https://doi.org/10.1080/14786419.2012.725397

Ojagbemi, A. and Gureje, O., 2020. The potential role of traditional medicine in the management of schizophrenia. *Current Psychiatry Reports*, 22, pp.1-7. https://doi.org/10.1007/s11920-020-01196-7

Okaiyeto, K. and Oguntibeju, O.O., 2021. African herbal medicines: Adverse effects and cytotoxic potentials with different therapeutic applications. *International Journal of Environmental Research and Public Health*, 18(11), p.5988, pp.1-20. https://doi.org/10.3390/ijerph18115988

Piechowska, P., Zawirska-Wojtasiak, R. and Mildner-Szkudlarz, S., 2019. Bioactive β-carbolines in food: A review. *Nutrients*, 11(4), p.814, pp.1-10. https://doi.org/10.3390/nu11040814

Pratama, O.A., Tunjung, W.A.S., Sutikno, S. and Daryono, B.S., 2019. Bioactive compound profile of melon leaf extract (Cucumis melo L.'Hikapel') infected by downy mildew. *Biodiversitas Journal of Biological Diversity*, 20(11), pp.3448-3453. https://doi.org/10.13057/biodiv/d201143

Rabiee, R., Hosseini Hooshiar, S., Ghaderi, A. and Jafarnejad, S., 2023. Schizophrenia, Curcumin and Minimizing Side Effects of Antipsychotic Drugs: Possible Mechanisms. *Neurochemical Research*, 48(3), pp.713-724. https://doi.org/10.1007/s11064-022-03798-4

Rabizadeh, F., Mirian, M.S., Doosti, R., Kiani-Anbouhi, R. and Eftekhari, E., 2022. Phytochemical Classification of Medicinal Plants Used in the Treatment of Kidney Disease Based on Traditional Persian Medicine. *Evidence-Based Complementary and Alternative Medicine*, 2022, ID.8022599, pp.1-13. https://doi.org/10.1155/2022/8022599

Rashed, K., 2020. Beta-sitosterol medicinal properties: A review article. *International Journal of Science Inventions Today*, 9, pp.208-212.

Savla, S.R., Laddha, A.P. and Kulkarni, Y.A., 2021. Pharmacology of apocynin: a natural acetophenone. *Drug Metabolism Reviews*, 53(4), pp.542-562. https://doi.org/10.1080/03602532.2021.1895203

Seritan, A.L., 2023. Advances in the Diagnosis and Management of Psychotic Symptoms in Neurodegenerative Diseases: A Narrative Review. *Journal of Geriatric Psychiatry and Neurology*, 36(6), pp.435-460. https://doi.org/10.1177/08919887231164357

Shafi, S., Ansari, H.R., Bahitham, W. and Aouabdi, S., 2019. The impact of natural antioxidants on the regenerative potential of vascular cells. *Frontiers in Cardiovascular Medicine*, 6, pp.1-9. https://doi.org/10.3389/fcvm.2019.00028

Shafri, M. and Abdul Manan, M.J., 2012. Therapeutic potential of the haruan (Channa striatus): from food to medicinal uses. *Malaysian Journal of Nutrition*, 18(1), pp.125-136.

Singh, S., Mandal, M.K., Masih, A., Saha, A., Ghosh, S.K., Bhat, H.R. and Singh, U.P., 2021. 1, 3, 5-Triazine: A versatile pharmacophore with diverse biological activities. *Archiv der Pharmazie*, 354(6), p.2000363. https://doi.org/10.1002/ardp.202000363

Sivasubramanian, R. and Brindha, P., 2013. In-vitro cytotoxic, antioxidant and GC-MS studies on Centratherum punctatum Cass. *International Journal of Pharmacy and Pharmaceutical Sciences*, 5(3), pp.364-367.

Spedding, M. F., Stein, D. J. and Sorsdahl, K., 2014. Task-shifting psychosocial interventions in public mental health: A review of the evidence in the South African context. *South African Health Review*, 2014(1), pp.73-87.

Stafford, G.I., Pedersen, P.D., Jäger, A.K. and Van Staden, J., 2007. Monoamine oxidase inhibition by southern African traditional medicinal plants. *South African Journal of Botany*, 73(3), pp.384-390. https://doi.org/10.1016/j.sajb.2007.03.001

Sudha, T.S. and Padmini, R., 2023. Evaluation Of Bioactive Compounds In Euphorbia Hirta Linn. Leaves Extract Using Gas Chromatographic And Mass Spectroscopic Techniques. *Journal of Pharmaceutical Negative Results*, pp.1988-1995. https://doi.org/10.47750/pnr.2023.14.S02.238%20%20

Sushmitha, H.S., Roy, C.L., Gogoi, D., Velagala, R.D., Nagarathna, A., Balasubramanian, S. and Rajadurai, M., 2018. Phytochemical and Pharmacological Studies on Hylocereus

undatus Seeds: An In Vitro Approach. *World Journal of Pharmacological Research*, 7(14), pp.986-1006.

Szabo, T., Volk, B. and Milen, M., 2021. Recent advances in the synthesis of β-carboline alkaloids. *Molecules*, 26(3), p.663. https://doi.org/10.3390/molecules26030663

Trevizan, L.N.F., do Nascimento, K.F., Santos, J.A., Kassuya, C.A.L., Cardoso, C.A.L., do Carmo Vieira, M., Moreira, F.M.F., Croda, J. and Formagio, A.S.N., 2016. Anti-inflammatory, antioxidant and anti-Mycobacterium tuberculosis activity of viridiflorol: The major constituent of Allophylus edulis (A. St.-Hil., A. Juss. & Cambess.) Radlk. *Journal of Ethnopharmacology*, 192, pp.510-515. https://doi.org/10.1016/j.jep.2016.08.053

World Health Organisation. 2022a, June 8. Mental Disorders [Fact Sheet]. https://www.who.int/news-room/fact-sheets/detail/mental-disorders#:~:text=A%20mental%20disorder%20is%20characterized,different%20types%20of%20mental%20disorders. (Accessed 5 March 2023).

World Health Organisation. 2022b, March 25. WHO establishes the Global Centre for Traditional Medicine in India. WHO. https://www.who.int/news/item/25-03-2022-who-establishes-the-global-centre-for-traditional-medicine-in-india. (Accessed 3 March 2023)

World Health Organisation. 2022c. World health statistics 2022: monitoring health for the SDGs, sustainable development goals. Geneva: World Health Organization; 2022. Licence: CC BY-NC-SA 3.0 IGO

Yff, B.T., Lindsey, K.L., Taylor, M.B., Erasmus, D.G. and Jäger, A.K., 2002. The pharmacological screening of Pentanisia prunelloides and the isolation of the antibacterial compound palmitic acid. *Journal of Ethnopharmacology*, 79(1), pp.101-107. https://doi.org/10.1016/S0378-8741(01)00380-4

Zekeya, N., Ibrahim, M., Mamiro, B., Ndossi, H., Kilonzo, M., Mkangara, M., Chacha, M., Chilongola, J. and Kideghesho, J., 2022. Potential of natural phenolic antioxidant compounds from Bersama abyssinica (Meliathacea) for treatment of chronic diseases. *Saudi Journal of Biological Sciences*, 29(6), p.103273. https://doi.org/10.1016/j.sjbs.2022.03.023

Prologue 4

The findings of the previous chapter indicated that all the seven medicinal plants utilised by the THPs for the treatment of schizophrenia and other mental illnesses are non-cytotoxic. These plants consisted of a number of bioactive compounds with some of these compounds containing antioxidant, neuroprotective and antipsychotic effects. In previous literature, *D. dregeana*, *B. disticha*, *A. hispida*, *A. monteiroae Oliv.* and *T. grandifolia* have been reported to treat mental illnesses. However, there is a paucity of studies reporting on the role of *S. mucronata*, and *C. miniata* in the treatment of these ailments. Of the two medicinal plants, *S. mucronata* was selected for testing in an animal model to attempt to close the gap in literature on this plant. *C. miniata* was not tested in this project as only one plant was permitted by the Animal Research Ethics Committee in order to reduce the number of experimental groups and animals used in this study. In the next paper, the effects of the methanol extract of *S. mucronata* were investigated in alleviating the negative symptoms of schizophrenia; i.e. asociality and anhedonia. Furthermore, the plant's potential effects were compared with the antipsychotic activity of risperidone. Findings are documented in the following Paper 5.

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CHAPTER 5

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Research paper

The antipsychotic potential of *Salix Mucronata* on ketamine-induced rats

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ABSTRACT

Salix mucronata is one of the herbal plants offered by the traditional health practitioners in KwaZulu-Natal, South Africa for the treatment of schizophrenia. This study aimed to investigate the effects of repeated administration of ketamine on social interaction, novelty and motivation in adult, male Sprague Dawley rats. It also aimed to investigate the potential of risperidone and the herbal extract of *S. mucronata* to reverse impairments that are induced by ketamine. Experimental rats (n=45) received a dose of ketamine at 30 mg/kg via intraperitoneal injection for 5 consecutive days. They were then allocated into their respective treatment groups and given risperidone (APD) and the herbal extract of *S. mucronata* (TM) at doses of 6 mg/kg and 5 mg/kg, respectively, for 7 consecutive days. Social behaviour was tested using the 3-chambered sociability test, and anhedonia was tested using the sucrose preference test. Ketamine induction elicited social withdrawal and reduced social novelty which were later successfully reversed by risperidone and *S. mucronata*. The rats showed reduced preference to sucrose post-induction and post-treatment. Ketamine and mild stress caused by scruff restraint elicited reduced weight gain for the animals. No differences were noted on brain mass between controls and experimental groups and also between risperidone and *S. mucronata* groups. However, reduced brain volume was noted in experimental groups. Dopamine and acetylcholine concentration levels were high in groups which received risperidone and *S. mucronata*. These findings highlight that the antipsychotic potential of *S. mucronata* is similar to risperidone.

Introduction

Approximately 1 in every 8 individuals are affected by mental disorders globally, with approximately 1 in 300 or almost 25 million individuals affected by schizophrenia (WHO, 2022; de Bartolomeis et al., 2023). Schizophrenia is classified as one of the most serious mental disorders due to its complicated behavioural and cognitive symptoms (Gao et al., 2023) and the reduced life expectancy of individuals diagnosed with this disorder, which is 10–25 years less compared to the general population (Sheybani-Arani et al., 2023). The symptoms of this disorder include hallucinations and delusions (positive symptoms), asociality, anhedonia, blunted affect and alogia (negative symptoms) and impairment of working memory, attention and executive function (cognitive symptoms) (Granger et al., 2023). According to Ngubane and De Gama (2023), the symptoms of schizophrenia may overlap with the symptoms observed in patients reported to be bewitched or who have an ancestral calling. This is in accordance with the study by van der Zeijst et al. (2021) who reported the aforementioned symptoms of schizophrenia as symptoms also observed in individuals with an ancestral calling.

Western treatment options for schizophrenia include medication, psychosocial rehabilitation, family interventions and psychoeducation (WHO, 2022). The medication includes antipsychotic drugs which are commonly classified into typical (first generation) and atypical (second generation). Typical antipsychotic drugs attenuate the positive symptoms whilst lacking the ability to improve the negative and cognitive symptoms of schizophrenia (Luther et al., 2023; Shettima et al., 2023). Atypical antipsychotic drugs exhibit the efficacy in alleviating both the positive and negative symptoms of this disorder (Sheybani-Arani et al., 2023). Although atypical antipsychotic drugs are reported to result in less severe side effects compared to the typical drugs, all antipsychotic drugs are associated with adverse side effects ranging from a dry mouth

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or mild sedation (minor side effects), to akathisia, constipation and sexual dysfunction (unpleasant effects), to acute dystonias (painful effects), to tardive dyskinesia or weight gain (disfiguring effects) and to agranulocytosis or myocarditis which are categorised as life-threatening side effects (Osacka et al., 2022; Sheybani-Arani et al., 2023). Hence, the sustained efforts in exploring alternative interventions particularly for the negative symptoms since they affect approximately 40 % of patients diagnosed with schizophrenia and prove to be the most challenging (Velligan and Rao, 2023).

Various models for the pathophysiology of schizophrenia exist; however, the dysregulation of dopamine (DA) is regarded as the primary neurotransmitter landmark and the nigrostriatal, mesolimbic, mesocortical and tuberoinfundibular pathways are the four major dopaminergic pathways (Sonnenschein et al., 2020; de Bartolomeis et al., 2023). Dopamine plays a primary role in reward processing and a dysregulation in the reward circuit results in anhedonia (Lambert et al., 2018; Sonnenschein et al., 2020). In the mesolimbic pathway, excess production of DA from the ventral tegmental area (VTA) to the limbic areas results in the development of the positive symptoms of schizophrenia (Patel et al., 2014). On the other hand, the negative symptoms of schizophrenia are associated with low levels of DA from the VTA to the cortex in the mesocortical pathway (Patel et al., 2014; Shettima et al., 2023; Velligan and Rao, 2023). The blockage of D2 receptor in the mesocortical, nigrostriatal, and tuberoinfundibular pathways has also been reported to aggravate the negative symptoms of schizophrenia (de Bartolomeis et al., 2023). Risperidone (RIS), one of the four atypical antipsychotic drugs, is established to successfully treat negative symptoms of schizophrenia through its antagonist role on D2 DA and 5-hydroxytryptamine (5-HT) 2A receptors (Nguyen et al., 2023; Ramos-Méndez et al., 2023). However, the side effects of this drug include elevated prolactin levels, akathisia and extrapyramidal symptoms (Lang et al., 2023). Hence, the exploration of alternative medicine is warranted. The dysfunction in cholinergic systems is also linked with the pathophysiology of schizophrenia as acetylcholine plays a significant role in functions such as memory and learning processes (which are impaired in this disorder) handled in the hippocampus (de Bartolomeis et al., 2023).

In a recent study by Ngubane and De Gama (2023), the findings suggested that the commonly used approach for treating mental illness, particularly schizophrenia was through the administration of an herbal concoction. In a subsequent study, which aimed to investigate the plants (their phytochemicals and cytotoxicity) used by the THPs in herbal concoctions, THPs at two muthi (traditional medicine) markets (Berea and Dalton) recommended seven medicinal plants used or prescribed for the treatment of schizophrenia. These herbal plants included: *Dioscorea dregeana* (Kunth) T. Durand (tuber), *Boophone disticha* (bulb), *Afroaster hispida* (*Aster bakerianus*) (roots), *Salix mucronata* (Thunb) (bark), *Artabotrys monteiroae Oliv.* (roots), *Trimeria grandifolia* (Hochst) Warb. subsp. (roots) and *Clivia miniata* (Lindl.) (roots). *D. dregeana* has been reported to treat insanity, hysteria, epilepsy and psychosis (Maroyi, 2022). *B. disticha* was reported to treat unspecified mental disorders; however, it is also known to induce hallucinations (Bonokwane et al., 2022). A. hispida was documented in the treatment of chronic headaches and psychiatric disturbances (Kose et al., 2015; Mugomeri et al., 2016; Kose, 2017). *A. monteiroae Oliv.* and *T. grandifolia* were reported to treat hysteria (Mhlongo and Van Wyk, 2019) and unspecified mental disorders (Masondo et al., 2019), respectively. *S. mucronata* has been reported to treat headaches by El-Sawi et al. (2020); however, this herbal plant has not been previously reported to treat schizophrenia. Hence, the aim of this study was to investigate and document the effects of *S. mucronata* and risperidone on impaired social interaction and anhedonia in a ketamine-induced rat model.

Materials and methods

Animals

This study was divided into three phases; viz. adaptation (pre-induction), induction and treatment phases. A total of 66 male adult Sprague-Dawley rats (weighing between 200 and 300 g), at the beginning of the ketamine injection regime) were obtained from the Biomedical Research Unit of the University of KwaZulu-Natal. The rats were divided into two main groups, a control (Ctrl) group which received sterile saline (n=21) and an experimental (Exp) group which received ketamine (n=45) during the induction phase. From these groups, the rats were further subdivided into six groups for the treatment phase; viz. $Ctrl^{SAL}$, $Ctrl^{APD}$, Ctrl™, Exp^{SAL}, Exp^{APD} and Exp™ (where; SAL = saline, APD = antipsychotic drug and TM = traditional medicine). The animals were single-housed in order to avoid contact with each other (Sanavi et al., 2022). However, the cages were placed close to each other to maintain auditory and olfactory contact between the animals, thus minimising stress and aggression (Becker and Grecksch, 2004). The housing conditions were set at a 12-h light/dark cycle with lights on from 6:00 AM to 6:00 PM and the room temperature was kept constant at relative humidity. All experiments were conducted during the light phase. The rats were afforded seven days of acclimatization, monitored and gently managed by the primary investigator daily. All stipulated research protocols were adhered to in accordance to the guidelines approved by the Animal Research Ethics Committee of the University of KwaZulu-Natal (Ethics Clearance number: AREC/000/5188/2023). This study also received permission from the Department of Agriculture, Land Reform and Rural Development of the Republic of South Africa (Permit number: SDAH-Epi-23021009580).

Treatment and drug preparation

After acclimatization to laboratory conditions, behavioural tests were conducted prior to the inducement of schizophrenia symptoms with ketamine. Ketamine, at 30 mg/kg, was diluted in 0.9 % saline and administered intraperitoneally to the experimental animals for five consecutive days to induce symptoms of schizophrenia (Becker et al., 2003). The ratio of ketamine-saline solution was calculated based on the weight of each rat for the duration of the induction phase. This solution was freshly prepared daily with the dose determined according to the weight of each rat. The control groups received sub-chronic doses of 0.9 % sterile saline (0.5 ml/kg) as the experimental group via intraperitoneal injection for five days. The animals were given 24–48 hours of withdrawal after induction.

The antipsychotic drug, risperidone (Rispacor) (RIS), and one of the herbal plants (*Salix mucronata*) used by the THPs in KwaZulu Natal for the treatment of schizophrenia symptoms, were used during the treatment phase. The duration of treatment was a week (seven days) based on the findings by Potkin et al. (2003), which indicated that improvements of symptoms in patients with schizophrenia can be observed in the first week of treatment with RIS at a dose of 6 mg/day. The animal equivalent doses for risperidone and *S. mucronata* were calculated based on the weight of the animals on each day of treatment. The stock solution for RIS was prepared by dissolving 5 mg of powdered RIS in 1000 µl of 1 % DMSO, the required dose was then diluted with sterile phosphate buffed saline (PBS) as RIS was insoluble and formed instant precipitation in water and normal saline. The stock solution of *S. mucronata* was also prepared by dissolving 5 mg of the methanol extract of this plant in 1000 µl of 1 % DMSO, distilled water was then used to dilute DMSO and as a vehicle for the required dose from this solution. The solutions were freshly prepared on each day of treatment and were administered orally via oral gavage.

Behavioural tests (BTs)

The 3-chambered sociability and sucrose preference tests were used to test the rats for asociality and anhedonia, respectively. This was conducted pre-induction (before inducement with ketamine on the experimental group), post-induction (24–48 hours after the last ketamine injection on day five) and post-treatment with saline (SAL), Risperidone (APD) and the herbal plant methanol extract of *S. mucronata* (Fig. 1).

Crawley's sociability test

Crawley's sociability test also known as the three-chambered paradigm test was used to assess social interaction behaviours (Sanavi et al., 2022). The Ugo Basile sociability apparatus (with transparent walls, partitions, and lids; 120×40×40(h) cm, with 2 grid cages) was used and the protocol was adapted from Sanavi et al.'s (2022) protocol. All rats, viz. test and the 2 strangers were of the same weight, age and sex. The apparatus were cleaned using tap water and 70 % ethanol between each test. The 3 phases of this experimental procedure are depicted in Fig. 2.

During the Sociability and Novelty phase, time spent by the test animal near or interacting (sniffing, touching, biting and/or scratching) with the caged animal/s or empty cage was recorded as Time (N), and the time spent by the test animal far or not interacting with the caged animals or empty cage was recorded as Time (F). And the total time spent in each chamber with the caged animals and the empty cage was also recorded. The object Recognition Index (RI) for sociability was calculated as follows:

$$RI = \frac{Stranger1 \quad (TN)}{Stranger1 \quad (TN) + Empty\ Chamber(TN)}$$

Where; Stranger 1 (TN) was the time spent by the test animal interacting with the caged Stranger 1 animal, and Empty Chamber (TN) was the time spent by the test animal exploring the empty cage during the sociability phase.

The RI for novelty was calculated as follows:

$$RI = \frac{Stranger2 \quad (TN)}{Stranger2 \quad (TN) + Stranger1 \quad (TN)}$$

Where: Stranger 1 (TN) was the time spent by the test animal interacting with the caged Stranger 1 animal/familiar animal from the sociability phase, and Stranger 2 (TN) was the time spent by the test animal interacting with the novel animal/Stranger 2 introduced during the novelty phase.

The closer the RI value to 1 the more social the animals are and the more they show preference for a novelty.

Sucrose preference test

The two-bottle methodology for assessing sucrose preference is an effective test that can be used in the investigation of anhedonia (Eagle et al., 2016). The two-bottle experiment allows for a comparison between behavioural preferences for water to a preference for the sucrose solution. Two 100 ml bottles were placed on either side of the home cages, one with normal drinking water and one with 1 % sucrose solution. The bottles were weighed for 4 consecutive days and the side of each bottle was alternated every day to avoid side bias. The sucrose preference percentage was calculated using the formula below:

$$\%\text{Sucrose Preference} = \frac{Consumed\ sucrose\ solution}{Total\ liquid\ consumption} \times \quad 100$$

Brain morphometry

After all procedures were completed, in order to avoid the interference of anaesthesia with the chemistry of the tissues, particularly neural tissue, rapid non-pharmacologic euthanasia (decapitation) was used to sacrifice the animals (Bekhbat et al., 2016). The brain was removed from the skull as per Collins et al. (2018) protocol. After removal from the skull, the brain was immediately weighed using a digital weighing balance (g) and measured using a digital Vernier caliper (mm). All measurements were conducted three times by one investigator for intra-observer reliability. A second investigator also conducted the measurements independently for inter-observer reliability. The measurements included: anterior-posterior length, lateral width and superior-inferior height (Fig. 3). All measurements were analysed for error in order to obtain the intra-observer coefficient (ICC) and Confidence Intervals (95 % CI). These measurements were then used to calculate the volume of the brain (Volume = Length × Width × Height). The frontal lobe, ventral tegmental area and the hippocampus on both the right and left sides were then dissected out from the brain for further biochemical analysis.

Dopamine and acetylcholine ELISA

Brain tissue samples [prefrontal cortex (PFC), ventral tegmental area (VTA) and hippocampus] were weighed and rinsed and homogenised using PBS. Thereafter, the homogenates were centrifuged at 5000xg for 5 minutes and the supernatant was collected and used for further analysis. The reagents for the ELISA assays were prepared as per Elabscience® user manuals for dopamine (Catalog no. E-EL-0046) and acetylcholine (Catalog no. E-EL-0081). The kit and all samples were brought to room temperature before they were used. A total of four plates were used, two dopamine plates for PFC and VTA samples, and two acetylcholine plates for PFC and hippocampus samples.

The assay procedure for each plate was as follows: 50 µL of the standard or sample was added to each well and was followed by immediate addition of 50 µL of Biotinylated Detection Ab to each well. Thereafter, the plate was incubated at 37°C for 45 minutes. After the contents of the plate were aspirated, the plate was washed 3 times with wash buffer solution. This was followed by the addition of 100 µL of HRP Conjugate to each well and the plate was incubated at 37°C for a further 30 minutes. After the contents of the plate were aspirated again, the plate was washed 5 times using a wash buffer solution. Thereafter, 90 µL of Substrate reagent was added to each well and the plate was incubated at 37°C for 15 minutes. Lastly after incubation, 50 µL of Stop solution

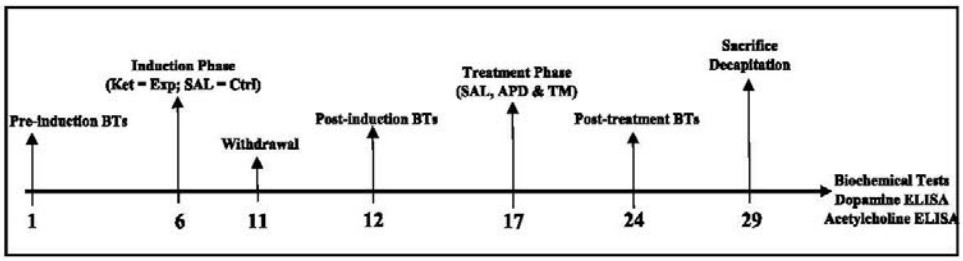


Fig. 1. Timeline of phases of the implementation procedure.

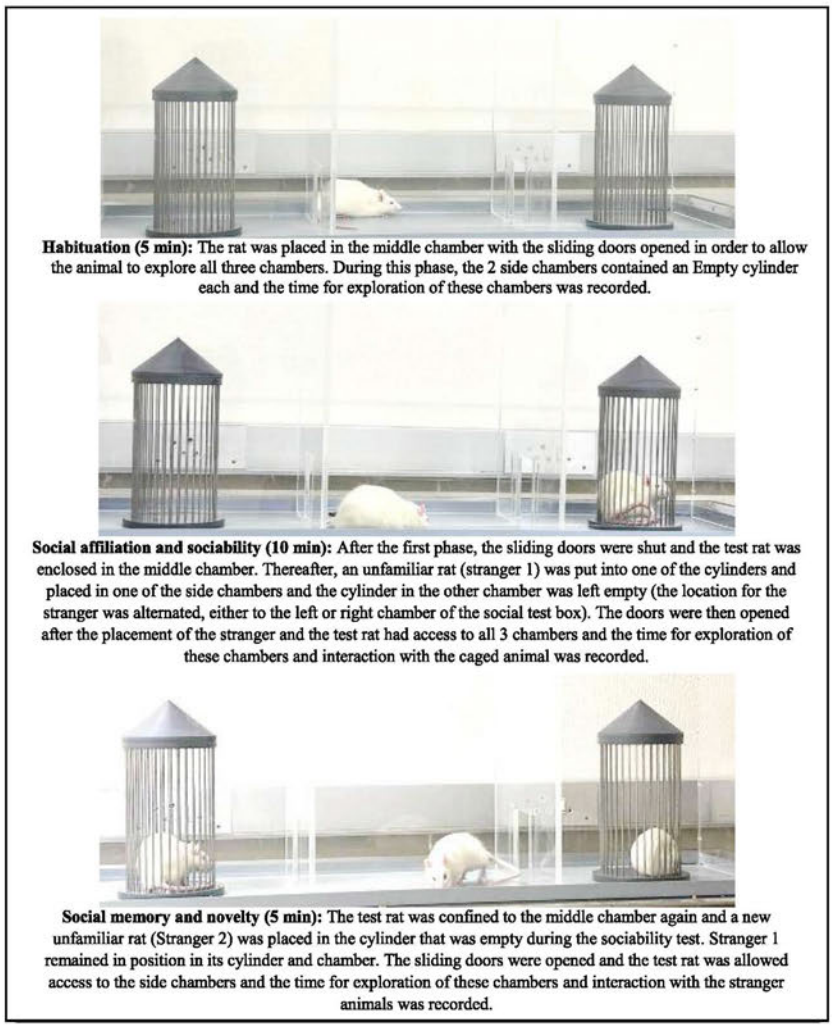


Fig. 2. Phases of the Crawley's sociability test.

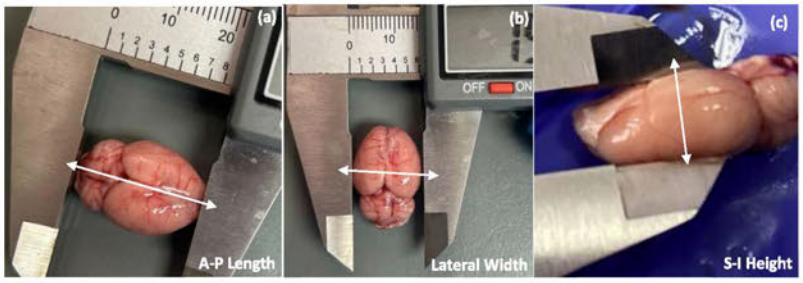


Fig. 3. Measurements of the brain: (a) length from the most anterior part of the frontal lobe to the most posterior part of the cerebellum, (b) width from the most lateral and convex part of one side of the brain to the most lateral and convex part on the other side, and (c) height from the most superior part of the brain to the most inferior part.

was added to each well and the plate was read immediately at 450 nm. The concentrations of dopamine (DA) and acetylcholine (ACh) present in each sample were determined using a standard curve.

Data analysis

A paired samples T-test was used to analyse the findings when comparing between pre- and post-induction data or when comparing post-induction with post-treatment findings on the same group. The One-way ANOVA test was used to analyse findings between all treatment groups during all phases of the experiment. The Two-way ANOVA test was used to assess the effect and interaction between ketamine treatment and drug effect. The Post Hoc test Tukey was applied for its most attraction properties. IBM SPSS version 28.0 for Windows software and Microsoft Excel (2016) were used for data analysis and for graph presentation, respectively. Values of $p<0.05$ were considered statistically significant.

Results

Effect of Ketamine, Risperidone and S. Mucronata on Weight Gain

During the adaptation/pre-induction phase, the average weight gained by the rats was 40 g, 49.29 g, 47 g, 54.13 g, 45.87 g and 43.33 g for $Ctrl^{SAL}$, $Ctrl^{APD}$, Ctrl™, Exp^{SAL}, Exp^{APD} and Exp™ groups, respectively. This dropped post-induction, the weight gain for $Ctrl^{SAL}$, $Ctrl^{APD}$ and Ctrl™ was 25 g, 19.29 and 17.71 g, respectively. The weight gained by the groups which received ketamine was lower compared to the control group ($p<0.001$), with the average weight gain for Exp^{SAL}=14.73 g, Exp^{APD}=12.53 g and Exp™=13.33 g. Post-treatment, the average weight gain increased for all groups except for $Ctrl^{APD}$ (18.43 g), with groups $Ctrl^{SAL}$, Ctrl™, Exp^{SAL}, Exp^{APD} and Exp™ gaining 32 g, 25.14 g, 30.27 g, 19.47 g and 27.67 g, respectively (Fig. 4). The overall weight gained by the experimental groups remained lower compared to the control groups (p=0.003).

Social interaction: sociability

Based on the findings from the sociability phase of the Crawley's sociability test, the time spent interacting with the caged animal (Stranger 1) reduced significantly post-induction for all animals which received ketamine (Fig. 5). Time (N) pre-induction for Exp^{SAL}, Exp^{APD} and Exp™ groups was 241.11 s, 215.46 s and 208.09 s, respectively; which decreased post-induction to 130.51 s, 126.47 s and 133.83 s, respectively. Post-induction, statistical significance was noted between Ctrl™ vs. EXP™ for Time (N) in chamber with Stranger 1 (p=0.003). Statistical significance was also noted between $Ctrl^{APD}$ vs Exp^{APD} for Time (F) in chamber with Stranger 1 post-induction (p=0.007). Post-treatment, Time (N) increased slightly for Exp^{SAL} to 140.13 s and increased significantly for Exp^{APD} and Exp™ to 180.24 s and 181.88, respectively. It was also noted that Time (N) for control groups also decreased post-induction; however, not as sharply as observed for the experimental groups. Time (N) pre-induction for $Ctrl^{SAL}$, $Ctrl^{APD}$ and Ctrl™ was 272.27 s, 264.67 and 306.28 s, respectively; post-induction this decreased to 213.55 s, 205.41 and 245.72, respectively. Post-treatment, Time (N) continued to decrease for all control groups to 187.74 s, 176.91 and 138.50 s for $Ctrl^{SAL}$, $Ctrl^{APD}$ and Ctrl™, respectively. Ctrl™ spent more time on chamber with an empty cage (302.89 s) compared to the total time spent on the chamber with Stranger 1 (215.95 s), and a statistical significance was noted between Ctrl™ and Exp™ (p=0.038) for total time spent in the chamber with an empty cage. The sociability RI decreased post-induction and decreased further post-treatment for all control groups, the decrease was particularly noticeable for Ctrl™. Pre-induction RI for $Ctrl^{SAL}$, $Ctrl^{APD}$ and Ctrl™ was 0.84, 0.83 and 0.87, respectively. Post-induction, RI for $Ctrl^{SAL}$, $Ctrl^{APD}$ and Ctrl™ was 0.77, 0.79 and 0.66, and post-treatment RI for these groups was 0.70, 0.68 and 0.58, respectively. For experimental groups, RI pre-induction for Exp^{SAL}, Exp^{APD} and Exp™ was 0.80, 0.70 and 0.70, respectively. Post induction and post-treatment the RI for these experimental groups was 0.79, 0.80 and 0.74, and 0.77, 0.84 and 0.77, respectively. For Exp^{APD} and Exp™, the RI post-treatment was higher compared to RI post-induction which was higher compared to RI pre-induction. The p-values for sociability RI were 0.388, 0.314 and 0.042 for pre-induction, post-induction and post-treatment phases, respectively. Statistical significance was noted in the post-treatment phase. Based on the Tukey post hoc test, No statistical significance was noted between groups in the pre- and post-induction phases, statistical significance was noted between Ctrl™ vs Exp^{APD} (p=0.027).

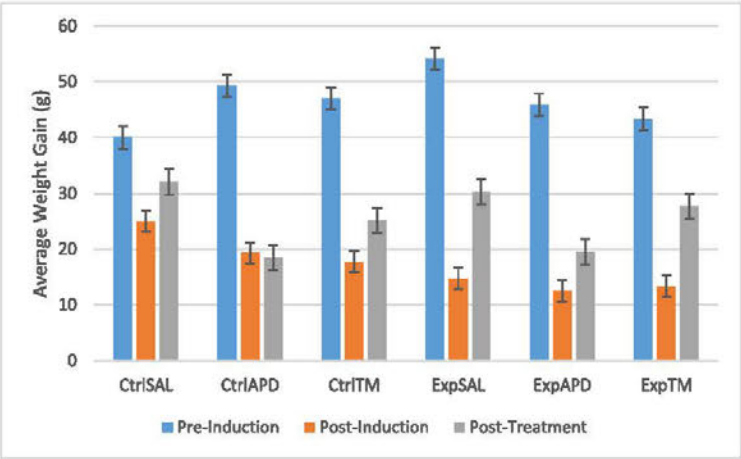


Fig. 4. Weight gain average pre-induction, post-induction and post-treatment. The weight mean difference between the different phases (pre- and post-induction, and post-treatment were determined in order document the weight gained by animals in their groups after each phase. The weight gained by the animals was calculated as follows: Pre-induction Adaptation Day 7 wt – adaptation Day 1 wt Post-induction Induction Day 5 wt – induction Day 1 wt Post-treatment Treatment Day 7 – treatment Day 1 wt No statistical significant interactions were noted for weight gained during pre- and post-induction, and post-treatment phases. The interactional effects were as follows: Pre-induction: $F(2,60)$ 0.910, p 0.408; Post-induction: $F(2,60)$ 0.202, p 0.818; Post-treatment: $F(2,60)$ 0.900, p 0.421.

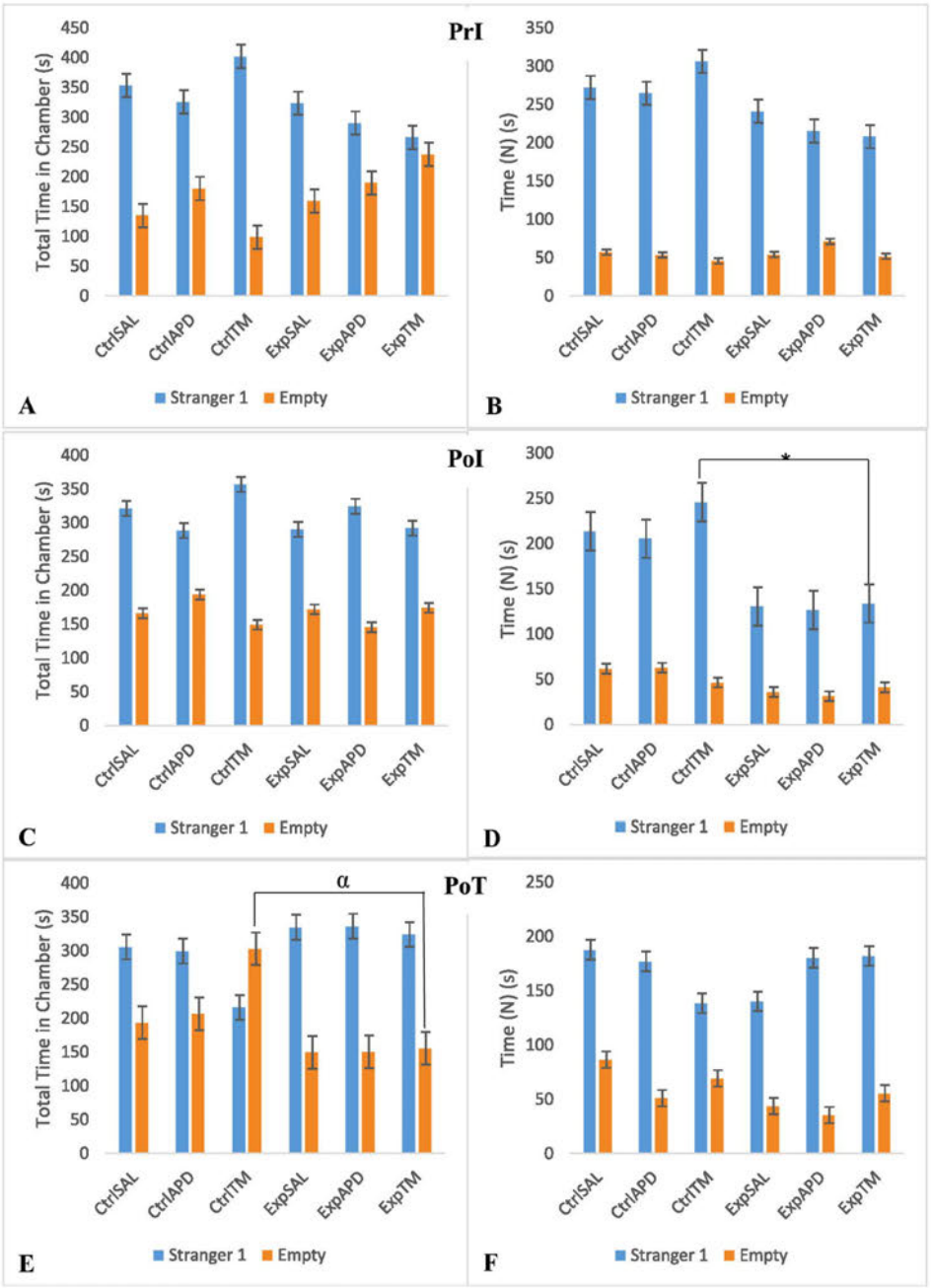
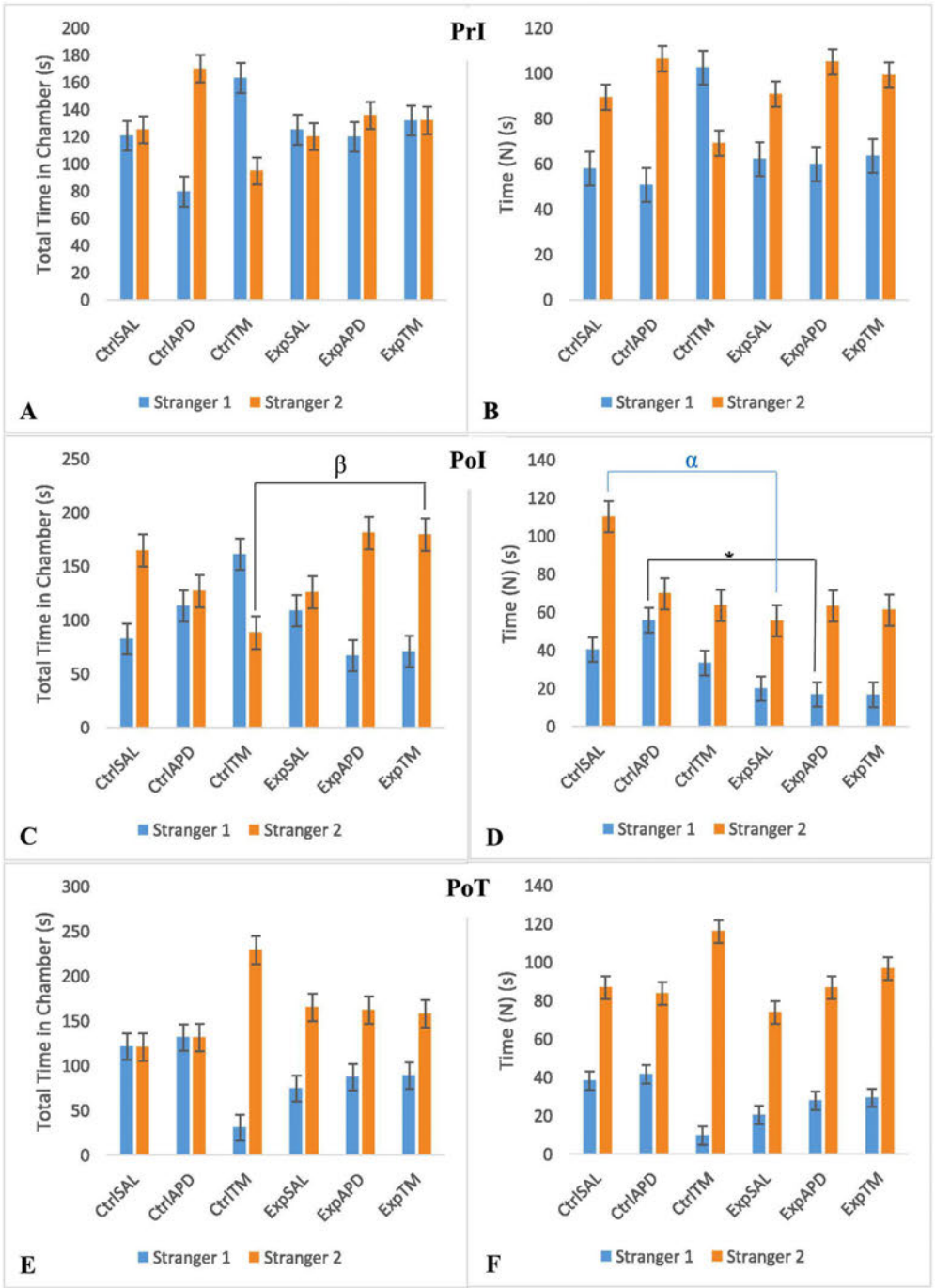


Fig. 5. Sociability phase of the 3-chambered sociability test; (PrI, A-B) pre-induction; (PoI, C-D) post- induction and (PoT, E-F) post-treatment. After 5 min of habituation, all animals (n=66) were allowed to explore the 3 chambers of the apparatus (one at a time); and a randomly selected Stranger 1 (S1) animal (1 of n=20 of animals which served as strangers and were not part of the test animals) was introduced in a cage within one of the chambers. The order of test animals to undergo the sociability test was random and the side (left or right) where Stranger 1 was introduced was also selected randomly. The other cage on the other side was left empty (E) during this phase. Findings in this Figure are presented for time spent interacting [TN, or Time (N)] with the empty cage or cage with S1 for the pre- and post-induction, and post-treatment phases. Total time (TT) spent in each chamber with these cages was also noted. Statistical significance was noted in post-induction Time (N) in chamber with Stranger 1 for Ctrl™ vs Exp™, p=0.003 (*), and in post-treatment total time spent in chamber with empty cage for Ctrl™ vs Exp™, p=0.038 (α). No statistical significant interaction was noted for the sociability phase; however, marginal significance (**) was noted for time spent interacting with S1 post-treatment. The interactional effects for this phase were as follows: Pre-induction: S1(TN) – $F(2,60)$=0.535, p=0.588; S1(TT) – $F(2,60)$=0.815, p=0.448; E (TN) - $F(2,60)$=0.159, p=0.853; E(TT) - $F(2,60)$=0.979, p=0.382 Post-induction: S1(TN) – $F(2,60)$=0.400, p=0.672; S1(TT) – $F(2,60)$=1.207, p=0.306; E(TN) - $F(2,60)$=0.914, p=0.406; E(TT) - $F(2,60)$=0.815, p=0.447 Post-treatment: S1(TN) – $F(2,60)$=2.590, p=0.083**; S1(TT) – $F(2,60)$=0.830, p=0.441; E(TN) - $F(2,60)$=1.069, p=0.350; E(TT) - $F(2,60)$=1.368, p=0.263.



(caption on next page)

Fig. 6. Novelty phase of the 3-chambered sociability test; (PrI, A-B) pre-induction; (PoI, C-D) post- induction and (PoT, E-F) post-treatment. After 10 min of the sociability test, all animals (n 66) were allowed to again explore the 3 chambers of the apparatus (one at a time); and a randomly selected Stranger 2 (S2) animal (also 1 of n 20 of animals which served as strangers and were not part of the test animals) was introduced in the cage which was previously empty during the sociability phase of the social interaction test. During the novelty phase (5 min), the test animal animals had access to explore or interact with either the familiar animal (Stranger 1/S1) or the novel animal (Stranger 2/S2). The test animals completed all phases of the social interaction test (habituation, sociability and novelty) before another randomly selected test animal was brought into the behavioural testing room to also complete the 3 phases. Findings in this Figure are presented for time spent interacting [TN, or Time (N)] with caged S1 and caged S2 for the pre- and post-induction, and post-treatment phases. Total time (TT) spent in each chamber with these cages was also noted. Statistical significance was noted in post-induction Time (N) in chamber with Stranger 1 for $Ctrl^{APD}$ vs Exp^{APD}, p 0.021 (*); post-induction Time (N) in chamber with Stranger 2 for $Ctrl^{SAL}$ vs Exp^{SAL}, p 0.029 (α); post-induction Total Time in chamber with Stranger 2 for Ctrl™ vs EXP™, p 0.047 (β). Statistical and marginal significant interactions (**) were noted post-induction and post-treatment for both time spent interacting with S1 and S2, and also total time spent in each chamber with the caged animals. The interactional effects for this phase were as follows: Pre-induction: S1(TN) – F(2,60) 0.972, p 0.384; S1(TT) – F(2,60) 0.820, p 0.445; S2(TN) - F(2,60) 0.429, p 0.653; S2(TT) - F(2,60) 0.942, p 0.396 Post-induction: S1(TN) – F(2,60) 1.017, p 0.368; S1(TT) – F(2,60) 3.508, p 0.036**; S2(TN) - F(2,60) 2.806, p 0.068**; S2(TT) - F(2,60) 4.750, p 0.012** Post-treatment: S1(TN) – F(2,60) 3.715, p 0.030**; S1 (TT) – F(2,60) 3.635, p 0.032; S2(TN) - F(2,60) 0.352, p 0.704; S2(TT) - F(2,60) 3.832, p 0.041**.

Social interaction: novelty

Based on the findings from the novelty phase of the Crawley's sociability test, all groups preferred interacting with Stranger 2 as opposed to Stranger 1 (Fig. 6). However, the time spent interacting with Stranger 2 decreased post-induction. Time (N) pre-induction for $Ctrl^{SAL}$, $Ctrl^{APD}$, Ctrl™, Exp^{SAL}, Exp^{APD} and Exp™ was 89.60 s, 106.62 s, 69.42 s, 91.04 s, 105.36 s and 99.48 s, respectively; post-induction this decreased to 69.98 s, 63.78 s, 55.73 s, 63.45 s and 61.39 s for $Ctrl^{APD}$, Ctrl™, Exp^{SAL}, Exp^{APD} and Exp™, respectively, while it increased to 110.41 s for $Ctrl^{SAL}$. Post-treatment, the time spent interacting with Stranger 2 decreased to 87.10 s for $Ctrl^{SAL}$ while it increased to 83.96 s, 116.28 s, 74.06 s, 87 s and 96.85 s for $Ctrl^{APD}$, Ctrl™, Exp^{SAL}, Exp^{APD} and Exp™, respectively. It was also noted that the time spent interacting with Stranger 1 also decreased post-induction for all groups. Time (N) for Stranger 1 pre-induction was 58.17 s, 50.86 s, 102.77, 62.34 s, 60.13 s and 63.76 s for $Ctrl^{SAL}$, $Ctrl^{APD}$, Ctrl™, Exp^{SAL}, Exp^{APD} and Exp™, respectively. Post-induction this decreased to 40.53 s, 33.51 s, 20.16 s, 16.94 s and 16.85 s for $Ctrl^{SAL}$, Ctrl™, Exp^{SAL}, Exp^{APD} and Exp™, respectively, but for $Ctrl^{APD}$ it slightly increased to 56.02 s. Overall, Time (N) decreased for the groups which received ketamine compared to the control groups. Post-treatment, Time (N) spent interacting with Stranger 2 for Exp^{APD} and Exp™ increased significantly to 28.08 s and 29.56 s, respectively, compared to Exp^{SAL} which showed no improvement with 20.63 s. Time (N) for $Ctrl^{SAL}$, $Ctrl^{APD}$ and Ctrl™ decreased to 38.51 s, 41.79 s and 9.92 s, respectively, and it decreased significantly for Ctrl™. The novelty RI increased post-induction for $Ctrl^{SAL}$ and Ctrl™ and decreased for $Ctrl^{APD}$. Post-treatment RI decreased for $Ctrl^{SAL}$, it stayed more or less the same for $Ctrl^{APD}$ and increase for Ctrl™. Pre-induction RI for $Ctrl^{SAL}$, $Ctrl^{APD}$ and Ctrl™ was 0.60, 0.70 and 0.37, respectively. Post-induction, RI for $Ctrl^{SAL}$, $Ctrl^{APD}$ and Ctrl™ was 0.76, 0.58 and 0.55, and post-treatment RI for these groups was 0.61, 0.56 and 0.94, respectively. For experimental groups, RI pre-induction for Exp^{SAL}, Exp^{APD} and Exp™ was 0.59, 0.70 and 0.59, respectively. Post induction and post-treatment the RI for these experimental groups was 0.69, 0.79 and 0.79, and 0.74, 0.72 and 0.77, respectively. For Exp^{SAL} RI increased post-induction and further increased post treatment. For Exp^{APD} and Exp™, the RI increased post-induction and then decreased post treatment. The p-values for novelty RI were 0.424, 0.098 and 0.072 for pre-induction, post-induction and post-treatment phases, respectively. Marginal significance was noted in the post-induction and post-treatment phases. Based on the Tukey post hoc test, No statistical significance was noted between groups in the pre- and post-induction phases, marginal significance was noted between Ctrl™ vs Exp^{APD} (p=0.057).

Sucrose preference

The preference for 1 % sucrose solution for both control and experimental groups decreased post-induction and further decreased post-treatment, with SP% for experimental groups remaining higher than that of control groups post-induction and post-treatment (Fig. 7). The SP % pre-induction for control and experimental groups was 61.26 % and 63.46 %, respectively; post-induction it decreased to 48.30 % and 56.58 %, respectively; and post-treatment it further decreased to 28.64 % and 33.05 %, respectively. Pre-induction, the SP% for $Ctrl^{SAL}$, $Ctrl^{APD}$, Ctrl™, Exp^{SAL}, Exp^{APD} and Exp™ was 58.18 %, 65.25 %, 60.36 %, 69.60 %, 63.68 % and 57.08 %, respectively. Post-induction, the SP% for $Ctrl^{SAL}$, $Ctrl^{APD}$, Ctrl™, Exp^{SAL}, Exp^{APD} and Exp™ was 31.89 %, 48.07 %, 64.93 %, 55.93 %, 51.90 % and 61.90 %,

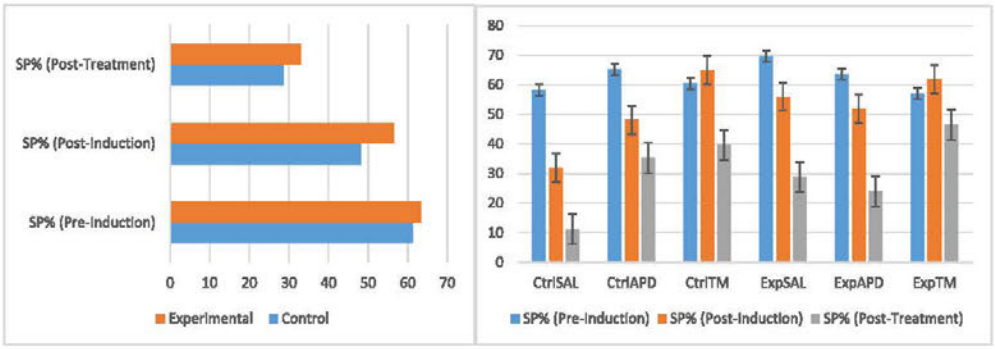


Fig. 7. Sucrose preference percentages (SP%). Anhedonia was measured using preference for normal drinking water over a sucrose solution. All test animals (n 66) were given 2 × 50 ml bottles (one with normal drinking and water and one with 1 % sucrose solution) in their home cages for 4 consecutive days. The sides which these bottles were placed was alternated every day. Liquid consumed was measured and the sucrose preference percentages (SP%) were calculated for each day. The sucrose preference test was done for all 3 phases of the experiment (pre- and post-induction, and post-treatment). No statistical significant interactions were noted for sucrose preference. The interactional effects for this phase were as follows: Pre-induction SP%: F(2,60) 0.412, p 0.664; Post-induction SP%: F(2,60) 0.701, p 0.500; Post-treatment SP%: F(2,60) 0.877, p 0.421.

respectively. Post- treatment, the SP% for $Ctrl^{SAL}$, $Ctrl^{APD}$, $Ctrl^{TM}$, Exp^{SAL}, Exp^{APD} and Exp^{TM} was 11.14 %, 35.18 %, 39.61 %, 28.87 %, 23.87 % and 46.42 %, respectively. No statistical significance was noted between all groups for sucrose preference (p-value range between all groups was 0.230–1.000).

Brain morphometry

The A-P length for $Ctrl^{SAL}$, $Ctrl^{APD}$, $Ctrl^{TM}$, Exp^{SAL}, Exp^{APD} and Exp^{TM} was 21.47 mm, 20.89 mm, 20.73 mm, 20.24 mm, 18.35 mm and 18.57 mm, respectively (intra-observer ICC=0.871; 95 % CI: 0.678–0.948; inter-observer ICC=0.935; 95 % CI: 0.875–0.971). The lateral width for $Ctrl^{SAL}$, $Ctrl^{APD}$, $Ctrl^{TM}$, Exp^{SAL}, Exp^{APD} and Exp^{TM} was 14.68 mm, 14.08 mm, 14.32 mm, 13.29 mm, 13.12 mm and 11.87 mm, respectively (intra-observer ICC=0.899; 95 % CI: 0.752–0.959; inter-observer ICC=0.946; 95 % CI: 0.896–0.976). The S-I height for $Ctrl^{SAL}$, $Ctrl^{APD}$, $Ctrl^{TM}$, Exp^{SAL}, Exp^{APD} and Exp^{TM} was 8.88 mm, 8.56 mm, 7.98 mm, 8.28 mm, 7.81 mm and 6.54 mm, respectively (intra-observer ICC=0.788; 95 % CI: 0.481–0.914; inter-observer ICC=0.910; 95 % CI: 0.822–0.960). No statistical significance was noted for all measurements between control and experimental groups which received the same treatment solutions (p-value range between all groups was 0.108–0.803). However, statistical significance was noted for $Ctrl^{SAL}$ vs. Exp^{APD} (p=0.024) and $Ctrl^{SAL}$ vs. Exp^{TM} (p=0.044) A-P length. There was no significant difference noted between the controls and experimental groups on brain mass (p- value range between all groups was 0.995–1.000); however, the brain volume varied significantly between these groups (Fig. 8). The brain mass for $Ctrl^{SAL}$, $Ctrl^{APD}$, $Ctrl^{TM}$, Exp^{SAL}, Exp^{APD} and Exp^{TM} was 1.96 g, 1.89 g, 1.92 g, 1.91 g, 1.97 g and 1.93 g, respectively. The calculated brain volume for $Ctrl^{SAL}$, $Ctrl^{APD}$, $Ctrl^{TM}$, Exp^{SAL}, Exp^{APD} and Exp^{TM} was 2.84 cm2, 2.61 cm2, 2.41 cm2, 2.24 cm2, 1.94 cm2 and 1.99 cm2, respectively.

Biochemical analysis

The DA concentration in the PFC was higher in both the control and experimental groups which received RIS and the *S. mucronata* extract with a statistical significant difference noted between the Exp^{SAL} and Exp^{TM} groups (p<0.001). The DA concentration in the VTA was also higher in the control and experimental groups which received RIS and the *S. mucronata extract*; however, there was no statistical significance noted between these groups (p-value range=0.065–1.000 between all groups). The ACh concentration in the PFC was higher in the control groups which received RIS and the *S. mucronata* extract with a statistical significant difference noted between $Ctrl^{SAL}$ and $Ctrl^{APD}$ (p=0.028). There was no difference noted for ACh concentration on the PFC in the experimental groups. There was also no difference noted in the ACh concentration in the hippocampus between all treatment groups; however, this concentration was lower in the Exp^{TM} group compared to all other groups.

Discussion

The modelling of a complete symptomatic spectrum of psychiatric disorders in animals remains a challenge for researchers; hence, animal models have been extensively used to enhance understanding of this human phenomenon; by reproducing these disorders, to understand their etio-pathology and study the effects of new treatments (Geyer and Markou, 1995). The limitation in using animal models is that laboratory animals lack higher mental function as they have a simpler brain cortical structure (Bialon and Wasik, 2022). Irrespectively, selected symptoms can be modelled successfully (Ellenbroek and Cools, 2000). In this study, a pharmacological animal model for schizophrenia symptoms was created using sub-chronic administration of ketamine. Exposure to chronic ketamine has been reported to reduce appetite and weight gain in both adult rats and humans (Parise et al., 2013), which was successfully done and explained by the decrease in weight gained by the animals post-induction in the current study. The control groups also showed a decrease in weight gained post-induction even though they were injected with sterile saline and not ketamine, this could be explained by the use of scruff restraint for intraperitoneal injection which is categorised as a mild stressor that animals do not adapt to (Lindsay et al., 2021). The scruff restraint could have also impaired their locomotor response (Lindsay et al., 2021), thus reducing their food consumption leading to lower average weight gain.

The administration of ketamine led to social withdrawal effects on the experimental groups in the current study, indicating a successful model for asociality. Control groups also showed signs of social withdrawal, which were not as significant as those in the experimental groups, which might have been induced by the aforementioned mild stress caused by scruff restraint (Lindsay et al., 2021). Social isolation could be another contributing factor to the change in behaviour observed in the control groups (Shangase et al., 2023). Post-treatment, a significant reverse in social withdrawal was noted in the experiment groups which received the RIS and *S. mucronata*. Contrarily, the control groups did not show improvement in social withdrawal post-treatment.

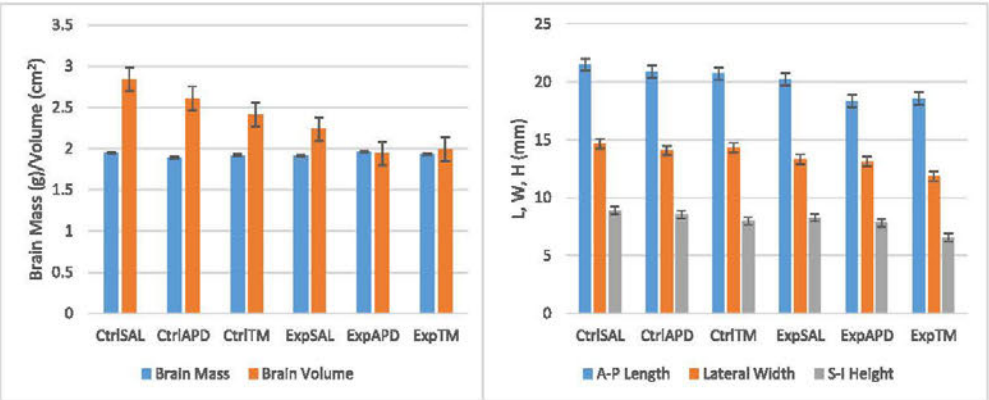


Fig. 8. Brain morphometry. After the test animals (n 66) were sacrificed, their brains were harvested and their mass, length (L), width (W) and height (H) were measure and the volume was calculated from L, W and H. No statistical significant interactions were noted for brain morphology. The interactional effects were as follows: Mass: F(2,60) 0.167, p 0.846; Volume: F(2,60) 0.217, p 0.805; Length: F(2,60) 0.490, p 0.615; Width: F(2,60) 0.820, p 0.445; Height: F(2,60) 0.461, p 0.633.

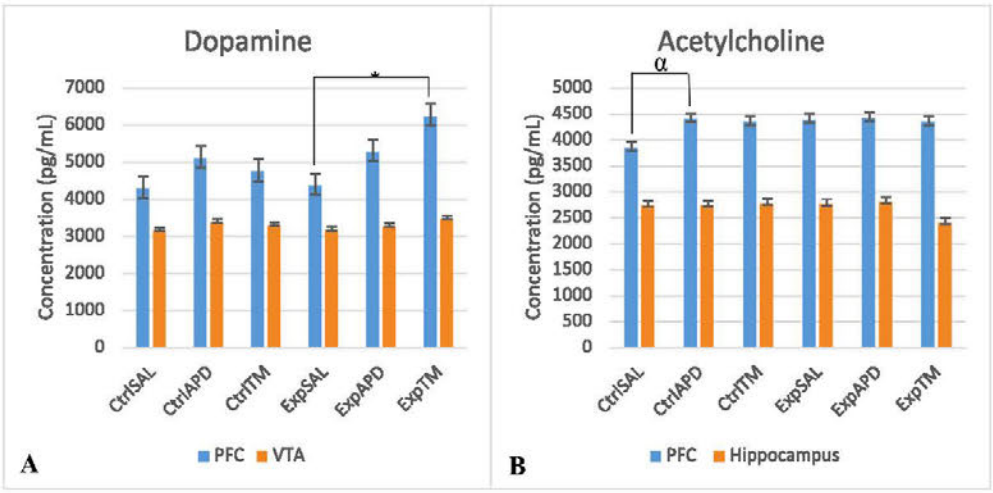


Fig. 9. Dopamine (in PFC and VTA) and Acetylcholine (in PFC and Hippocampus) concentrations. Key: After the brain was measured for all test animals (n 66), the prefrontal cortex, ventral tegmental area and hippocampi were harvested from both sides of the brains. ELISA kits were used to determine the dopamine and acetylcholine concentrations from the harvested parts of the brain. Statistical significance was noted for DA PFC Exp^{SAL} vs Exp^{TM}, $p<0.001$ (*), and for ACh PFC $Ctrl^{SAL}$ vs $Ctrl^{APD}$, p 0.028 (α).Statistical significant interaction was noted for the PFC dopamine concentration and marginal significance was noted for PFC acetylcholine concentration. The interactional effects were as follows: DA_PFC: F(2,60) 2.606, p 0.082**; DA_VTA: F(2,60) 1.258, p 0.321; ACh_PFC: F(2,60) 4.181, p 0.020**; ACh_Hippo: F(2,60) 0.579, p 0.564.

Snigdha and Neill (2008) also reported that selected antipsychotics alleviate social deficits which were induced by PCP in this study. Pre-induction, all animals showed preference for social novelty; however, the time spent interacting with Stranger 2 significantly decreased for the experimental groups post-induction. The time spent interacting with Stranger 1 also decreased post-induction. Post-treatment, a significant increase in the preference for social novelty was noted for the experimental groups which received RIS and *S. mucronata*. Furthermore, antipsychotics such as clozapine have also been reported to reverse deficits of social novelty discrimination in a study by Harich et al. (2007). Social withdrawal from Stranger 2 or social novelty improved post-treatment for the control groups which received RIS and *S. mucronata*, and the control group which received saline showed no improvement in social novelty.

Post-induction, sucrose preference for all groups decreased with the SP% for experimental groups being higher compared to control groups. This suggests that the groups which received ketamine were less anhedonic compared to the rats which did not receive ketamine. Sucrose preference continued to decrease for all groups post-treatment; however, the SP% for both the control and experimental groups which received *S. mucronata* was higher compared to other groups and the SP% for RIS receiving animals was only higher in controls. This suggests that the herbal extract had a slight potential in alleviating anhedonia which has not been reported previously.

According to Wulff et al. (2023), following chronic stress, the preference of a sucrose solution diminishes in stress-naive rodents. This might explain the continued decrease in the sucrose preference post-induction and post-treatment for all groups as all animals in the current study were exposed to some level of stress, contributed to by isolation and handling during the induction and treatment phases and also during behavioural tests. The further decrease in sucrose preference post-treatment can also be explained by findings by Gres et al. (2023) stating that antipsychotic medication can induce anhedonia by increasing the sensitivity to DA in the reward pathway. Nevertheless, a single administration of a sub-anaesthetic dose of ketamine has been reported to possess antidepressant effects in treatment-resistant depressed patients (Zanos and Gould, 2018); thus, the anhedonic-state in the experimental groups post- induction might have been alleviated by the sub-chronic administration of ketamine in the current study. Antidepressants influence anhedonia as this symptom is common in depressive disorders and is also one of the persistent negative symptoms of schizophrenia (Gres et al., 2023).

There was no difference between the brain mass across all control and experimental groups. However, a decrease in brain volume was noted in all experimental groups which received ketamine. The control and experimental groups which received saline during the treatment period had a higher brain volume compared to the groups which received RIS and *S. mucronata*. These findings are similar to the observations reported by Veijola *et al.* (2014) whereby the reduction in brain volume in schizophrenia was noted and both atypical and typical antipsychotic medications were reported to also contribute to these reductions. The whole brain volume reduction was also reported in clinical high risk patients for psychosis in a meta- analysis by Vissink et al. (2022). It was also noted that the A-P length, lateral width and S-I height measurements were also lower for experimental groups compared to control groups. The control and experimental groups which received saline during treatment had higher A-P length compared to the groups which received RIS and *S. mucronata*. The experimental group which received *S. mucronata* had the lowest lateral width, while the control and experimental groups which received *S. mucronata* had the lowest S-I height. This suggests that *S. mucronata* also contributed to reduction of the brain volume as similar to RIS.

Cognitive function is regulated by the cortical DA projections and processes, while flexible behaviour, attention and working memory, are influenced by DA in the PFC (Sesack and Carr, 2002). Dopamine functionality is determined by the origin of the dopamine neurons, and neurons originating from the VTA are associated with reward prediction error (Ott and Nieder, 2019). Through the mesocortical dopamine pathway, the midbrain dopamine neurons in the VTA are projected to the PFC (Ott and Nieder, 2019). The dopamine concentration in the PFC for control and experimental groups which received RIS and *S. mucronata* were higher compared to the groups which received saline in the current study. This suggests that RIS and *S. mucronata* increased the levels of DA in the PFC which is in accordance with the findings reported by Mork et al. (2009). The negative and cognitive symptoms of schizophrenia are associated with the dopaminergic hypofunction in the

PFC and second-generation APDs have been previously reported to increase the DA levels in the PFC in order to alleviate these symptoms (Mork et al., 2009). Minor differences were noted in the DA concentration in the VTA across all control and experiment groups.

The control groups which received RIS and *S. mucronata* also had higher ACh concentrations in the PFC compared to the control groups; however, no differences in this concentration were noted in the experimental groups. This suggests that RIS and *S. mucronata* are capable of increasing the ACh release in the PFC, even though this was only noted in healthy rats which did not receive ketamine in the current study. These findings concur with reports by Ichikawa et al. (2002) who demonstrated that RIS and other atypical antipsychotic drugs; such as olanzapine, ziprasidone and clozapine increased the levels of ACh in the PFC. The findings in this study showing increased ACh levels in the PFC support the noted slight increased sucrose intake in the groups which received RIS and *S. mucronata* during post-treatment behavioural tests. ACh levels in the hippocampus were not affected by RIS in the current study; however, the *S. mucronata* showed a decrease in the ACh concentration in the hippocampus of ketamine-induced rats. These findings differed from Shirazi-Southall et al. (2002) who reported that second-generation antipsychotic drugs; such as RIS, olanzapine and clozapine, increase the levels of ACh in the rat hippocampus. However, Shirazi-Southall et al. (2002) had also reported RIS and ziprasidone to produce a moderate increase of the ACh compared to olanzapine and clozapine which produced a robust increase of this neurotransmitter.

Conclusions, limitations and recommendations

The current study provides evidence that the administration of repeated doses of ketamine in rats induces deficits in sociability and social novelty. The current study also provides evidence of the antipsychotic potential of the herbal plant, *Salix mucronata* as animals treated with this extract reversed social and novelty deficits caused by ketamine in a similar manner as RIS. The potential effects of *S. mucronata* to alleviate the negative symptoms of schizophrenia was also confirmed by the increase of DA and ACh levels in the prefrontal cortex; however, memory deficits were not affected by either the RIS and *S. mucronata*, as no improvements were noted in ACh levels in the hippocampus. These findings support the use of *S. mucronata* as a traditional medicine approaches in the management of negative symptoms of schizophrenia.

The limitations of this study included the lack of ability to track memory for reward location with the employed sucrose preference test protocol, this is recommended for future studies as it will provide clear insight on the role of RIS and *S. mucronata* in memory. Additionally, the daily food and water intake of the rats was not tracked, thus the reasons for reduced weight gain post induction can only be hypothesized based on previous literature reports. Furthermore, the isolation of animals influenced their social behaviour. Thus, isolation interfered with the activity of both risperidone and *S. mucronata*. Therefore, future studies are recommended to use models that limit social isolation. Furthermore, it is also recommended for future research to investigate different pathways of the neurotransmitters linked to the pathophysiology of schizophrenia and also investigate how the herbal plants, and their bioactive compounds, may affect these pathways and the projection of different neurons.

CRediT authorship contribution statement

Musa Mabandla: Conceptualization, Supervision. **Brenda De Gama**: Conceptualization, Supervision, Writing – review & editing. **Ntombifuthi Princess Ngubane**: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Validation, Visualization, Writing – original draft.

Ethical considerations

All research protocols in this study were adhered to in accordance to the guidelines approved by the Animal Research Ethics Committee of the University of KwaZulu-Natal (Ethics Clearance number: AREC/000/5188/2023). This study also received permission from the Department of Agriculture, Land Reform and Rural Development of the Republic of South Africa (Permit number: SDAH-Epi-23021009580).

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Declaration of Competing Interest

The authors have no conflict of interest to disclose.

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References

de Bartolomeis, A., Ciccarelli, M., De Simone, G., Mazza, B., Barone, A., Vellucci, L., 2023. Canonical and non-canonical antipsychotics' dopamine-related mechanisms of present and next generation molecules: a systematic review on translational highlights for treatment response and treatment-resistant schizophrenia. Int. J. Mol. Sci. 24 (6), 1–57. https://doi.org/10.3390/ijms24065945.

Becker, A., Grecksch, G., 2004. Ketamine-induced changes in rat behaviour: a possible animal model of schizophrenia. Test of predictive validity. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 28 (8), 1267–1277. https://doi.org/10.1016/j.pnpbp.2004.06.019.

Becker, A., Peters, B., Schroeder, H., Mann, T., Huether, G., Grecksch, G., 2003. Ketamine-induced changes in rat behaviour: a possible animal model of schizophrenia. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 27 (4), 687–700. https://doi.org/10.1016/S0278-5846(03)00080-0.

Bekhbat, M., Merrill, L., Kelly, S.D., Lee, V.K., Neigh, G.N., 2016. Brief anesthesia by isoflurane alters plasma corticosterone levels distinctly in male and female rats: implications for tissue collection methods. Behav. Brain Res. 305, 122–125. https://doi.org/10.1016/j.bbr.2016.03.003.

Bialon, M., Wasik, A., 2022. Advantages and limitations of animal schizophrenia models. Int. J. Mol. Sci. 23 (11), 1–32. https://doi.org/10.3390/ijms23115968.

Bonolkwane, M.B., Lekhooa, M., Struwig, M., Aremu, A.O., 2022. Antidepressant effects of South African plants: an appraisal of ethnobotanical surveys, ethnopharmacological and phytochemical studies. Front. Pharmacol. 13, 895286 https://doi.org/10.3389/fphar.2022.895286.

Collins, S.C., Wagner, C., Gagliardi, L., Kretz, P.F., Fischer, M.C., Kessler, P., Kannan, M., Yalcin, B., 2018. A method for parasagittal sectioning for neuroanatomical quantification of brain structures in the adult mouse. Curr. Protoc. Mouse Biol. 8 (3), e48 https://doi.org/10.1002/cpmo.48.

van der Zeijst, M.C., Veling, W., Makhathini, E.M., Mtshemla, S., Mbatha, N.D., Shabalala, S.S., Susser, I., Burns, J.K., Susser, E., Hoek, H.W., 2021. Psychopathology among apprentice traditional health practitioners: a quantitative study from rural KwaZulu-Natal, South Africa. Transcult. Psychiatry 58 (4), 486–498. https://doi.org/10.1177/1363461520949672.

Eagle, A.L., Mazei-Robison, M., Robison, A.J., 2016. Sucrose preference test to measure stress-induced anhedonia. Bio-Protoc. 6 (11) pp. e1822-e1822.

Ellenbroek, B.A., Cools, A.R., 2000. Animal models for the negative symptoms of schizophrenia. Behav. Pharmacol., 11(3-4) 223–233.

El-Sawi, S.A., Maamoun, A.A., Salama, A.H., Maamoun, M.A., Farghaly, A.A., 2020. Application of micro-emulsion formulation in improving the antiproliferative performance of Salix mucronata (Thunb) leaves with chemical investigation of the

active extract. Acta Ecol. Sin. 40 (4), 339–346. https://doi.org/10.1016/j.chnaes.2020.04.004.
Gao, Z., Li, B., Guo, X., Bai, W., Kou, C., 2023. The association between schizophrenia and white blood cells count: a bidirectional two-sample Mendelian randomization study. BMC Psychiatry 23 (1), 1–7. https://doi.org/10.1186/s12888-023-04760-6.
Geyer, M.A., Markou, A., 1995. Animal models of psychiatric disorders. Psychopharmacol.: Fourth Gener. Prog. 787–798.
Granger, K.T., Sand, M., Caswell, S., Lizarraga-Valderrama, L.R., Barnett, J.H., Moran, P. M., 2023. A new era for schizophrenia drug development–Lessons for the future. Drug Discov. Today 28 (7), 1–8. https://doi.org/10.1016/j.drudis.2023.103603.
Gres, A., Sagud, M., Dickov, A., 2023. The effect of vortioxetine on anhedonia in patients with schizophrenia. Int. J. Psychiatry Med. 139–152. https://doi.org/10.1177/00912174231199925.
Harich, S., Gross, G., Bespalov, A., 2007. Stimulation of the metabotropic glutamate 2/3 receptor attenuates social novelty discrimination deficits induced by neonatal phencyclidine treatment. Psychopharmacology 192, 511–519. https://doi.org/10.1007/s00213-007-0742-y.
Ichikawa, J., Dai, J., O'Laughlin, I.A., Fowler, W.L., Meltzer, H.Y., 2002. Atypical, but not typical, antipsychotic drugs increase cortical acetylcholine release without an effect in the nucleus accumbens or striatum. Neuropsychopharmacology 26 (3), 325–339. https://doi.org/10.1016/S0893-133X(01)00312-8.
Kose, L.E.S., 2017. Evaluation of commonly used medicinal plants of Maseru District in Lesotho for their ethnobotanical uses, antimicrobial properties and phytochemical compositions. University of Johannesburg, South Africa.
Kose, L.S., Moteetee, A., Van Vuuren, S., 2015. Ethnobotanical survey of medicinal plants used in the Maseru district of Lesotho. J. Ethnopharmacol. 170, 184–200. https://doi.org/10.1016/j.jep.2015.04.047.
Lambert, C., Da Silva, S., Ceniti, A.K., Rizvi, S.J., Foussias, G., Kennedy, S.H., 2018. Anhedonia in depression and schizophrenia: a transdiagnostic challenge. CNS Neurosci. Ther. 24 (7), 615–623. https://doi.org/10.1111/cns.12854.
Lang, X., Xue, M., Zang, X., Wu, F., Xiu, M., Zhang, X., 2023. Efficacy of low-dose risperidone in combination with sertraline in first-episode drug-naïve patients with schizophrenia: a randomized controlled open-label study. J. Transl. Med. 21 (1), 615–623. https://doi.org/10.1111/cns.12854.
Lindsay, A., Holm, J., Razzoli, M., Bartolomucci, A., Ervasti, J.M., Lowe, D.A., 2021. Some dystrophy phenotypes of dystrophin-deficient mdx mice are exacerbated by mild, repetitive daily stress. FASEB J. 35 (4), 1–11. https://doi.org/10.1096/fj.202002500R.
Luther, L., Jarvis, S.A., Spilka, M.J., Strauss, G.P., 2023. Global reward processing deficits predict negative symptoms transdiagnostically and transphasically in a severe mental illness-spectrum sample. Eur. Arch. Psychiatry Clin. Neurosci. 2023, 1–12. https://doi.org/10.1007/s00406-023-01714-7.
Maroyi, A., 2022. Medicinal uses, phytochemistry and pharmacological properties of Dioscorea dregeana (Kunth) T. Durand & Schinz. Med. Plants Int. J. Phytomedicines Relat. Ind. 14 (1), 57–63. https://doi.org/10.5958/0975-6892.2022.00006.5.
Masondo, N.A., Stafford, G.I., Aremu, A.O., Makunga, N.P., 2019. Acetylcholinesterase inhibitors from southern African plants: an overview of ethnobotanical, pharmacological potential and phytochemical research including and beyond Alzheimer's disease treatment. South Afr. J. Bot. 120, 39–64. https://doi.org/10.1016/j.sajb.2018.09.011.
Mhlongo, L.S., Van Wyk, B.E., 2019. Zulu medicinal ethnobotany: new records from the amandawe area of KwaZulu-Natal, South Africa. South Afr. J. Bot. 122, 266–290. https://doi.org/10.1016/j.sajb.2019.02.012.
Mork, A., Witten, L.M., Arnt, J., 2009. Effect of sertindole on extracellular dopamine, acetylcholine, and glutamate in the medial prefrontal cortex of conscious rats: a comparison with risperidone and exploration of mechanisms involved. Psychopharmacology 206, 39–49. https://doi.org/10.1007/s00213-009-1578-4.
Mugomeri, E., Chatanga, P., Raditladi, T., Makara, M., Tarirai, C., 2016. Ethnobotanical study and conservation status of local medicinal plants: towards a repository and monograph of herbal medicines in Lesotho. Afr. J. Tradit., Complement. Altern. Med. 13 (1), 143–156. https://doi.org/10.4314/ajtcam.v13i1.20.
Ngubane, N.P., De Gama, B.Z., 2023. A quantitative evaluation of traditional health practitioners' perspectives on mental disorders in KwaZulu-Natal: knowledge, diagnosis, and treatment practices. J. Spiritual. Ment. Health 2023, 1–21. https://doi.org/10.1080/19349637.2023.2194561.
Nguyen, H.D., Jo, W.H., Hoang, N.H.M., Kim, M.S., 2023. Risperidone ameliorated 1, 2-Diacetylbenzene-induced cognitive impairments in mice via activating prolactin signaling pathways. Int. Immunopharmacol. 115, 109726 https://doi.org/10.1016/j.intimp.2023.109726.
Osacka, J., Kiss, A., Pitnik, Z., 2022. Possible involvement of apoptosis in the antipsychotics side effects: a mini-review. Clin. Exp. Pharmacol. Physiol. 49 (8), 836–847. https://doi.org/10.1111/1440-1681.13671.
Ott, T., Nieder, A., 2019. Dopamine and cognitive control in prefrontal cortex. Trends Cogn. Sci. 23 (3), 213–234. https://doi.org/10.1016/j.tics.2018.12.006.
Parise, E.M., Alcantara, L.F., Warren, B.L., Wright, K.N., Hadad, R., Sial, O.K., Kroeck, K. G., Iñiguez, S.D., Bolaños-Guzmán, C.A., 2013. Repeated ketamine exposure induces an enduring resilient phenotype in adolescent and adult rats. Biol. Psychiatry 74 (10), 750–759. https://doi.org/10.1016/j.biopsych.2013.04.027.
Patel, K.R., Cherian, J., Gohil, K., Atkinson, D., 2014. Schizophrenia: overview and treatment options. Pharm. Ther. 39 (9), 638–645.
Potkin, S.G., Saha, A.R., Kujawa, M.J., Carson, W.H., Ali, M., Stock, E., Stringfellow, J., Ingenito, G., Marder, S.R., 2003. Aripiprazole, an antipsychotic with a novel mechanism of action, and risperidone vs placebo in patients with schizophrenia and schizoaffective disorder. Arch. Gen. Psychiatry 60 (7), 681–690. https://doi.org/10.1001/archpsyc.60.7.681.
Ramos-Méndez, M.A., Tovilla-Zárate, C.A., Juárez-Rojop, I.E., López-Narváez, M.L., Genis-Mendoza, A.D., González-Castro, T.B., Martínez-Magaña, J.J., Villar-Juárez, G. E., Bellizzia-Rodríguez, M., Castillo-Avila, R.G., Hernández-Díaz, Y., 2023. Increased Interleukins: effect of risperidone in individuals with schizophrenia-a systematic review. SN Compr. Clin. Med. 5 (1), 1–11. https://doi.org/10.1007/s42399-023-01479-z.
Sanavi, M.A., Ghazvini, H., Zargari, M., Ghalehnoei, H., Hosseini-Khah, Z., 2022. Effects of clozapine and risperidone antipsychotic drugs on the expression of CACNA1C and behavioral changes in rat 'Ketamine model of schizophrenia. Neurosci. Lett. 770, 136354 https://doi.org/10.1016/j.neulet.2021.136354.
Sesack, S.R., Carr, D.B., 2002. Selective prefrontal cortex inputs to dopamine cells: implications for schizophrenia. Physiol. Behav. 77 (4-5), 513–517. https://doi.org/10.1016/S0031-9384(02)00931-9.
Shangase, K.B., Luvuno, M., Mabandla, M.V., 2023. Investigating the Robustness of a Rodent "Double Hit"(Post-Weaning Social Isolation and NMDA Receptor Antagonist) Model as an Animal Model for Schizophrenia: a systematic review. Brain Sci. 13 (6), 1–16. https://doi.org/10.3390/brainsci13060848.
Shettima, F.B., Sheikh, T.L., Wakil, M.A., Abdulaziz, M., Rabebbe, I.B., O Abdulmalik, J., 2023. Prevalence and correlates of extrapyramidal side effects among patients with schizophrenia spectrum disorders on typical and atypical antipsychotics. Arch. Psychiatry Res.: Int. J. Psychiatry Relat. Sci. 59 (2), 225–240. https://doi.org/10.20471/dec.2023.59.02.06.
Sheybani-Arani, M.H., Khajavi-Mayvan, F., Asl, A.S., Hamzeie, P., Namazi, S., Seddigh, H., Massoudifar, A., Moayedi, F., 2023. Investigating the relationship between the duration of hospitalization and receiving various types of typical and atypical antipsychotic drugs in patients with schizophrenia: a cross-sectional study. Clin. Schizophr. Relat. Psychoses 17 (20), 1–6.
Shirazi-Southall, S., Rodriguez, D.E., Nomikos, G.G., 2002. Effects of typical and atypical antipsychotics and receptor selective compounds on acetylcholine efflux in the hippocampus of the rat. Neuropsychopharmacology 26 (5), 583–594. https://doi.org/10.1016/S0893-133X(01)00400-6.
Snigdha, S., Neill, J.C., 2008. Efficacy of antipsychotics to reverse phencyclidine-induced social interaction deficits in female rats—a preliminary investigation. Behav. Brain Res. 187 (2), 489–494. https://doi.org/10.1016/j.bbr.2007.10.012.
Sonnenschein, S.F., Gomes, F.V., Grace, A.A., 2020. Dysregulation of midbrain dopamine system and the pathophysiology of schizophrenia. Front. Psychiatry 11, 613. https://doi.org/10.3389/fpsyt.2020.00613.
Veijola, J., Guo, J.Y., Moilanen, J.S., Jääskeläinen, E., Miettunen, J., Kyllönen, M., Haapea, M., Huhtaniska, S., Alaräisänen, A., Mäki, P., Kiviniemi, V., 2014. Longitudinal changes in total brain volume in schizophrenia: relation to symptom severity, cognition and antipsychotic medication. PloS One 9 (7), 486–498. https://doi.org/10.1177/1363461520949672.
Velligan, D.I., Rao, S., 2023. Schizophrenia: salient symptoms and pathophysiology. J. Clin. Psychiatry 84 (1), 1–2. https://doi.org/10.4088/JCP.MS21078COM7.
Vissink, C.E., Winter-van Rossum, I., Cannon, T.D., Fusar-Poli, P., Kahn, R.S., Bossong, M.G., 2022. Structural brain volumes of individuals at clinical high risk for psychosis: a meta-analysis. Biol. Psychiatry Glob. Open Sci. 2 (2), 147–152. https://doi.org/10.1016/j.bpsgos.2021.09.002.
World Health Organisation. (2022). Mental Disorders [Fact Sheet]. (https://www.who.int/news-room/fact-sheets/detail/mental-disorders#:~:text=A%20mental%20disorder%20is%20characterized,different%20types%20of%20mental%20disorders). (Accessed 22 September 2023).
Wulff, A.B., Cooper, P., Kodjo, E., Abel, E., Thompson, S.M., 2023. How sucrose preference is gained and lost: an in-depth analysis of drinking behavior during the sucrose preference test in mice. ENeuro 10 (9), 1–18. https://doi.org/10.1523/ENEURO.0195-23.2023.
Zanos, P., Gould, T., 2018. Mechanisms of ketamine action as an antidepressant. Mol. Psychiatry 23 (4), 801–811. https://doi.org/10.1038/mp.2017.255.

Prologue 5

The following final chapter provides a brief overview of the study, its aim and objectives and how each objective was achieved. It also provides a Table presenting papers which emanated from each objective of the study. Additionally, it provides a summary of the main findings collated from the previous chapters of this thesis. A summary of the limitations of the current study and recommendations for future research is also provided. Chapter 6 also provides a brief interpretation and conclusion of the main findings of the study.

CHAPTER 6

6.1 A Brief Overview of the Study

Traditional or alternative treatment approaches are increasingly becoming an area of interest globally for different ailments, particularly mental illness. This is contributed to by various factors which include dissatisfaction with biomedical medicine due to how it tends to ignore culture and spirituality when treating its patients, adverse side effects, and high costs associated with biomedical medications. Different population groups perceive illness (its cause and required methods of treatment) as per their cultural and spiritual beliefs in the traditional healing context. Thus, the same ailment may be explained and treated differently by different cultural groups. Hence, this study aimed to document methods of diagnosis and treatment used by THPs in KwaZulu-Natal for the management of mental illnesses, particularly schizophrenia. Schizophrenic symptoms overlap with different culture-bound syndromes, particularly ufufunyane, witchcraft and an ancestral calling which cannot be treated by biomedical medicine. Hence, the focus of this disorder in this study. There is still scepticism towards traditional medicine due to the paucity of details regarding the composition and safety of the herbal plants used in medicinal concoctions which are part of the treatment approach used by the THPs. This study therefore also aimed to document the phytochemicals and cytotoxicity of the herbal plants used by the THPs in KwaZulu-Natal to treat culture-bound syndromes which may be equated to schizophrenia based on their symptoms. Additionally, it aimed to investigate the antipsychotic potential of *S. mucronata* (one of the aforementioned herbal plants) and compared its effects to the antipsychotic drug, risperidone. The objectives of this study were achieved through Papers 2, 3, 5, and Manuscript 4 (Table 1).

Table 1: Papers Emanating from the Objectives of the Study

Objectives	Papers
To quantitatively investigate (through the administration of questionnaires) and document the methods of diagnosis and treatment used by THPs to diagnose mental illnesses, particularly schizophrenia.	**Paper 2** **Method:** Administration of questionnaires to THPs
To qualitatively investigate further (through focus group discussions) and document the THPs' perspective on how the explanation of mental disorders (viz. their cause, methods of diagnosis and treatment), particularly schizophrenia, is influenced by cultural beliefs.	**Paper 3** **Method:** Conduction of focus group discussions
To investigate the phytochemicals and cytotoxicity of the medicinal plants used by THPs to treat schizophrenia.	**Manuscript 4** **Methods:** GC-MS analysis and MTT Assay
To investigate and compare the effectiveness of the medicinal plants used by THPs with the currently used antipsychotic drug risperidone in the treatment of schizophrenia through a pharmacological animal model. Further, to document behavioural changes, brain mass and volume, and changes in the levels of dopamine and acetylcholine on the hippocampus and prefrontal cortex.	**Paper 5** **Methods:** Pharmacological schizophrenia model

6.2 Synthesis

Western-defined psychiatric illnesses such as schizophrenia tend to pose complexity in their description and understanding as they also possess supernatural causation explanations with their symptoms which also overlap with conditions which are culture-bound in the African traditional healing context (Molot, 2017). Particularly, the negative symptoms of schizophrenia are reported to overlap with the symptoms presented by patients who are thought to be bewitched, to have an ancestral calling or *ufufunyana* (van der Zeijst *et al.,* 2021). Also, the explanation of the causation of illness varies between the two entities (biomedical and traditional healing), hence, the methods used to diagnose and treat these illnesses are expected to vary.

The first component of this study aimed to document the knowledge and understanding of THPs on mental disorders, particularly schizophrenia which was the focus of this study. It also aimed to document the methods used by the THPs to diagnose and treat schizophrenia to reflect their variability from biomedical medicine. These findings were presented in Papers 2 and 3 of this thesis. With regard to knowledge of mental disorders, only a few of the THPs at the Harry Gwala District Municipality in KwaZulu-Natal had an accurate knowledge of mental disorders in relation to Western-psychiatry description of these disorders. The THPs' understanding and description of mental disorders could be influenced by the supernatural powers believed to play a role in the aetiology of these disorders and their holistic perception of mental illness which are not considered in a biomedical healing settings (Marques *et al.,* 2021).

The methods of diagnosing mental illness also vary between the two entities, with THPs taking a number of factors (such as biological, socio-cultural and spiritual) into consideration when diagnosing their patients. This differs from the biomedical setting where the Diagnostic and Statistical Manual of Mental Disorders (DSM-5), which only focuses on the symptoms presented by the patients, is the key tool used to diagnose mental illness. Based on the findings presented in Papers 2 and 3 of this thesis, the common methods of diagnosis used by THPs included divination through consultation with ancestors and seeking information from the family members or from the individuals accompanying the patient. Consultation with the ancestors may be accompanied by the burning of incense and/or throwing of bones (Kpobi *et al.,* 2019; Shange and Ross 2022). In this study, burning of incense was also reported to be used independently to soothe and calm the patients. The prevalent symptoms noted by the THPs in their patients diagnosed with mental illness included aggression, unresponsiveness and also being incoherent. Other symptoms included the inability to walk or talk, visual and auditory hallucinations, lack of sleep, not eating, stress, calling out names of people unseen by others, being fearful, screams, epileptic attacks, forgetfulness and alogia. Delusions and hallucinations, asociality, alogia, anhedonia, and lack of memory and attention correspond with the symptoms of schizophrenia as defined in Western psychiatry (APA, 2013; Koster *et al.,* 2014; Deng and Xu, 2017).

The cornerstone treatment for schizophrenia in the biomedical setting is the administration of antipsychotic drugs, such as risperidone for the negative symptoms of this disorder (Stroup *et al.,* 2000; Sanz-Fuentenebro *et al.,* 2013; Nguyen *et al.,* 2023; Ramos-Méndez, 2023). However, approximately 40% of patients diagnosed with schizophrenia inadequately respond to the antipsychotic medications (Lowe *et al.,* 2018), and these medications are associated with adverse side effects ranging from mild to severe and unbearable, and in some instances may be fatal (Deng and Xu, 2017; Stroup and Gray, 2018; Crellin *et al.,* 2022). Hence the exploration of alternative treatment methods such as traditional

approaches in this study. The administration of a medicinal concoction was the prevalent traditional method of treatment for schizophrenia in the current study. This concoction is prepared using one or more herbal plants, but the information on these plants was not shared with the investigator in this study as this knowledge was regarded as sacred by the THPs from the HGDM based on the knowledge being gifted to them by their ancestors. However, the THPs recommended the investigator to visit the *muthi* market (Zulu pharmacy) for more information on the herbal plants used for the treatment of mental illnesses. Nonetheless, the safety of the herbal plants used remains questionable as little is known and understood in this area. The safety and efficacy of using both biomedical and traditional medicine concurrently is also questionable (Green and Makhubu, 1984; Freeman and Motsei, 1992; Moshabela *et al.,* 2016). Thus, the second component of this study aimed to investigate the phytochemistry and cytotoxicity effects of the herbal plants used by the THPs for the treatment of schizophrenia (presented in Manuscript 4 of this thesis). This component also aimed to investigate the antipsychotic potential of one of the herbal plants used for the treatment of schizophrenia in comparison to risperidone (presented in Paper 5 in this thesis).

A total of seven herbal plants were offered by the THPs at the Berea and Dalton *muthi* markets (in Durban, KwaZulu-Natal); viz. *Dioscorea dregeana*, *Boophone disticha*, *Afroaster hispida*, *Salix mucronata*, *Artabotrys monteiroae Oliv.*, *Trimeria grandifolia* and *Clivia miniata* for the treatment of mental disorders, including schizophrenia. The GC-MS analysis was used for the identification of the bioactive compounds in each plant and the MTT assay was used to assess the cytotoxic effect of these plants. Based on the findings of this study, the identification of compounds such as .beta.-Carboline which possesses sedative, anxiolytic, anticonvulsant, neuroprotective and anti-inflammatory activity (Piechowska *et al.,* 2019; Szabo *et al.,* 2021), sitosterols with activity in psychosis and insanity (Maroyi, 2022), Octadecanoic acid reported to be used to treat schizophrenia (Sushmitha *et al.,* 2018), Erucic acid which possesses regulatory and protective effects on the nervous system (Gao *et al.,* 2022), .beta.-Amyrin with antidepressant effects (Nogueira *et al.,* 2019), and cis-Linoleic acid (also referred to as 9,12-Octadecadienoic acid) which possesses anti-inflammatory properties (Fagbemi *et al.,* 2021) supports the use of these herbal plants in the treatment of schizophrenia, thus increasing the likelihood of the use of traditional medicine, particularly herbal medicine, as a treatment option for mental disorders. The scepticism on the safety of herbal medicine was diminished for the herbal plants used in this study as their different extracts (i.e. methanol, ethanol and DCM extracts) showed increased cell viability at varying concentrations, thus indicating a positive effect on cell proliferation and non-cytotoxicity.

S. mucronata was selected from the seven aforementioned herbal plants for further investigation. The antipsychotic potential of this plant was investigated in a pharmacological animal model of schizophrenia. Firstly, a sub-chronic dose of ketamine was used to induce asociality and anhedonia, thereby mimicking the negative symptoms of schizophrenia. Sociability of the animals was tested using the 3-chambered sociability apparatus, while anhedonia was tested using the sucrose preference test. After the induction of schizophrenia, the animals were then treated with risperidone and a methanol extract of *S. mucronata*. The deficits in social behaviour [i.e. social withdrawal with familiar (Stranger 1) and novel (Stranger 2) rat] caused ketamine were reversed by both RIS and *S. mucronata*. With the dysregulation in DA and ACh levels, in structures such as the PFC, VTA and hippocampus, reported to have a primary role in the pathophysiology of schizophrenia, the levels of these neurotransmitters were increased in the PFC post-treatment with *S. mucronata*. These findings support the use of the bark of *S. mucronata* by the THPs in the treatment of *ufufunyana* schizophrenia as they provide evidence of the antipsychotic potential of this herbal plant.

6.3 Conclusions, Limitations and Recommendations

This study concludes that THPs and traditional medicine have a positive role and potential in the treatment of mental disorders, particularly the negative symptoms of schizophrenia. The findings of the study demonstrated that the THPs play an important part in diagnosing and treating culture-bound syndromes which lead to mental illness, particularly schizophrenia. Additionally, the association of cultural and spiritual beliefs with traditional healing regarding the explanation and treatment of illness was emphasised in this study. The potential of traditional medicine in the treatment of schizophrenia is demonstrated by the antipsychotic effects of the methanol extract of *S. mucronata*. Furthermore, the herbal plants offered in KwaZulu-Natal for the treatment of mental disorders do not possess cytotoxic effects, and are thus safe for consumption.

Notwithstanding the fact that promising research findings emerged from this thesis, the study experienced limitations in its different phases and components. Regarding the first component of the study, it only focused on only one municipality out of eleven in the KwaZulu-Natal (KZN) province. Additionally, the sample in this component presented approximately only 25% of the registered THPs in the Harry Gwala District Municipality. Thus, the findings from this component cannot be generalised to the other municipalities in the province. Therefore, it is recommended that the explanation of causal factors for mental illness, as well as the methods used by the THPs in the other municipalities of KZN to diagnose and treat mental disorders be explored. This will assist in the provision of a more accurate consensus regarding the influence of cultural and spiritual beliefs on the understanding, diagnosis and

treatment of illness in the traditional healing context. Furthermore, some of the information was considered sacred by the THPs due its association with the ancestors. Thus, extensive details on the approaches used to diagnose and treat illnesses (i.e. preparation medicinal concoctions, details on the herbal plants used and where these plants are collected) were not obtained. Nonetheless, it is recommended that the perspectives of patients and caregivers also be explored in order to obtain a broad understanding of the extent of the influence of cultural beliefs and ancestral spirits in mental illness. Another factor that can be considered as a weakness regarding the findings obtained from the first component of this study is the variability observed in the methods of diagnosis and treatment for mental illness and other ailments. According to the World Health Organisation, building trust and incorporating traditional medicine and practices into mainstream healthcare requires ensuring their safety, regulation, and standardization. However, this might be a challenge due the variability noted in traditional healing practices.

In the second component of the study, the different phytochemicals constituted in the seven herbal plants recommended by the THPs at the *muthi* markets for the treatment of schizophrenic symptoms were identified. However, activity of each of the phytochemical compounds in alleviating mental illnesses was not investigated and documented. It can only be hypothesised for *S. mucronata* that Octadecanoic acid is involved in the mechanism of alleviating schizophrenic symptoms because this compound was previously reported to be involved in the treatment of schizophrenia by Sushmitha *et al.,* (2018). Hence, it is recommended that future studies investigate the antipsychotic effects of the identified bioactive compounds in the seven herbal plants for a better understanding of their mechanistic effects in the treatment of schizophrenia. Regarding the cytotoxicity of the herbal plants, cytotoxic activity was tested in only liver cells, and these plants might indicate toxicity in other cells (for example, they could be neurotoxic). Thus, it is recommended that future studies investigate the cytotoxic effects of these plants in other cell cultures and also in vivo. Furthermore, in the animal model, the daily food and water intake of the rats was not tracked, thus the reasons for reduced weight gain post induction can only be hypothesised based on previous literature reports. Additionally, the isolation of animals proved to have an influence on the social behaviour of the rats, meaning that isolation interfered with the activity of both risperidone and *S. mucronata* to a certain extent. Therefore, it is recommended that a model that limits social isolation be used in future studies. Furthermore, memory for reward location with the sucrose preference test used in this study was not monitored, and this remains an area for future studies. Additionally, it is recommended that future research also investigates different pathways of the neurotransmitters linked to the pathophysiology of schizophrenia and also investigate how the herbal plants, and their bioactive compounds, may affect these pathways and the projection of different neurons.

6.4 References

1. American Psychiatric Association. 2013. Diagnostic and statistical manual of mental disorders: DSM-5 (5th ed). Washington, D.C.: American Psychiatric Publishing.
2. Crellin, N.E., Priebe, S., Morant, N., Lewis, G., Freemantle, N., Johnson, S., Horne, R., Pinfold, V., Kent, L., Smith, R. and Darton, K., 2022. An analysis of views about supported reduction or discontinuation of antipsychotic treatment among people with schizophrenia and other psychotic disorders. *BMC Psychiatry*, 22(1), pp.1-11.
3. Deng, H. and Xu, J., 2017. Wendan decoction (Traditional Chinese medicine) for schizophrenia. *Cochrane Database of Systematic Reviews*, (6), pp.1-92.
4. Fagbemi, K.O., Aina, D.A. and Olajuyigbe, O.O., 2021. Soxhlet extraction versus hydrodistillation using the clevenger apparatus: A comparative study on the extraction of a volatile compound from Tamarindus indica seeds. *The Scientific World Journal*, 2021, pp.1-8.
5. Freeman, M. and Motsei, M., 1992. Planning health care in South Africa—is there a role for traditional healers?. *Social Science and Medicine*, 34(11), pp.1183-1190.
6. Gao, L., Li, H., Li, B., Shao, H., Yu, X., Miao, Z., Zhang, L., Zhu, L. and Sheng, H., 2022. Traditional uses, phytochemistry, transformation of ingredients and pharmacology of the dried seeds of Raphanus sativus L.(Raphani Semen), A comprehensive review. *Journal of Ethnopharmacology*, 294, pp.1-21.
7. Green, E.C. and Makhubu, L., 1984. Traditional healers in Swaziland: toward improved cooperation between the traditional and modern health sectors. *Social Science and Medicine*, 18(12), pp.1071-1079.
8. Koster, L.S., Carbon, M. and Correll, C.U., 2014. Emerging drugs for schizophrenia: an update. *Expert Opinion on Emerging Drugs*, 19(4), pp.511-531.
9. Kpobi, L.N., Swartz, L. and Omenyo, C.N., 2019. Traditional herbalists' methods of treating mental disorders in Ghana. *Transcultural Psychiatry*, 56(1), pp.250-266.
10. Lowe, P., Krivoy, A., Porffy, L., Henriksdottir, E., Eromona, W. and Shergill, S.S., 2018. When the drugs don't work: treatment-resistant schizophrenia, serotonin and serendipity. *Therapeutic Advances in Psychopharmacology*, 8(1), pp.63-70.
11. Maroyi, A., 2022. Medicinal uses, phytochemistry and pharmacological properties of Dioscorea dregeana (Kunth) T. Durand & Schinz. *Medicinal Plants-International Journal of Phytomedicines and Related Industries*, 14(1), pp.57-63.
12. Marques, B., Freeman, C. and Carter, L., 2021. Adapting traditional healing values and beliefs into therapeutic cultural environments for health and well-being. *International Journal of Environmental Research and Public Health*, 19(1), pp.1-19.
13. Molot, M., 2017. Discourses of Psychiatry and Culture: The Interface Between Western and Traditional Medicine in the Treatment of Mental Illness. Independent Study Project (ISP) Collection. 2582. https://digitalcollections.sit.edu/isp_collection/2582

14. Moshabela, M., Zuma, T. and Gaede, B., 2016. Bridging the gap between biomedical and traditional health practitioners in South Africa. *South African Health Review*, 2016(1), pp.83-92.
15. Ngubane, N.P. and De Gama, B.Z., 2023. A quantitative evaluation of traditional health practitioners' perspectives on mental disorders in KwaZulu-Natal: knowledge, diagnosis, and treatment practices. *Journal of Spirituality in Mental Health*, 2023, pp.1-21.
16. Nguyen, H.D., Jo, W.H., Hoang, N.H.M. and Kim, M.S., 2023. Risperidone ameliorated 1, 2-Diacetylbenzene-induced cognitive impairments in mice via activating prolactin signaling pathways. *International Immunopharmacology*, 115, pp.1-19.
17. Nogueira, A.O., Oliveira, Y.I.S., Adjafre, B.L., de Moraes, M.E.A. and Aragao, G.F., 2019. Pharmacological effects of the isomeric mixture of alpha and beta amyrin from Protium heptaphyllum: a literature review. *Fundamental and Clinical Pharmacology*, 33(1), pp.4-12.
18. Piechowska, P., Zawirska-Wojtasiak, R. and Mildner-Szkudlarz, S., 2019. Bioactive β-carbolines in food: A review. *Nutrients*, 11(4), pp.1-10.
19. Ramos-Méndez, M.A., Tovilla-Zárate, C.A., Juárez-Rojop, I.E., López-Narváez, M.L., Genis-Mendoza, A.D., González-Castro, T.B., Martínez-Magaña, J.J., Villar-Juárez, G.E., Bellizzia-Rodríguez, M., Castillo-Avila, R.G. and Hernández-Díaz, Y., 2023. Increased Interleukins: Effect of Risperidone in Individuals with Schizophrenia—a Systematic Review. *SN Comprehensive Clinical Medicine*, 5(1), pp.1-11.
20. Sanz-Fuentenebro, J., Taboada, D., Palomo, T., Aragües, M., Ovejero, S., Del Alamo, C. and Molina, V., 2013. Randomized trial of clozapine vs. risperidone in treatment-naïve first-episode schizophrenia: results after one year. *Schizophrenia Research*, 149(1-3), pp.156-161.
21. Shange, S. and Ross, E., 2022. "The Question Is Not How but Why Things Happen": South African Traditional Healers' Explanatory Model of Mental Illness, Its Diagnosis and Treatment. *Journal of Cross-Cultural Psychology*, 53(5), pp.503-521.
22. Stroup, T.S., A. Lieberman, J., S. Swartz, M. and McEvoy, J.P., 2000. Comparative effectiveness of antipsychotic drugs in schizophrenia. *Dialogues in Clinical Neuroscience*, 2(4), pp.373-379.
23. Sushmitha, H.S., Roy, C.L., Gogoi, D., Velagala, R.D., Nagarathna, A., Balasubramanian, S. and Rajadurai, M., 2018. Phytochemical and Pharmacological Studies on Hylocereus undatus Seeds: An In Vitro Approach. *World Journal of Pharmacological Research*, 7(14), pp.986-1006.
24. Szabo, T., Volk, B. and Milen, M., 2021. Recent advances in the synthesis of β-carboline alkaloids. *Molecules*, 26(3), pp.1-54
25. van der Zeijst, M.C., Veling, W., Makhathini, E.M., Mtshemla, S., Mbatha, N.D., Shabalala, S.S., Susser, I., Burns, J.K., Susser, E. and Hoek, H.W., 2021. Psychopathology among apprentice traditional health practitioners: A quantitative study from rural KwaZulu-Natal, South Africa. *Transcultural Psychiatry*, 58(4), pp.486-498.

APPENDICES

Appendix 1: Gatekeepers permission (Department of Health and Harry Gwala District)

KWAZULU-NATAL PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

DIRECTORATE:
AFRICAN TRADITIONAL MEDICINE

Private Bag X454, PIETERMARITZBURG, 3200
Moses Mabhida Building, 330 Langalibalele Street, Pietermaritzburg, 3200
Tel: 033 3953096 Fax: 033 3952515 Email Lindokuhle.khubonj@kznhealth.gov.za

27 July 2021

To Whom It May Concern

SUPPORT LETTER TO CONDUCT RESEARCH AMONG TRADITIONAL HEALTH PRACTITIONERS (THPs) IN THE KWAZULU-NATAL PROVINCE

This is to confirm that the Department of Health was approached by Doctoral student, Miss Ntombifuthi Princess Ngubane (209511770) regarding her research on: **The Role of Traditional Health Practitioners and the Effect of Traditional Medicine in the Treatment of Negative Symptoms of Schizophrenia in EThekwini District, KwaZulu Natal, South Africa.** The researcher seeks to administer questionnaires to THPs to investigate and document their understanding, identification and classification of mental illnesses especially schizophrenia. The study will also document the approaches used by THPs to treat and manage schizophrenia.

The objectives of the study are outlined as being:

(a) To document the methods of diagnosis used by traditional health practitioners to identify and classify different mental illnesses, especially the scales they use for the evaluation of symptoms associated with schizophrenia.
(b) To document the indigenous treatment approaches used to treat and manage schizophrenia, particularly the negative symptoms.
(c) To test the toxicity and effectiveness of the traditional medicines used by traditional health practitioners to treat the negative symptoms of schizophrenia and to compare these with classical drug clozapine which is currently used for treatment of schizophrenia.
(d) To document any anatomical and physiological changes on the brain that may occur due to schizophrenia and also changes observed after treatment with traditional medicines.

The Directorate of African Traditional Medicine supports Miss Ntombifuthi Princess Ngubane's proposed research study.

Yours Sincerely

[redacted]

Vukani Enliven Khoza
Director: ATM



HARRY GWALA TRADITIONAL HEALTH PRACTITIONERS

-[redacted]0-2021

To Whom It May Concern

PERMISSION TO CONDUCT RESEARCH AMONG TRADITIONAL HEALTH PRACTITIONERS (THPs) IN THE KWAZULU-NATAL PROVINCE

This is to confirm that the Harry Gwala Health District was approached by Doctoral student, Miss Ntombifuthi Princess Ngubane (209511770) regarding her research on: **The Role of Traditional Health Practitioners and the Effect of Traditional Medicine in the Treatment of Negative Symptoms of Schizophrenia in KwaZulu Natal, South Africa.** The researcher seeks to administer questionnaires to the THPs and to also conduct focus groups with the THPs to investigate and document their understanding, identification and classification of mental illnesses, particularly schizophrenia. The study will also document the approaches used by THPs to treat and manage schizophrenia.

The objectives of the study are outlined as being:

(a) To document the methods of diagnosis used by traditional health practitioners to identify and classify different mental illnesses, especially the scales they use for the evaluation of symptoms associated with schizophrenia.

(b) To document the indigenous treatment approaches used to treat and manage schizophrenia, particularly the negative symptoms.

(c) To test the toxicity and effectiveness of the traditional medicines used by traditional health practitioners to treat the negative symptoms of schizophrenia and to compare these with classical drug which is currently used for treatment of schizophrenia.

District Health Coordinator: Mrs. N. Khwela ([redacted])
District Municipality Coordinator: Mrs. M. Hlongwa ([redacted])

(d) To document any anatomical and physiological changes on the brain that may occur due to schizophrenia and also changes observed after treatment with traditional medicines.

The Harry Gwala Health District has no objection to Miss Ntombifuthi Princess Ngubane undertaking this proposed research study.

Yours Sincerely

[redacted]

District Traditional Health Practitioners Chairperson

District Health Coordinator: Mrs. N. Khwela ([redacted]
District Municipality Coordinator: Mrs. M. Hlongwa ([redacted]

Appendix 2: HSSREC Ethics Approval (and amended approval)

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

11 August 2021

Miss Ntombifuthi Princess Ngubane (209511770)
School Of Lab Med & Medical Sc
Westville Campus

Dear Miss Ngubane,

Protocol reference number: HSSREC/00002944/2021
Project title: The Role of Traditional Health Practitioners and Traditional Medicine in the Treatment of the Negative Symptoms of Schizophrenia
Degree: PhD

Approval Notification – Expedited Application

This letter serves to notify you that your application received on 10 June 2021 in connection with the above, was reviewed by the Humanities and Social Sciences Research Ethics Committee (HSSREC) and the protocol has been granted **FULL APPROVAL with the following condition.**

1. **Obtain clearance from AREC for respective laboratory work**

Any alteration/s to the approved research protocol i.e. Questionnaire/Interview Schedule, Informed Consent Form, Title of the Project, Location of the Study, Research Approach and Methods must be reviewed and approved through the amendment/modification prior to its implementation. In case you have further queries, please quote the above reference number. PLEASE NOTE: Research data should be securely stored in the discipline/department for a period of 5 years.

This approval is valid until 11 August 2022.
To ensure uninterrupted approval of this study beyond the approval expiry date, a progress report must be submitted to the Research Office on the appropriate form 2 - 3 months before the expiry date. A close-out report to be submitted when study is finished.

All research conducted during the COVID-19 period must adhere to the national and UKZN guidelines.

HSSREC is registered with the South African National Research Ethics Council (REC-040414-040).

Yours sincerely,

[redacted]

Professor Dipane Hlalele (Chair)

/dd

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

04 November 2021

Ntombifuthi Princess Ngubane (209511770)
School Of Lab Med & Medical Sc
Westville Campus

Dear NP Ngubane,

Protocol reference number: HSSREC/00002944/2021
Project title: The Role of Traditional Health Practitioners and Traditional Medicine in the Treatment of the Negative Symptoms of Schizophrenia

Approval Notification – Amendment Application

This letter serves to notify you that your application and request for an amendment received on 02 November 2021 has now been approved as follows:

- Change in research site

Any alterations to the approved research protocol i.e. Questionnaire/Interview Schedule, Informed Consent Form; Title of the Project, Location of the Study must be reviewed and approved through an amendment /modification prior to its implementation. In case you have further queries, please quote the above reference number.

PLEASE NOTE: Research data should be securely stored in the discipline/department for a period of 5 years.

All research conducted during the COVID-19 period must adhere to the national and UKZN guidelines.

Best wishes for the successful completion of your research protocol.

Yours faithfully

[redacted]

.....
Professor Dipane Hlalele (Chair)

/dd

Appendix 3: AREC Ethics Approval

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

06 April 2023

Ms Ntombifuthi Princess Ngubane (209511770)
School of Laboratory Medicine & Medical Sciences
Westville

Dear Ms Ntombifuthi Princess Ngubane,

Protocol reference number: AREC/00005188/2023
Project title: The Role of Traditional Health Practitioners and the Effect of Traditional Medicine in the Treatment of Negative Symptoms of Schizophrenia in KwaZulu Natal, South Africa

Full Approval

This letter serves to notify you that your revised application was received on 10 March 2023. The Animal Research Ethics Committee has accepted the documents submitted, and **FULL APPROVAL** for the protocol has been granted.

This approval is valid for one year from 12 April 2023.

To ensure uninterrupted approval of this study beyond the approval expiry date, a progress report must be submitted to the Research Office on the appropriate form 2 - 3 months before the expiry date.

Please note: There must be adherence to national and institutional regulations and guidelines at all times. Researchers will be personally responsible and liable for non-adherence to national regulations. If in doubt, please contact the Research Ethics Chair and/or the University Dean of Research for advice.

Please note: Any Veterinary and Para-Veterinary procedures must be conducted by a SAVC registered VET or SAVC authorized person.

Any alteration/s to the approved research protocol, i.e Title of Project, Location of the Study, Research Approach and Methods must be reviewed and approved through the amendment/modification prior to its implementation. In case you have further queries, please quote the above reference number.

Please note: Research data should be securely stored in the discipline/department for a period of 5 years.

I take this opportunity of wishing you everything of the best with your study.

Yours faithfully

[redacted]

Dr Dalene Vosloo, PhD, Pr. Sci. Nat.
Chair: Animal Research Ethics Committee

/kr

cc Supervisor: Dr Brenda De Gama, Dr Musa Mabandla

Animal Research Ethics Committee (AREC)
Ms Karen Reinertsen (Administrator)
Westville Campus, Govan Mbeki Building
Postal Address: Private Bag X54001, Durban 4000
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Website: http://research.ukzn.ac.za/Research-Ethics/Animal-Ethics.aspx

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Appendix 4: Section 20 Permit

agriculture, land reform & rural development

Department:
Agriculture, Land Reform and Rural Development
REPUBLIC OF SOUTH AFRICA

Directorate Animal Health, Department of Agriculture, Land Reform and Rural Development
Private Bag X138, Pretoria 0001

Enquiries: Ms Mama Laing • Tel: +27 12 319 7442 • Fax: +27 12 319 7470 • E-mail: MarnaL@dalrrd.gov.za
Reference: 12/11/1/5 (2744ZY)

Responsible person: Miss Ntombifuthi Princess Ngubane
Institution: University of KwaZulu-Natal - Westville Campus, University Road, Westville 3629
Email: Ngubanen8@ukzn.ac.za

Dear Miss Ntombifuthi Princess Ngubane

PERMISSION TO DO RESEARCH IN TERMS OF SECTION 20 OF THE ANIMAL DISEASES ACT, 1984 (ACT NO 35 OF 1984)

Title of research project / study: "The Role of Traditional Health Practitioners and the Effect of Traditional Medicine in the Treatment of the Negative Symptoms of Schizophrenia in KwaZulu Natal, South Africa"

Your application, received 2022-11-01, requesting permission under Section 20 of the Animal Diseases Act, 1984 (Act No 35 of 1984) to perform the research project or study stipulated above, refers.

1. Based on the information provided in your application, the Director of Animal Health has no objection to this study. The study may continue if statement 1.1 to 1.6 hereunder (as applicable) are, and remain, accurate. Should the scope of your research project change in any way you are required to inform the Section 20 Secretariat and may not proceed with any activities until written permission to do so have been granted by the National Director: Animal Health.

    1.1. No work will be done with controlled and notifiable animal diseases (list can be obtained / requested from this office), which includes any animal diseases which do not occur in South Africa;
    1.2. No imported material of animal origin or imported animal pathogens will be utilized in the study;
    1.3. No samples that originate from a biobank will be used in the study;
    1.4. No clinical studies will be performed in the target species, either in a laboratory or in the field;
    1.5. The areas where the samples are to be collected are not under restriction for controlled or notifiable diseases to which the species of animal, from which the samples are obtained, is susceptible;
    1.6. No samples or products that have not been passed as fit for human consumption will be obtained from an abattoir.

2. In addition to the conditions mentioned in point 1, you are responsible for ensuring that your research project or study complies with all or part of the following, as applicable:

2.1. Permission to perform research under Section 20 of the Animal Diseases Act, 1984 (Act no 35 of 1984) does not relieve the researcher of any responsibility which may be placed on him/her by any other Act of the Republic of South Africa, including the Veterinary and Para-Veterinary Professions Act, 1982 (Act No 19 of 1982), the Fertilizers, Farm Feeds, Agricultural Remedies and Stock Remedies Act, 1947 (Act No 36 of 1947), the Medicines and Related Substances Control Act, 1965 (Act No 101 of 1965), the Genetically Modified Organisms Act, 1997 (Act No 15 of 1997) and the National Environmental Management: Biodiversity Act, 2004 (Act No 10 of 2004);

2.2. No part of the study may begin until valid ethical approval has been obtained in writing from the relevant South African authority;

2.3. All biological or potentially infectious material must be packaged and transported in accordance with International Air Transport Association (IATA) requirements and the National Road Traffic Act, 1996 (Act No. 93 of 1996);

2.4. Any incidence or suspected incidence of a controlled or notifiable disease in terms of the Animal Diseases Act, 1984 (Act No 35 of 1984), must be reported immediately to the responsible state veterinarian;

2.5. Samples or material may not be outsourced or used for further/other research without prior written approval from the Director of Animal Health, except for headless embalmed carcasses of Sprague-Dawley rats to be stored at the Department of Clinical Anatomy, UKZN;

2.6. All potentially infectious material utilised or generated during or by the study is to be destroyed at completion of the study and only a registered waste disposal company may be used for the removal of waste generated during or by the study;

2.7. Records must be kept for five years for auditing purposes.

Written permission from the Director of Animal Health must be obtained prior to any deviation from the conditions. Application must be sent in writing to MarnaL@dalrrd.gov.za.

Failure to obtain written permission as above may be considered a contravention of the Animal Diseases Act, 1984 (Act No 35 of 1984).

Expiry date of this permit: 30 November 2025

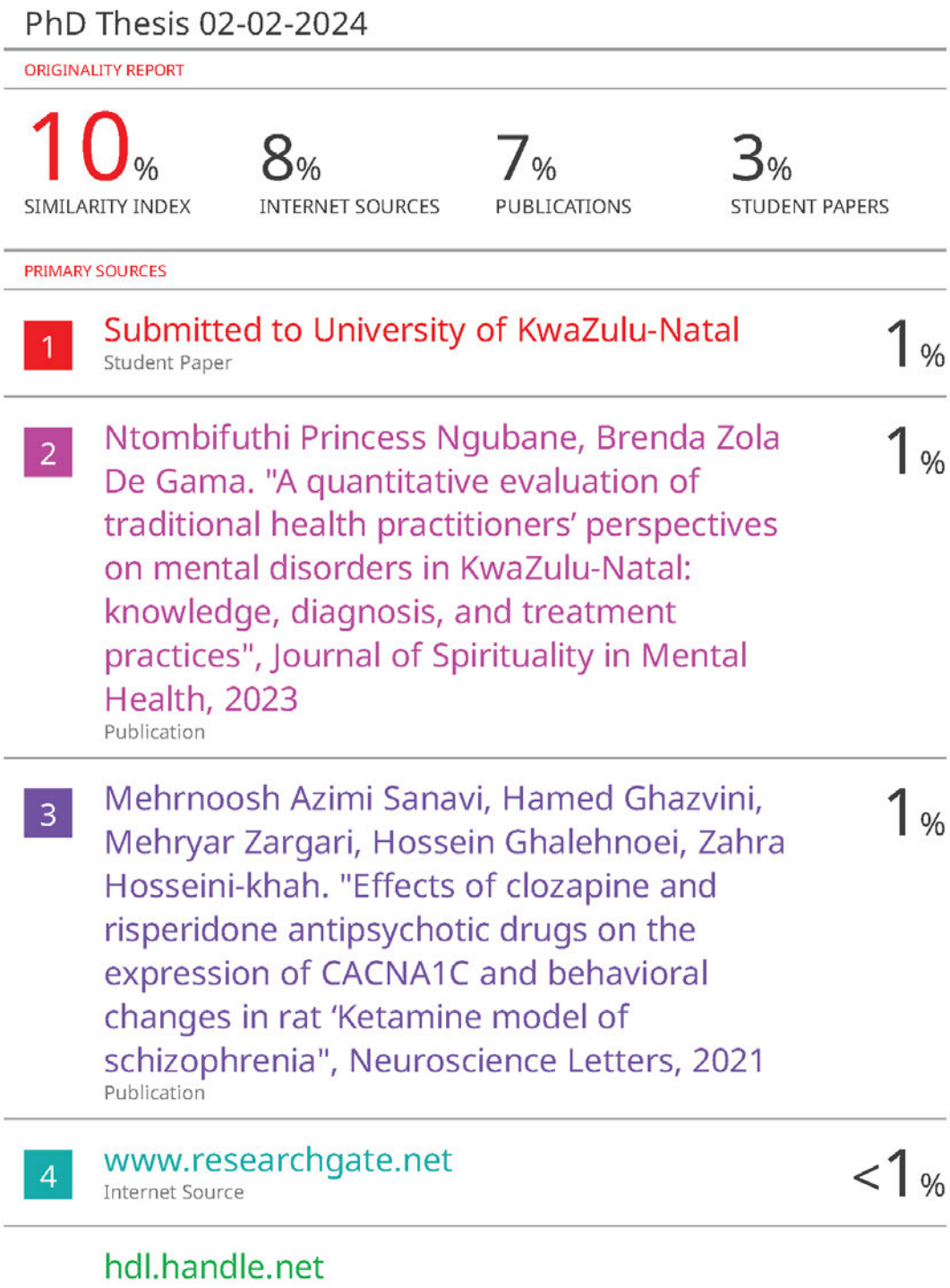
Kind regards,

[redacted]

Dr Mpho Mapi
DIRECTOR: ANIMAL HEALTH
Date: 2023 -02- 10

SUBJECT: Permission to do research in terms of Section 20 of the Animal Diseases Act, 1984 (Act No 35 of 1984)

Appendix 5: Turnitin Report



PhD Thesis 02-02-2024

ORIGINALITY REPORT

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SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

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Durban
4000
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From: John Boughey
Date: 02/02/24

Item	Unit Cost (R)	Total (R)
English Language editing of Ph D thesis **The Role of Traditional Health Practitioners and the Effect of Salix mucronata on the Treatment of Negative Symptoms of Schizophrenia in KwaZulu Natal, South Africa.** 18,000 words (excluding lists of figures, tables, and contents, References, Manuscripts, and Appendices) @ 20c per word)	**R 0.20 per word**	**3,600. 00**

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Appendix 7: Raw Data for Component One of the Study

7.1. Questionnaire Coding

The Role of Indigenous Healers and the Effect of Indigenous Medicine in the Treatment of the Negative Symptoms of Schizophrenia in KwaZulu Natal

QUESTIONNAIRE

SECTION A: SOCIO-DEMORAPHIC INFORMATION

Demographic Details

D1 Participant No: (1-100) D2 Sex: (F=1; M=2) D3 Age:

Youth (15-34 years) = 1
Middle Age (35-59 years) = 2
Senior/Elderly (60 years and over) = 3

D4 Address or Region: Kokstad=1; Umzimkhulu=2; Buhlebezwe=3; Dr. Nkzn Zuma=4

D3 Do you classify yourself as: Inyanga ☐ 1
Isangoma ☐ 2
Umthandazi ☐ 3
Other ☐ 4

Combination = 5

D6 If other is selected, please specify: String

D7 Period of practice (as healer):

Entry Level (0-5 years) = 1
Mid-Level (6-15 years) = 2
Senior Level (16-30 years) = 3
Expert Level (Over 30 years) = 4
No response = 5

D8 Area of specialization/traditional healing/traditional medicine:

Group 1 (Head, Mental illnesses, Stroke and Epilepsy) = 1
Group 2 (General, Ukuhlola, Performs Rituals, Removes Spells and Initiation) = 2
Group 3 (Aches, Wounds, Shingles, Umeqo and Feet) = 3
Group 4 (Abdomen, Maternity related issues and Ilumbo)
Not Specified = 5

QUESTIONS:

SECTION B: KNOWLEDGE OF MENTAL HEALTH DISORDERS AND THEIR TREATMENT

Q1 Do you know what mental health disorders are? Yes (1); No (2) No response=3

Q2 What is your understanding of a mental health disorder?

(a) Condition that affects one's thinking, mood and behaviour (1)

(b) Condition whereby one loses their mind (2)

(c) There is no such thing as mental health disorders (3)

(d) Other (4)

(e) Don't know (4)

Combination/All = 4

Q3 In your traditional healing practice have you diagnosed an individual suffering from a mental disorder before? Yes (1); No (2) No response=2

Q4 Which mental disorder/s have you diagnosed before?

Anxiety disorder ☐ (1)

Schizophrenia ☐ (2)

Substance use disorder ☐ (3)

Major depressive disorder ☐ (4)

Other ☐ (5)

2+3=6	1+2+4=6
No response=5	1+2=6
1+3=6	1+2+3=6
1+4=6	
2+4=6	

Q4.1 If other, please specify: NA = 1; Not Specified = 2; Specified (Aches, Stroke & Epilepsy) = 3

Q5 How do you diagnose the mental disorder/s selected above?

(a) Consult with ancestors for guidance (1)

(b) Ask patient about their background for guidance (2)

(c) Read patient for signs and guidance (3)

(d) Use patient's symptoms for guidance (4)

(e) Other (5)

1+2+3=6	1+4=6
1+2+4=6	All=6
2+3+4=6	2+3=6
1+3=6	3+4=6
No response=5	1+2=6

Q6 How do you treat your patients in general?

(a) Pray for them (1)

(b) Take them for a cleansing bath (2)

(c) Give them a medicinal concoction (3)

(d) Refer them to a pharmacy (4)

(e) Other (5)

1+2+3=6	All=6
1+3=6	No response=5
1+4=6	2+3=6
1+3+4=6	1+2+4=6
1+2=6	

Q7 Is your treatment option effective? Yes (1); No (2); Don't know (3); No response (3)

Q8 How do you know that your treatment option is effective?

(a) Do follow-up (1)

(b) Confident in my treatment option (2)

(c) Other (3); 1+2=4; No response=3

Q9 What is traditional medicine?

(a) A medicinal concoction made from indigenous plants (1)

(b) The cheaper treatment option (2)

(c) Similar to pharmacological medicine (3)

(d) Other (4)

1+3=4	1+2=4
No response=4	All=4

SECTION C: KNOWLEDGE OF SCHIZOPHRENIA AND ITS TREATMENT

Q10 Do you know a mental health disorder called schizophrenia? Yes (1); No (2) No response = 2

Q11 What is your understanding of schizophrenia?

(a) Sign that the person with this disorder has an ancestral calling (1)

(b) Sign that the person with this disorder has been bewitched (2)

(c) Craziness (3)

(d) Losing touch with reality (4)

(e) Other (5)

3+4=6	1+3=6
No response=5	2+3+4=6
1+2+4=6	1+4=6

Q12 Are delusions, hallucinations, hostility, suspiciousness, grandiosity and conceptual disorganization symptoms of schizophrenia? Yes (1); No (2); Don't know (3); No response (3)

13 Is the lack of motivation, lack of enjoyment and social interactions, asociality, anhedonia and alogia symptoms of schizophrenia? Yes (1); No (2); Don't know (3); No response (3)

Q14 Do you treat or manage schizophrenia? Yes (1); No (2); No response (2)

Q15 How do you treat or manage schizophrenia?

(a) Give patient a medicinal concoction (1)

(b) Take patient for cleansing bath (2)

(c) Pray for the patient (3)

(d) Perform a ritual on the patient (4)

(e) Other (5)

1+2=6	1+3+4=6
1+3=6	1+2+4=6
1+2+3=6	3+4=6
No response=5	1+4=6
All=6	

7.2. Questionnaire Data Entry

SPSS Qestionnaire Data Entry Final 18-02-2022.sav

	D1	D2	D3	D4	D5	D6
1	2.00	1.00	2.00	1.00	5.00	N/A
2	3.00	1.00	2.00	1.00	5.00	N/A
3	4.00	1.00	2.00	1.00	5.00	N/A
4	5.00	1.00	2.00	1.00	5.00	N/A
5	6.00	1.00	2.00	2.00	3.00	N/A
6	7.00	1.00	2.00	1.00	2.00	N/A
7	8.00	1.00	1.00	2.00	2.00	N/A
8	10.00	2.00	2.00	2.00	2.00	N/A
9	13.00	1.00	2.00	2.00	2.00	N/A
10	14.00	1.00	2.00	2.00	5.00	N/A
11	15.00	1.00	3.00	2.00	5.00	N/A
12	16.00	1.00	2.00	2.00	2.00	N/A
13	17.00	1.00	1.00	2.00	3.00	N/A
14	20.00	2.00	2.00	2.00	5.00	N/A
15	22.00	1.00	1.00	2.00	5.00	N/A
16	24.00	2.00	1.00	2.00	1.00	N/A
17	25.00	2.00	2.00	2.00	3.00	N/A
18	26.00	3.00	2.00	2.00	5.00	N/A
19	27.00	1.00	1.00	2.00	5.00	N/A
20	28.00	1.00	2.00	2.00	5.00	N/A
21	29.00	1.00	2.00	2.00	2.00	N/A
22	30.00	2.00	3.00	3.00	5.00	N/A
23	31.00	1.00	2.00	3.00	2.00	N/A
24	32.00	2.00	2.00	3.00	3.00	N/A
25	33.00	1.00	2.00	3.00	2.00	N/A
26	34.00	1.00	3.00	3.00	2.00	N/A
27	35.00	1.00	3.00	3.00	2.00	N/A
28	36.00	1.00	2.00	3.00	5.00	N/A
29	37.00	2.00	2.00	3.00	5.00	N/A
30	38.00	1.00	2.00	3.00	2.00	N/A
31	39.00	1.00	2.00	3.00	2.00	N/A
32	40.00	1.00	3.00	3.00	5.00	N/A
33	41.00	1.00	2.00	3.00	5.00	N/A
34	42.00	1.00	2.00	3.00	2.00	N/A
35	43.00	1.00	1.00	3.00	2.00	N/A
36	44.00	1.00	2.00	3.00	2.00	N/A
37	45.00	1.00	2.00	3.00	2.00	N/A
38	46.00	2.00	1.00	3.00	2.00	N/A
39	48.00	2.00	1.00	3.00	4.00	Ithwasa

SPSS Qestionnaire Data Entry Final 18-02-2022.sav

	Q4.1	Q5	Q6	Q7	Q8	Q9
1	1	6.00	6.00	1.00	4.00	1.00
2	1	3.00	2.00	1.00	2.00	1.00
3	1	6.00	6.00	1.00	4.00	1.00
4	1	6.00	6.00	1.00	1.00	1.00
5	1	2.00	1.00	1.00	1.00	1.00
6	1	1.00	4.00	1.00	1.00	4.00
7	1	6.00	6.00	1.00	4.00	1.00
8	1	6.00	6.00	1.00	4.00	1.00
9	1	6.00	6.00	3.00	4.00	1.00
10	1	6.00	6.00	1.00	4.00	1.00
11	1	6.00	6.00	1.00	4.00	1.00
12	1	6.00	6.00	3.00	4.00	1.00
13	1	3.00	4.00	3.00	3.00	4.00
14	1	6.00	6.00	1.00	1.00	1.00
15	1	2.00	1.00	3.00	3.00	1.00
16	1	5.00	1.00	1.00	3.00	1.00
17	1	1.00	1.00	1.00	2.00	1.00
18	1	6.00	6.00	1.00	2.00	1.00
19	1	4.00	6.00	1.00	2.00	1.00
20	1	5.00	6.00	1.00	1.00	1.00
21	1	5.00	5.00	3.00	4.00	1.00
22	1	6.00	6.00	1.00	4.00	1.00
23	1	6.00	3.00	1.00	1.00	1.00
24	1	5.00	5.00	1.00	3.00	4.00
25	1	3.00	3.00	1.00	4.00	1.00
26	1	6.00	3.00	3.00	3.00	4.00
27	1	5.00	3.00	1.00	2.00	1.00
28	1	6.00	6.00	1.00	4.00	4.00
29	1	6.00	6.00	1.00	4.00	4.00
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31	1	1.00	3.00	1.00	1.00	1.00
32	1	6.00	6.00	1.00	4.00	1.00
33	3	1.00	3.00	1.00	1.00	1.00
34	1	1.00	3.00	3.00	4.00	3.00
35	1	6.00	6.00	1.00	4.00	4.00
36	1	1.00	6.00	1.00	4.00	1.00
37	1	4.00	3.00	1.00	1.00	1.00
38	1	3.00	3.00	1.00	1.00	1.00
39	1	5.00	5.00	1.00	1.00	1.00

SPSS Qestionnaire Data Entry Final 18-02-2022.sav

	Q10	Q11	Q12	Q13	Q14	Q15
1	1.00	6.00	1.00	2.00	1.00	6.00
2	1.00	4.00	2.00	1.00	2.00	2.00
3	1.00	4.00	1.00	3.00	1.00	6.00
4	1.00	4.00	2.00	1.00	2.00	1.00
5	2.00	6.00	1.00	3.00	2.00	5.00
6	2.00	5.00	3.00	3.00	2.00	5.00
7	2.00	6.00	1.00	3.00	1.00	6.00
8	2.00	4.00	1.00	2.00	1.00	6.00
9	2.00	6.00	3.00	3.00	2.00	5.00
10	2.00	6.00	3.00	1.00	2.00	6.00
11	2.00	5.00	3.00	3.00	2.00	5.00
12	2.00	6.00	3.00	3.00	2.00	6.00
13	1.00	6.00	1.00	1.00	1.00	5.00
14	1.00	5.00	3.00	3.00	1.00	6.00
15	1.00	4.00	1.00	1.00	2.00	5.00
16	2.00	4.00	2.00	1.00	2.00	1.00
17	1.00	4.00	2.00	2.00	2.00	1.00
18	2.00	5.00	3.00	3.00	2.00	5.00
19	1.00	4.00	1.00	1.00	1.00	1.00
20	1.00	4.00	1.00	1.00	2.00	1.00
21	2.00	5.00	3.00	3.00	2.00	5.00
22	2.00	4.00	1.00	1.00	1.00	6.00
23	1.00	4.00	1.00	1.00	1.00	1.00
24	1.00	6.00	1.00	1.00	2.00	6.00
25	1.00	4.00	1.00	1.00	1.00	1.00
26	2.00	6.00	1.00	1.00	1.00	1.00
27	2.00	4.00	2.00	1.00	2.00	1.00
28	1.00	6.00	1.00	1.00	1.00	6.00
29	1.00	6.00	1.00	1.00	1.00	6.00
30	1.00	6.00	1.00	1.00	1.00	6.00
31	1.00	6.00	1.00	1.00	1.00	6.00
32	1.00	4.00	2.00	1.00	1.00	1.00
33	1.00	6.00	1.00	1.00	2.00	1.00
34	2.00	5.00	3.00	3.00	2.00	5.00
35	1.00	6.00	1.00	2.00	2.00	6.00
36	1.00	6.00	1.00	2.00	2.00	1.00
37	1.00	4.00	1.00	1.00	1.00	1.00
38	1.00	4.00	1.00	1.00	1.00	1.00
39	2.00	4.00	1.00	1.00	1.00	1.00

SPSS Qestionnaire Data Entry Final 18-02-2022.sav

	D1	D2	D3	D4	D5	D6
40	56.00	1.00	2.00	4.00	2.00	N/A
41	57.00	1.00	1.00	4.00	2.00	N/A
42	58.00	1.00	3.00	4.00	2.00	N/A
43	59.00	1.00	3.00	4.00	2.00	N/A
44	60.00	1.00	2.00	4.00	5.00	N/A
45	61.00	1.00	2.00	4.00	5.00	N/A
46	62.00	1.00	2.00	4.00	5.00	N/A
47	63.00	1.00	2.00	4.00	5.00	N/A
48	64.00	1.00	3.00	4.00	5.00	N/A
49	65.00	1.00	2.00	4.00	2.00	N/A
50	66.00	1.00	3.00	4.00	5.00	N/A
51	67.00	2.00	3.00	4.00	1.00	N/A
52	68.00	1.00	3.00	4.00	2.00	N/A
53	70.00	1.00	2.00	2.00	2.00	N/A
54	71.00	1.00	2.00	4.00	3.00	N/A
55	72.00	1.00	1.00	4.00	2.00	N/A
56	73.00	1.00	2.00	4.00	5.00	N/A
57	74.00	1.00	2.00	4.00	2.00	N/A
58	75.00	2.00	2.00	4.00	2.00	N/A
59	76.00	2.00	1.00	4.00	2.00	N/A
60	77.00	2.00	1.00	4.00	2.00	N/A
61	78.00	2.00	1.00	4.00	2.00	N/A
62	79.00	2.00	2.00	4.00	2.00	N/A
63	80.00	2.00	1.00	4.00	2.00	N/A
64	81.00	1.00	1.00	4.00	2.00	N/A
65	82.00	1.00	1.00	4.00	2.00	N/A
66	83.00	2.00	2.00	3.00	2.00	N/A
67	84.00	1.00	1.00	3.00	3.00	N/A
68	85.00	1.00	2.00	3.00	2.00	N/A
69	86.00	1.00	2.00	3.00	2.00	N/A
70	87.00	1.00	2.00	3.00	2.00	N/A
71	88.00	1.00	2.00	3.00	2.00	N/A
72	89.00	1.00	2.00	3.00	3.00	N/A
73	90.00	1.00	2.00	3.00	2.00	N/A
74	91.00	1.00	2.00	3.00	2.00	N/A
75	92.00	2.00	1.00	3.00	2.00	N/A
76	93.00	1.00	2.00	3.00	2.00	N/A
77	94.00	1.00	3.00	3.00	3.00	N/A
78	95.00	1.00	2.00	3.00	2.00	N/A

SPSS Qestionnaire Data Entry Final 18-02-2022.sav

	D7	D8	Q1	Q2	Q3	Q4
40	2.00	5	1.00	4.00	2.00	5.00
41	1.00	5	1.00	1.00	1.00	2.00
42	3.00	5	2.00	1.00	2.00	5.00
43	1.00	5	1.00	1.00	2.00	5.00
44	3.00	5	1.00	4.00	1.00	6.00
45	3.00	5	1.00	4.00	2.00	1.00
46	2.00	5	1.00	2.00	2.00	5.00
47	1.00	5	1.00	4.00	1.00	6.00
48	3.00	5	1.00	2.00	2.00	4.00
49	1.00	5	1.00	4.00	1.00	2.00
50	1.00	5	1.00	4.00	2.00	1.00
51	1.00	4	2.00	3.00	2.00	5.00
52	4.00	5	1.00	2.00	1.00	5.00
53	1.00	4	2.00	2.00	2.00	5.00
54	3.00	5	1.00	2.00	1.00	1.00
55	1.00	5	1.00	4.00	1.00	1.00
56	2.00	1	1.00	4.00	1.00	6.00
57	1.00	5	1.00	4.00	2.00	1.00
58	3.00	2	2.00	4.00	1.00	2.00
59	2.00	2	2.00	1.00	1.00	6.00
60	2.00	5	1.00	1.00	1.00	6.00
61	1.00	2	1.00	2.00	2.00	2.00
62	4.00	2	1.00	2.00	1.00	4.00
63	1.00	2	1.00	1.00	1.00	2.00
64	1.00	5	1.00	4.00	1.00	1.00
65	2.00	5	1.00	2.00	1.00	2.00
66	3.00	2	1.00	2.00	1.00	2.00
67	1.00	5	1.00	4.00	2.00	1.00
68	3.00	5	1.00	2.00	1.00	3.00
69	1.00	5	1.00	4.00	2.00	1.00
70	2.00	5	2.00	4.00	2.00	1.00
71	2.00	5	1.00	2.00	2.00	2.00
72	2.00	5	1.00	2.00	1.00	2.00
73	1.00	5	2.00	1.00	2.00	5.00
74	2.00	5	2.00	1.00	2.00	2.00
75	1.00	1	1.00	1.00	2.00	2.00
76	1.00	5	2.00	2.00	1.00	2.00
77	3.00	1	1.00	2.00	1.00	5.00
78	2.00	3	1.00	2.00	2.00	2.00

SPSS Qestionnaire Data Entry Final 18-02-2022.sav

	Q4.1	Q5	Q6	Q7	Q8	Q9
40	1	1.00	6.00	3.00	2.00	4.00
41	1	6.00	6.00	1.00	4.00	1.00
42	1	1.00	3.00	1.00	1.00	3.00
43	1	5.00	3.00	3.00	1.00	1.00
44	1	6.00	6.00	1.00	1.00	1.00
45	1	1.00	6.00	1.00	4.00	1.00
46	1	5.00	5.00	3.00	4.00	1.00
47	1	6.00	6.00	1.00	3.00	4.00
48	1	1.00	6.00	1.00	2.00	1.00
49	1	6.00	3.00	1.00	4.00	1.00
50	1	3.00	6.00	1.00	2.00	1.00
51	1	5.00	6.00	1.00	1.00	1.00
52	1	1.00	3.00	3.00	2.00	1.00
53	1	5.00	6.00	1.00	4.00	1.00
54	1	6.00	6.00	1.00	4.00	1.00
55	1	6.00	6.00	1.00	4.00	1.00
56	1	6.00	6.00	1.00	4.00	4.00
57	1	6.00	6.00	1.00	4.00	4.00
58	1	5.00	3.00	1.00	4.00	4.00
59	1	1.00	5.00	1.00	1.00	1.00
60	1	6.00	3.00	1.00	1.00	1.00
61	1	1.00	3.00	1.00	1.00	1.00
62	1	1.00	3.00	1.00	4.00	1.00
63	1	6.00	3.00	1.00	2.00	1.00
64	1	6.00	6.00	1.00	4.00	1.00
65	1	1.00	3.00	1.00	1.00	1.00
66	1	1.00	3.00	1.00	1.00	1.00
67	1	6.00	6.00	1.00	4.00	1.00
68	1	5.00	2.00	1.00	3.00	1.00
69	1	3.00	6.00	1.00	4.00	1.00
70	1	2.00	3.00	1.00	2.00	1.00
71	1	4.00	3.00	1.00	2.00	1.00
72	1	3.00	1.00	1.00	1.00	1.00
73	2	5.00	5.00	1.00	2.00	1.00
74	1	6.00	6.00	1.00	4.00	1.00
75	1	4.00	4.00	1.00	1.00	1.00
76	1	3.00	3.00	1.00	1.00	1.00
77	3	1.00	1.00	1.00	1.00	1.00
78	1	3.00	3.00	1.00	1.00	1.00

SPSS Qestionnaire Data Entry Final 18-02-2022.sav

	Q10	Q11	Q12	Q13	Q14	Q15
40	2.00	6.00	3.00	3.00	1.00	6.00
41	1.00	4.00	1.00	3.00	1.00	1.00
42	2.00	5.00	3.00	3.00	2.00	5.00
43	2.00	5.00	3.00	3.00	2.00	5.00
44	1.00	4.00	1.00	1.00	2.00	1.00
45	2.00	5.00	3.00	3.00	2.00	5.00
46	2.00	5.00	3.00	3.00	2.00	5.00
47	2.00	5.00	3.00	3.00	2.00	5.00
48	1.00	4.00	2.00	1.00	1.00	6.00
49	1.00	4.00	2.00	1.00	2.00	1.00
50	1.00	4.00	1.00	2.00	1.00	6.00
51	2.00	5.00	3.00	3.00	2.00	5.00
52	2.00	5.00	3.00	3.00	2.00	5.00
53	2.00	5.00	3.00	3.00	2.00	5.00
54	1.00	4.00	1.00	1.00	2.00	5.00
55	1.00	4.00	1.00	1.00	2.00	5.00
56	1.00	6.00	1.00	1.00	1.00	6.00
57	2.00	5.00	3.00	3.00	2.00	5.00
58	2.00	5.00	3.00	3.00	2.00	5.00
59	1.00	4.00	1.00	2.00	1.00	1.00
60	1.00	4.00	2.00	1.00	1.00	1.00
61	1.00	4.00	1.00	1.00	2.00	1.00
62	1.00	4.00	1.00	2.00	2.00	1.00
63	1.00	6.00	1.00	1.00	1.00	6.00
64	1.00	4.00	1.00	1.00	2.00	5.00
65	1.00	4.00	2.00	1.00	2.00	4.00
66	1.00	3.00	2.00	2.00	1.00	1.00
67	1.00	4.00	1.00	1.00	2.00	5.00
68	1.00	4.00	1.00	1.00	1.00	5.00
69	1.00	4.00	1.00	2.00	2.00	1.00
70	2.00	5.00	3.00	3.00	2.00	5.00
71	2.00	5.00	3.00	3.00	2.00	5.00
72	1.00	3.00	1.00	1.00	1.00	5.00
73	2.00	5.00	3.00	3.00	2.00	5.00
74	2.00	5.00	3.00	3.00	2.00	5.00
75	1.00	4.00	1.00	1.00	2.00	1.00
76	1.00	4.00	2.00	2.00	1.00	1.00
77	2.00	5.00	3.00	3.00	2.00	5.00
78	1.00	4.00	1.00	1.00	1.00	1.00

SPSS Qestionnaire Data Entry Final 18-02-2022.sav

	D1	D2	D3	D4	D5	D6
79	96.00	1.00	2.00	4.00	2.00	N/A
80	97.00	1.00	1.00	3.00	2.00	N/A
81	98.00	1.00	2.00	3.00	2.00	N/A
82	99.00	1.00	2.00	3.00	2.00	N/A
83	100.00	2.00	1.00	3.00	3.00	N/A
84	47.00	1.00	2.00	2.00	2.00	N/A
85	49.00	2.00	3.00	2.00	1.00	N/A
86	50.00	1.00	3.00	2.00	2.00	N/A
87	55.00	2.00	3.00	2.00	2.00	N/A
88	51.00	1.00	2.00	1.00	2.00	N/A
89	52.00	1.00	2.00	1.00	2.00	N/A
90	53.00	1.00	2.00	1.00	2.00	N/A
91	54.00	2.00	1.00	1.00	2.00	N/A
92	69.00	1.00	1.00	1.00	2.00	N/A
93	101.00	1.00	2.00	1.00	2.00	N/A
94	102.00	2.00	1.00	1.00	2.00	N/A
95	103.00	2.00	1.00	1.00	2.00	N/A
96	104.00	1.00	2.00	1.00	2.00	N/A
97	105.00	2.00	2.00	1.00	2.00	N/A
98	106.00	1.00	2.00	1.00	2.00	N/A
99	107.00	1.00	3.00	1.00	3.00	N/A
100	108.00	1.00	2.00	1.00	2.00	N/A

SPSS Qestionnaire Data Entry Final 18-02-2022.sav

	D7	D8	Q1	Q2	Q3	Q4
79	1.00	5	1.00	2.00	2.00	5.00
80	1.00	5	1.00	2.00	1.00	2.00
81	2.00	5	1.00	1.00	1.00	2.00
82	3.00	5	1.00	1.00	1.00	2.00
83	1.00	5	2.00	2.00	1.00	4.00
84	3.00	5	1.00	1.00	1.00	6.00
85	3.00	5	1.00	1.00	1.00	6.00
86	3.00	5	1.00	1.00	1.00	6.00
87	4.00	5	1.00	1.00	1.00	6.00
88	1.00	5	1.00	2.00	1.00	2.00
89	2.00	5	1.00	4.00	1.00	2.00
90	1.00	5	1.00	2.00	1.00	2.00
91	1.00	5	1.00	3.00	1.00	6.00
92	1.00	5	1.00	2.00	1.00	2.00
93	1.00	5	1.00	2.00	1.00	2.00
94	2.00	5	1.00	2.00	1.00	2.00
95	2.00	5	1.00	2.00	1.00	2.00
96	2.00	5	1.00	4.00	1.00	2.00
97	3.00	5	1.00	2.00	1.00	2.00
98	2.00	5	1.00	2.00	1.00	2.00
99	2.00	5	1.00	1.00	1.00	6.00
100	2.00	5	1.00	5.00	1.00	2.00

SPSS Qestionnaire Data Entry Final 18-02-2022.sav

	Q4.1	Q5	Q6	Q7	Q8	Q9
79	3	4.00	3.00	1.00	2.00	1.00
80	1	1.00	3.00	1.00	2.00	1.00
81	1	3.00	3.00	1.00	2.00	1.00
82	1	3.00	3.00	1.00	1.00	1.00
83	1	6.00	1.00	1.00	2.00	1.00
84	1	6.00	3.00	1.00	2.00	1.00
85	1	6.00	3.00	1.00	2.00	1.00
86	1	6.00	3.00	1.00	2.00	1.00
87	1	6.00	3.00	1.00	2.00	1.00
88	1	1.00	3.00	1.00	1.00	1.00
89	1	3.00	2.00	1.00	2.00	1.00
90	1	1.00	3.00	1.00	1.00	1.00
91	1	1.00	2.00	1.00	2.00	1.00
92	1	1.00	3.00	1.00	2.00	1.00
93	1	1.00	2.00	1.00	1.00	1.00
94	1	1.00	2.00	1.00	2.00	1.00
95	1	1.00	3.00	1.00	1.00	1.00
96	1	1.00	2.00	1.00	2.00	1.00
97	1	4.00	4.00	1.00	1.00	1.00
98	1	1.00	2.00	1.00	2.00	1.00
99	1	5.00	6.00	1.00	1.00	1.00
100	1	1.00	3.00	1.00	2.00	1.00

SPSS Qestionnaire Data Entry Final 18-02-2022.sav

	Q10	Q11	Q12	Q13	Q14	Q15
79	1.00	4.00	2.00	2.00	2.00	1.00
80	1.00	4.00	1.00	2.00	1.00	1.00
81	2.00	5.00	3.00	3.00	2.00	5.00
82	2.00	5.00	3.00	3.00	2.00	5.00
83	2.00	5.00	3.00	3.00	2.00	5.00
84	1.00	6.00	1.00	3.00	1.00	1.00
85	1.00	6.00	1.00	3.00	1.00	1.00
86	1.00	6.00	1.00	2.00	1.00	1.00
87	1.00	6.00	1.00	3.00	1.00	1.00
88	1.00	4.00	3.00	1.00	1.00	1.00
89	1.00	4.00	1.00	1.00	1.00	1.00
90	1.00	4.00	1.00	1.00	1.00	1.00
91	1.00	4.00	1.00	1.00	1.00	2.00
92	1.00	4.00	1.00	1.00	1.00	1.00
93	1.00	4.00	1.00	1.00	1.00	1.00
94	1.00	4.00	1.00	1.00	1.00	1.00
95	1.00	4.00	1.00	3.00	1.00	1.00
96	1.00	4.00	1.00	1.00	1.00	1.00
97	1.00	4.00	1.00	1.00	2.00	1.00
98	1.00	3.00	1.00	1.00	1.00	1.00
99	1.00	5.00	1.00	1.00	1.00	6.00
100	1.00	4.00	1.00	3.00	1.00	1.00

7.3. Focused Transcripts for Focus Group Discussions

Since the start of your practice, how many patients have you diagnosed with mental health disorders? Common age and gender profile?		
Kokstad		
Group 1	P4	I have treated 4 who have recovered and continued with their normal lives. The first one was a 38 year old man, the other one was a 18year old young woman and the other 2 were 16 and 19 year old young men.
	P14	I have treated 7 and they have not been mentally disturbed since then. Their condition was to a point that they were chained as they were destroying everything in their households. The 7 patients were boys who just completed Grade 12/school.
	P7	The patients I'm certain recovered are 2 which I have treated and they are still fine to date. They are continuing with their studies as they were school kids. The last patient I treated was writing his finals in Grade 12, I'm not sure of his age. The other patients I have treated, one was approximately 30-32 years old and the other one was a man approximately 28 years old.
	P13	I have treated a school kid who also recovered. Other patients I have treated include 2 kids who were attending crèche and one 14 year old girl.
	P16	I have treated 2 who have not returned, which means they recovered and are still okay. One patient was a 50 year old man and the other one was a 25 year old man.
	P3	Those I have treated were taking drugs. There were 3 of them and 1 who came in last week is still with me, they could say their name when they came in but now they can even play with others. The first one was a 16 year old kid.
Group 2	P(n) male	I have diagnosed both boys and girls. When we were growing up, we used to do ukuphosa (form of witchcraft) on girls which created ufufunyana on them. In now days, even woman can do this type of witchcraft on men.
uMzimkhulu		
	P2	It is usually young people between the ages of 15 to 30 years who suffer from mental disorders and it is usually females.
	P6	It usually females who are from the age of 15 years and above.
	P7	It is usually females from the ages of 15, 16 and above 34 years.
	P1	It usually starts in children form the age of 15 years. Sometimes also in older people above 50 years due to stress caused by troublesome grandchildren.
	P3	Schizophrenia is common in young kids around the ages of 16, 17, 18 - 20 years.
	P4	Schizophrenia is common in girls between the ages of 15 - 20 years.
	P12	The onset of mental disorders usually occurs in kids at the stage of adolescents. And other people who suffer from mental disorders are old people between the ages of 50 - 60 years due to being abused by their children. It is usually women who suffer from mental disorders
Buhlebezwe		

	P13	I've treated a 28 year old and a 15 year old girl with schizophrenia.
	P5	Those I've treated were high school kids who were 16, 17 and 18 years old. In some cases, older people between the ages of 40-50years old also come in due to mental disorders.
	P15	One of my patients who had schizophrenia was an 18 year old girl.
NDZ		
	P12	Mental disorders are common in males in the age of 50 and above.
	P2	Mental disorders can affect people in age of 40 or younger.
	P3	Mental illness can affect a child who is 10 years old.
	P9	Mental disorders usually affect males.

Apart from your patients, which gender and age groups commonly suffer from mental disorders?		
Buhlebezwe		
	P14	I've seen a 14 year old boy
	P(n)	I usually see males between the ages of 35-40 years of age
	P10	I usually see males between the ages of 30-40 years of age.

What is/has been the cause of the mental disorders in the patients you have treated and what was the diagnosis?		
Kokstad		
Group 1		
	P4	Of the 4 that I treated, drugs were the cause mental illness in 2. Once they recover they are able to say which drugs they were using. 1 was ufufunyana and the other seemed to have had the illness in them.
	P14	Most of those I have treated had ufufunyana, into efakiwe, umeqo, babevutheliswa. The cause of mental illness in the 2 patients isilwane sihamba nalo ufufunyana.
	P7	2 had schizophrenia and the cause was the use of drugs in the other one.
	P13	The other patient had seizures.
	P16	One of my patients were abused by their wife until they were not okay mentally. Another patient had seizures which were caused by isilwane.

	P3	My patients were suffering from ufufunyana. The first one ubehlushwa abantu abadala (had an ancestral calling), this started when they were young and this was ignored in their family until the ancestors took away their sanity.
Group 2	P(n) male	What cause schizophrenia/ufufunyana in girls was ukuphosa ngohlobo oluthize. Ufufunyana is not hereditary
	P(n) female	Schizophrenia/ufufunyana olufakwe ngendlela emdaka besides ukuphoswa.
	P(n) female2	Only diabetes or asthma are hereditary, not ufufunyana
uMzimkhulu		
	P2	The cause of mental illness in my patient was defaulting in their HIV treatment. In some cases it is caused by ukubhiddana kwezinto emsamo and the ancestors
	P7	Schizophrenia caused by ukuphambana kwezinto emsamo and not accepting the gifts that your ancestor give to you. In others mental illness is caused by too much stress leading to depression. Others default from their HIV treatment and then say they have an ancestral calling when that is not the case.
	P1	In some case when family members do not have the same beliefs and follow different religions, kwakumele kwenziwe umsebenzi kagogo for one of the patients for them to recover; however, their mother and sister refused because they do not believe in such until they became mentally ill. Grandparents get depressed because of misbehaved grandchildren.
	P3	I have diagnosed schizophrenia and as I was questioning the patient's parents I discovered that the patient defaulted from their HIV treatment. It happens that when you default from HIV treatment you become mentally ill. Sometimes mental illness is due to umsamo ongalinganga, sometimes ufufunyana is due to izinto zabantu (bewitchment) and ancestors.
	P4	The problem was with the ancestors and akukho abazali asebeke bakulungisa okuqondene nomsamo kanti umntwana wayezelwe embethe.
	P12	There are different types of mental illness, there is stress and a person with stress is not aggressive, whereas a crazy person is aggressive. Another person who has mental illness ufakwa izizwe, ufufunyana. There are different types of ufufunyana, there is izimungulu, imidlenyane ana amabutho amnyama which is what causes mental illness in a person.
Buhlebezwe		
	P11	I've diagnosed ufufnyana and other mental illnesses. It is easy for us THPs to diagnose ufufunyana because if ulumbambile walihlukumeza luyakhuluma and a person with ufufunyana is aggressive. And a person who has ufufunyana does not wander around as this disorder affects them at particular time, olunye ufufunyana luthuswa umbani wezulu.
	P8	In all mental illnesses there is a spirit that enters their body and that spirit goes straight to the head and cause mental illness. Ufufunyana can be caused by young men bewitching woman for love, in some cases it is caused by abathakazthi (witches/wizards)

		who do not want to see that person succeed. They then use imithi that evokes evil spirits in the victim and these sprits then clash with the good spirits living inside of this person and when these spirits fight, they cause mental disturbance.
	P5	A person becomes mentally ill because of imimoya yokuthi uthakathiwe (spirits from bewitchment), they are just crazy and some mental illness has to do with ancestors. In some cases a person can have ancestors fighting for them, maybe that person is using a surname that is not theirs and the ancestors from these surnames end up fighting over them.
	P20	An unclear ancestry and using an incorrect surname can cause mental disturbance.
NDZ		
	P13	There is a person who is just crazy, there is ufufunyana which is inflicted and there is mental illness a THP cannot treat. There are mental illnesses in my knowledge, ufufunyana, madness and izizwe (spirit possession).
	P2	In some cases it is just confusion, in cases it is heredity and in some cases it requires ukuthi umuntu umphehlele amagobongo and that it how you will know if the illness has to do with ancestors.
	P3	If there was someone in the family with this illness you will find that the child will also have it. In some cases you would find that a child is mentally ill because there are things (traditionally/culturally) that need to be done for them. In some cases igobongo and in other cases they have to be THPs.
	P9	In some cases it is caused by stress.

What were the common symptoms?		
Kokstad		
Group1	P14	The one who had schizophrenia had seen something.
	P7	One who had schizophrenia was chasing and killing chickens and also said they were seeing some people. Another one who had schizophrenia saw people who were chasing them.
	P13	They were seeing people we did not see.
	P16	When you were sitting and chatting with the one who had stress, you could see that they were far away with thoughts and you had to shake him for him to pay attention.
	P14	One patient exited class through the window and the other person banging himself on his car saying he no longer wants it and this was due to bewitchment.
	P13	One child could not walk and speak due to the illness.

	P3	The symptoms for different mental illnesses are never the same. But in some instances they may be similar. In some cases you will find that a patient is carried to you and they hear sounds ringing in their ears. Some cannot speak, some are violent and hit everything around them.
Group 2	P(n) male	Ufufunyana can show no symptoms until a certain time.
	P(n) female	A person with schizophrenia becomes cheeky and aggressive.
uMzimkhulu		
	P2	Aggressive to a point that they tied and carried to me. And some scream and beat other kids at school.
	P3	The patient was tied up and in pain. They were said to be aggressive, they teared their clothes, destroyed everything in the house, they were not eating or sleeping. (mental illness due to defaulting from HIV treatment)
	P4	They were calling out people's names.
	P12	A crazy person is aggressive, but a depressed person is not. A person with mental illness also has seizures. And a person with stress experiences headaches, ubanamahlombe asindayo and they hear sounds.
	P	
Buhlebezwe		
	P11	Some talk non-stop, some look shocked (with their eyes out), some don't eat, they are unresponsive, incoherent and they move around a lot.
	P8	They are incoherent, there is a spirit in them that then goes to their brain and makes them talk things that don't make any sense. They are also forgetful. A person with ufufunyana screams, sometimes they do not want to speak while they have the ability to speak, ufufunyana limits the ability of a person to speak as they can tell a person who to speak to and vice versa
	P13	A person with ufufunyana does not want to eat, they want to eat inedible leaves.
NDZ		
	P13	A person with izizwe cries or screams out of the blue, calls a name of that person and then goes to that person. As they do this, they are disturbed mentally. A person with ufufunyana screams, does things sitting one place and does not speak much. And there is a mad person who speaks a lot of things which do not make sense, in most cases THPs cannot treat a mad person.

Methods of diagnosis		
Kokstad		
Group 1	P3	Uyasihlola – you communicate with the patients ancestors in order to understand the cause of illness. You first consult empephweni with the ancestors, that where you will get the answers and how you need to help your patient.
	P14	You first find out from the people who bring in the patient what is wrong with the patient. You then consult with the ancestors and they will give you the diagnosis. In some case you do not need to consult with the ancestors as you can tell the person is not mentally ill but needs counselling and some illnesses need doctors and not THPs.
uMzimkhulu		
	P6	I consult with ancestors as the patient comes in and they advise on what is wrong with the patient and how I should treat them.

Treatment		
Kokstad		
Group 1	P4	You first need to cleanse the patient before you can start with treatment. You also need to monitor the timing when the illness kicks.
	P16	Stressed patients need counselling.
	P3	When the illness has to do with ancestors, appropriate rituals must be done.
	P14	Other mental illnesses need to be treated by doctors, the one we treat is ufufunyana. Uyamkhamela umdoma ngomuthi to calm them down. When it comes to ufufunyana we use traditional medicines which we get from the forest. We do not buy our medicines at Muthi market as medicinal plants are not the same from different parts of the country. We are confident in our medicinal plants as we do not have red soil in our region. We learn about the different herbs during initiation. For treatment of ufufunyana, sibhemisa ikhala using powdered muthi from the forest. Focusing on ufufunyana, the traditional medicine is inhaled through the nose using powdered herbs. Ufufunyana can also be removed through induced vomiting and enema with traditional medicine.
	P13	Sometimes there no need to even use herbal remedies on a patient. With just water and prayer they can be treated.

Group 2	P(n) male	You use a medicinal concoction to treat schizophrenia. Schizophrenia can be treated as long as you know the cause.
	P(n) female 2	There is no standard duration of treatment, it depends on each patient's situation.
	P(n) female	Medication is administered through inhalation, enema and drinking, It depends on the techniques used by each healer.
uMzimkhulu		
	P12	As there are different symptoms, the modes of treatment are also different. In some cases a mentally ill person does not require to be treated with pills but with traditional medicines (ubulawu) when the illness has to do with ancestors and umsamo ungalungile. A person with umdlenyane is treated by ukuthelwa emakhaleni (sniffing), apahalaziswe (vomiting), amiselwe ukhamba lofufunyana. It is hard for us to give out the herbs that we use, maybe it would be better if the researchers or government gives us for the information we give on these herbal plants. Or if the herbal plants get tested at the lab and they succeed, the product crated from it has to have my name as a healer and I should be paid for its use.
	P7	Even when the governments sees that biomedical medicine (which they charge money for) is not working, they will never come to the THPs because they perceive biomedical medicine to be superior to traditional healing.
Buhlebesizwe		
	P8	I burn muthi with coal for a person with ufufunyana and I make them inhale the smoke to provoke the spirits in them to start speaking.
	P13	We burn muthi for a person with ufufunyana until the spirits speak.
	P11	There many ways to treat mental illness, and we as THPs do not the same treatment approaches. The treatment approaches include burning umuthi for the patient to inhale, enema and induced vomiting. We also use a technique where things need to come through the nose from the patient's head. Steaming and a cleansing bath can also be used, but we have to calm the patient down first before you them for treatment. Even when the patients show the same symptoms, but it never happens that the same treatment method is used on them. The duration of treatment depends on how strong the witches' muthi is. Not only do the other ethnic groups also have ancestors, the Indians particularly also bewitch each other, they have strong and dark muthi.
	P5	We do not use the same treatment methods. I first consult with the ancestors, and then use enema and then the aggression in a patient gets better. Sometimes an herbal concoction is squeezed inside the nose of a patient, when you put this muthi in their nose in some cases bugs, i.e. a beetle would come out from the nose. After these come out the patient then gets better. There

		after you can take the patient outside to the river or mountain and into hills for further treatment. Steaming and induced vomiting can also be used. The bugs that come out from a patient's nose are alive and they need to be caught because if they escape, the patient will never recover from their mental illness. These bugs must be used in the patient's muthi from treatment. You cannot have a standard treatment duration for all patients when they are bewitched because the witches do not use the same approaches when they are bewitching these patients. Traditional healing is not only for a specific ethnic group, we also use our traditional medicine to treat white people, Indians, and other ethnic groups. Other ethnic groups also have ancestors, here at iXopo we have a traditional healer who is White.
	P4	You cannot predict the treatment duration, it depends on the situation of the patient and how quickly they get better.
NDZ		
	P14	Usually we are advised by our ancestors on the treatment method that we should use. But usually for mentally ill patients, medication is administered through inhalation to try and calm the patient, steaming, induced vomiting and enema are also used.
	P(n)	I offer counselling to mentally ill patients before moving on to muthi. But it is impossible to have a talk with some of the mentally ill patients because sometimes they do not speak, I use muthi to try and calm them down in order to try and talk to them.
	P13	I use izilwane which I get from the mountain or from Berea muthi market before moving on to muthi.

Ufufunyana, Bewitchment or an ancestral calling, how do you differentiate between different mental illnesses?		
Kokstad		
Group 1	P3	Sometimes the symptoms you experience when you have an ancestral calling may be mistaken for ufufunyana by other THPs. In my experience before I became a THP, I had an ancestral calling but some THPs diagnosed the symptoms I had as ufufunyana.
	P14	A calling never presents itself exactly as ufufunyana as it a gift given to you by your ancestors, they are just not the same. It's easy to identify a person with ufufunyana, a person with a calling, the calling distinguishes itself by certain signs or symptoms. For example, a woman might not like men (young or old). And a calling is something you are born with, and when you reach the stage of womanhood where you are eligible for marriage, you man will just reject you even when you have 6 children with him. It also depends on whether family traditions have been kept well, if not, they will need to kept before you can start with your initiation.
	P13	I will give you an example for a case of ufufunyana. Some people bayaphoswa (bewitched) by young men. And then you will find that ufufunyana is now in this person until they end up going through initiation as ufufunyana can even lead to infertility.

		Sometimes you will find that there are spirits who died through accidents, and one family member might end up going through initiation when all that needs to be done is sort out this spirit and cleanse it.
Group 2	P(n) female2	There is an overlap of symptoms (i.e. forgetfulness) between these conditions. And this makes it hard to tell if a patient has ufufunyana or a calling just from the presented symptoms. What helps and make the diagnosis clear is consulting the ancestors.
Buhlebezwe		
	P11	Ufufunyana is said to be easy to pick up because once you torture the spirit possessing the patient it will speak. A person with ufufunyana is aggressive and a person with ufufunyana does not move around a lot because ufufunyana shows itself at certain times, some of ufufunyana is scared of lightning and if the patient is terrified by something ufufunyana will show up in them.
NDZ		
	P13	There is just madness and then ufufunyana which is manmade. Some mental illness a THP cannot treat. The symptoms in categorising different mental illnesses. I would say there are 3 categories of mental illness, ufufunyana, madness and izizwe. When you have an ancestral calling, your brain does not as well as that of a normal person, you hallucinate (you see things other people don't see), which means you are not in this world but a different world (spiritual world), but you remember everything that happens in the spiritual world and then other people think you are crazy. Sometimes it feels like you are living under water. We can also pick up from what people are saying when we are speaking to them, and from what they say you can tell their diagnosis.

Follow-up + Records		
Kokstad		
Group 1	P4	When a patient comes to you, you want to know their history of treatment and where they have been before coming to you, this is important information. Because knowing what they have been given previously helps understand where you will need to start with your form of treatment.
	P14	I do keep records and also it is important to know if the patients has medication/s they are taking and they have had a medical check-up for other diseases. You also need to know their treatment history, where have they been, what have they be given and what are they currently taking. You need to follow-up on all these things before you start treating the patient.
uMzimkhulu		
	P2	We do have books where we keep all records of our patients (their names, surnames, contact details and address). We also keep medical and treatment history of the patients. We also do follow-up on our patients and find out how the treatment process is going.

	P11	It is important to ask a patient upon arrival if they have been receiving treatment somewhere else and then from the treatment history you can tell how long you are going to be with the patient if you have to treat them while you stay with them.
	P7	It is important to have treatment history of your patients as a THP, which also gives you clues of the type of illness they have.
	P8	Before I can start treating a patient I need to know if they have been to a clinic, if not, they need to start there and only if they do not get help from the clinic I can start treating that person.
	P1	Some patients know that when they go to the clinics they are going to be asked a lot of questions regarding their health, whereas if they go to THP, they will be assisted without any questions asked. The danger in this is that the patient might be taking certain pills and when I give them my muthi which will affect the efficacy of the pills. If you these patients about clinic, they then doubt your abilities as a THP. So, knowing the patient's medical history is important in deciding on the treatment approach you will use as a healer. If the patient refuses with this information, then we cannot help them.
	P4	We did not pay attention to these things before, until we were advised by the department of health. We even follow-up on patients now and if you can see that they are not getting any better, you then send them to the clinic. We even encourage some to take their biomedical medication.
	P3	Some patients come to THPs and request particular muthi, you as a healer then have to ask if there's any treatment they are taking. Because if I just give them my muthi only to find that they are taking other treatments as well, should they then dies, I (the healer) will be held liable for their death.
	P12	I need to ask the patient about their health status and if they have been anywhere else for treatment. And I have to give my muthi to someone who healthy.
Buhlebesizwe		
	P11	Some of us do keep records as per the requirements from the department of health. We have a register where we record details of all patients we receive per day. But I usually do not ask many things from patients with simple illness, I ensure that I get medical history for patients who are critically ill. It is important to know if the person is showing the presented symptoms for the first time, what other illnesses have they had previously and what help, and why did they bring the patient to me this time around and not where they were previously treated.
NDZ		
	P14	According to my knowledge, you have to have records of all patients who come to your house (note their name, surname and contact details). Even though I am not sure if we all do this, but I know we all do pay attention on the medical history of the patient (where have they previously received treatment, how were they treated). We keep records so that even when the patient has left after treatment our ancestors remember them and be able to communicate with them.

Referrals + Working together with biomedical healthcare facilities or medication		
Kokstad		
Group 1	P14	When you can see that the patient is not fit to received traditional medicine, you take them to a medical doctor. Because there is nothing you can do to a person with an affected (or injured) brain. When a patient has pre-existing conditions such as HIV and diabetes, we cannot make that person stop taking their medication or we would killing that person. So, a person can receive both traditional and biomedical treatment at the same time is such cases.
Group 2	P(n) male	We do referrals. Because when the patient comes, you do not just give them muthi, we first have to take them for a medical check-up for pre-existing diseases. Because giving them muthi might make these diseases worse.
	P(n) female	I second P(n) male. Because sometimes a patient might come to you experiencing loss of power/energy and we cannot administer any medication on a patient in that state. So they need to get at least a drip before muthi is administered to them.
	P(n) female2	Even when the illness is caused by the ancestors, the patient has to go to the clinic first to check their health status. This will ensure that I do not give a lot of chores to the patient even when they have to collect or take their medication, I need to know these things so I can also remind them to take their medication. Ufufunyana can never be treated through biomedical approaches, it can only be treated through traditional healing. Because using both treatment methods will be confusing, biomedical medication will only manage symptoms for a particular time and now as a healer you do not know whether your muthi is treating the patient
uMzimkhulu		
	P12	Biomedical medication works, but you cannot mix it with traditional medicine. Even though traditional medicine is usually the second option, so we let them take biomedical medicine and then we follow with our muthi. We give biomedical medicine time though so that it can also do its job before we give them our muthi.
	P2	When a person defaults from their HIV treatment you need to send or refer them to a clinic where they will advise what needs to be done. We also refer a person with ufunyane to the clinic, especially if they do not eat, they need drips for energy because we cannot use umuthi on a weak person. Sometimes we take our patients to clinics to have them checked for underlying illnesses before we can use munthi on them.
	P6	If I cannot treat the person I refer them to the clinic where they get treated.
	P3	I could not treat the patient as they were defaulting from their HIV treatment and so I had to refer them to a clinic. I could only calm the patient down.

	P7	There is no relationship between biomedical medicine and traditional healing because biomedical doctors would rather cut a patient's limb if they are failing to treat wounds due to diabetes, they would never send the patients for traditional healing because they see THPs as mad people.
Buhlebesizwe		
	P8	We do refer our patients to biomedical facilities when we can see that they are getting worse instead of better. In one case a patient had seizures, and when these started they had to be rushed to hospital or clinic for help. So there is somewhat a relationship between biomedical medicine and traditional healing in this sense.
NDZ		
	P14	There are situations that forces you to refer a patient to biomedical facilities, and we (THPs) help where we can. When treating some diseases we can work together with biomedical medicine and there are diseases we as THPs just cannot treat, even if we try in the end the patient has to get pills. We can collaborate in treating mental illness because in some cases you will find that a mentally ill patient is experiencing loss of power/energy and we do not have idrip as THPs, the patient then has to go to a biomedical facility.
	P13	A collaboration is possible between biomedical and traditional medicines because our bodies cannot survive with only traditional medicine in nowadays (where there are a lot of new diseases).

Appendix 8: Raw Data for Component Two of the Study

Note that for the phytochemistry and cytotoxicity data:

Where plants are indicated by 1-7:

1	*D. dregeana*
2	*B. disticha* A
3	*A. hispida*
4	*S. mucronata*
5	*A. monteiroae Oliv*
6	*T. grandifolia*
7	*C. miniata*

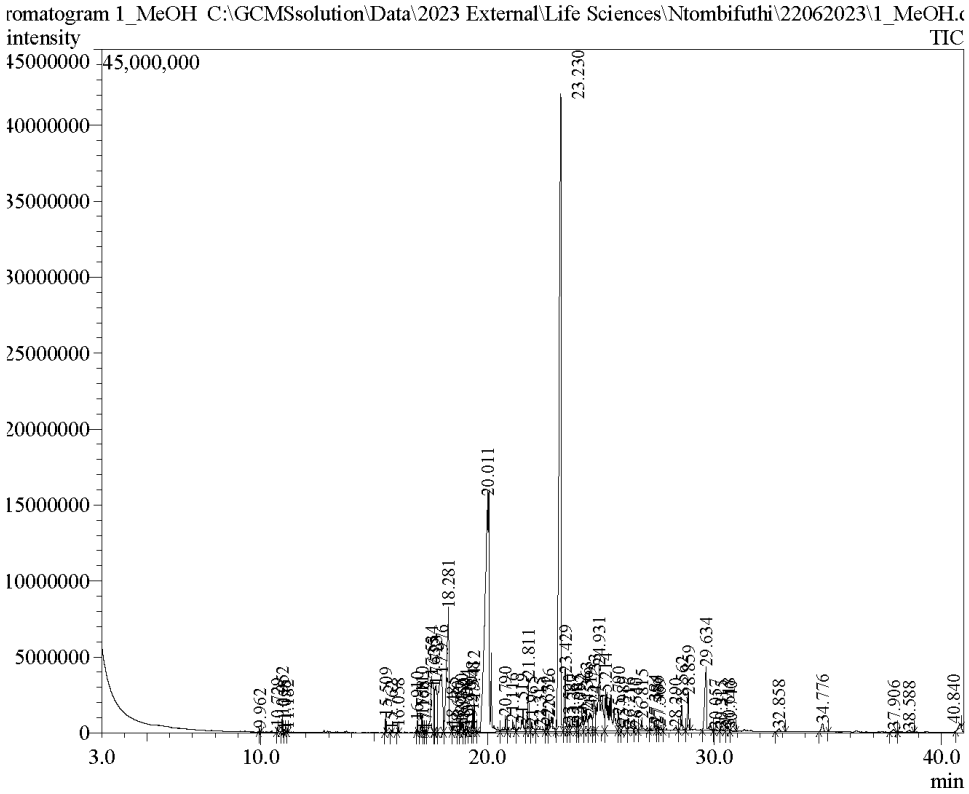
Solvents used for extractions are indicated as:

MeOH	Methanol
EtOH	Ethanol
DCM	Dichloromethane

8.1. Phytochemistry

Sample Information

Analyzed by	: Unathi
Analyzed	: 2023-06-22 05:27:16 PM
Sample Type	: Unknown
Level #	: 1
Sample Name	: 1_MeOH
Sample ID	: 1_MeOH
IS Amount	: [1]=1
Sample Amount	: 1
Dilution Factor	: 1
Vial #	: 2
Injection Volume	: 3.00
Data File	: C:\GCMSsolution\Data\2023 External\Life Sciences\Ntombifuthi\22062023\1_MeC
Org Data File	: C:\GCMSsolution\Data\2023 External\Life Sciences\Ntombifuthi\22062023\1_MeC
Method File	: C:\GCMSsolution\Methods\2023 Ibrahim.qgm
Org Method File	: C:\GCMSsolution\Methods\2023 Ibrahim.qgm
Report File	:
Tuning File	: C:\GCMSsolution\System\Tune1\2023 Tuning files\17042023.qgt
Modified by	: Unathi
Modified	: 2023-06-22 06:08:17 PM



Peak Report TIC

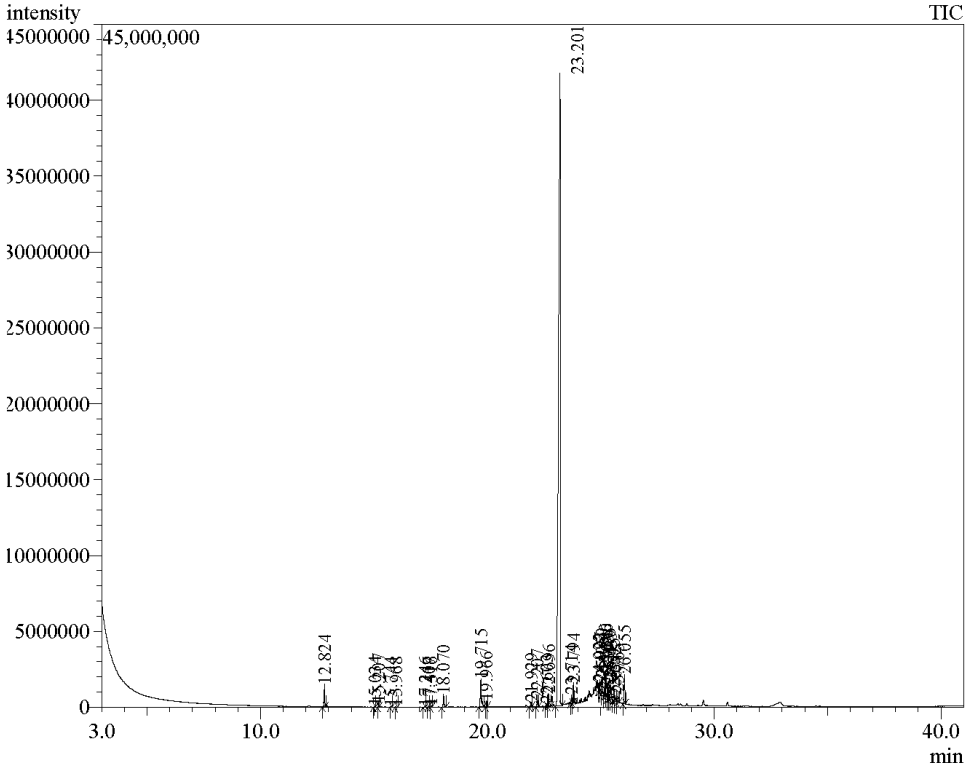
Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	eight%
1	9.962	9.920	10.020	404952	0.04	210141	0.17
2	10.729	10.695	10.770	725772	0.08	319581	0.25
3	10.952	10.905	11.005	1898955	0.20	1071325	0.84
4	11.058	11.015	11.100	342872	0.04	157613	0.12
5	11.182	11.150	11.230	339147	0.04	204096	0.16
6	15.509	15.460	15.545	1835931	0.20	1058218	0.83
7	15.762	15.710	15.795	352023	0.04	179198	0.14
8	16.058	16.010	16.110	643421	0.07	340563	0.27
9	16.910	16.875	16.940	1339926	0.14	762773	0.60
10	17.110	16.995	17.145	2932917	0.32	1100542	0.87
11	17.168	17.145	17.220	447661	0.05	134200	0.11
12	17.272	17.220	17.315	483355	0.05	134622	0.11
13	17.534	17.315	17.625	30023865	3.23	3709209	2.92
14	17.635	17.625	17.670	7675282	0.83	2914274	2.29
15	17.723	17.670	17.790	19954779	2.15	3127987	2.46
16	17.976	17.790	18.070	46000303	4.95	3819476	3.00
17	18.281	18.070	18.430	57042441	6.13	8042867	6.32
18	18.485	18.430	18.660	2072545	0.22	355265	0.28
19	18.680	18.660	18.760	366415	0.04	115619	0.09
20	18.870	18.760	18.930	4511172	0.49	593497	0.47
21	18.950	18.930	19.010	770244	0.08	361465	0.28
22	19.084	19.010	19.140	2048456	0.22	802136	0.63
23	19.270	19.140	19.310	1137529	0.12	365780	0.29
24	19.348	19.310	19.380	2652848	0.29	1474166	1.16
25	19.412	19.380	19.540	3738988	0.40	1988011	1.56
26	20.011	19.540	20.565	209537907	22.53	15280763	12.01
27	20.790	20.565	20.865	4177684	0.45	900957	0.71
28	21.116	20.865	21.265	5186057	0.56	634805	0.50
29	21.519	21.265	21.690	9341533	1.00	730185	0.57
30	21.811	21.690	21.890	11918665	1.28	3177465	2.50
31	21.965	21.890	22.115	3334278	0.36	512607	0.40
32	22.315	22.115	22.540	3931346	0.42	268272	0.21
33	22.632	22.540	22.665	1885158	0.20	403456	0.32
34	22.716	22.665	23.015	8709224	0.94	1011808	0.80
35	23.230	23.015	23.340	258683187	27.81	41331990	32.50
36	23.429	23.340	23.515	13880635	1.49	3133296	2.46
37	23.590	23.515	23.640	2686252	0.29	416769	0.33
38	23.767	23.640	23.915	6328111	0.68	609249	0.48
39	23.985	23.915	24.015	2459901	0.26	609094	0.48
40	24.092	24.015	24.240	7370444	0.79	483554	0.38
41	24.363	24.240	24.440	9835868	1.06	1403584	1.10
42	24.519	24.440	24.640	11442740	1.23	1154824	0.91
43	24.733	24.640	24.765	10320706	1.11	1816980	1.43
44	24.931	24.765	25.165	43315295	4.66	3900567	3.07
45	25.214	25.165	25.740	38914742	4.18	2403297	1.89
46	25.790	25.740	25.890	5428621	0.58	1144506	0.90
47	25.989	25.890	26.165	4645448	0.50	336347	0.26
48	26.216	26.165	26.465	3102208	0.33	257876	0.20
49	26.540	26.465	26.640	1431228	0.15	250245	0.20
50	26.815	26.640	27.115	4258428	0.46	781356	0.61
51	27.384	27.115	27.440	2564418	0.28	434169	0.34
52	27.486	27.440	27.540	1182460	0.13	359075	0.28
53	27.569	27.540	27.740	1200854	0.13	210654	0.17
54	28.290	27.740	28.415	1982962	0.21	185075	0.15
55	28.562	28.415	28.740	6436055	0.69	1599037	1.26

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	eight%
56	28.859	28.740	28.940	8528315	0.92	2298912	1.81
57	29.634	29.465	29.965	23883267	2.57	4064964	3.20
58	30.057	29.965	30.240	626726	0.07	110074	0.09
59	30.315	30.240	30.465	529501	0.06	89734	0.07
60	30.618	30.490	30.690	1160355	0.12	273665	0.22
61	30.740	30.690	30.965	361876	0.04	61322	0.05
62	32.858	32.690	33.140	1313005	0.14	186682	0.15
63	34.776	34.615	35.040	3577299	0.38	513461	0.40
64	37.906	37.715	38.065	1416039	0.15	153217	0.12
65	38.588	38.065	38.815	1413063	0.15	98259	0.08
66	40.840	40.640	40.890	2063728	0.22	253734	0.20
				930107388	100.00	127188510	100.00

Sample Information

Analyzed by	: Unathi
Analyzed	: 2023-06-22 06:16:46 PM
Sample Type	: Unknown
Level #	: 1
Sample Name	: 2_MeOH
Sample ID	: 2_MeOH
IS Amount	: [1]=1
Sample Amount	: 1
Dilution Factor	: 1
Vial #	: 3
Injection Volume	: 3.00
Data File	: C:\GCMSsolution\Data\2023 External\Life Sciences\Ntombifuthi\22062023\2_MeC
Org Data File	: C:\GCMSsolution\Data\2023 External\Life Sciences\Ntombifuthi\22062023\2_MeC
Method File	: C:\GCMSsolution\Methods\2023 Ibrahim.qgm
Org Method File	: C:\GCMSsolution\Methods\2023 Ibrahim.qgm
Report File	:
Tuning File	: C:\GCMSsolution\System\Tune1\2023 Tuning files\17042023.qgt
Modified by	: Unathi
Modified	: 2023-06-22 06:57:47 PM

romatogram 2_MeOH C:\GCMSsolution\Data\2023 External\Life Sciences\Ntombifuthi\22062023\2_MeOH.q



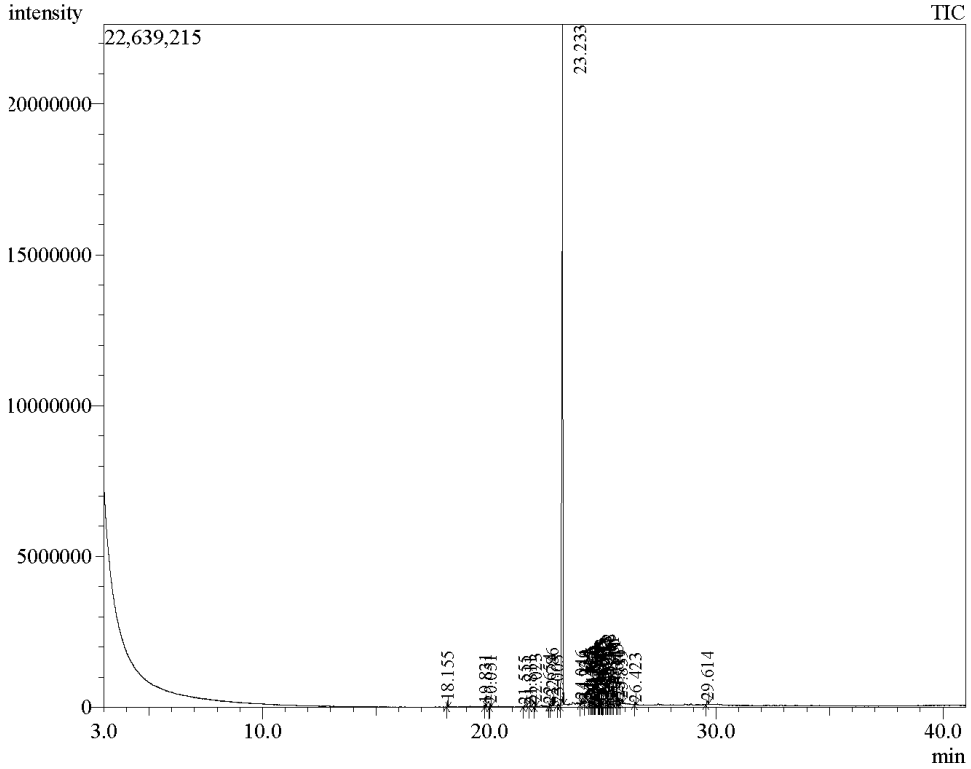
Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	eight%
1	12.824	12.735	12.875	2917473	1.15	1474793	2.33
2	15.034	14.980	15.080	99190	0.04	66407	0.11
3	15.207	15.165	15.245	432670	0.17	262807	0.42
4	15.744	15.715	15.810	52898	0.02	29209	0.05
5	15.968	15.930	16.055	112862	0.04	56873	0.09
6	17.246	17.175	17.285	142607	0.06	64865	0.10
7	17.407	17.370	17.440	72662	0.03	44357	0.07
8	17.516	17.485	17.575	161699	0.06	85107	0.13
9	18.070	17.990	18.190	1833453	0.72	854256	1.35
10	19.715	19.630	19.860	6133757	2.41	1865500	2.95
11	19.966	19.925	20.025	701793	0.28	395808	0.63
12	21.929	21.855	21.985	541882	0.21	277304	0.44
13	22.207	22.135	22.275	1089752	0.43	518925	0.82
14	22.603	22.550	22.640	347769	0.14	162470	0.26
15	22.696	22.660	22.820	1584488	0.62	853056	1.35
16	23.201	23.005	23.310	210198092	82.59	41683302	65.92
17	23.714	23.665	23.770	1250520	0.49	569045	0.90
18	23.794	23.750	23.840	2314649	0.91	1296191	2.05
19	24.922	24.895	24.950	824439	0.32	568644	0.90
20	24.997	24.950	25.030	1678665	0.66	621168	0.98
21	25.059	25.030	25.095	2034383	0.80	1070565	1.69
22	25.190	25.135	25.190	1924701	0.76	1543803	2.44
23	25.246	25.215	25.275	2777234	1.09	1459246	2.31
24	25.303	25.280	25.335	1624773	0.64	910847	1.44
25	25.378	25.340	25.410	2777063	1.09	1382653	2.19
26	25.439	25.400	25.480	2463727	0.97	1248567	1.97
27	25.575	25.520	25.605	1880384	0.74	880722	1.39
28	25.642	25.610	25.690	887472	0.35	399306	0.63
29	25.765	25.710	25.820	1458075	0.57	663941	1.05
30	26.055	25.985	26.110	4195907	1.65	1924215	3.04
				254515039	100.00	63233952	100.00

Sample Information

Analyzed by	: Unathi
Analyzed	: 2023-06-22 07:06:06 PM
Sample Type	: Unknown
Level #	: 1
Sample Name	: 3_MeOH
Sample ID	: 3_MeOH
IS Amount	: [1]=1
Sample Amount	: 1
Dilution Factor	: 1
Vial #	: 4
Injection Volume	: 3.00
Data File	: C:\GCMSsolution\Data\2023 External\Life Sciences\Ntombifuthi\22062023\3_MeC
Org Data File	: C:\GCMSsolution\Data\2023 External\Life Sciences\Ntombifuthi\22062023\3_MeC
Method File	: C:\GCMSsolution\Methods\2023 Ibrahim.qgm
Org Method File	: C:\GCMSsolution\Methods\2023 Ibrahim.qgm
Report File	:
Tuning File	: C:\GCMSsolution\System\Tune1\2023 Tuning files\17042023.qgt
Modified by	: Unathi
Modified	: 2023-06-22 07:47:06 PM

romatogram 3_MeOH C:\GCMSsolution\Data\2023 External\Life Sciences\Ntombifuthi\22062023\3_MeOH.q



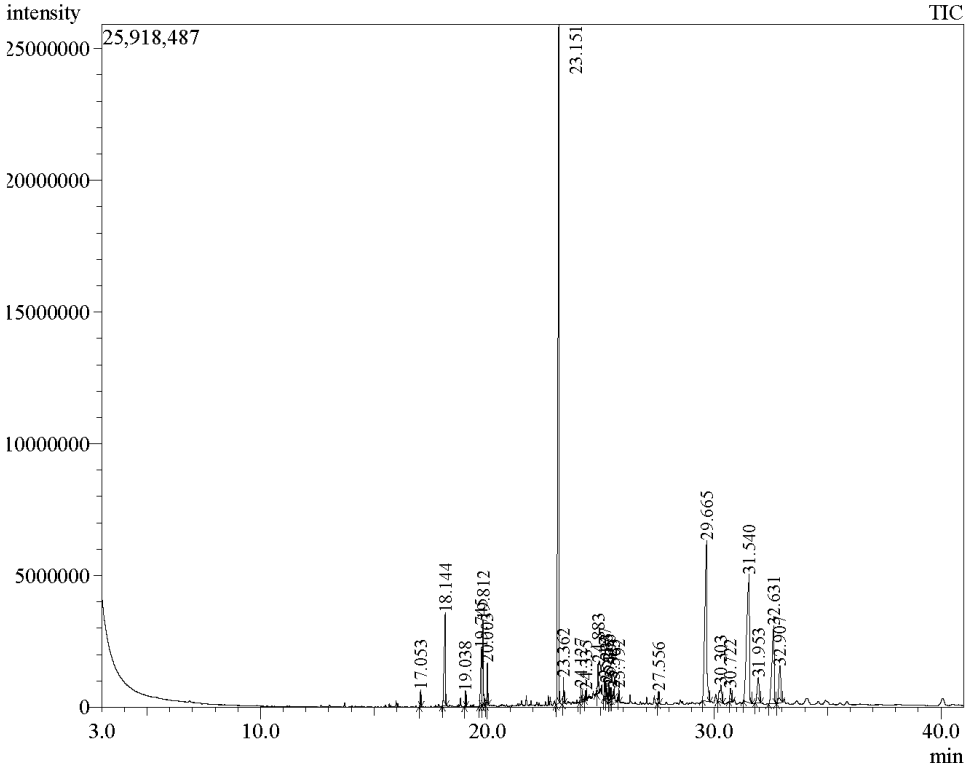
Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	eight%
1	18.155	18.115	18.205	359718	0.40	197130	0.62
2	19.831	19.800	19.870	164557	0.18	98601	0.31
3	20.051	20.015	20.090	193261	0.22	106923	0.34
4	21.555	21.495	21.650	148442	0.17	57629	0.18
5	21.811	21.740	21.870	151215	0.17	56369	0.18
6	22.023	21.990	22.060	192734	0.21	113956	0.36
7	22.654	22.620	22.685	126248	0.14	72598	0.23
8	22.786	22.685	22.830	532087	0.59	301063	0.95
9	23.005	22.985	23.125	147084	0.16	43328	0.14
10	23.233	23.125	23.295	66977481	74.71	22547838	70.85
11	24.046	24.010	24.080	298523	0.33	154274	0.48
12	24.224	24.195	24.260	201303	0.22	104064	0.33
13	24.415	24.385	24.445	204157	0.23	107588	0.34
14	24.465	24.445	24.495	123926	0.14	56643	0.18
15	24.525	24.495	24.545	393566	0.44	190368	0.60
16	24.570	24.545	24.595	545660	0.61	238055	0.75
17	24.610	24.595	24.630	387658	0.43	200763	0.63
18	24.650	24.630	24.685	623851	0.70	264585	0.83
19	24.759	24.685	24.785	1233707	1.38	304751	0.96
20	24.809	24.785	24.830	599722	0.67	251416	0.79
21	24.851	24.830	24.865	561040	0.63	306381	0.96
22	24.898	24.865	24.920	1108432	1.24	410402	1.29
23	24.943	24.920	24.970	1134156	1.27	489995	1.54
24	24.995	24.970	25.020	978276	1.09	440484	1.38
25	25.072	25.020	25.105	1796039	2.00	449564	1.41
26	25.133	25.105	25.170	1409837	1.57	566576	1.78
27	25.190	25.170	25.210	407306	0.45	193304	0.61
28	25.262	25.210	25.290	1736108	1.94	654913	2.06
29	25.316	25.290	25.350	1479215	1.65	642809	2.02
30	25.376	25.350	25.415	865489	0.97	354470	1.11
31	25.451	25.415	25.480	1190596	1.33	587790	1.85
32	25.511	25.480	25.595	1412970	1.58	470285	1.48
33	25.645	25.595	25.680	774068	0.86	317009	1.00
34	25.711	25.680	25.775	420912	0.47	158248	0.50
35	25.839	25.775	25.885	275892	0.31	132673	0.42
36	26.423	26.395	26.475	131859	0.15	68744	0.22
37	29.614	29.555	29.675	363804	0.41	111483	0.35
				89650899	100.00	31823072	100.00

Sample Information

Analyzed by	: Unathi
Analyzed	: 2023-06-22 09:35:34 PM
Sample Type	: Unknown
Level #	: 1
Sample Name	: 4_MeOH
Sample ID	: 4_MeOH
IS Amount	: [1]=1
Sample Amount	: 1
Dilution Factor	: 1
Vial #	: 5
Injection Volume	: 3.00
Data File	: C:\GCMSsolution\Data\2023 External\Life Sciences\Ntombifuthi\22062023\4_MeC
Org Data File	: C:\GCMSsolution\Data\2023 External\Life Sciences\Ntombifuthi\22062023\4_MeC
Method File	: C:\GCMSsolution\Methods\2023 Ibrahim.qgm
Org Method File	: C:\GCMSsolution\Methods\2023 Ibrahim.qgm
Report File	:
Tuning File	: C:\GCMSsolution\System\Tune1\2023 Tuning files\17042023.qgt
Modified by	: Unathi
Modified	: 2023-06-22 10:16:34 PM

romatogram 4_MeOH C:\GCMSsolution\Data\2023 External\Life Sciences\Ntombifuthi\22062023\4_MeOH.q



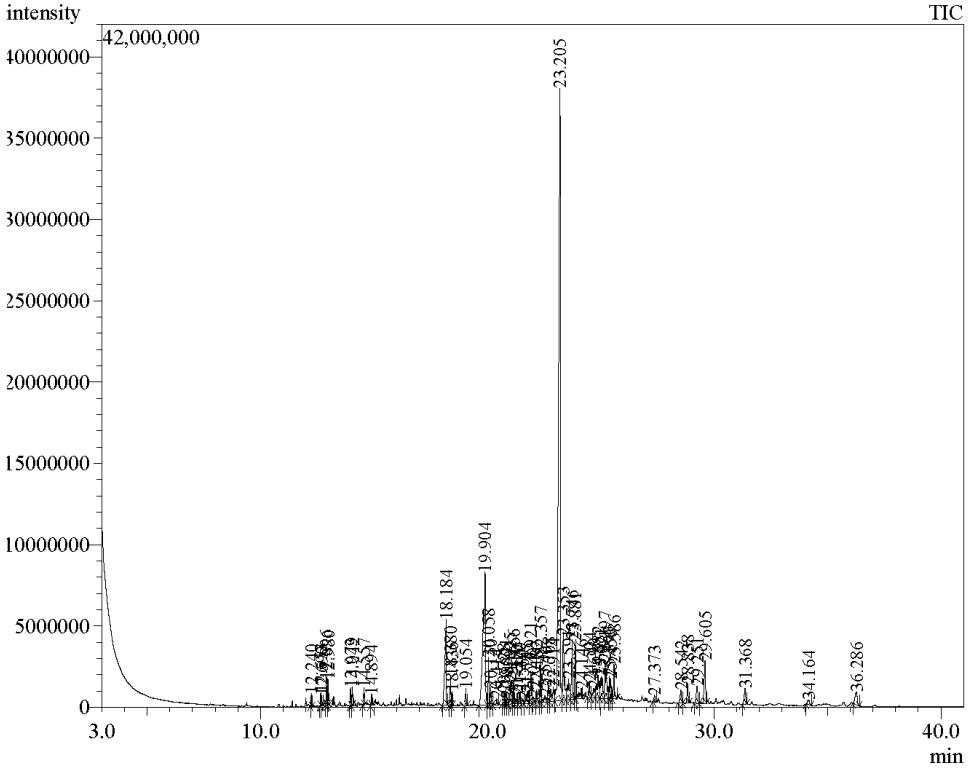
Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	eight%
1	17.053	17.005	17.090	1249391	0.50	647656	1.05
2	18.144	18.020	18.195	13800281	5.53	3518520	5.70
3	19.038	19.000	19.075	1009171	0.40	590523	0.96
4	19.745	19.650	19.770	8412984	3.37	2176011	3.53
5	19.812	19.770	19.865	8332324	3.34	3254913	5.28
6	20.003	19.925	20.050	3189059	1.28	1607224	2.60
7	23.151	23.030	23.200	81864920	32.81	25770963	41.77
8	23.362	23.315	23.405	1895757	0.76	1000267	1.62
9	24.127	24.090	24.180	1080826	0.43	547885	0.89
10	24.335	24.300	24.375	730298	0.29	362778	0.59
11	24.883	24.830	24.935	2867319	1.15	1177689	1.91
12	25.177	25.135	25.205	1625180	0.65	793063	1.29
13	25.233	25.205	25.265	1311837	0.53	607830	0.99
14	25.366	25.330	25.400	1268026	0.51	586701	0.95
15	25.427	25.400	25.470	860361	0.34	455344	0.74
16	25.563	25.470	25.605	1046189	0.42	367747	0.60
17	25.792	25.765	25.840	645962	0.26	416103	0.67
18	27.556	27.510	27.600	1110001	0.44	476884	0.77
19	29.665	29.485	29.820	37788565	15.15	6101904	9.89
20	30.303	30.170	30.390	4210299	1.69	655352	1.06
21	30.722	30.665	30.815	2107232	0.84	500268	0.81
22	31.540	31.300	31.685	38989163	15.63	4780338	7.75
23	31.953	31.815	32.065	5664139	2.27	952935	1.54
24	32.631	32.405	32.735	20445223	8.20	2949300	4.78
25	32.907	32.765	33.005	7975859	3.20	1402199	2.27
				249480366	100.00	61700397	100.00

Sample Information

Analyzed by	: Unathi
Analyzed	: 2023-06-22 10:25:15 PM
Sample Type	: Unknown
Level #	: 1
Sample Name	: 5_MeOH
Sample ID	: 5_MeOH
IS Amount	: [1]=1
Sample Amount	: 1
Dilution Factor	: 1
Vial #	: 6
Injection Volume	: 3.00
Data File	: C:\GCMSsolution\Data\2023 External\Life Sciences\Ntombifuthi\22062023\5_MeC
Org Data File	: C:\GCMSsolution\Data\2023 External\Life Sciences\Ntombifuthi\22062023\5_MeC
Method File	: C:\GCMSsolution\Methods\2023 Ibrahim.qgm
Org Method File	: C:\GCMSsolution\Methods\2023 Ibrahim.qgm
Report File	:
Tuning File	: C:\GCMSsolution\System\Tune1\2023 Tuning files\17042023.qgt
Modified by	: Unathi
Modified	: 2023-06-22 11:06:16 PM

romatogram 5_MeOH C:\GCMSsolution\Data\2023 External\Life Sciences\Ntombifuthi\22062023\5_MeOH.q



Peak Report TIC

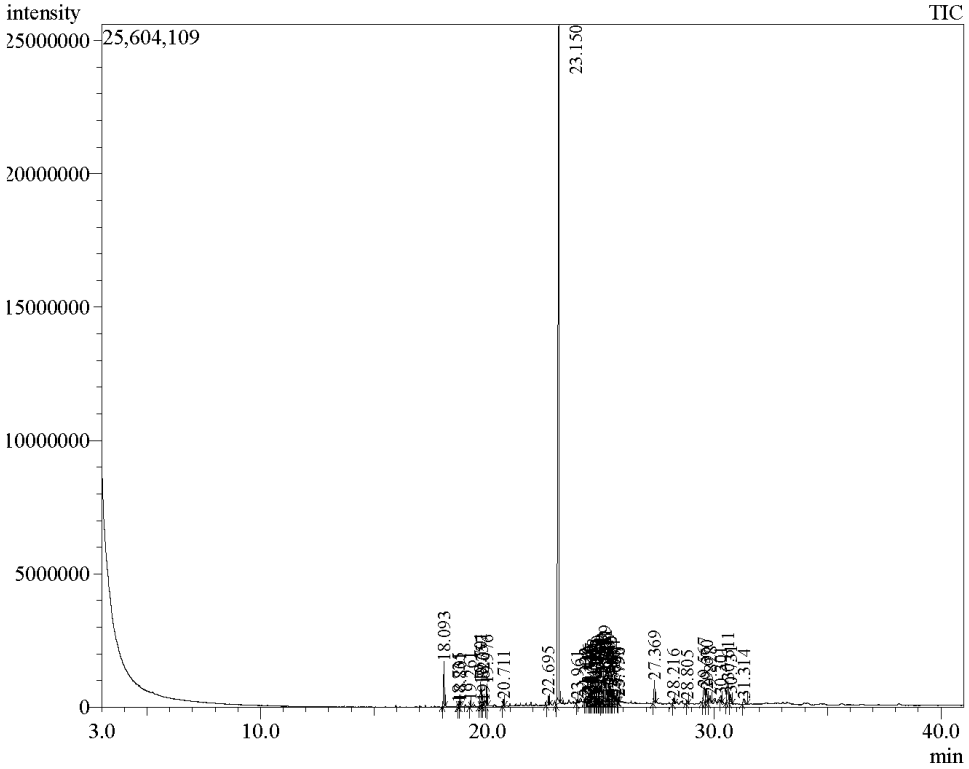
Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	eight%
1	12.240	12.205	12.290	1373568	0.28	786562	0.69
2	12.653	12.615	12.675	1604519	0.33	809779	0.71
3	12.690	12.675	12.765	1369527	0.28	638506	0.56
4	12.896	12.855	12.945	2923455	0.60	1596727	1.39
5	12.980	12.945	13.025	2794863	0.57	1564810	1.36
6	13.973	13.930	14.015	2147734	0.44	1136367	0.99
7	14.049	14.015	14.070	2088543	0.43	1147247	1.00
8	14.557	14.500	14.595	2568825	0.53	1081021	0.94
9	14.894	14.850	14.920	1386768	0.28	688912	0.60
10	18.184	18.025	18.250	23365316	4.79	5262111	4.59
11	18.380	18.315	18.410	3636022	0.75	1829983	1.59
12	18.436	18.410	18.480	1447540	0.30	889481	0.78
13	19.054	19.000	19.120	2287274	0.47	1058500	0.92
14	19.904	19.635	20.000	57790270	11.86	7869057	6.86
15	20.058	20.000	20.110	6652107	1.36	2939953	2.56
16	20.150	20.110	20.240	2201063	0.45	1052946	0.92
17	20.414	20.240	20.490	1370544	0.28	382838	0.33
18	20.728	20.650	20.770	1727418	0.35	719484	0.63
19	20.821	20.770	20.850	2093302	0.43	1004872	0.88
20	20.890	20.850	20.930	1065303	0.22	375721	0.33
21	21.025	20.940	21.120	4595573	0.94	1326608	1.16
22	21.166	21.120	21.210	3410171	0.70	1487504	1.30
23	21.310	21.210	21.350	2151100	0.44	552465	0.48
24	21.420	21.350	21.450	2143127	0.44	724657	0.63
25	21.560	21.510	21.630	1172199	0.24	271453	0.24
26	21.745	21.630	21.840	2865449	0.59	633192	0.55
27	21.921	21.840	21.990	5928349	1.22	1975036	1.72
28	22.040	21.990	22.120	1909688	0.39	380347	0.33
29	22.182	22.120	22.290	2391353	0.49	780460	0.68
30	22.357	22.290	22.390	6309729	1.29	2767761	2.41
31	22.540	22.390	22.640	2750431	0.56	381072	0.33
32	22.708	22.640	22.760	3860052	0.79	1001369	0.87
33	22.914	22.760	22.970	5574512	1.14	842552	0.73
34	23.205	22.970	23.300	186869634	38.34	36738345	32.02
35	23.353	23.300	23.480	17250661	3.54	4151328	3.62
36	23.591	23.480	23.670	8283421	1.70	1255404	1.09
37	23.746	23.670	23.830	11491493	2.36	3496779	3.05
38	23.881	23.830	23.920	7835308	1.61	3464917	3.02
39	24.146	23.920	24.190	3629746	0.74	567948	0.49
40	24.504	24.420	24.570	3538902	0.73	920430	0.80
41	24.690	24.570	24.760	4162689	0.85	602243	0.52
42	24.882	24.760	24.960	8867714	1.82	1364647	1.19
43	25.021	24.960	25.140	8565027	1.76	1313377	1.14
44	25.197	25.140	25.350	11688299	2.40	2222835	1.94
45	25.390	25.350	25.420	3215222	0.66	1382046	1.20
46	25.450	25.420	25.520	2881828	0.59	1197059	1.04
47	25.586	25.520	25.720	6296907	1.29	2054290	1.79
48	27.373	27.310	27.450	1183905	0.24	410485	0.36
49	28.542	28.420	28.630	3435383	0.70	794010	0.69
50	28.838	28.630	28.910	3793067	0.78	1177619	1.03
51	29.251	29.140	29.350	4900583	1.01	1039391	0.91
52	29.605	29.350	29.670	11346055	2.33	2585865	2.25
53	31.368	31.240	31.450	4191150	0.86	962529	0.84
54	34.164	34.060	34.290	1636022	0.34	278876	0.24
55	36.286	36.120	36.410	5396246	1.11	808328	0.70

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	eight%
				487414956	100.00	114748104	100.00

Sample Information

Analyzed by	: Unathi
Analyzed	: 2023-06-22 11:14:57 PM
Sample Type	: Unknown
Level #	: 1
Sample Name	: 6_MeOH
Sample ID	: 6_MeOH
IS Amount	: [1]=1
Sample Amount	: 1
Dilution Factor	: 1
Vial #	: 7
Injection Volume	: 3.00
Data File	: C:\GCMSsolution\Data\2023 External\Life Sciences\Ntombifuthi\22062023\6_MeC
Org Data File	: C:\GCMSsolution\Data\2023 External\Life Sciences\Ntombifuthi\22062023\6_MeC
Method File	: C:\GCMSsolution\Methods\2023 Ibrahim.qgm
Org Method File	: C:\GCMSsolution\Methods\2023 Ibrahim.qgm
Report File	:
Tuning File	: C:\GCMSsolution\System\Tune1\2023 Tuning files\17042023.qgt
Modified by	: Unathi
Modified	: 2023-06-22 11:55:58 PM

romatogram 6_MeOH C:\GCMSsolution\Data\2023 External\Life Sciences\Ntombifuthi\22062023\6_MeOH.q



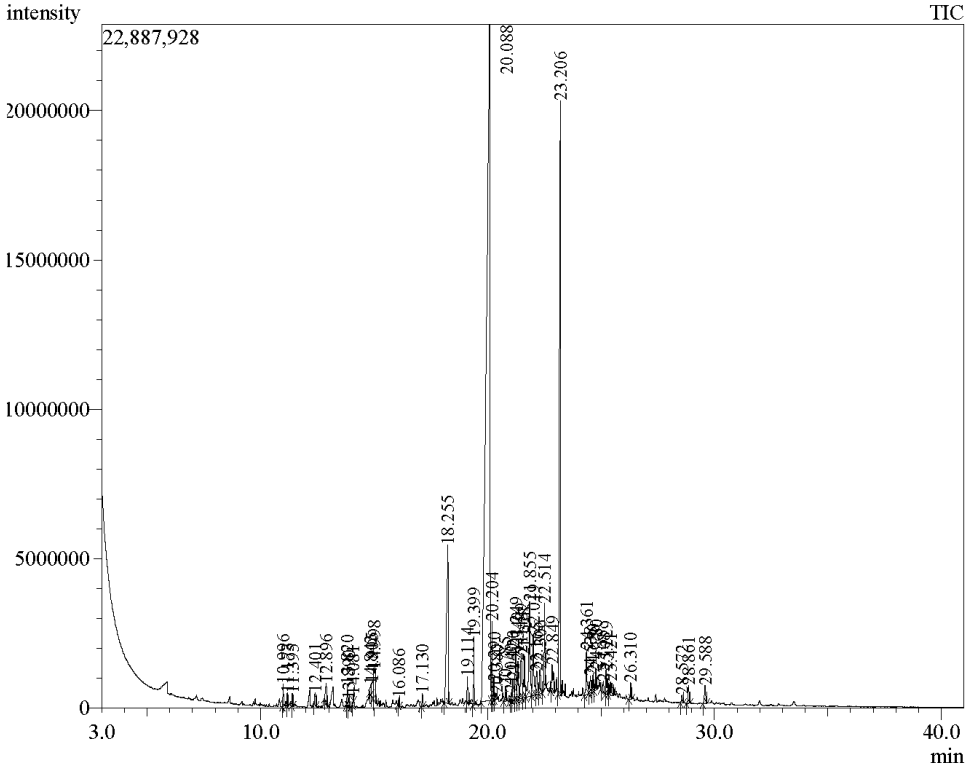
Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	eight%
1	18.093	18.015	18.145	4133533	3.06	1689566	3.70
2	18.735	18.705	18.765	249923	0.18	155037	0.34
3	18.801	18.765	18.840	339290	0.25	195096	0.43
4	19.261	19.225	19.295	373711	0.28	226929	0.50
5	19.701	19.645	19.725	1951923	1.44	915598	2.00
6	19.757	19.725	19.790	1631949	1.21	794748	1.74
7	19.820	19.790	19.850	359596	0.27	160971	0.35
8	19.976	19.930	20.020	1506010	1.11	823371	1.80
9	20.711	20.675	20.755	448776	0.33	259227	0.57
10	22.695	22.600	22.735	583339	0.43	354967	0.78
11	23.150	23.030	23.215	81866178	60.54	25462053	55.72
12	23.961	23.925	23.995	286794	0.21	144313	0.32
13	24.332	24.300	24.360	361486	0.27	163113	0.36
14	24.440	24.360	24.460	582023	0.43	216786	0.47
15	24.486	24.460	24.510	678380	0.50	295693	0.65
16	24.530	24.510	24.545	438017	0.32	242714	0.53
17	24.567	24.545	24.600	729189	0.54	309582	0.68
18	24.668	24.600	24.700	1679247	1.24	492034	1.08
19	24.723	24.700	24.740	638325	0.47	305894	0.67
20	24.760	24.740	24.785	810730	0.60	332882	0.73
21	24.812	24.785	24.835	1239713	0.92	500552	1.10
22	24.860	24.835	24.885	1448916	1.07	630644	1.38
23	24.912	24.885	24.940	1371409	1.01	529247	1.16
24	24.994	24.940	25.025	2231818	1.65	539487	1.18
25	25.049	25.025	25.080	1646948	1.22	716908	1.57
26	25.110	25.080	25.130	696501	0.52	238120	0.52
27	25.179	25.130	25.205	2528945	1.87	1006809	2.20
28	25.231	25.205	25.270	2188909	1.62	879598	1.92
29	25.294	25.270	25.335	1080625	0.80	469238	1.03
30	25.369	25.335	25.400	1551527	1.15	723857	1.58
31	25.430	25.400	25.460	1360990	1.01	602075	1.32
32	25.480	25.460	25.510	408400	0.30	187580	0.41
33	25.564	25.510	25.605	1186126	0.88	437823	0.96
34	25.627	25.605	25.700	529449	0.39	220438	0.48
35	25.755	25.730	25.775	253272	0.19	148784	0.33
36	25.790	25.775	25.825	259483	0.19	177466	0.39
37	27.369	27.310	27.425	2080031	1.54	859822	1.88
38	28.216	28.170	28.265	577560	0.43	215022	0.47
39	28.805	28.770	28.865	396131	0.29	137593	0.30
40	29.567	29.475	29.625	2948398	2.18	590358	1.29
41	29.670	29.625	29.730	1868208	1.38	504043	1.10
42	29.828	29.730	29.895	1123876	0.83	274358	0.60
43	30.301	30.250	30.355	666783	0.49	206679	0.45
44	30.611	30.525	30.665	3545869	2.62	821328	1.80
45	30.731	30.665	30.805	1525074	1.13	343137	0.75
46	31.314	31.240	31.465	857701	0.63	197075	0.43
				135221081	100.00	45698615	100.00

Sample Information

Analyzed by	: Unathi
Analyzed	: 2023-06-23 01:43:51 AM
Sample Type	: Unknown
Level #	: 1
Sample Name	: 7_MeOH
Sample ID	: 7_MeOH
IS Amount	: [1]=1
Sample Amount	: 1
Dilution Factor	: 1
Vial #	: 8
Injection Volume	: 3.00
Data File	: C:\GCMSsolution\Data\2023 External\Life Sciences\Ntombifuthi\22062023\7_MeC
Org Data File	: C:\GCMSsolution\Data\2023 External\Life Sciences\Ntombifuthi\22062023\7_MeC
Method File	: C:\GCMSsolution\Methods\2023 Ibrahim.qgm
Org Method File	: C:\GCMSsolution\Methods\2023 Ibrahim.qgm
Report File	:
Tuning File	: C:\GCMSsolution\System\Tune1\2023 Tuning files\17042023.qgt
Modified by	: Unathi
Modified	: 2023-06-23 02:24:51 AM

romatogram 7_MeOH C:\GCMSsolution\Data\2023 External\Life Sciences\Ntombifuthi\22062023\7_MeOH.c



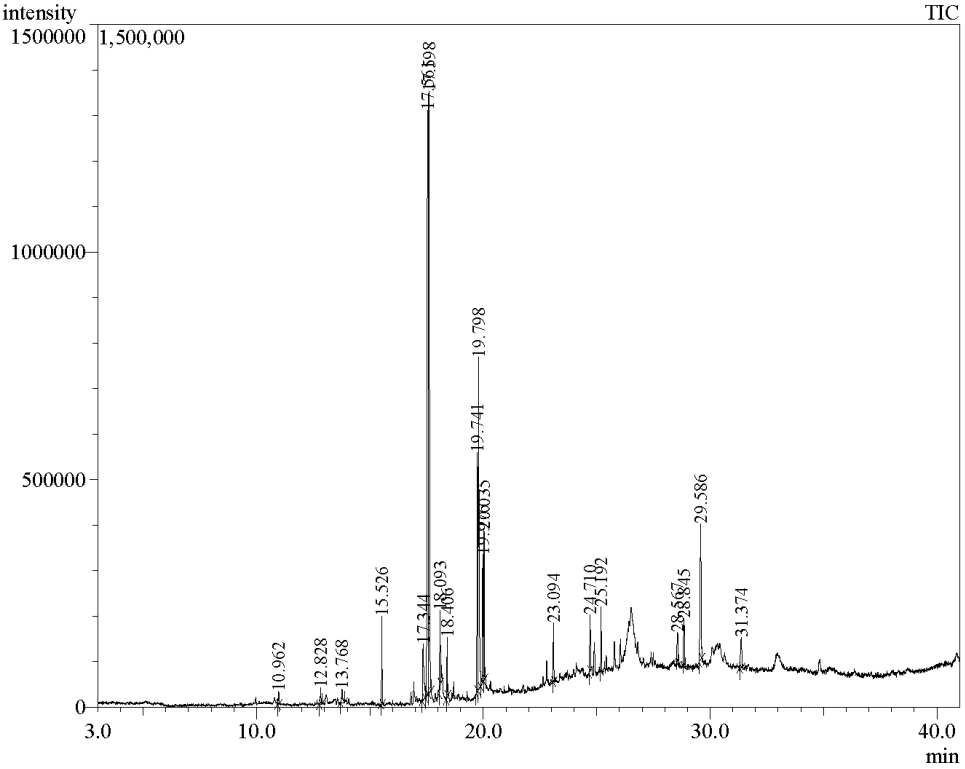
Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	eight%
1	10.996	10.955	11.035	1299403	0.29	749719	0.89
2	11.173	11.140	11.210	697294	0.15	436794	0.52
3	11.395	11.365	11.435	677837	0.15	426410	0.50
4	12.401	12.355	12.455	1185484	0.26	406739	0.48
5	12.896	12.820	12.940	2179776	0.48	691615	0.82
6	13.820	13.775	13.855	1759127	0.39	736789	0.87
7	13.890	13.855	13.920	654691	0.15	249896	0.30
8	14.081	14.050	14.105	454417	0.10	278813	0.33
9	14.842	14.805	14.865	688729	0.15	305619	0.36
10	14.905	14.865	14.965	2788204	0.62	477718	0.57
11	14.998	14.965	15.080	5868848	1.30	1039764	1.23
12	16.086	16.055	16.120	423176	0.09	261657	0.31
13	17.130	17.090	17.165	778561	0.17	432969	0.51
14	18.255	18.100	18.310	25674001	5.70	5303579	6.28
15	19.114	19.065	19.195	1811650	0.40	863211	1.02
16	19.399	19.350	19.435	3685247	0.82	2277550	2.70
17	20.088	19.720	20.160	241992648	53.69	22520734	26.65
18	20.204	20.160	20.250	5033664	1.12	2457414	2.91
19	20.290	20.250	20.310	1435574	0.32	681876	0.81
20	20.380	20.310	20.430	908043	0.20	212115	0.25
21	20.785	20.710	20.830	1014930	0.23	460511	0.55
22	21.040	20.990	21.080	816390	0.18	259334	0.31
23	21.120	21.080	21.190	3164999	0.70	698057	0.83
24	21.249	21.190	21.290	6290632	1.40	1769071	2.09
25	21.340	21.290	21.400	3952227	0.88	1235248	1.46
26	21.489	21.400	21.550	6362319	1.41	1499848	1.78
27	21.628	21.550	21.690	4941106	1.10	1407442	1.67
28	21.855	21.690	21.950	13181145	2.92	3034091	3.59
29	22.019	21.950	22.110	8022227	1.78	2056123	2.43
30	22.165	22.110	22.260	3598298	0.80	776392	0.92
31	22.306	22.260	22.420	3105056	0.69	772039	0.91
32	22.514	22.420	22.580	5663682	1.26	2684485	3.18
33	22.849	22.790	22.960	4299704	0.95	890711	1.05
34	23.206	23.100	23.260	62705115	13.91	19205856	22.73
35	24.361	24.260	24.470	4726007	1.05	1472513	1.74
36	24.538	24.470	24.610	2269292	0.50	541607	0.64
37	24.650	24.610	24.700	1310090	0.29	578820	0.69
38	24.780	24.700	24.830	1570605	0.35	658875	0.78
39	25.108	25.000	25.190	1510273	0.34	363823	0.43
40	25.229	25.190	25.320	2870743	0.64	832468	0.99
41	25.421	25.320	25.550	2967418	0.66	444809	0.53
42	26.310	26.230	26.360	1326073	0.29	564623	0.67
43	28.572	28.510	28.650	949532	0.21	279766	0.33
44	28.861	28.770	28.940	1870948	0.42	596190	0.71
45	29.588	29.490	29.680	2266548	0.50	601288	0.71
				450751733	100.00	84494971	100.00

Sample Information

Analyzed by	: Unathi
Analyzed	: 2023-06-26 08:49:08 AM
Sample Type	: Unknown
Level #	: 1
Sample Name	: 1_EtOH
Sample ID	: 1_EtOH
IS Amount	: [1]=1
Sample Amount	: 1
Dilution Factor	: 1
Vial #	: 2
Injection Volume	: 5.00
Data File	: C:\GCMSsolution\Data\2023 External\Life Sciences\Ntombifuthi\22062023\1_EtO
Org Data File	: C:\GCMSsolution\Data\2023 External\Life Sciences\Ntombifuthi\22062023\1_EtO
Method File	: C:\GCMSsolution\Methods\2023 Ibrahim.qgm
Org Method File	: C:\GCMSsolution\Methods\2023 Ibrahim.qgm
Report File	:
Tuning File	: C:\GCMSsolution\System\Tune1\2023 Tuning files\17042023.qgt
Modified by	: Unathi
Modified	: 2023-06-26 09:30:08 AM

omatogram 1_EtOH C:\GCMSsolution\Data\2023 External\Life Sciences\Ntombifuthi\22062023\1_EtOH a.Q



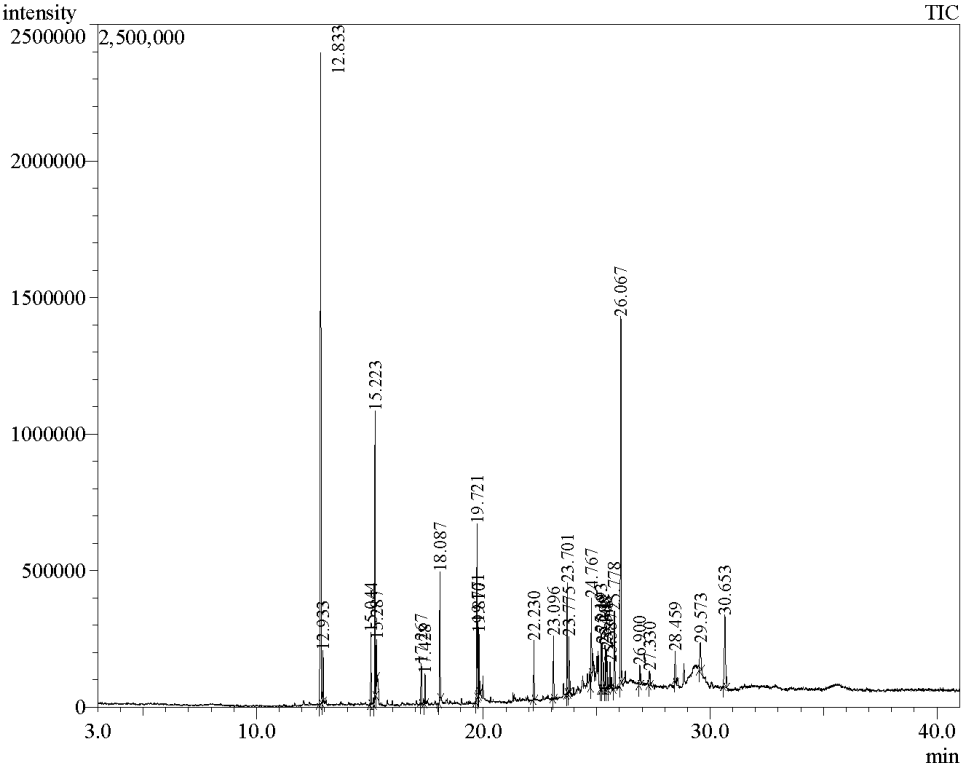
Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	eight%
1	10.962	10.930	11.010	56480	0.33	28673	0.47
2	12.828	12.770	12.885	80866	0.48	37135	0.61
3	13.768	13.705	13.890	110992	0.65	30522	0.50
4	15.526	15.485	15.570	341806	2.01	193009	3.16
5	17.344	17.305	17.435	429940	2.53	123039	2.01
6	17.561	17.435	17.580	5058060	29.82	1282771	20.98
7	17.598	17.580	17.705	3591689	21.17	1322230	21.62
8	18.093	18.055	18.130	338053	1.99	170651	2.79
9	18.406	18.370	18.445	224211	1.32	133016	2.18
10	19.741	19.680	19.765	1246953	7.35	525017	8.59
11	19.798	19.765	19.885	1843773	10.87	729138	11.92
12	19.976	19.885	20.010	606181	3.57	281060	4.60
13	20.035	20.010	20.075	534704	3.15	331473	5.42
14	23.094	23.060	23.135	211882	1.25	128486	2.10
15	24.710	24.655	24.745	281968	1.66	125594	2.05
16	25.192	25.160	25.225	241820	1.43	141452	2.31
17	28.567	28.525	28.620	178346	1.05	63901	1.04
18	28.845	28.805	28.910	304418	1.79	101139	1.65
19	29.586	29.515	29.645	1015995	5.99	299586	4.90
20	31.374	31.310	31.425	266204	1.57	67341	1.10
				16964341	100.00	6115233	100.00

Sample Information

Analyzed by	: Unathi
Analyzed	: 2023-06-26 09:41:47 AM
Sample Type	: Unknown
Level #	: 1
Sample Name	: 2_EtOH
Sample ID	: 2_EtOH
IS Amount	: [1]=1
Sample Amount	: 1
Dilution Factor	: 1
Vial #	: 3
Injection Volume	: 5.00
Data File	: C:\GCMSsolution\Data\2023 External\Life Sciences\Ntombifuthi\22062023\2_EtO
Org Data File	: C:\GCMSsolution\Data\2023 External\Life Sciences\Ntombifuthi\22062023\2_EtO
Method File	: C:\GCMSsolution\Methods\2023 Ibrahim.qgm
Org Method File	: C:\GCMSsolution\Methods\2023 Ibrahim.qgm
Report File	:
Tuning File	: C:\GCMSsolution\System\Tune1\2023 Tuning files\17042023.qgt
Modified by	: Unathi
Modified	: 2023-06-26 10:22:47 AM

omatogram 2_EtOH C:\GCMSsolution\Data\2023 External\Life Sciences\Ntombifuthi\22062023\2_EtOH a.Q



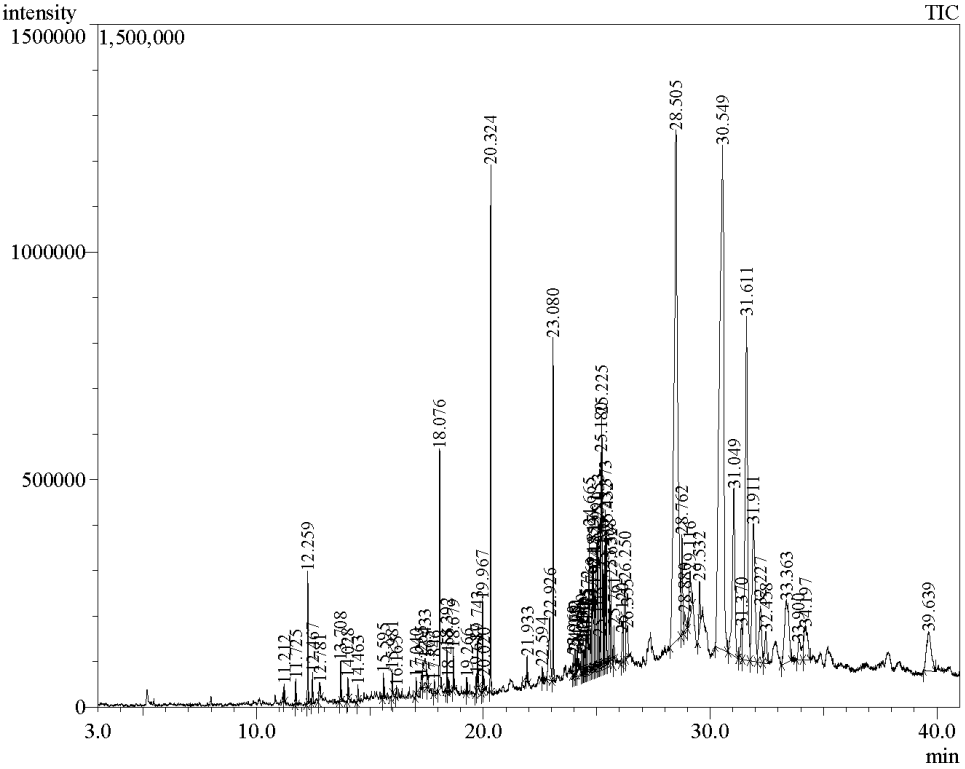
Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	eight%
1	12.833	12.755	12.880	5526750	26.05	2382065	23.16
2	12.933	12.880	12.970	312582	1.47	190629	1.85
3	15.044	15.005	15.085	418282	1.97	265108	2.58
4	15.223	15.180	15.255	1719985	8.11	1058199	10.29
5	15.287	15.260	15.310	230833	1.09	167123	1.62
6	17.267	17.220	17.305	310100	1.46	146239	1.42
7	17.428	17.400	17.465	174982	0.82	108596	1.06
8	18.087	18.040	18.135	860987	4.06	472349	4.59
9	19.721	19.670	19.750	1314309	6.19	641271	6.23
10	19.771	19.750	19.790	501149	2.36	270918	2.63
11	19.810	19.790	19.850	408102	1.92	227084	2.21
12	22.230	22.190	22.275	392609	1.85	216549	2.11
13	23.096	23.060	23.140	390845	1.84	229691	2.23
14	23.701	23.660	23.740	732846	3.45	405877	3.95
15	23.775	23.740	23.820	400291	1.89	207216	2.01
16	24.767	24.725	24.800	546907	2.58	298252	2.90
17	25.193	25.155	25.225	463642	2.19	206787	2.01
18	25.249	25.225	25.285	355279	1.67	166270	1.62
19	25.388	25.350	25.420	310551	1.46	161135	1.57
20	25.448	25.420	25.490	282061	1.33	143791	1.40
21	25.587	25.490	25.625	199591	0.94	95989	0.93
22	25.778	25.740	25.840	588809	2.78	282101	2.74
23	26.067	26.005	26.110	2859668	13.48	1344175	13.07
24	26.900	26.865	26.955	177984	0.84	72756	0.71
25	27.330	27.290	27.375	139062	0.66	52127	0.51
26	28.459	28.425	28.505	254345	1.20	111352	1.08
27	29.573	29.520	29.630	365733	1.72	100346	0.98
28	30.653	30.585	30.735	978200	4.61	262257	2.55
				21216484	100.00	10286252	100.00

Sample Information

Analyzed by	: Unathi
Analyzed	: 2023-06-23 08:03:07 AM
Sample Type	: Unknown
Level #	: 1
Sample Name	: 3_EtOH
Sample ID	: 3_EtOH
IS Amount	: [1]=1
Sample Amount	: 1
Dilution Factor	: 1
Vial #	: 11
Injection Volume	: 3.00
Data File	: C:\GCMSsolution\Data\2023 External\Life Sciences\Ntombifuthi\22062023\3_EtO
Org Data File	: C:\GCMSsolution\Data\2023 External\Life Sciences\Ntombifuthi\22062023\3_EtO
Method File	: C:\GCMSsolution\Methods\2023 Ibrahim.qgm
Org Method File	: C:\GCMSsolution\Methods\2023 Ibrahim.qgm
Report File	:
Tuning File	: C:\GCMSsolution\System\Tune1\2023 Tuning files\17042023.qgt
Modified by	: Unathi
Modified	: 2023-06-23 08:44:08 AM

hromatogram 3_EtOH C:\GCMSsolution\Data\2023 External\Life Sciences\Ntombifuthi\22062023\3_EtOH.qg



Peak Report TIC

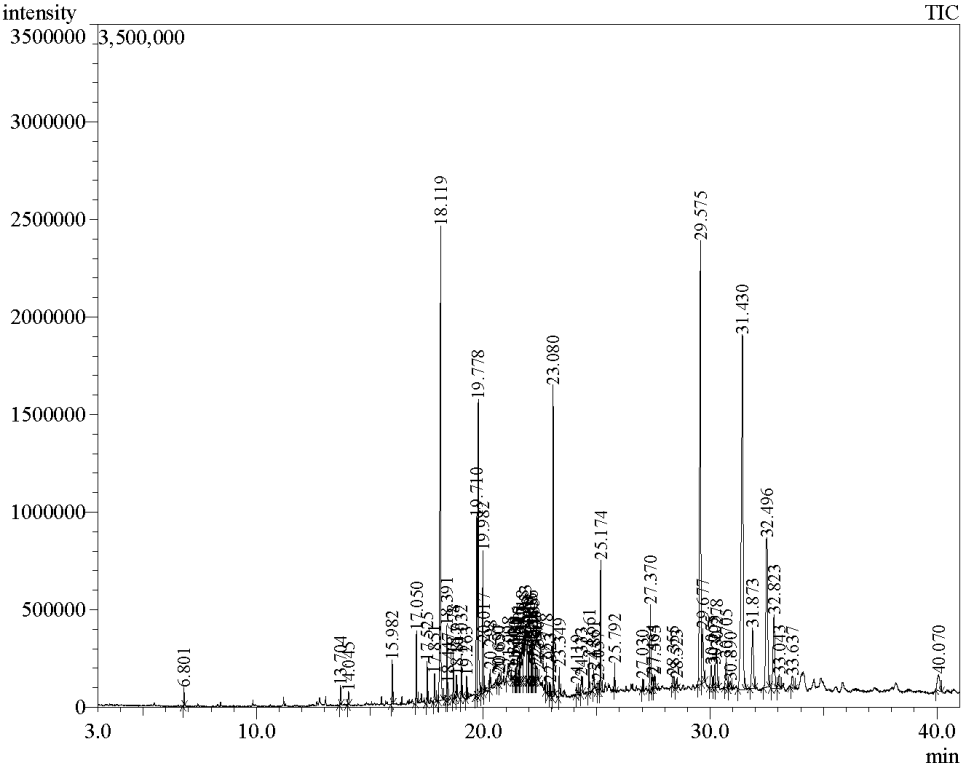
Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	eight%
1	11.212	11.195	11.245	56069	0.08	38030	0.27
2	11.725	11.695	11.780	113936	0.17	56077	0.40
3	12.259	12.210	12.305	501446	0.74	291408	2.10
4	12.467	12.420	12.505	126663	0.19	68105	0.49
5	12.781	12.720	12.835	125154	0.18	37720	0.27
6	13.708	13.665	13.750	163870	0.24	89093	0.64
7	14.028	13.995	14.075	98082	0.14	50545	0.36
8	14.463	14.430	14.500	60050	0.09	36790	0.26
9	15.595	15.555	15.640	97465	0.14	56871	0.41
10	15.981	15.950	16.010	97483	0.14	58598	0.42
11	16.165	16.135	16.200	38309	0.06	25186	0.18
12	17.040	17.005	17.080	80777	0.12	41898	0.30
13	17.265	17.235	17.320	90883	0.13	24856	0.18
14	17.433	17.320	17.500	540058	0.80	64098	0.46
15	17.565	17.500	17.585	107752	0.16	17762	0.13
16	17.846	17.810	17.880	61525	0.09	29397	0.21
17	18.076	18.000	18.150	1079129	1.59	528770	3.80
18	18.392	18.360	18.430	167349	0.25	96590	0.69
19	18.455	18.430	18.495	61537	0.09	31680	0.23
20	18.679	18.650	18.720	136917	0.20	91721	0.66
21	19.266	19.245	19.300	56144	0.08	34535	0.25
22	19.687	19.650	19.710	69546	0.10	40787	0.29
23	19.743	19.710	19.795	201790	0.30	107786	0.78
24	19.967	19.925	20.000	364412	0.54	202093	1.45
25	20.020	20.000	20.060	56066	0.08	30822	0.22
26	20.324	20.280	20.365	1862660	2.75	1156656	8.32
27	21.933	21.895	21.985	96986	0.14	52504	0.38
28	22.594	22.570	22.640	52374	0.08	30964	0.22
29	22.926	22.805	23.035	738377	1.09	137705	0.99
30	23.080	23.035	23.140	1290316	1.90	753344	5.42
31	23.960	23.930	24.000	96574	0.14	44948	0.32
32	24.042	24.000	24.060	132480	0.20	53629	0.39
33	24.075	24.060	24.115	66158	0.10	41078	0.30
34	24.151	24.115	24.180	100235	0.15	55154	0.40
35	24.349	24.305	24.375	164027	0.24	63233	0.45
36	24.390	24.375	24.415	86657	0.13	45179	0.33
37	24.430	24.415	24.460	110050	0.16	44908	0.32
38	24.479	24.460	24.500	97125	0.14	53753	0.39
39	24.525	24.500	24.545	154318	0.23	70905	0.51
40	24.572	24.545	24.605	313547	0.46	109484	0.79
41	24.665	24.605	24.710	787814	1.16	312817	2.25
42	24.769	24.710	24.780	314303	0.46	135132	0.97
43	24.817	24.780	24.840	637825	0.94	227029	1.63
44	24.859	24.840	24.890	478670	0.71	220140	1.58
45	24.914	24.890	24.945	427047	0.63	155569	1.12
46	24.991	24.945	25.025	910743	1.34	270973	1.95
47	25.053	25.025	25.090	741873	1.10	313106	2.25
48	25.105	25.090	25.135	124060	0.18	62794	0.45
49	25.180	25.135	25.200	973584	1.44	466828	3.36
50	25.225	25.200	25.270	1416732	2.09	548654	3.95
51	25.296	25.270	25.330	394029	0.58	206054	1.48
52	25.373	25.330	25.405	669563	0.99	335887	2.42
53	25.432	25.405	25.475	526157	0.78	287270	2.07
54	25.568	25.475	25.595	406306	0.60	204745	1.47
55	25.632	25.595	25.690	442134	0.65	182777	1.32

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	eight%
56	25.761	25.720	25.795	136424	0.20	79493	0.57
57	26.120	26.075	26.190	404751	0.60	65814	0.47
58	26.250	26.190	26.290	776321	1.15	174537	1.26
59	26.355	26.290	26.410	352715	0.52	64410	0.46
60	28.505	28.225	28.710	12422045	18.33	1121326	8.07
61	28.762	28.710	28.840	905428	1.34	220437	1.59
62	28.880	28.840	29.020	225980	0.33	44096	0.32
63	29.116	29.020	29.235	661722	0.98	103413	0.74
64	29.532	29.460	29.590	377429	0.56	107486	0.77
65	30.549	30.225	30.770	14557463	21.49	1107054	7.97
66	31.049	30.810	31.250	2830368	4.18	366172	2.63
67	31.370	31.250	31.430	416876	0.62	70150	0.50
68	31.611	31.430	31.760	5891710	8.70	753004	5.42
69	31.911	31.760	32.080	2788815	4.12	301428	2.17
70	32.227	32.080	32.330	944964	1.39	121887	0.88
71	32.458	32.330	32.580	461330	0.68	69293	0.50
72	33.363	33.160	33.590	1847129	2.73	137615	0.99
73	33.900	33.810	34.100	464126	0.69	36301	0.26
74	34.197	34.100	34.420	885786	1.31	74136	0.53
75	39.639	39.420	39.940	1234058	1.82	85603	0.62
				67750576	100.00	13898092	100.00

Sample Information

Analyzed by	: Unathi
Analyzed	: 2023-06-23 08:52:22 AM
Sample Type	: Unknown
Level #	: 1
Sample Name	: 4_EtOH
Sample ID	: 4_EtOH
IS Amount	: [1]=1
Sample Amount	: 1
Dilution Factor	: 1
Vial #	: 12
Injection Volume	: 3.00
Data File	: C:\GCMSsolution\Data\2023 External\Life Sciences\Ntombifuthi\22062023\4_EtO
Org Data File	: C:\GCMSsolution\Data\2023 External\Life Sciences\Ntombifuthi\22062023\4_EtO
Method File	: C:\GCMSsolution\Methods\2023 Ibrahim.qgm
Org Method File	: C:\GCMSsolution\Methods\2023 Ibrahim.qgm
Report File	:
Tuning File	: C:\GCMSsolution\System\Tune1\2023 Tuning files\17042023.qgt
Modified by	: Unathi
Modified	: 2023-06-23 09:33:22 AM

hromatogram 4_EtOH C:\GCMSsolution\Data\2023 External\Life Sciences\Ntombifuthi\22062023\4_EtOH.qg



Peak Report TIC

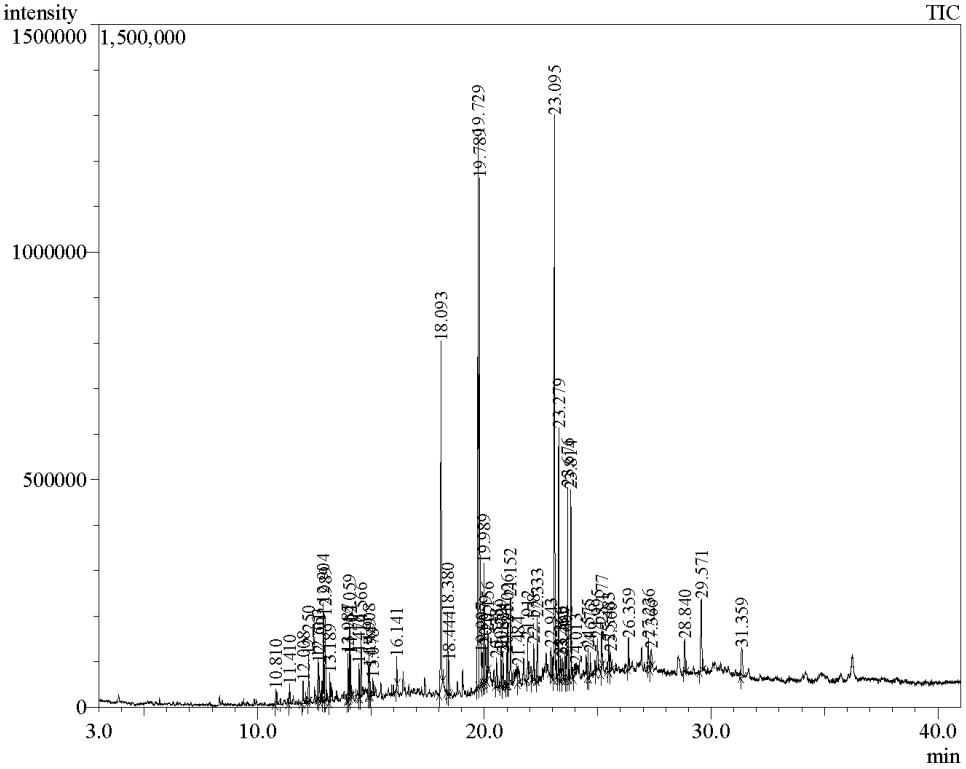
Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	eight%
1	6.801	6.765	6.835	147654	0.21	92800	0.43
2	13.704	13.660	13.740	170158	0.25	99075	0.46
3	14.045	14.005	14.080	112339	0.16	70148	0.33
4	15.982	15.930	16.030	393583	0.57	225482	1.05
5	17.050	17.005	17.125	712824	1.03	370507	1.72
6	17.525	17.485	17.590	418222	0.61	214017	1.00
7	17.851	17.805	17.935	351374	0.51	145819	0.68
8	18.119	18.030	18.240	7475818	10.83	2433083	11.31
9	18.391	18.345	18.420	629635	0.91	381885	1.78
10	18.447	18.420	18.490	208477	0.30	90190	0.42
11	18.678	18.640	18.710	362709	0.53	232344	1.08
12	18.817	18.785	18.875	235752	0.34	112720	0.52
13	19.032	18.995	19.085	397392	0.58	224704	1.04
14	19.263	19.225	19.310	188465	0.27	117578	0.55
15	19.710	19.645	19.735	2224983	3.22	918634	4.27
16	19.778	19.735	19.880	3676199	5.33	1517083	7.05
17	19.982	19.880	20.005	1656040	2.40	729817	3.39
18	20.017	20.005	20.055	394131	0.57	255826	1.19
19	20.298	20.275	20.340	196646	0.28	103195	0.48
20	20.650	20.605	20.685	129589	0.19	41827	0.19
21	20.697	20.685	20.800	207328	0.30	53125	0.25
22	21.098	21.030	21.225	586808	0.85	76132	0.35
23	21.330	21.305	21.395	123103	0.18	18320	0.09
24	21.410	21.395	21.430	124301	0.18	60339	0.28
25	21.450	21.430	21.475	187565	0.27	77576	0.36
26	21.516	21.475	21.530	339482	0.49	125750	0.58
27	21.545	21.530	21.580	359821	0.52	121773	0.57
28	21.601	21.580	21.640	393290	0.57	145841	0.68
29	21.718	21.640	21.740	787916	1.14	211293	0.98
30	21.853	21.740	21.915	1971600	2.86	237434	1.10
31	21.940	21.915	21.965	487538	0.71	178003	0.83
32	21.990	21.965	22.005	344809	0.50	153803	0.72
33	22.060	22.005	22.095	861721	1.25	174411	0.81
34	22.105	22.095	22.120	295308	0.43	197989	0.92
35	22.136	22.120	22.175	636038	0.92	214032	1.00
36	22.185	22.175	22.230	490637	0.71	172271	0.80
37	22.270	22.230	22.280	329370	0.48	106378	0.49
38	22.310	22.280	22.335	267861	0.39	84193	0.39
39	22.390	22.335	22.445	261314	0.38	56205	0.26
40	22.778	22.740	22.825	248405	0.36	152484	0.71
41	22.923	22.895	22.955	124386	0.18	67642	0.31
42	23.080	23.035	23.125	2678877	3.88	1597119	7.43
43	23.349	23.310	23.425	322550	0.47	153221	0.71
44	24.119	24.085	24.180	123293	0.18	53864	0.25
45	24.333	24.300	24.390	266525	0.39	85115	0.40
46	24.661	24.620	24.715	352158	0.51	182292	0.85
47	24.861	24.830	24.900	170540	0.25	99015	0.46
48	25.057	25.015	25.125	171233	0.25	54168	0.25
49	25.174	25.125	25.260	1290569	1.87	675757	3.14
50	25.792	25.755	25.835	229091	0.33	136548	0.63
51	27.030	26.995	27.080	121072	0.18	60774	0.28
52	27.370	27.300	27.430	1124129	1.63	432011	2.01
53	27.464	27.430	27.510	177600	0.26	72847	0.34
54	27.555	27.510	27.605	203970	0.30	69396	0.32
55	28.355	28.315	28.425	192837	0.28	55941	0.26

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	eight%
56	28.523	28.475	28.565	152883	0.22	49096	0.23
57	29.575	29.445	29.630	8565766	12.41	2271270	10.56
58	29.677	29.630	29.785	1174478	1.70	277806	1.29
59	30.055	29.965	30.120	486867	0.71	115329	0.54
60	30.205	30.120	30.240	485522	0.70	114683	0.53
61	30.278	30.240	30.380	834383	1.21	202317	0.94
62	30.705	30.635	30.795	647057	0.94	151439	0.70
63	30.890	30.795	30.950	189469	0.27	41226	0.19
64	31.430	31.230	31.550	10419791	15.10	1820980	8.47
65	31.873	31.770	31.980	1521515	2.20	309224	1.44
66	32.496	32.360	32.625	4185795	6.07	766369	3.56
67	32.823	32.695	32.940	2010643	2.91	378461	1.76
68	33.043	32.940	33.140	380796	0.55	71935	0.33
69	33.637	33.555	33.745	352577	0.51	62155	0.29
70	40.070	39.950	40.220	666490	0.97	82006	0.38
				69009067	100.00	21504092	100.00

Sample Information

Analyzed by	: Unathi
Analyzed	: 2023-06-27 10:55:16 AM
Sample Type	: Unknown
Level #	: 1
Sample Name	: 5 EtOH
Sample ID	: 5 EtOH
IS Amount	: [1]=1
Sample Amount	: 1
Dilution Factor	: 1
Vial #	: 1
Injection Volume	: 8.00
Data File	: C:\GCMSsolution\Data\2023 External\Life Sciences\Ntombifuthi\22062023\5 EtOI
Org Data File	: C:\GCMSsolution\Data\2023 External\Life Sciences\Ntombifuthi\22062023\5 EtOI
Method File	: C:\GCMSsolution\Methods\2023 Ibrahim.qgm
Org Method File	: C:\GCMSsolution\Methods\2023 Ibrahim.qgm
Report File	:
Tuning File	: C:\GCMSsolution\System\Tune1\2023 Tuning files\17042023.qgt
Modified by	: Unathi
Modified	: 2023-06-27 11:36:16 AM

hromatogram 5 EtOH C:\GCMSsolution\Data\2023 External\Life Sciences\Ntombifuthi\22062023\5 EtOH.QG



Peak Report TIC

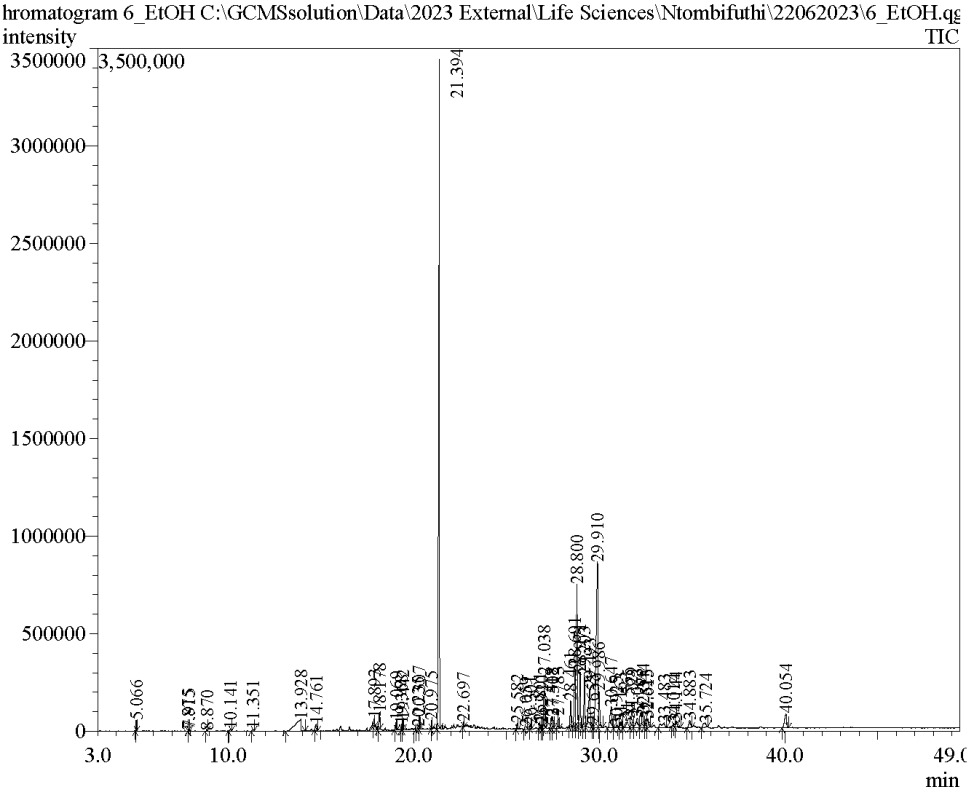
Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	eight%
1	10.810	10.765	10.880	80747	0.36	34314	0.33
2	11.410	11.370	11.445	67717	0.30	42507	0.41
3	12.008	11.960	12.125	123140	0.55	50307	0.49
4	12.250	12.215	12.280	162566	0.72	103724	1.01
5	12.663	12.630	12.685	173946	0.77	90185	0.88
6	12.701	12.685	12.780	175150	0.78	81983	0.80
7	12.904	12.865	12.955	369091	1.64	212572	2.07
8	12.989	12.955	13.045	348302	1.55	186460	1.81
9	13.189	13.155	13.210	107590	0.48	65363	0.64
10	13.987	13.950	14.025	176475	0.78	102192	0.99
11	14.059	14.025	14.080	281047	1.25	165135	1.61
12	14.102	14.080	14.135	156133	0.69	93968	0.91
13	14.476	14.450	14.515	124612	0.55	75670	0.74
14	14.566	14.515	14.605	346416	1.54	143101	1.39
15	14.908	14.870	14.930	172223	0.76	94943	0.92
16	14.949	14.930	14.990	103316	0.46	63565	0.62
17	15.078	14.990	15.110	109720	0.49	39284	0.38
18	16.141	16.115	16.175	103870	0.46	67525	0.66
19	18.093	18.015	18.175	1906227	8.46	762325	7.41
20	18.380	18.340	18.420	367787	1.63	178902	1.74
21	18.444	18.420	18.490	128677	0.57	74235	0.72
22	19.729	19.655	19.755	2974395	13.20	1212846	11.80
23	19.789	19.755	19.880	2734222	12.14	1115846	10.85
24	19.907	19.880	19.950	199596	0.89	70177	0.68
25	19.989	19.950	20.020	513789	2.28	264151	2.57
26	20.059	20.020	20.115	217254	0.96	81743	0.80
27	20.156	20.115	20.190	178178	0.79	96134	0.94
28	20.538	20.500	20.595	146898	0.65	62561	0.61
29	20.729	20.695	20.775	138063	0.61	81000	0.79
30	20.824	20.790	20.865	149735	0.66	75638	0.74
31	20.995	20.950	21.010	144424	0.64	70891	0.69
32	21.026	21.010	21.065	252641	1.12	135672	1.32
33	21.152	21.065	21.225	521464	2.32	191534	1.86
34	21.484	21.455	21.565	142230	0.63	38252	0.37
35	21.912	21.880	21.940	166301	0.74	89013	0.87
36	22.178	22.085	22.225	174441	0.77	80914	0.79
37	22.333	22.295	22.370	246515	1.09	140966	1.37
38	22.943	22.895	22.990	175195	0.78	61879	0.60
39	23.095	23.040	23.165	2461445	10.93	1239004	12.05
40	23.279	23.210	23.320	1006405	4.47	552764	5.38
41	23.366	23.320	23.420	149907	0.67	53155	0.52
42	23.461	23.420	23.505	121157	0.54	49081	0.48
43	23.602	23.570	23.630	127623	0.57	54034	0.53
44	23.676	23.630	23.715	786977	3.49	421909	4.10
45	23.814	23.740	23.865	785870	3.49	416001	4.05
46	24.013	23.935	24.050	106264	0.47	27593	0.27
47	24.575	24.540	24.620	138760	0.62	65430	0.64
48	24.676	24.620	24.715	110839	0.49	52430	0.51
49	24.995	24.905	25.035	167929	0.75	70566	0.69
50	25.177	25.140	25.215	214223	0.95	99282	0.97
51	25.483	25.450	25.525	137101	0.61	66132	0.64
52	25.560	25.525	25.625	119455	0.53	44236	0.43
53	26.359	26.315	26.400	143564	0.64	70346	0.68
54	27.236	27.195	27.280	127121	0.56	62490	0.61
55	27.366	27.320	27.410	130205	0.58	42152	0.41

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	eight%
56	28.840	28.795	28.895	194201	0.86	71243	0.69
57	29.571	29.500	29.640	558955	2.48	159093	1.55
58	31.359	31.310	31.430	276995	1.23	66438	0.65
				22525089	100.00	10280856	100.00

Sample Information

Analyzed by	: Unathi
Analyzed	: 2023-06-23 12:28:36 PM
Sample Type	: Unknown
Level #	: 1
Sample Name	: 6_EtOH
Sample ID	: 6_EtOH
IS Amount	: [1]=1
Sample Amount	: 1
Dilution Factor	: 1
Vial #	: 14
Injection Volume	: 3.00
Data File	: C:\GCMSsolution\Data\2023 External\Life Sciences\Ntombifuthi\22062023\6_EtO
Org Data File	: C:\GCMSsolution\Data\2023 External\Life Sciences\Ntombifuthi\22062023\6_EtO
Method File	: C:\GCMSsolution\Methods\2023 Emmanuel.qgm
Org Method File	: C:\GCMSsolution\Methods\2023 Emmanuel.qgm
Report File	:
Tuning File	: C:\GCMSsolution\System\Tune1\2023 Tuning files\17042023.qgt
Modified by	: Unathi
Modified	: 2023-06-23 01:18:00 PM

hromatogram 6_EtOH C:\GCMSsolution\Data\2023 External\Life Sciences\Ntombifuthi\22062023\6_EtOH.qg



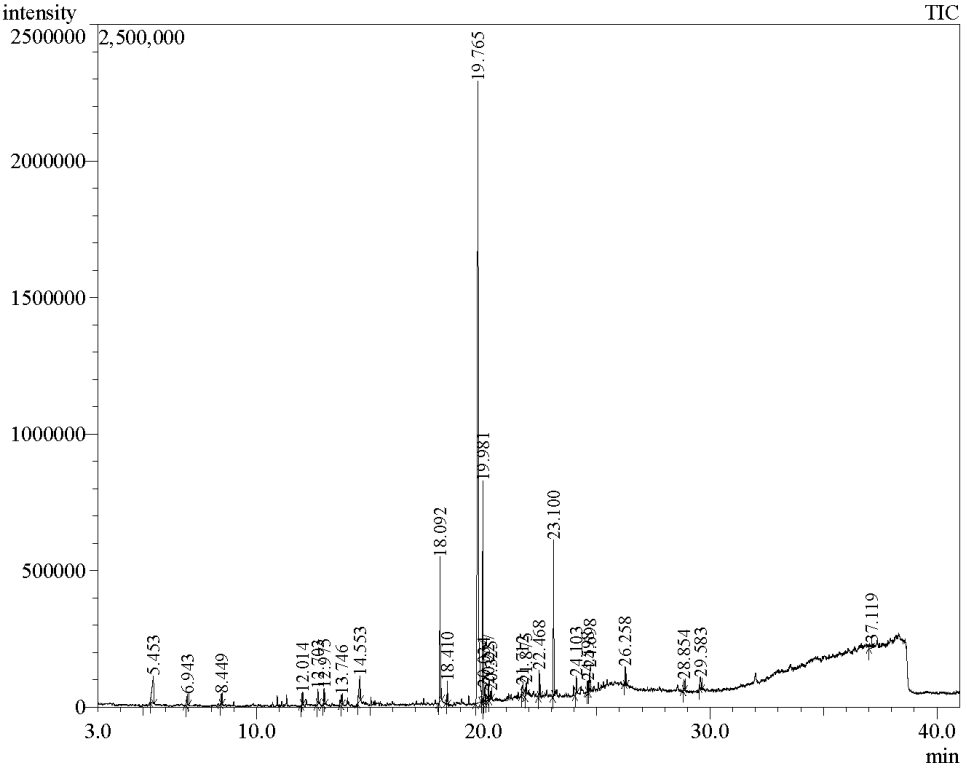
Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	eight%
1	5.066	5.030	5.105	76568	0.21	54875	0.67
2	7.875	7.850	7.875	1189	0.00	1754	0.02
3	7.915	7.870	7.960	23268	0.06	9439	0.11
4	8.870	8.785	8.905	13732	0.04	4539	0.06
5	10.141	10.045	10.220	33607	0.09	9917	0.12
6	11.351	11.270	11.425	37316	0.10	9370	0.11
7	13.928	13.110	14.170	1277015	3.46	60746	0.74
8	14.761	14.670	14.850	163838	0.44	32904	0.40
9	17.893	17.815	17.915	136876	0.37	43840	0.53
10	18.178	18.085	18.210	311951	0.85	84922	1.03
11	19.069	18.985	19.115	133828	0.36	48026	0.58
12	19.368	19.330	19.400	76662	0.21	31231	0.38
13	19.442	19.400	19.485	92910	0.25	52887	0.64
14	20.235	20.140	20.280	52345	0.14	12483	0.15
15	20.307	20.280	20.355	127935	0.35	70515	0.86
16	20.975	20.945	21.010	71433	0.19	44745	0.54
17	21.394	21.270	21.440	11612363	31.49	3430841	41.76
18	22.697	22.655	22.735	54984	0.15	27100	0.33
19	25.582	25.520	25.640	96114	0.26	30179	0.37
20	26.059	25.955	26.155	95574	0.26	11824	0.14
21	26.301	26.255	26.370	59651	0.16	17610	0.21
22	26.801	26.735	26.860	106934	0.29	34859	0.42
23	26.920	26.860	26.965	64272	0.17	19643	0.24
24	27.038	26.965	27.130	906649	2.46	272924	3.32
25	27.418	27.345	27.460	222905	0.60	59424	0.72
26	27.502	27.460	27.570	210573	0.57	60511	0.74
27	27.783	27.700	27.860	248008	0.67	63155	0.77
28	28.461	28.365	28.535	524294	1.42	133138	1.62
29	28.691	28.575	28.725	1432406	3.88	309618	3.77
30	28.800	28.725	28.875	3096556	8.40	731659	8.91
31	28.971	28.875	29.070	1227296	3.33	281392	3.42
32	29.203	29.100	29.285	1030828	2.80	263502	3.21
33	29.493	29.285	29.600	1449150	3.93	202035	2.46
34	29.655	29.600	29.710	84401	0.23	16738	0.20
35	29.910	29.710	29.955	5794234	15.71	841193	10.24
36	29.986	29.955	30.195	865781	2.35	186335	2.27
37	30.647	30.455	30.775	800696	2.17	108479	1.32
38	30.925	30.775	31.075	103993	0.28	9920	0.12
39	31.163	31.075	31.285	195212	0.53	28608	0.35
40	31.565	31.285	31.665	549847	1.49	43731	0.53
41	31.729	31.665	31.855	361720	0.98	55836	0.68
42	32.063	31.855	32.185	426713	1.16	46558	0.57
43	32.344	32.185	32.425	449812	1.22	70865	0.86
44	32.523	32.425	32.575	258541	0.70	42285	0.51
45	32.615	32.575	32.775	252433	0.68	43432	0.53
46	33.483	33.175	33.635	325123	0.88	21925	0.27
47	34.014	33.905	34.075	92027	0.25	16527	0.20
48	34.144	34.075	34.385	195862	0.53	30700	0.37
49	34.883	34.735	35.005	235535	0.64	37112	0.45
50	35.724	35.505	35.835	243987	0.66	21563	0.26
51	40.054	39.845	40.205	569750	1.55	72760	0.89
				36874697	100.00	8216174	100.00

Sample Information

Analyzed by	: Unathi
Analyzed	: 2023-06-26 12:48:12 PM
Sample Type	: Unknown
Level #	: 1
Sample Name	: 7_EtOH
Sample ID	: 7_EtOH
IS Amount	: [1]=1
Sample Amount	: 1
Dilution Factor	: 1
Vial #	: 5
Injection Volume	: 5.00
Data File	: C:\GCMSsolution\Data\2023 External\Life Sciences\Ntombifuthi\22062023\7_EtO
Org Data File	: C:\GCMSsolution\Data\2023 External\Life Sciences\Ntombifuthi\22062023\7_EtO
Method File	: C:\GCMSsolution\Methods\2023 Ibrahim.qgm
Org Method File	: C:\GCMSsolution\Methods\2023 Ibrahim.qgm
Report File	:
Tuning File	: C:\GCMSsolution\System\Tune1\2023 Tuning files\17042023.qgt
Modified by	: Unathi
Modified	: 2023-06-26 01:29:12 PM

omatogram 7_EtOH C:\GCMSsolution\Data\2023 External\Life Sciences\Ntombifuthi\22062023\7_EtOH a.Q

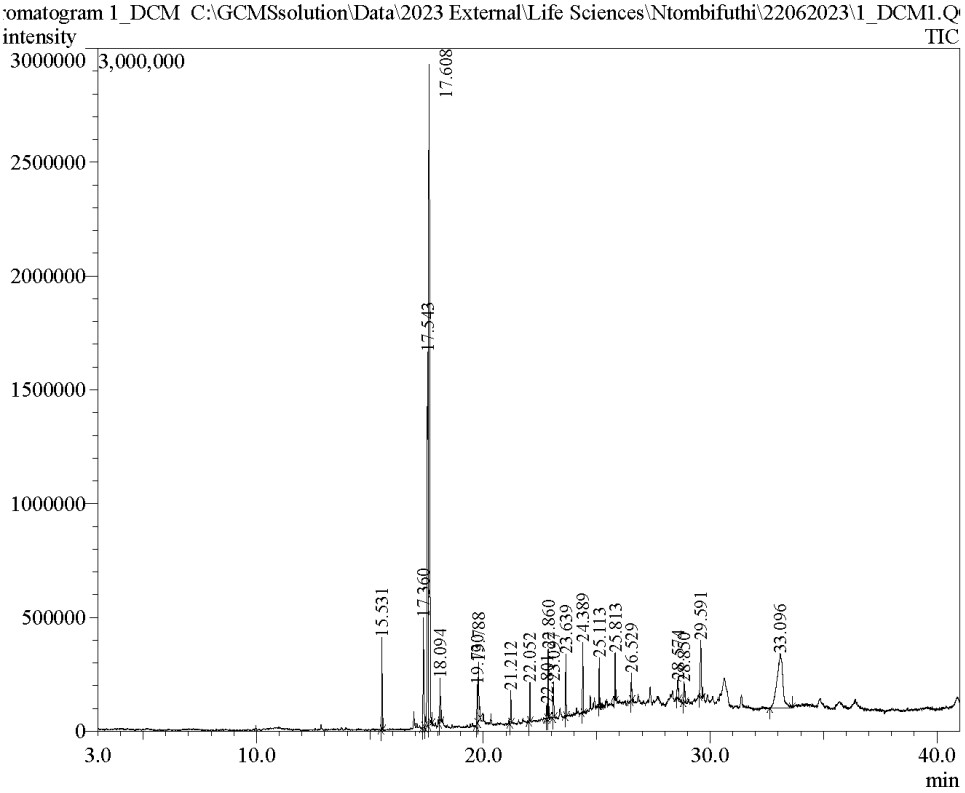


Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	eight%
1	5.453	5.305	5.490	487983	3.42	101721	1.88
2	6.943	6.885	7.005	84819	0.59	39718	0.73
3	8.449	8.410	8.485	72000	0.50	38613	0.71
4	12.014	11.975	12.050	84866	0.59	45618	0.84
5	12.703	12.660	12.745	111605	0.78	56329	1.04
6	12.975	12.935	13.030	110455	0.77	58002	1.07
7	13.746	13.730	13.795	75098	0.53	37221	0.69
8	14.553	14.450	14.600	319125	2.24	93274	1.72
9	18.092	18.035	18.170	1013707	7.11	531224	9.79
10	18.410	18.380	18.450	125583	0.88	81597	1.50
11	19.765	19.665	19.925	7743093	54.27	2276637	41.97
12	19.981	19.925	20.020	1499496	10.51	807478	14.89
13	20.034	20.020	20.070	104476	0.73	51306	0.95
14	20.227	20.125	20.255	147506	1.03	57857	1.07
15	20.325	20.255	20.365	90734	0.64	31351	0.58
16	21.712	21.685	21.770	94014	0.66	36424	0.67
17	21.875	21.840	21.920	87640	0.61	46862	0.86
18	22.468	22.430	22.505	178800	1.25	98973	1.82
19	23.100	23.060	23.140	957279	6.71	572764	10.56
20	24.103	24.045	24.140	127708	0.90	64363	1.19
21	24.598	24.560	24.630	86772	0.61	41063	0.76
22	24.698	24.630	24.745	202131	1.42	87488	1.61
23	26.258	26.220	26.300	130552	0.92	66396	1.22
24	28.854	28.805	28.895	119232	0.84	40137	0.74
25	29.583	29.530	29.635	138331	0.97	43370	0.80
26	37.119	37.010	37.140	74291	0.52	18655	0.34
				14267296	100.00	5424441	100.00

Sample Information

Analyzed by	: Unathi
Analyzed	: 2023-06-26 07:59:44 AM
Sample Type	: Unknown
Level #	: 1
Sample Name	: 1_DCM
Sample ID	: 1_DCM
IS Amount	: [1]=1
Sample Amount	: 1
Dilution Factor	: 1
Vial #	: 16
Injection Volume	: 5.00
Data File	: C:\GCMSsolution\Data\2023 External\Life Sciences\Ntombifuthi\22062023\1_DCM
Org Data File	: C:\GCMSsolution\Data\2023 External\Life Sciences\Ntombifuthi\22062023\1_DCM
Method File	: C:\GCMSsolution\Methods\2023 Ibrahim.qgm
Org Method File	: C:\GCMSsolution\Methods\2023 Ibrahim.qgm
Report File	:
Tuning File	: C:\GCMSsolution\System\Tune1\2023 Tuning files\17042023.qgt
Modified by	: Unathi
Modified	: 2023-06-26 08:40:44 AM



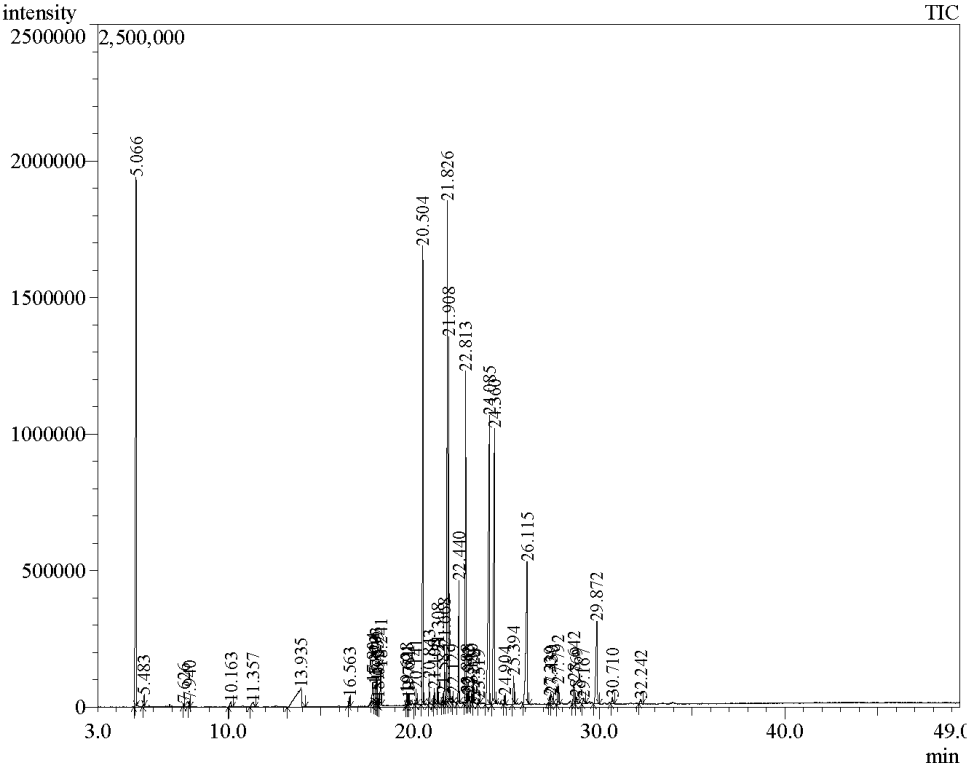
Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	eight%
1	15.531	15.490	15.575	700910	2.49	406061	4.68
2	17.360	17.310	17.415	1119219	3.98	483058	5.56
3	17.543	17.415	17.565	4753276	16.89	1641356	18.90
4	17.608	17.565	17.730	11072286	39.35	2900636	33.40
5	18.094	18.050	18.145	375379	1.33	195083	2.25
6	19.730	19.685	19.755	408872	1.45	173819	2.00
7	19.788	19.755	19.870	728424	2.59	261566	3.01
8	21.212	21.175	21.250	227963	0.81	144455	1.66
9	22.052	22.015	22.085	285761	1.02	172416	1.99
10	22.801	22.765	22.825	123924	0.44	65034	0.75
11	22.860	22.825	22.895	499175	1.77	302676	3.49
12	23.097	23.060	23.135	263838	0.94	157196	1.81
13	23.639	23.605	23.675	455726	1.62	271644	3.13
14	24.389	24.335	24.425	582834	2.07	310103	3.57
15	25.113	25.080	25.160	370476	1.32	216050	2.49
16	25.813	25.775	25.845	334458	1.19	203247	2.34
17	26.529	26.490	26.570	221726	0.79	121947	1.40
18	28.574	28.520	28.635	241973	0.86	80095	0.92
19	28.850	28.810	28.920	278081	0.99	88769	1.02
20	29.591	29.520	29.665	860183	3.06	249182	2.87
21	33.096	32.635	33.630	4230151	15.04	239097	2.75
				28134635	100.00	8683490	100.00

Sample Information

Analyzed by	: Unathi
Analyzed	: 2023-06-23 05:07:26 PM
Sample Type	: Unknown
Level #	: 1
Sample Name	: 2_DCM
Sample ID	: 2_DCM
IS Amount	: [1]=1
Sample Amount	: 1
Dilution Factor	: 1
Vial #	: 17
Injection Volume	: 3.00
Data File	: C:\GCMSsolution\Data\2023 External\Life Sciences\Ntombifuthi\22062023\2_DCM
Org Data File	: C:\GCMSsolution\Data\2023 External\Life Sciences\Ntombifuthi\22062023\2_DCM
Method File	: C:\GCMSsolution\Methods\2023 Emmanuel.qgm
Org Method File	: C:\GCMSsolution\Methods\2023 Emmanuel.qgm
Report File	:
Tuning File	: C:\GCMSsolution\System\Tune1\2023 Tuning files\17042023.qgt
Modified by	: Unathi
Modified	: 2023-06-23 05:56:50 PM

hromatogram 2_DCM C:\GCMSsolution\Data\2023 External\Life Sciences\Ntombifuthi\22062023\2_DCM.qg



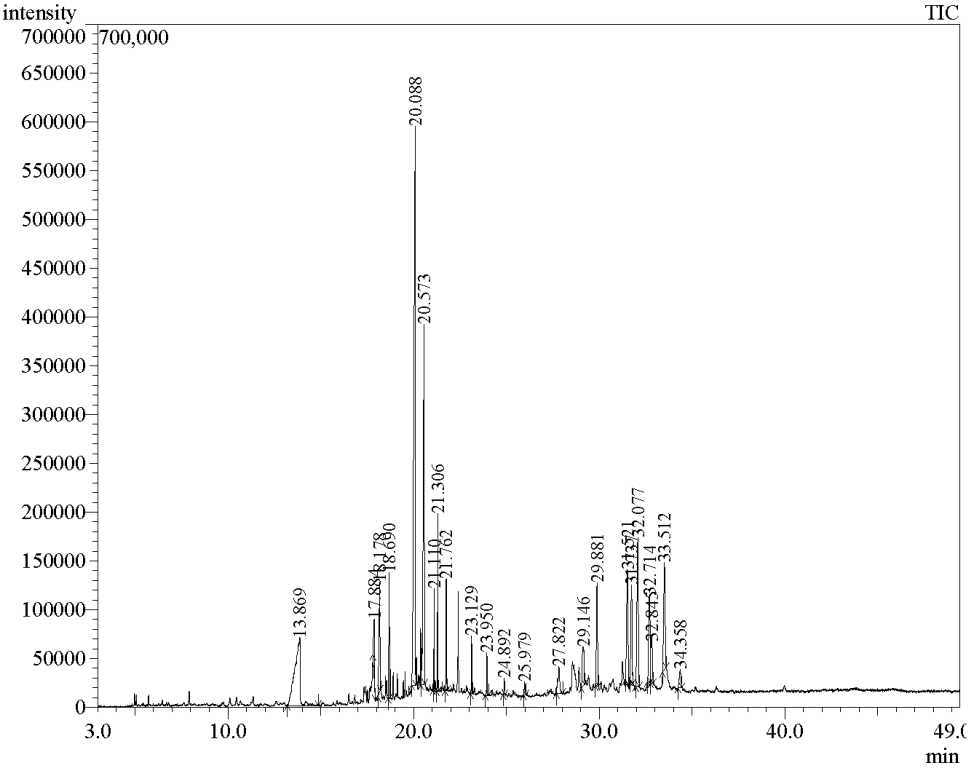
Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	eight%
1	5.066	4.950	5.085	6194917	14.98	1935408	14.49
2	5.483	5.425	5.510	80260	0.19	37595	0.28
3	7.626	7.595	7.675	23668	0.06	10285	0.08
4	7.940	7.880	7.980	55430	0.13	20876	0.16
5	10.163	10.040	10.275	86421	0.21	18719	0.14
6	11.357	11.205	11.495	146900	0.36	17341	0.13
7	13.935	13.210	14.170	1500654	3.63	70934	0.53
8	16.563	16.490	16.615	105877	0.26	33096	0.25
9	17.804	17.715	17.850	333025	0.81	71507	0.54
10	17.929	17.850	17.950	333157	0.81	67143	0.50
11	17.993	17.950	18.015	284867	0.69	98398	0.74
12	18.039	18.015	18.070	149977	0.36	83843	0.63
13	18.095	18.070	18.130	31065	0.08	21990	0.16
14	18.241	18.140	18.285	458444	1.11	136637	1.02
15	19.628	19.585	19.655	85282	0.21	48119	0.36
16	19.691	19.655	19.725	74584	0.18	42302	0.32
17	19.751	19.725	19.790	39033	0.09	23773	0.18
18	20.141	20.090	20.195	113142	0.27	56934	0.43
19	20.504	20.420	20.560	4040145	9.77	1676940	12.55
20	20.843	20.800	20.885	169135	0.41	91844	0.69
21	21.108	21.080	21.150	93840	0.23	58562	0.44
22	21.308	21.265	21.350	283701	0.69	189543	1.42
23	21.583	21.525	21.630	63333	0.15	33547	0.25
24	21.668	21.630	21.715	367731	0.89	204601	1.53
25	21.826	21.715	21.860	4591613	11.10	1833667	13.72
26	21.908	21.860	21.975	2416724	5.84	1335962	10.00
27	22.129	22.090	22.155	57919	0.14	32142	0.24
28	22.440	22.350	22.480	959174	2.32	448130	3.35
29	22.813	22.730	22.860	2901037	7.02	1211842	9.07
30	22.888	22.860	22.920	76673	0.19	29532	0.22
31	22.942	22.920	22.990	42024	0.10	21319	0.16
32	23.130	23.095	23.175	63624	0.15	35298	0.26
33	23.222	23.185	23.255	40268	0.10	21418	0.16
34	23.519	23.480	23.575	44887	0.11	17191	0.13
35	24.085	23.875	24.135	5016746	12.13	1058673	7.92
36	24.360	24.205	24.435	4162827	10.07	1008009	7.54
37	24.904	24.855	24.970	76467	0.18	32043	0.24
38	25.394	25.325	25.470	284818	0.69	104272	0.78
39	26.115	25.890	26.225	2653252	6.42	518053	3.88
40	27.339	27.275	27.375	95472	0.23	27144	0.20
41	27.430	27.375	27.525	133117	0.32	33975	0.25
42	27.792	27.705	27.865	266698	0.64	71667	0.54
43	28.642	28.535	28.715	357361	0.86	84466	0.63
44	28.769	28.715	28.905	119470	0.29	25489	0.19
45	29.167	29.075	29.265	91213	0.22	19980	0.15
46	29.872	29.685	30.005	1591805	3.85	301549	2.26
47	30.710	30.625	30.815	98679	0.24	22542	0.17
48	32.242	32.135	32.345	92630	0.22	16764	0.13
				41349086	100.00	13361064	100.00

Sample Information

Analyzed by	: Unathi
Analyzed	: 2023-06-23 06:03:15 PM
Sample Type	: Unknown
Level #	: 1
Sample Name	: 3_DCM
Sample ID	: 3_DCM
IS Amount	: [1]=1
Sample Amount	: 1
Dilution Factor	: 1
Vial #	: 18
Injection Volume	: 3.00
Data File	: C:\GCMSsolution\Data\2023 External\Life Sciences\Ntombifuthi\22062023\3_DCM
Org Data File	: C:\GCMSsolution\Data\2023 External\Life Sciences\Ntombifuthi\22062023\3_DCM
Method File	: C:\GCMSsolution\Methods\2023 Emmanuel.qgm
Org Method File	: C:\GCMSsolution\Methods\2023 Emmanuel.qgm
Report File	:
Tuning File	: C:\GCMSsolution\System\Tune1\2023 Tuning files\17042023.qgt
Modified by	: Unathi
Modified	: 2023-06-23 06:52:40 PM

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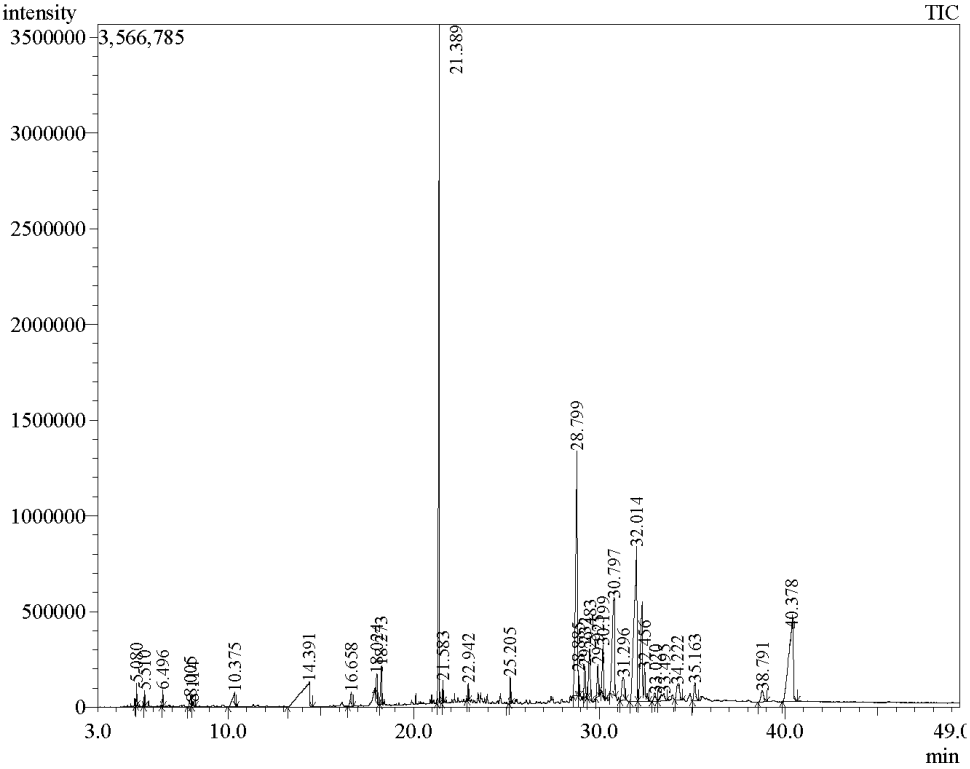
Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	eight%
1	13.869	13.210	14.875	1459399	12.02	69578	2.62
2	17.884	17.815	17.940	177829	1.47	62357	2.35
3	18.178	18.085	18.210	385324	3.17	112668	4.24
4	18.690	18.635	18.715	275850	2.27	129113	4.86
5	20.088	19.905	20.130	3083892	25.41	573079	21.58
6	20.573	20.410	20.620	1567423	12.91	369045	13.90
7	21.110	21.075	21.150	154489	1.27	104534	3.94
8	21.306	21.235	21.340	318988	2.63	181967	6.85
9	21.762	21.710	21.830	192032	1.58	114126	4.30
10	23.129	23.065	23.175	110331	0.91	59459	2.24
11	23.950	23.875	24.055	93509	0.77	43532	1.64
12	24.892	24.835	24.930	41576	0.34	17088	0.64
13	25.979	25.920	26.065	43982	0.36	13306	0.50
14	27.822	27.705	28.045	177223	1.46	27480	1.03
15	29.146	29.045	29.230	276390	2.28	40571	1.53
16	29.881	29.790	29.970	448684	3.70	105934	3.99
17	31.521	31.415	31.610	600182	4.95	117743	4.43
18	31.737	31.650	31.815	476279	3.92	99733	3.76
19	32.077	31.965	32.170	760523	6.27	152828	5.76
20	32.714	32.600	32.775	525655	4.33	88924	3.35
21	32.845	32.775	32.920	248675	2.05	42919	1.62
22	33.512	33.410	33.615	604429	4.98	110218	4.15
23	34.358	34.250	34.460	114189	0.94	18939	0.71
				12136853	100.00	2655141	100.00

Sample Information

Analyzed by	: Unathi
Analyzed	: 2023-06-23 06:59:02 PM
Sample Type	: Unknown
Level #	: 1
Sample Name	: 4_DCM
Sample ID	: 4_DCM
IS Amount	: [1]=1
Sample Amount	: 1
Dilution Factor	: 1
Vial #	: 19
Injection Volume	: 3.00
Data File	: C:\GCMSsolution\Data\2023 External\Life Sciences\Ntombifuthi\22062023\4_DCM
Org Data File	: C:\GCMSsolution\Data\2023 External\Life Sciences\Ntombifuthi\22062023\4_DCM
Method File	: C:\GCMSsolution\Methods\2023 Emmanuel.qgm
Org Method File	: C:\GCMSsolution\Methods\2023 Emmanuel.qgm
Report File	:
Tuning File	: C:\GCMSsolution\System\Tune1\2023 Tuning files\17042023.qgt
Modified by	: Unathi
Modified	: 2023-06-23 07:48:26 PM

hromatogram 4_DCM C:\GCMSsolution\Data\2023 External\Life Sciences\Ntombifuthi\22062023\4_DCM.qg

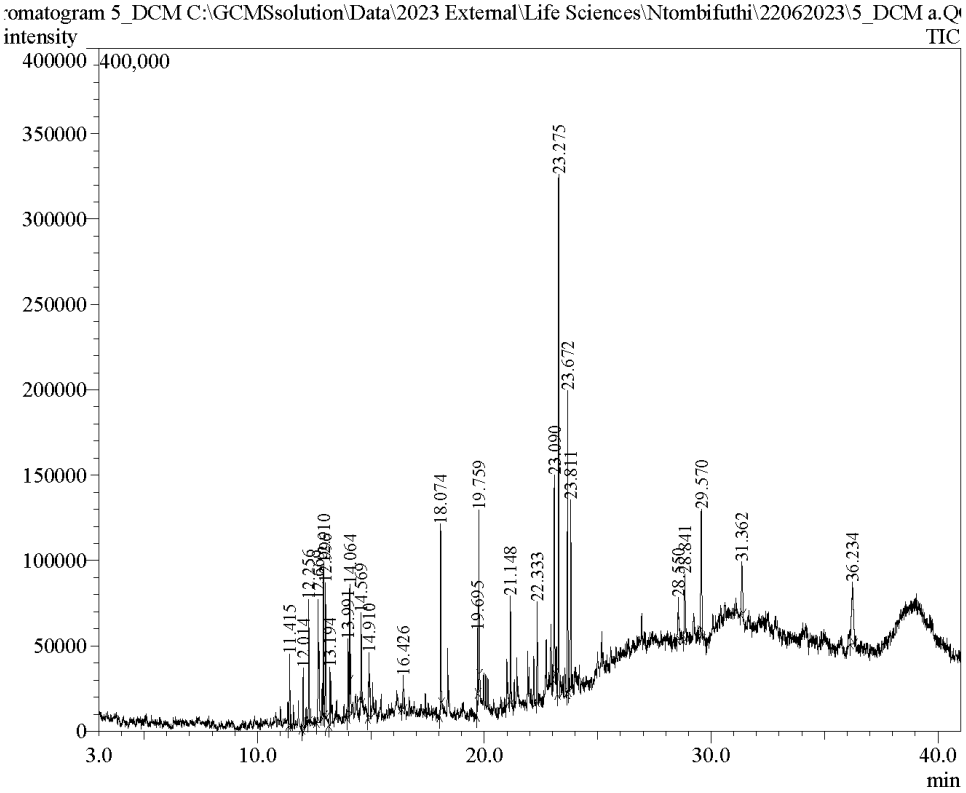


Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	eight%
1	5.080	5.050	5.115	168282	0.28	117259	1.25
2	5.510	5.470	5.550	152480	0.25	82093	0.87
3	6.496	6.450	6.540	174273	0.29	85405	0.91
4	8.005	7.845	8.065	225399	0.37	51332	0.55
5	8.114	8.055	8.265	63856	0.10	22096	0.23
6	10.375	10.010	10.460	709357	1.16	77491	0.82
7	14.391	13.215	14.575	4429050	7.25	131764	1.40
8	16.658	16.465	16.750	395074	0.65	75399	0.80
9	18.024	17.950	18.085	455776	0.75	122507	1.30
10	18.273	18.165	18.315	740791	1.21	200542	2.13
11	21.389	21.270	21.425	10875782	17.79	3545248	37.68
12	21.583	21.530	21.620	228056	0.37	113889	1.21
13	22.942	22.890	22.980	197117	0.32	90147	0.96
14	25.205	25.150	25.260	381430	0.62	138920	1.48
15	28.799	28.585	28.860	7977196	13.05	1280137	13.61
16	28.885	28.860	28.940	350134	0.57	128344	1.36
17	29.232	29.130	29.330	792871	1.30	159662	1.70
18	29.483	29.345	29.595	1651865	2.70	264851	2.81
19	29.925	29.820	30.015	848972	1.39	169273	1.80
20	30.199	30.090	30.310	1240081	2.03	257475	2.74
21	30.797	30.570	30.885	4468744	7.31	508397	5.40
22	31.296	31.125	31.435	1087512	1.78	119115	1.27
23	32.014	31.655	32.095	8373120	13.70	802107	8.53
24	32.456	32.095	32.695	4709281	7.71	157054	1.67
25	33.020	32.845	33.145	327589	0.54	40739	0.43
26	33.495	33.145	33.645	539217	0.88	38022	0.40
27	34.222	34.070	34.495	911204	1.49	80911	0.86
28	35.163	35.020	35.345	660764	1.08	93860	1.00
29	38.791	38.545	39.020	579777	0.95	58401	0.62
30	40.378	39.870	40.670	7403326	12.11	396429	4.21
				61118376	100.00	9408869	100.00

Sample Information

Analyzed by	: Unathi
Analyzed	: 2023-06-26 02:47:28 PM
Sample Type	: Unknown
Level #	: 1
Sample Name	: 5_DCM
Sample ID	: 5_DCM
IS Amount	: [1]=1
Sample Amount	: 1
Dilution Factor	: 1
Vial #	: 6
Injection Volume	: 5.00
Data File	: C:\GCMSsolution\Data\2023 External\Life Sciences\Ntombifuthi\22062023\5_DCM
Org Data File	: C:\GCMSsolution\Data\2023 External\Life Sciences\Ntombifuthi\22062023\5_DCM
Method File	: C:\GCMSsolution\Methods\2023 Ibrahim.qgm
Org Method File	: C:\GCMSsolution\Methods\2023 Ibrahim.qgm
Report File	:
Tuning File	: C:\GCMSsolution\System\Tune1\2023 Tuning files\17042023.qgt
Modified by	: Unathi
Modified	: 2023-06-26 03:28:28 PM



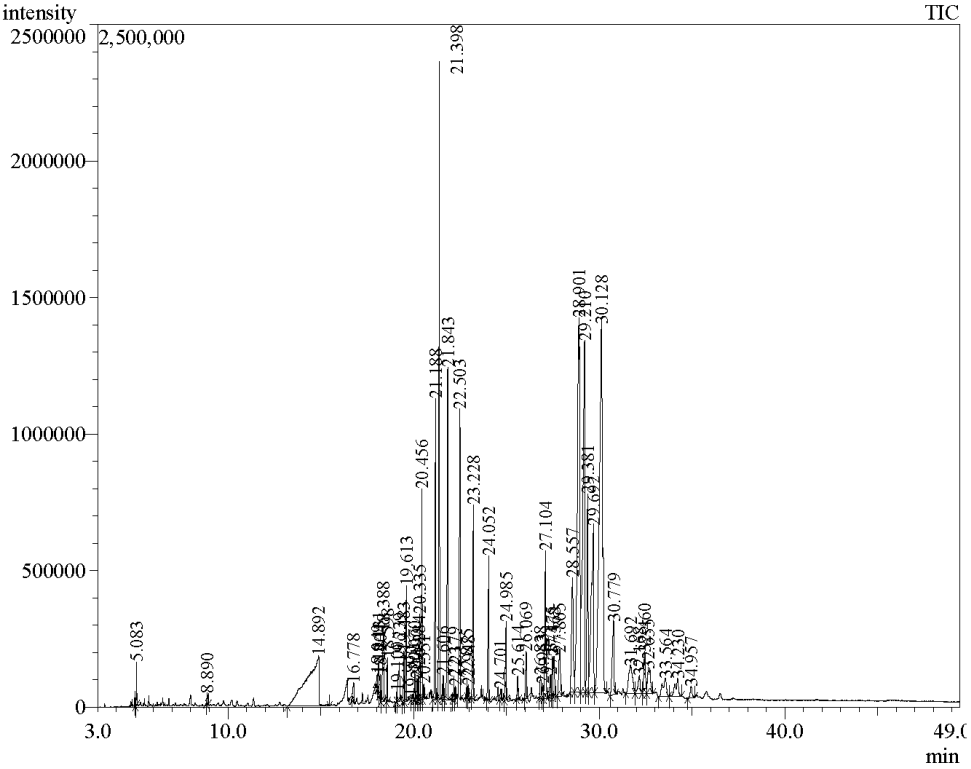
Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	eight%
1	11.415	11.365	11.475	77437	1.93	42286	2.19
2	12.014	11.955	12.085	63542	1.58	35941	1.86
3	12.256	12.225	12.290	109575	2.73	71357	3.69
4	12.669	12.605	12.775	226703	5.64	72107	3.73
5	12.910	12.875	12.945	149796	3.73	88807	4.59
6	12.996	12.945	13.035	141513	3.52	78539	4.06
7	13.194	13.145	13.200	50100	1.25	34094	1.76
8	13.991	13.950	14.030	84024	2.09	46267	2.39
9	14.064	14.035	14.090	93710	2.33	64291	3.32
10	14.569	14.540	14.610	93058	2.32	50789	2.63
11	14.910	14.865	15.000	118137	2.94	38686	2.00
12	16.426	16.355	16.475	48563	1.21	19587	1.01
13	18.074	18.035	18.120	199123	4.95	109054	5.64
14	19.695	19.670	19.735	86020	2.14	45319	2.34
15	19.759	19.735	19.790	170840	4.25	102359	5.29
16	21.148	21.120	21.195	116428	2.90	62015	3.21
17	22.333	22.300	22.370	106263	2.64	59150	3.06
18	23.090	23.040	23.130	275365	6.85	117810	6.09
19	23.275	23.230	23.320	556431	13.85	305547	15.80
20	23.672	23.635	23.710	319500	7.95	176987	9.15
21	23.811	23.710	23.855	220198	5.48	110964	5.74
22	28.550	28.500	28.615	65966	1.64	24863	1.29
23	28.841	28.775	28.910	113816	2.83	38549	1.99
24	29.570	29.525	29.625	226039	5.62	71733	3.71
25	31.362	31.300	31.420	104528	2.60	30530	1.58
26	36.234	36.145	36.330	202100	5.03	36239	1.87
				4018775	100.00	1933870	100.00

Sample Information

Analyzed by	: Unathi
Analyzed	: 2023-06-23 10:42:06 PM
Sample Type	: Unknown
Level #	: 1
Sample Name	: 6_DCM
Sample ID	: 6_DCM
IS Amount	: [1]=1
Sample Amount	: 1
Dilution Factor	: 1
Vial #	: 21
Injection Volume	: 3.00
Data File	: C:\GCMSsolution\Data\2023 External\Life Sciences\Ntombifuthi\22062023\6_DCM
Org Data File	: C:\GCMSsolution\Data\2023 External\Life Sciences\Ntombifuthi\22062023\6_DCM
Method File	: C:\GCMSsolution\Methods\2023 Emmanuel.qgm
Org Method File	: C:\GCMSsolution\Methods\2023 Emmanuel.qgm
Report File	:
Tuning File	: C:\GCMSsolution\System\Tune1\2023 Tuning files\17042023.qgt
Modified by	: Unathi
Modified	: 2023-06-23 11:31:30 PM

hromatogram 6_DCM C:\GCMSsolution\Data\2023 External\Life Sciences\Ntombifuthi\22062023\6_DCM.qg



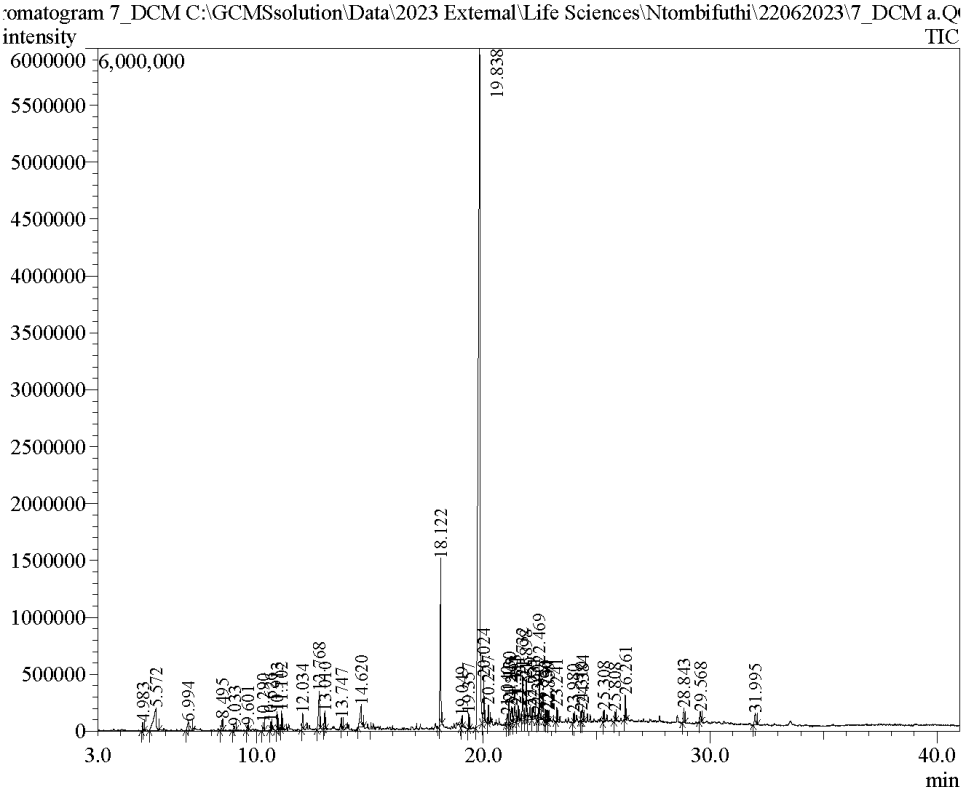
Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	eight%
1	5.083	5.030	5.110	215933	0.21	159134	0.84
2	8.890	8.845	8.950	114771	0.11	40146	0.21
3	14.892	13.210	15.485	8459840	8.14	184071	0.97
4	16.778	16.695	16.820	238277	0.23	66138	0.35
5	18.049	17.990	18.065	138426	0.13	50090	0.26
6	18.131	18.065	18.155	444367	0.43	119364	0.63
7	18.204	18.155	18.240	262528	0.25	89884	0.47
8	18.388	18.260	18.425	1162278	1.12	259387	1.36
9	18.598	18.545	18.625	358961	0.35	160559	0.84
10	19.100	19.055	19.125	111456	0.11	39054	0.21
11	19.239	19.125	19.270	773024	0.74	145501	0.76
12	19.483	19.410	19.525	531279	0.51	186603	0.98
13	19.613	19.525	19.645	1032404	0.99	423530	2.23
14	19.890	19.870	20.025	97035	0.09	14123	0.07
15	20.070	20.025	20.100	245036	0.24	108398	0.57
16	20.190	20.100	20.230	226574	0.22	77175	0.41
17	20.284	20.230	20.300	277087	0.27	124189	0.65
18	20.335	20.300	20.370	633367	0.61	312716	1.64
19	20.456	20.370	20.485	1961957	1.89	769164	4.04
20	20.551	20.485	20.620	153547	0.15	55486	0.29
21	21.188	21.040	21.220	2971969	2.86	1104235	5.80
22	21.398	21.310	21.430	5857087	5.64	2336962	12.28
23	21.606	21.565	21.650	184122	0.18	87306	0.46
24	21.843	21.650	21.875	3423704	3.29	1218727	6.40
25	22.179	22.115	22.205	161646	0.16	84223	0.44
26	22.237	22.205	22.275	96998	0.09	46822	0.25
27	22.503	22.385	22.530	3335268	3.21	1060551	5.57
28	22.875	22.840	22.905	110547	0.11	69953	0.37
29	22.948	22.910	23.000	102035	0.10	48266	0.25
30	23.228	23.110	23.265	2469447	2.38	712061	3.74
31	24.052	23.935	24.090	1780150	1.71	529178	2.78
32	24.701	24.630	24.750	134860	0.13	39559	0.21
33	24.985	24.895	25.020	1029174	0.99	286476	1.51
34	25.614	25.550	25.680	302192	0.29	86264	0.45
35	26.069	25.975	26.130	664554	0.64	171206	0.90
36	26.838	26.770	26.905	321046	0.31	84350	0.44
37	26.963	26.905	27.005	197583	0.19	54385	0.29
38	27.104	27.005	27.215	2177749	2.10	541516	2.85
39	27.371	27.300	27.405	280503	0.27	76642	0.40
40	27.475	27.405	27.515	614823	0.59	147234	0.77
41	27.568	27.515	27.660	659867	0.63	148798	0.78
42	27.865	27.750	27.985	1056134	1.02	157561	0.83
43	28.557	28.425	28.670	2666943	2.57	418742	2.20
44	28.901	28.670	29.005	13071970	12.58	1371962	7.21
45	29.210	29.005	29.265	9880228	9.51	1277926	6.71
46	29.381	29.265	29.435	3181246	3.06	725246	3.81
47	29.692	29.435	29.755	5466732	5.26	614678	3.23
48	30.128	29.755	30.390	16469301	15.85	1323428	6.95
49	30.779	30.590	30.890	1725386	1.66	250693	1.32
50	31.692	31.440	31.940	1480375	1.42	100827	0.53
51	32.168	31.940	32.315	584535	0.56	63737	0.33
52	32.460	32.315	32.565	1001507	0.96	150524	0.79
53	32.655	32.565	32.890	1019246	0.98	86587	0.45
54	33.564	33.190	33.765	942713	0.91	65977	0.35
55	34.230	33.765	34.440	779324	0.75	61889	0.33

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	eight%
56	34.957	34.765	35.140	298099	0.29	41766	0.22
				103937210	100.00	19030969	100.00

Sample Information

Analyzed by	: Unathi
Analyzed	: 2023-06-26 03:36:58 PM
Sample Type	: Unknown
Level #	: 1
Sample Name	: 7_DCM
Sample ID	: 7_DCM
IS Amount	: [1]=1
Sample Amount	: 1
Dilution Factor	: 1
Vial #	: 2
Injection Volume	: 5.00
Data File	: C:\GCMSsolution\Data\2023 External\Life Sciences\Ntombifuthi\22062023\7_DCM
Org Data File	: C:\GCMSsolution\Data\2023 External\Life Sciences\Ntombifuthi\22062023\7_DCM
Method File	: C:\GCMSsolution\Methods\2023 Ibrahim.qgm
Org Method File	: C:\GCMSsolution\Methods\2023 Ibrahim.qgm
Report File	:
Tuning File	: C:\GCMSsolution\System\Tune1\2023 Tuning files\17042023.qgt
Modified by	: Unathi
Modified	: 2023-06-26 04:17:57 PM



Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	eight%
1	4.983	4.935	5.070	120372	0.22	71781	0.52
2	5.572	5.270	5.700	1766347	3.30	197470	1.42
3	6.994	6.880	7.170	316826	0.59	81912	0.59
4	8.495	8.380	8.515	346537	0.65	101516	0.73
5	9.033	8.940	9.090	130118	0.24	40341	0.29
6	9.601	9.535	9.645	99111	0.19	47070	0.34
7	10.290	10.220	10.355	117784	0.22	69578	0.50
8	10.626	10.575	10.675	115809	0.22	75847	0.55
9	10.913	10.875	10.955	265935	0.50	161771	1.16
10	11.102	11.070	11.145	251166	0.47	160621	1.16
11	12.034	11.985	12.070	300196	0.56	139477	1.00
12	12.768	12.670	12.830	1170847	2.19	328604	2.37
13	13.010	12.955	13.060	381679	0.71	151150	1.09
14	13.747	13.715	13.815	163566	0.31	82571	0.59
15	14.620	14.480	14.725	1013545	1.89	196942	1.42
16	18.122	18.045	18.185	3788982	7.07	1466347	10.56
17	19.049	19.010	19.085	187368	0.35	100737	0.73
18	19.357	19.325	19.395	222005	0.41	139345	1.00
19	19.838	19.670	19.955	31611627	59.01	6018967	43.34
20	20.024	19.955	20.095	909345	1.70	394874	2.84
21	20.227	20.180	20.295	343573	0.64	156133	1.12
22	21.040	21.005	21.105	350554	0.65	81762	0.59
23	21.130	21.105	21.190	619361	1.16	201620	1.45
24	21.243	21.190	21.315	557672	1.04	146459	1.05
25	21.393	21.315	21.465	651736	1.22	158097	1.14
26	21.541	21.465	21.595	356447	0.67	137375	0.99
27	21.732	21.690	21.825	1017030	1.90	369588	2.66
28	21.898	21.825	21.955	894912	1.67	354007	2.55
29	22.059	21.955	22.150	487927	0.91	113069	0.81
30	22.231	22.150	22.290	429755	0.80	108863	0.78
31	22.390	22.365	22.435	129346	0.24	56453	0.41
32	22.469	22.435	22.550	946661	1.77	497085	3.58
33	22.730	22.685	22.760	203940	0.38	97761	0.70
34	22.794	22.760	22.830	221904	0.41	93214	0.67
35	22.859	22.830	22.900	161607	0.30	94328	0.68
36	23.241	23.195	23.290	227447	0.42	125959	0.91
37	23.980	23.930	24.015	160053	0.30	89180	0.64
38	24.310	24.275	24.345	204334	0.38	91497	0.66
39	24.384	24.345	24.450	346174	0.65	151831	1.09
40	25.308	25.270	25.345	229277	0.43	106881	0.77
41	25.808	25.765	25.845	154183	0.29	84576	0.61
42	26.261	26.215	26.315	423200	0.79	217759	1.57
43	28.843	28.785	28.900	390301	0.73	131166	0.94
44	29.568	29.520	29.640	302281	0.56	90873	0.65
45	31.995	31.910	32.085	480647	0.90	105102	0.76
				53569487	100.00	13887559	100.00

8.2. Cytotoxicity

Cytotoxicity – MTT Assay Raw Data Collection Sheets

Methanol 48hr												
	1	2	3	4	5	6	7	8	9	10	11	12
Plants	C	1 (100%)	1 (50%)	2 (100%)	2 (50%)	3 (100%)	3 (50%)	4 (100%)	4 (50%)	5 (100%)	5 (50%)	C
A	0,075	0,038	0,05	0,029	0,042	0,05	0,116	0,127	0,098	0,108	0,1	0,206
B	0,063	0,072	0,036	0,044	0,058	0,05	0,163	0,123	0,109	0,079	0,078	0,263
C	0,064	0,042	0,048	0,039	0,073	0,087	0,133	0,167	0,148	0,077	0,181	0,33
D	0,073	0,045	0,085	0,065	0,074	0,092	0,087	0,134	0,133	0,112	0,066	0,246
E	0,039	0,042	0,072	0,055	0,063	0,072	0,118	0,136	0,108	0,08	0,102	0,157
F	0,05	0,057	0,083	0,046	0,067	0,081	0,056	0,116	0,177	0,196	0,18	0,13
G	0,07	0,048	0,052	0,053	0,063	0,095	0,097	0,126	0,109	0,221	0,206	0,085
H	0,066	0,053	0,042	0,046	0,049	0,064	0,094	0,091	0,084	0,14	0,103	0,183
Mean	**0,0625**	**0,0496**	**0,0585**	**0,0471**	**0,061**	**0,074**	**0,108**	**0,128**	**0,121**	**0,127**	**0,127**	**0,2**

Methanol 48hr												
	1	2	3	4	5	6	7	8	9	10	11	12
Plants	6 (100%)		6 (50%)		7 (100%)		7 (50%)		C	C	C	C
A	0,138	0,069	0,082	0,06	0,052	0,038	0,05	0,034	0,089	0,046	0,073	0,049
B	0,087	0,095	0,087	0,09	0,051	0,053	0,08	0,044	0,102	0,072	0,094	0,045
C	0,112	0,087	0,098	0,079	0,065	0,064	0,089	0,064	0,12	0,06	0,107	0,064
D	0,105	0,085	0,104	0,091	0,063	0,064	0,1	0,049	0,079	0,056	0,053	0,077
E	0,085	0,122	0,083	0,105	0,049	0,058	0,103	0,07	0,138	0,065	0,106	0,07
F	0,113	0,116	0,207	0,084	0,052	0,055	0,09	0,065	0,114	0,055	0,123	0,074
G	0,095	0,102	0,117	0,077	0,048	0,053	0,065	0,081	0,137	0,084	0,08	0,065
H	0,115	0,11	0,087	0,102	0,056	0,048	0,064	0,07	0,081	0,045	0,061	0,054
Mean	**0,106**	**0,098**	**0,108**	**0,086**	**0,055**	**0,054**	**0,080**	**0,0596**	**0,108**	**0,060**	**0,087**	**0,062**
												0,079

Methanol 24hr															
		1	2	3	4	5	6	Mean	7	8	9	10	11	12	Mean
		100%							50%						
A	1	0,088	0,102	0,072	0,077	0,098	0,063	**0,083**	0,084	0,075	0,074	0,08	0,067	0,064	**0,074**
B	2	0,07	0,049	0,046	0,062	0,07	0,053	**0,058**	0,058	0,053	0,083	0,054	0,07	0,056	**0,062**
C	3	0,066	0,06	0,039	0,08	0,075	0,056	**0,063**	0,062	0,041	0,053	0,055	0,074	0,048	**0,056**
D	4	0,115	0,114	0,088	0,113	0,13	0,098	**0,1096**	0,12	0,106	0,059	0,051	0,068	0,058	**0,077**
E	5	0,067	0,098	0,043	0,15	0,109	0,07	**0,0895**	0,08	0,064	0,084	0,067	0,047	0,058	**0,067**
F	6	0,163	0,128	0,112	0,143	0,173	0,093	**0,135**	0,143	0,124	0,08	0,126	0,106	0,092	**0,112**
G	7	0,067	0,061	0,057	0,066	0,056	0,054	**0,060**	0,123	0,046	0,118	0,071	0,058	0,067	**0,081**
H	C	0,076	0,076	0,054	0,083	0,084	0,079	-	0,082	0,047	0,057	0,087	0,063	0,058	**0,071**

Ethanol 24hr															
		1	2	3	4	5	6	Mean	7	8	9	10	11	12	Mean
		100%							50%						
A	1	0,168	0,045	0,185	0,221	0,186	0,072	**0,146**	0,295	0,192	0,329	0,189	0,113	0,062	**0,197**
B	2	0,117	0,083	0,077	0,066	0,061	0,145	**0,092**	0,069	0,183	0,383	0,12	0,068	0,046	**0,145**
C	3	0,198	0,044	0,05	0,061	0,086	0,079	**0,086**	0,08	0,206	0,324	0,063	0,055	0,045	**0,129**
D	4	0,114	0,112	0,079	0,093	0,127	0,183	**0,118**	0,144	0,157	0,097	0,15	0,107	0,059	**0,119**
E	5	0,052	0,084	0,094	0,144	0,098	0,141	**0,102**	0,154	0,182	0,284	0,172	0,15	0,044	**0,164**
F	6	0,127	0,108	0,103	0,119	0,146	0,161	**0,127**	0,144	0,151	0,296	0,149	0,097	0,376	**0,202**
G	7	0,064	0,048	0,045	0,088	0,064	0,136	**0,074**	0,127	0,091	0,253	0,172	0,088	0,042	**0,129**
H	C	0,056	0,049	0,046	0,114	0,112	0,19	-	0,173	0,138	0,272	0,093	0,076	0,044	**0.114**

Ethanol 48hr												
	1	2	3	4	5	6	7	8	9	10	11	12
Plants	1 (100%)	1 (50%)	2 (100%)	2 (50%)	3 (100%)	3 (50%)	4 (100%)	4 (50%)	5 (100%)	5 (50%)	C	C
A	0,252	0,052	0,079	0,077	0,068	0,047	0,117	0,09	0,085	0,073	0,144	0,233
B	0,075	0,045	0,043	0,059	0,058	0,047	0,161	0,101	0,103	0,158	0,077	0,127
C	0,059	0,118	0,059	0,047	0,916	0,063	0,176	0,117	0,183	0,218	0,095	0,139
D	0,068	0,121	0,085	0,105	0,099	0,061	0,154	0,109	0,115	0,105	0,087	0,164
E	0,151	0,151	0,061	0,053	0,098	0,074	0,195	0,128	0,185	0,288	0,138	0,135
F	0,152	0,111	0,047	0,048	0,046	0,061	0,157	0,184	0,077	0,234	0,102	0,224
G	0,061	0,05	0,104	0,052	0,089	0,111	0,155	0,152	0,091	0,237	0,087	0,214
H	0,133	0,125	0,065	0,039	0,059	0,054	0,118	0,13	0,098	0,103	0,149	0,221
Mean	**0,119**	**0,097**	**0,068**	**0,06**	**0,179**	**0,065**	**0,154**	**0,126**	**0,117**	**0,177**	**0,1099**	**0,182**

Ethanol 48hr												
	1	2	3	4	5	6	7	8	9	10	11	12
Plants	6 (100%)		6 (50%)		7 (100%)		7 (50%)		C	C	C	C
A	0,106	0,092	0,088	0,087	0,041	0,047	0,047	0,086	0,108	0,138	0,064	0,071
B	0,086	0,095	0,087	0,054	0,048	0,043	0,043	0,078	0,074	0,069	0,081	0,08
C	0,078	0,096	0,088	0,085	0,043	0,055	0,069	0,11	0,092	0,109	0,118	0,104
D	0,094	0,1	0,093	0,062	0,048	0,05	0,055	0,102	0,102	0,096	0,075	0,086
E	0,078	0,075	0,094	0,081	0,049	0,047	0,06	0,075	0,102	0,041	0,066	0,097
F	0,074	0,077	0,063	0,055	0,04	0,051	0,053	0,098	0,092	0,083	0,08	0,062
G	0,129	0,086	0,067	0,057	0,043	0,045	0,058	0,095	0,098	0,07	0,088	0,1
H	0,072	0,092	0,067	0,048	0,037	0,042	0,061	0,099	0,093	0,067	0,122	0,114
Mean	**0,0896**	**0,089**	**0,081**	**0,066**	**0,044**	**0,048**	**0,056**	**0,093**	**0,095**	**0,084**	**0,087**	**0,089**

DCM 48hr												
	1	2	3	4	5	6	7	8	9	10	11	12
Plants	1 (100%)	1 (50%)	2 (100%)	2 (50%)	3 (100%)	3 (50%)	4 (100%)	4 (50%)	5 (100%)	5 (50%)	C	C
A	0,149	0,141	0,073	0,081	0,114	0,15	0,145	0,187	0,135	0,151	0,055	0,101
B	0,231	0,188	0,092	0,116	0,248	0,179	0,146	0,177	0,141	0,224	0,091	0,093
C	0,08	0,122	0,075	0,108	0,264	0,286	0,161	0,142	0,111	0,216	0,066	0,099
D	0,092	0,108	0,061	0,12	0,304	0,21	0,154	0,065	0,114	0,237	0,07	0,081
E	0,119	0,246	0,079	0,126	0,11	0,151	0,204	0,174	0,099	0,203	0,048	0,052
F	0,099	0,174	0,087	0,126	0,246	0,174	0,168	0,202	0,138	0,32	0,055	0,065
G	0,097	0,181	0,099	0,136	0,371	0,223	0,169	0,199	0,147	0,328	0,056	0,07
H	0,195	0,168	0,081	0,146	0,237	0,242	0,1	0,185	0,129	0,321	0,063	0,088
Mean	**0,133**	**0,166**	**0,081**	**0,1199**	**0,237**	**0,202**	**0,156**	**0,166**	**0,127**	**0,25**	**0,063**	**0,0811**

DCM 48hr												
	1	2	3	4	5	6	7	8	9	10	11	12
Plants	6 (100%)		6 (50%)		7 (100%)		7 (50%)		C	C	C	C
A	0,167	0,166	0,133	0,087	0,121	0,192	0,149	0,168	0,13	0,064	0,1	0,087
B	0,169	0,135	0,129	0,126	0,123	0,167	0,238	0,21	0,129	0,102	0,131	0,077
C	0,168	0,118	0,183	0,131	0,121	0,158	0,301	0,186	0,171	0,11	0,076	0,083
D	0,166	0,136	0,186	0,213	0,168	0,157	0,291	0,256	0,116	0,13	0,161	0,096
E	0,132	0,174	0,175	0,203	0,127	0,144	0,214	0,158	0,129	0,073	0,134	0,069
F	0,164	0,148	0,164	0,172	0,137	0,125	0,276	0,14	0,139	0,095	0,137	0,068
G	0,086	0,158	0,188	0,157	0,12	0,187	0,162	0,166	0,125	0,104	0,144	0,079
H	0,142	0,138	0,136	0,102	0,092	0,14	0,13	0,163	0,103	0,077	0,075	0,085
Mean	**0,149**	**0,147**	**0,162**	**0,149**	**0,126**	**0,159**	**0,220**	**0,181**	**0,130**	**0,094**	**0,1197**	**0,0805**

DCM 24hr															
		1	2	3	4	5	6	Mean	7	8	9	10	11	12	
		100%							50%						
A	1	0,119	0,096	0,113	0,057	0,135	0,245	**0,128**	0,088	0,081	0,049	0,078	0,068	0,063	**0,071**
B	2	0,096	0,074	0,094	0,071	0,054	0,201	**0,098**	0,143	0,06	0,055	0,09	0,099	0,093	**0,09**
C	3	0,2	0,127	0,158	0,075	0,114	0,321	**0,166**	0,102	0,095	0,089	0,077	0,17	0,066	**0,0998**
D	4	0,194	0,085	0,13	0,057	0,1	0,199	**0,128**	0,083	0,093	0,079	0,089	0,195	0,103	**0,107**
E	5	0,071	0,061	0,099	0,061	0,082	0,183	**0,093**	0,119	0,13	0,141	0,187	0,189	0,154	**0,153**
F	6	0,188	0,099	0,067	0,078	0,088	0,217	**0,123**	0,097	0,109	0,074	0,094	0,077	0,069	**0,092**
G	7	0,19	0,18	0,19	0,083	0,078	0,124	**0,141**	0,084	0,092	0,078	0,095	0,071	0,08	**0.083**
H	C	0,154	0,136	0,114	0,093	0,071	0,127	-	0,075	0,053	0,046	0,1	0,086	0,065	**0.093**

8.3. Animal Work

Animal Work Data Codes

Identifier	Code	Description
ID	-	Animal number (1-66)
I (induction) GROUP	1	Control (Saline)
	2	Experimental (Ketamine)
T (treatment) GROUP	1	Control (SAL)
	2	Control (APD)
	3	Control (TM)
	4	Experimental (SAL)
	5	Experimental (APD)
	6	Experimental (TM)
WEIGHT AD 1	-	Weight in grams – Adaptation Day 1
WEIGHT AD 7	-	Weight in grams – Adaptation Day 7
WEIGHT PBT 1	-	Weight in grams – Post behavioural Tests 1
WEIGHT ID 1	-	Weight in grams – Induction Day 1
WEIGHT ID 5	-	Weight in grams – Induction Day 5
WEIGHT PBT 2	-	Weight in grams – Post behavioural Tests 2
WEIGHT TD 1	-	Weight in grams – Treatment Day 1
WEIGHT TD 7	-	Weight in grams – Treatment Day 7
WEIGHT PBT 3	-	Weight in grams – Post behavioural Tests 3
HCL	-	Habituation phase left chamber
HFL	-	Habituation phase left chamber (frequency)
HTL	-	Habituation phase left chamber (time in seconds)
HCM	-	Habituation phase middle chamber
HFM	-	Habituation phase middle chamber (frequency)
HTM	-	Habituation phase middle chamber (time in seconds)
HCR	-	Habituation phase right chamber
HFR	-	Habituation phase right chamber (frequency)
HTR	-	Habituation phase right chamber (time in seconds)

SCS1	-	Sociability phase Stranger 1 chamber
SFS1	-	Sociability phase Stranger 1 chamber (frequency)
STS1N	-	Sociability phase Stranger 1 chamber (time in seconds – near/interaction)
STS1F	-	Sociability phase Stranger 1 chamber (time in seconds – far/no interaction)
SCM	-	Sociability phase middle chamber
SFM	-	Sociability phase middle chamber (frequency)
STM	-	Sociability phase middle chamber (time in seconds)
SCE	-	Sociability phase Empty chamber
SFE	-	Sociability phase Empty chamber (frequency)
STEN	-	Sociability phase Empty chamber (time in seconds – near/interaction)
STEF	-	Sociability phase Empty chamber (time in seconds – far/no interaction)
NCS1	-	Novelty phase Stranger 1 chamber
NFS1	-	Novelty phase Stranger 1 chamber (frequency)
NTS1N	-	Novelty phase Stranger 1 chamber (time in seconds – near/interaction)
NTS1F	-	Novelty phase Stranger 1 chamber (time in seconds – far/no interaction)
NCM	-	Novelty phase middle chamber
NFM	-	Novelty phase middle chamber (frequency)
NTM	-	Novelty phase middle chamber (time in seconds)
NCS2	-	Novelty phase Stranger 2 chamber
NFS2	-	Novelty phase Stranger 2 chamber (frequency)
NTS2N	-	Novelty phase Stranger 2 chamber (time in seconds – near/interaction)
NTS2F	-	Novelty phase Stranger 2 chamber (time in seconds – far/no interaction)
TLC D1	-	Total liquid consumption (g) for Day 1
TLC D2	-	Total liquid consumption (g) for Day 2
TLC D3	-	Total liquid consumption (g) for Day 3
TLC D4	-	Total liquid consumption (g) for Day 4
SP D1	-	Sucrose preference % for Day 1

SP D2	-	Sucrose preference % for Day 2
SP D3	-	Sucrose preference % for Day 3
SP D4	-	Sucrose preference % for Day 4
BMo	-	Brain Morphometry
BMa	-	Brain mass (g)
BV	-	Brain volume (cm^2)
APL	-	Anterior-posterior length (mm)
LW	-	Lateral width (mm)
SIH	-	Superior-inferior height (mm)
HR	-	Hippocampus mass – right
HL	-	Hippocampus mass – left
PR	-	Prefrontal cortex mass – right
PL	-	Prefrontal cortex mass – left
SR	-	VTA mass – right
SL	-	VTA mass – left
DA_PFC	-	DA ELISA Abs for Prefrontal cortex
DA_VTA	-	DA ELISA Abs for ventral tegmental area
ACh_PFC	-	ACh ELISA Abs for Prefronatal Cortex
ACh_Hippo	-	ACh ELISA Abs for Hippocampus

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	ID	IGROUP	TGROUP	WEIGHTAD1
1	1	1	1	214.00
2	2	1	1	169.00
3	3	1	1	177.00
4	4	1	2	192.00
5	5	1	2	215.00
6	6	1	2	192.00
7	7	1	3	180.00
8	8	1	3	178.00
9	9	1	3	190.00
10	10	2	4	182.00
11	11	2	4	218.00
12	12	2	4	193.00
13	13	2	4	167.00
14	14	2	4	185.00
15	15	2	5	174.00
16	16	2	5	215.00
17	17	2	5	183.00
18	18	2	5	178.00
19	19	2	5	169.00
20	20	2	6	176.00
21	21	2	6	197.00
22	22	2	6	208.00
23	23	2	6	232.00
24	24	2	6	218.00
25	25	1	1	160.00
26	26	2	5	182.00
27	27	2	6	166.00
28	28	2	6	184.00
29	29	2	6	185.00
30	30	2	6	173.00
31	31	2	4	161.00
32	32	2	4	169.00
33	33	2	4	187.00
34	34	2	4	185.00
35	35	2	4	190.00
36	36	2	5	181.00
37	37	1	1	161.00
38	38	2	5	183.00

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	WEIGHTAD7	AVG_ADW	WEIGHPBT1	WEIGHTID1
1	203.00	208.50	263.00	263.00
2	226.00	197.50	264.00	264.00
3	238.00	207.50	271.00	271.00
4	238.00	215.00	259.00	259.00
5	268.00	241.50	291.00	291.00
6	244.00	218.00	278.00	278.00
7	233.00	206.50	275.00	275.00
8	234.00	206.00	273.00	273.00
9	241.00	215.50	286.00	286.00
10	245.00	213.50	283.00	283.00
11	274.00	246.00	326.00	326.00
12	254.00	223.50	299.00	299.00
13	234.00	200.50	288.00	288.00
14	237.00	211.00	281.00	281.00
15	223.00	198.50	261.00	261.00
16	261.00	238.00	314.00	314.00
17	233.00	208.00	280.00	280.00
18	236.00	207.00	275.00	275.00
19	223.00	196.00	278.00	278.00
20	227.00	201.50	266.00	266.00
21	244.00	220.50	274.00	274.00
22	246.00	227.00	287.00	287.00
23	269.00	250.50	305.00	305.00
24	254.00	236.00	296.00	296.00
25	194.00	177.00	225.00	225.00
26	217.00	199.50	257.00	257.00
27	201.00	183.50	249.00	249.00
28	225.00	204.50	269.00	269.00
29	224.00	204.50	257.00	257.00
30	212.00	192.50	248.00	248.00
31	193.00	177.00	236.00	236.00
32	212.00	190.50	262.00	262.00
33	231.00	209.00	276.00	276.00
34	217.00	201.00	256.00	256.00
35	230.00	210.00	285.00	285.00
36	218.00	199.50	264.00	264.00
37	188.00	174.50	225.00	225.00
38	230.00	206.50	288.00	288.00

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	WEIGHTID5	AVG_IDW	WEIGHTPBT2	WEIGHTTD1
1	329.00	296.00	347.00	347.00
2	271.00	267.50	292.00	292.00
3	279.00	275.00	300.00	300.00
4	269.00	264.00	278.00	278.00
5	307.00	299.00	323.00	323.00
6	283.00	280.50	291.00	291.00
7	278.00	276.50	295.00	295.00
8	290.00	281.50	303.00	303.00
9	299.00	292.50	315.00	315.00
10	281.00	282.00	296.00	296.00
11	324.00	325.00	343.00	343.00
12	305.00	302.00	332.00	332.00
13	290.00	289.00	308.00	308.00
14	283.00	282.00	308.00	308.00
15	263.00	262.00	278.00	278.00
16	316.00	315.00	335.00	335.00
17	277.00	278.50	299.00	299.00
18	276.00	275.50	294.00	294.00
19	266.00	272.00	296.00	296.00
20	262.00	264.00	282.00	282.00
21	262.00	268.00	282.00	282.00
22	286.00	286.50	308.00	308.00
23	305.00	305.00	329.00	329.00
24	295.00	295.50	314.00	314.00
25	245.00	235.00	262.00	262.00
26	272.00	264.50	288.00	288.00
27	259.00	254.00	286.00	286.00
28	286.00	277.50	307.00	307.00
29	276.00	266.50	297.00	297.00
30	266.00	257.00	286.00	286.00
31	273.00	254.50	301.00	301.00
32	289.00	275.50	330.00	330.00
33	306.00	291.00	330.00	330.00
34	273.00	264.50	300.00	300.00
35	317.00	301.00	339.00	339.00
36	286.00	275.00	312.00	312.00
37	254.00	239.50	290.00	290.00
38	314.00	301.00	345.00	345.00

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	WEIGHTTD7	AVG_TW	WEIGHTPBT3	HFLPrl
1	385.00	366.00	411.00	4.00
2	343.00	317.50	347.00	1.00
3	350.00	325.00	367.00	4.00
4	310.00	294.00	321.00	1.00
5	357.00	340.00	376.00	4.00
6	311.00	301.00	327.00	2.00
7	332.00	313.50	346.00	3.00
8	341.00	322.00	349.00	2.00
9	349.00	332.00	367.00	.00
10	332.00	314.00	346.00	1.00
11	399.00	371.00	410.00	5.00
12	377.00	354.50	397.00	4.00
13	366.00	337.00	386.00	3.00
14	355.00	331.50	378.00	1.00
15	312.00	295.00	322.00	4.00
16	360.00	347.50	375.00	.00
17	319.00	309.00	330.00	.00
18	326.00	310.00	333.00	4.00
19	338.00	317.00	365.00	4.00
20	314.00	298.00	330.00	.00
21	316.00	299.00	332.00	1.00
22	341.00	324.50	356.00	1.00
23	370.00	349.50	390.00	5.00
24	359.00	336.50	369.00	2.00
25	276.00	269.00	299.00	1.00
26	297.00	292.50	308.00	3.00
27	310.00	298.00	335.00	2.00
28	331.00	319.00	345.00	2.00
29	321.00	309.00	337.00	2.00
30	304.00	295.00	317.00	.00
31	319.00	310.00	339.00	4.00
32	345.00	337.50	364.00	6.00
33	346.00	338.00	364.00	2.00
34	321.00	310.50	325.00	5.00
35	354.00	346.50	373.00	5.00
36	328.00	320.00	341.00	4.00
37	314.00	302.00	336.00	3.00
38	355.00	350.00	373.00	4.00

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	HTLPrI	HFMPrI	HTMPrI	HFRPrI
1	67.58	11.00	162.30	5.00
2	11.69	2.00	14.27	2.00
3	67.51	9.00	86.49	5.00
4	27.16	3.00	152.19	2.00
5	88.80	7.00	60.56	4.00
6	48.34	4.00	162.84	2.00
7	64.91	7.00	111.85	5.00
8	280.96	2.00	20.04	.00
9	.00	3.00	147.36	3.00
10	16.29	2.00	36.69	2.00
11	65.91	8.00	81.14	5.00
12	90.25	9.00	55.23	6.00
13	56.74	6.00	91.31	6.00
14	114.08	3.00	22.64	2.00
15	82.71	8.00	98.52	4.00
16	.00	1.00	32.74	1.00
17	.00	.00	.00	1.00
18	57.31	8.00	157.37	5.00
19	87.21	8.00	51.66	5.00
20	.00	1.00	7.82	2.00
21	27.53	2.00	38.27	1.00
22	16.93	4.00	41.57	3.00
23	79.64	8.00	66.66	5.00
24	59.60	3.00	198.82	1.00
25	19.41	2.00	64.02	1.00
26	61.11	6.00	76.58	4.00
27	53.91	4.00	81.19	2.00
28	159.33	4.00	89.56	2.00
29	47.13	5.00	131.31	2.00
30	.00	3.00	51.40	3.00
31	52.53	10.00	80.78	6.00
32	20.68	10.00	73.91	9.00
33	43.12	4.00	80.18	2.00
34	101.71	10.00	70.41	6.00
35	81.12	10.00	64.46	5.00
36	84.04	8.00	63.39	4.00
37	50.51	7.00	55.83	4.00
38	42.08	7.00	59.59	4.00

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	HTRPrl	SFS1Prl	STS1NPrl	STS1FPrl
1	126.63	4.00	242.29	80.15
2	276.60	9.00	262.86	39.73
3	115.84	8.00	317.38	58.04
4	121.41	2.00	54.08	.00
5	151.24	2.00	347.26	49.64
6	89.18	6.00	368.68	54.53
7	123.41	9.00	351.30	29.61
8	.00	5.00	296.56	43.45
9	153.31	5.00	302.96	36.21
10	249.24	7.00	220.19	44.69
11	155.59	9.00	234.90	73.01
12	155.66	10.00	344.08	21.12
13	151.95	10.00	314.17	60.82
14	162.05	6.00	223.24	113.42
15	123.25	1.00	436.44	163.56
16	269.00	.00	.00	.00
17	300.00	2.00	499.87	98.00
18	85.86	6.00	209.07	72.65
19	162.10	3.00	164.68	25.97
20	294.01	3.00	295.63	152.33
21	235.15	.00	.00	.00
22	242.32	5.00	342.07	102.26
23	154.11	3.00	122.30	.00
24	42.08	.00	.00	.00
25	216.92	5.00	192.87	42.18
26	162.65	8.00	206.80	39.29
27	155.88	3.00	317.30	134.72
28	51.33	6.00	214.52	23.62
29	121.89	7.00	248.30	65.51
30	249.04	10.00	247.33	24.53
31	167.09	9.00	246.66	20.04
32	155.00	5.00	350.15	116.40
33	176.32	2.00	368.12	214.31
34	128.40	10.00	208.22	44.75
35	154.69	8.00	239.49	62.95
36	152.89	6.00	199.13	79.36
37	187.43	4.00	373.26	168.86
38	198.67	3.00	106.92	.00

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	STS1TTPrl	SFMPrl	STMPrl	SFEPrl
1	322.44	8.00	72.34	5.00
2	302.59	15.00	109.02	7.00
3	375.42	12.00	111.41	5.00
4	54.08	3.00	16.90	2.00
5	396.90	3.00	35.20	1.00
6	423.21	9.00	121.57	3.00
7	380.91	12.00	91.22	4.00
8	340.01	9.00	80.36	4.00
9	339.17	7.00	173.55	3.00
10	264.88	9.00	292.84	2.00
11	307.91	18.00	182.92	8.00
12	365.20	15.00	134.68	6.00
13	374.99	12.00	167.91	4.00
14	336.66	10.00	125.35	3.00
15	600.00	.00	.00	.00
16	.00	1.00	13.07	1.00
17	597.87	1.00	4.67	.00
18	281.74	11.00	214.70	4.00
19	190.65	8.00	61.04	4.00
20	447.96	6.00	84.76	3.00
21	.00	.00	.00	1.00
22	444.33	7.00	91.63	2.00
23	122.30	3.00	21.54	2.00
24	.00	1.00	73.64	1.00
25	235.05	12.00	97.78	6.00
26	246.09	11.00	167.76	4.00
27	452.02	4.00	94.81	2.00
28	238.14	13.00	111.77	8.00
29	313.81	10.00	105.47	7.00
30	271.86	18.00	169.41	8.00
31	266.70	14.00	191.16	8.00
32	466.55	9.00	77.29	4.00
33	582.43	1.00	18.18	.00
34	252.97	13.00	147.43	8.00
35	302.44	12.00	175.46	5.00
36	278.49	13.00	361.44	7.00
37	542.12	3.00	34.39	1.00
38	106.92	5.00	478.28	1.00

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	STENPrI	STEFPrI	STETTPrI	NFS1PrI
1	23.42	184.65	208.67	3.00
2	50.43	141.18	191.61	3.00
3	80.04	36.40	116.44	2.00
4	15.74	513.79	529.53	3.00
5	.00	167.55	167.55	.00
6	55.09	.00	55.09	1.00
7	26.83	101.47	128.30	6.00
8	71.32	109.91	181.23	4.00
9	87.82	.00	87.82	1.00
10	43.54	.00	43.54	2.00
11	78.95	44.84	123.79	1.00
12	65.33	35.70	101.03	3.00
13	37.65	20.00	57.65	4.00
14	45.26	93.03	138.29	1.00
15	.00	.00	.00	2.00
16	.00	588.41	588.41	2.00
17	.00	.00	.00	2.00
18	91.23	12.67	103.90	1.00
19	131.16	217.46	348.62	4.00
20	68.60	.00	68.60	1.00
21	.00	600.00	600.00	3.00
22	65.20	.00	65.20	1.00
23	2.65	453.99	456.64	3.00
24	.00	526.77	526.77	.00
25	156.64	110.77	267.41	1.00
26	92.11	134.57	226.68	3.00
27	47.55	6.23	53.78	2.00
28	104.64	145.35	249.99	1.00
29	83.70	97.34	181.04	1.00
30	102.34	54.66	157.00	2.00
31	116.37	31.15	147.52	3.00
32	44.84	11.69	56.53	2.00
33	.00	.00	.00	1.00
34	107.52	91.90	199.42	3.00
35	73.76	48.38	122.14	6.00
36	101.80	91.27	193.07	4.00
37	23.23	.00	23.23	1.00
38	15.19	.00	15.19	.00

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	NTS1NPrl	NTS1FPrl	NFS1TTPrl	NFMPrl
1	45.70	35.20	80.90	6.00
2	30.52	36.10	66.62	7.00
3	98.55	114.54	213.09	2.00
4	43.22	.00	43.22	4.00
5	.00	.00	.00	3.00
6	103.42	.00	103.42	1.00
7	61.23	13.83	75.06	5.00
8	99.75	39.30	139.05	3.00
9	156.15	.00	156.15	1.00
10	22.18	18.36	40.54	4.00
11	.00	7.94	7.94	3.00
12	61.03	29.92	90.95	5.00
13	72.25	.00	72.25	5.00
14	176.91	123.72	300.63	.00
15	99.21	105.85	205.06	3.00
16	130.57	30.63	161.20	5.00
17	191.18	72.47	263.65	2.00
18	20.99	105.31	126.30	2.00
19	123.40	.00	123.40	2.00
20	19.04	5.33	24.37	1.00
21	72.54	20.93	93.47	7.00
22	268.94	.00	268.94	.00
23	34.64	14.51	49.15	4.00
24	.00	.00	.00	1.00
25	23.40	.00	23.40	4.00
26	81.82	56.90	138.72	3.00
27	149.29	79.14	228.43	2.00
28	117.48	72.07	189.55	2.00
29	63.48	178.17	241.34	1.00
30	143.54	30.67	174.21	4.00
31	39.99	24.35	64.34	9.00
32	104.40	102.34	206.74	2.00
33	63.14	237.23	300.37	.00
34	49.10	24.99	74.09	5.00
35	130.28	55.58	185.86	6.00
36	54.54	13.66	68.21	6.00
37	70.57	110.73	181.30	5.00
38	.00	.00	.00	2.00

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	NTMPrl	NFS2Prl	NTS2NPrl	NTS2FPrl
1	66.83	3.00	109.72	37.71
2	51.11	3.00	73.94	80.43
3	4.96	2.00	73.20	.00
4	29.29	3.00	172.51	55.55
5	68.27	3.00	95.85	136.31
6	10.37	1.00	109.45	77.22
7	44.59	9.00	162.75	21.93
8	31.86	4.00	105.05	24.47
9	144.37	.00	.00	.00
10	28.38	2.00	188.00	45.88
11	118.76	2.00	160.40	20.78
12	12.10	5.00	183.67	14.81
13	115.32	4.00	108.51	4.67
14	.00	.00	.00	.00
15	15.87	2.00	81.94	.00
16	52.45	2.00	70.03	18.43
17	37.70	.00	.00	.00
18	14.34	2.00	101.57	58.34
19	14.01	4.00	162.84	.00
20	4.07	2.00	249.47	26.96
21	84.18	3.00	80.60	46.79
22	.00	1.00	32.73	.00
23	64.83	3.00	137.13	3.26
24	4.47	2.00	238.87	57.15
25	37.12	4.00	165.87	74.46
26	20.16	2.00	118.10	23.32
27	8.66	1.00	46.33	16.92
28	18.82	1.00	79.98	11.88
29	8.24	2.00	50.52	.00
30	48.64	2.00	64.41	13.00
31	95.59	6.00	103.52	36.96
32	5.95	2.00	84.63	.00
33	.00	.00	.00	.00
34	70.86	5.00	108.55	46.79
35	35.73	3.00	75.16	3.11
36	25.75	4.00	154.97	51.32
37	35.36	4.00	64.89	18.76
38	207.64	2.00	92.74	.00

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	NCS2TTPrI	HFLPoI	HTLPoI	HFMPoI
1	147.43	5.00	83.33	10.00
2	154.37	5.00	87.11	9.00
3	73.20	3.00	81.75	7.00
4	228.06	2.00	27.11	5.00
5	232.16	1.00	27.86	4.00
6	186.67	2.00	86.80	3.00
7	184.68	3.00	62.39	7.00
8	129.52	3.00	94.51	8.00
9	.00	1.00	9.35	3.00
10	233.88	1.00	24.74	5.00
11	181.18	5.00	95.46	9.00
12	198.48	4.00	109.22	7.00
13	113.18	5.00	100.57	9.00
14	.00	1.00	63.19	4.00
15	81.94	2.00	47.90	7.00
16	88.46	.00	.00	1.00
17	.00	6.00	95.43	10.00
18	159.91	1.00	50.68	4.00
19	162.84	1.00	69.93	3.00
20	276.43	2.00	96.46	4.00
21	127.39	2.00	42.86	5.00
22	32.73	3.00	40.87	9.00
23	140.39	4.00	113.41	10.00
24	296.02	2.00	223.62	3.00
25	240.33	1.00	16.14	3.00
26	141.42	3.00	88.17	7.00
27	63.25	3.00	120.39	6.00
28	91.86	4.00	115.70	9.00
29	50.52	3.00	185.18	5.00
30	77.41	1.00	18.61	4.00
31	140.48	3.00	70.84	7.00
32	84.63	6.00	79.89	11.00
33	.00	1.00	24.03	3.00
34	155.34	5.00	107.77	10.00
35	78.27	4.00	69.45	9.00
36	206.29	3.00	44.99	8.00
37	83.65	1.00	9.22	4.00
38	92.74	3.00	64.11	6.00

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	HTMPol	HFRPol	HTRPol	SFS1Pol
1	97.32	5.00	119.61	5.00
2	66.24	5.00	147.04	9.00
3	46.72	5.00	148.42	8.00
4	84.03	4.00	188.91	9.00
5	73.24	3.00	204.92	5.00
6	90.81	1.00	144.34	7.00
7	110.53	4.00	127.27	6.00
8	50.37	4.00	156.22	7.00
9	40.91	3.00	250.13	7.00
10	64.35	3.00	211.24	6.00
11	77.41	5.00	140.11	7.00
12	104.80	4.00	86.29	10.00
13	70.27	4.00	129.39	6.00
14	107.52	3.00	129.69	6.00
15	77.63	4.00	174.73	5.00
16	19.80	1.00	284.57	5.00
17	86.35	4.00	118.44	13.00
18	49.00	2.00	200.66	5.00
19	54.01	2.00	176.41	4.00
20	55.80	2.00	148.02	3.00
21	42.02	3.00	215.99	8.00
22	72.91	6.00	186.67	11.00
23	79.99	5.00	104.03	7.00
24	53.76	1.00	32.20	5.00
25	29.20	2.00	255.58	5.00
26	60.19	3.00	151.99	7.00
27	56.53	3.00	126.82	11.00
28	91.41	5.00	93.28	9.00
29	41.58	2.00	80.44	8.00
30	60.39	2.00	221.36	7.00
31	87.01	4.00	142.47	4.00
32	100.58	7.00	119.79	6.00
33	26.48	2.00	250.23	5.00
34	94.16	5.00	98.31	7.00
35	84.91	5.00	146.27	10.00
36	74.34	5.00	180.99	8.00
37	50.58	3.00	194.72	6.00
38	131.07	3.00	105.16	4.00

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	STS1NPol	STS1FPol	SCS1TTPol	SFMPol
1	226.84	93.31	320.15	10.00
2	221.71	182.23	403.94	13.00
3	223.00	51.49	274.49	14.00
4	202.65	67.04	269.69	12.00
5	285.84	41.13	326.97	8.00
6	208.61	63.00	271.61	10.00
7	272.46	102.15	374.61	11.00
8	359.09	68.29	427.38	8.00
9	144.87	156.45	301.32	13.00
10	208.59	166.10	374.69	9.00
11	136.52	211.92	348.44	13.00
12	108.23	142.46	250.69	14.00
13	161.93	144.19	306.12	10.00
14	112.14	149.04	261.18	12.00
15	131.54	208.98	340.52	9.00
16	87.30	180.42	267.72	10.00
17	158.92	185.49	344.41	19.00
18	127.32	131.82	259.14	8.00
19	212.18	175.79	387.97	6.00
20	116.53	285.58	402.11	4.00
21	146.98	176.97	323.95	14.00
22	173.98	164.31	338.29	18.00
23	192.67	160.70	353.37	12.00
24	82.36	64.02	146.38	10.00
25	209.57	171.31	380.88	10.00
26	101.60	182.74	284.34	9.00
27	169.81	67.14	236.95	21.00
28	216.83	167.50	384.33	12.00
29	138.29	33.47	171.76	16.00
30	118.47	199.96	318.45	10.00
31	77.99	118.46	196.45	10.00
32	133.34	232.71	366.05	14.00
33	118.47	103.03	221.50	11.00
34	118.35	129.82	248.17	12.00
35	174.54	151.31	325.85	15.00
36	114.42	281.58	396.00	13.00
37	223.27	91.02	314.29	11.00
38	53.17	25.25	78.42	7.00

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	STMPol	SFEPol	STENPol	STEFPol
1	131.73	5.00	43.99	104.69
2	93.45	6.00	77.60	19.77
3	96.79	7.00	160.08	67.98
4	114.77	6.00	96.57	119.12
5	148.26	4.00	65.53	59.46
6	141.66	4.00	115.46	71.56
7	110.36	4.00	86.54	33.01
8	87.17	3.00	68.46	17.21
9	162.88	6.00	56.28	80.51
10	164.96	3.00	15.80	44.72
11	104.16	7.00	27.14	120.00
12	152.05	6.00	14.76	182.65
13	145.96	5.00	85.56	66.49
14	133.05	6.00	28.70	177.20
15	69.29	3.00	7.74	182.59
16	103.85	5.00	38.83	189.89
17	148.79	7.00	57.17	49.47
18	124.59	3.00	82.41	134.11
19	141.66	2.00	13.25	56.79
20	65.55	2.00	22.36	110.19
21	139.00	6.00	10.68	126.49
22	157.29	7.00	6.57	98.04
23	115.10	5.00	75.32	59.26
24	74.48	4.00	24.77	354.65
25	88.66	4.00	40.37	90.60
26	193.07	3.00	8.78	62.52
27	185.09	10.00	83.89	93.36
28	157.67	3.00	34.37	23.76
29	177.31	7.00	66.50	184.75
30	170.83	3.00	28.83	82.21
31	314.05	4.00	14.37	75.55
32	83.68	7.00	34.82	115.70
33	122.84	6.00	65.48	190.03
34	186.92	5.00	56.13	109.01
35	133.11	7.00	48.24	92.86
36	143.96	5.00	8.12	49.18
37	129.15	5.00	44.77	111.91
38	266.86	3.00	45.01	210.09

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	SCETTPol	NFS1Pol	NTS1NPol	NTS1FPol	NCS1TTPol
1	148.68	2.00	36.36	42.35	78.71
2	97.37	4.00	80.75	26.83	107.58
3	228.06	3.00	57.79	9.09	66.88
4	215.69	2.00	62.84	142.80	205.64
5	124.99	4.00	157.12	94.52	251.64
6	187.02	2.00	90.81	60.11	150.92
7	119.55	4.00	21.37	110.91	132.28
8	85.67	1.00	7.13	.00	7.13
9	136.79	1.00	106.87	122.26	229.13
10	60.52	4.00	31.91	97.32	129.23
11	147.15	2.00	3.21	26.07	29.28
12	197.41	5.00	44.18	98.22	142.40
13	152.05	2.00	6.63	61.10	67.73
14	205.90	3.00	19.85	115.81	135.66
15	190.33	3.00	23.43	50.59	74.02
16	228.72	1.00	7.45	31.35	38.80
17	106.64	4.00	16.59	49.46	66.05
18	216.52	1.00	.00	.00	.00
19	70.04	2.00	15.95	66.72	82.67
20	132.55	1.00	10.14	27.77	37.91
21	137.17	3.00	17.48	172.55	190.03
22	104.61	2.00	20.63	22.10	42.73
23	134.58	2.00	46.13	94.21	140.34
24	379.42	3.00	24.33	93.98	118.31
25	130.97	2.00	19.22	11.52	30.74
26	71.30	4.00	35.88	104.31	140.19
27	177.25	2.00	18.99	15.02	34.01
28	58.13	2.00	26.50	19.58	46.08
29	251.25	3.00	6.98	55.43	62.41
30	111.04	3.00	24.46	94.64	119.10
31	89.92	1.00	4.11	16.19	20.30
32	150.52	4.00	14.23	89.54	103.77
33	255.51	3.00	45.04	176.93	221.97
34	165.14	2.00	26.10	13.58	39.68
35	141.10	5.00	28.47	107.65	136.12
36	57.30	2.00	10.74	66.86	77.60
37	156.68	.00	.00	.00	.00
38	255.10	3.00	21.32	18.94	40.26

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	NFMPol	NTMPol	NFS2Pol	NTS2NPol
1	6.00	49.31	4.00	165.00
2	4.00	60.41	3.00	120.25
3	5.00	22.16	4.00	171.51
4	3.00	21.49	1.00	53.33
5	4.00	48.67	.00	.00
6	5.00	94.97	3.00	43.74
7	6.00	75.73	3.00	87.09
8	2.00	25.37	2.00	189.68
9	2.00	54.55	1.00	16.66
10	6.00	73.18	2.00	76.87
11	7.00	79.17	5.00	115.87
12	7.00	48.59	6.00	52.61
13	4.00	94.38	2.00	60.64
14	4.00	31.25	2.00	46.70
15	6.00	54.51	3.00	71.26
16	3.00	21.70	3.00	39.69
17	7.00	81.92	3.00	42.61
18	3.00	31.72	2.00	60.90
19	6.00	57.44	4.00	86.28
20	5.00	75.21	3.00	91.01
21	5.00	33.65	2.00	51.47
22	7.00	65.21	5.00	98.19
23	6.00	52.88	4.00	70.84
24	7.00	76.43	4.00	54.19
25	8.00	144.46	5.00	75.89
26	10.00	63.18	6.00	46.83
27	7.00	65.03	5.00	40.87
28	6.00	51.05	4.00	94.39
29	6.00	24.68	3.00	43.45
30	7.00	71.98	3.00	34.43
31	3.00	114.03	2.00	33.58
32	6.00	31.80	4.00	64.20
33	5.00	20.92	2.00	40.75
34	7.00	63.48	4.00	96.78
35	9.00	67.93	4.00	37.33
36	7.00	63.95	5.00	60.88
37	3.00	36.28	3.00	60.56
38	7.00	69.17	5.00	62.81

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	NTS2FPol	NCS2TTPol	HFLPoT	HTLPoT
1	7.39	172.39	3.00	47.83
2	12.13	132.38	4.00	69.57
3	39.67	211.18	4.00	110.77
4	19.86	73.19	5.00	141.82
5	.00	.00	2.00	228.07
6	10.92	54.66	1.00	268.85
7	5.22	92.31	3.00	114.75
8	77.86	267.54	2.00	33.69
9	.00	16.66	1.00	24.24
10	21.03	97.90	1.00	277.17
11	75.86	191.73	2.00	31.95
12	56.55	109.16	3.00	120.19
13	77.48	138.12	3.00	102.71
14	87.49	134.19	1.00	31.13
15	100.48	171.74	1.00	105.31
16	200.16	239.85	2.00	208.65
17	109.75	152.36	3.00	60.09
18	141.33	202.23	1.00	27.25
19	73.12	159.40	1.00	195.16
20	96.16	187.17	1.00	13.64
21	25.07	76.54	2.00	192.57
22	94.08	192.27	3.00	79.50
23	39.19	110.03	3.00	82.39
24	91.86	146.05	2.00	57.40
25	54.17	130.06	2.00	49.57
26	49.91	96.74	3.00	132.15
27	160.36	201.23	2.00	78.23
28	108.37	202.76	3.00	60.54
29	169.75	213.32	4.00	112.75
30	74.81	109.24	1.00	23.53
31	133.19	166.77	1.00	42.00
32	100.90	165.10	4.00	74.60
33	17.07	57.82	2.00	267.26
34	101.43	198.21	3.00	112.17
35	58.94	96.27	4.00	69.80
36	97.81	158.69	2.00	169.23
37	203.56	264.21	3.00	57.23
38	128.26	191.07	2.00	91.76

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	HFMPoT	HTMPoT	HFRPoT	HTRPoT
1	6.00	145.68	3.00	106.86
2	8.00	69.50	4.00	167.59
3	9.00	57.59	4.00	131.73
4	9.00	75.03	4.00	83.50
5	3.00	46.85	1.00	25.43
6	1.00	31.68	.00	.00
7	7.00	90.63	3.00	107.17
8	7.00	59.01	5.00	207.62
9	3.00	40.92	2.00	235.24
10	1.00	23.22	.00	.00
11	4.00	35.64	2.00	232.83
12	7.00	79.80	4.00	100.41
13	7.00	67.89	4.00	129.76
14	2.00	25.57	1.00	243.68
15	3.00	56.91	1.00	137.09
16	3.00	49.19	1.00	42.57
17	9.00	90.51	5.00	149.74
18	3.00	117.07	2.00	156.24
19	2.00	67.35	1.00	37.83
20	3.00	70.41	2.00	216.04
21	4.00	27.26	2.00	80.54
22	6.00	103.66	3.00	117.28
23	7.00	58.39	4.00	163.56
24	5.00	54.27	3.00	190.09
25	4.00	21.94	2.00	228.80
26	6.00	36.84	3.00	131.49
27	5.00	36.58	3.00	185.60
28	5.00	95.82	2.00	114.09
29	8.00	77.11	4.00	110.49
30	4.00	42.98	3.00	234.20
31	2.00	154.39	1.00	104.09
32	8.00	43.03	4.00	182.62
33	2.00	33.33	.00	.00
34	8.00	65.06	4.00	123.09
35	9.00	67.06	5.00	163.37
36	4.00	34.19	2.00	97.09
37	7.00	93.00	4.00	149.55
38	4.00	133.02	2.00	77.93

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	SFS1PoT	STS1NPoT	STS1FPoT	SCS1TTPoT
1	7.00	200.33	80.90	281.23
2	8.00	184.23	60.84	245.07
3	8.00	248.74	110.57	359.31
4	8.00	201.91	69.79	271.70
5	5.00	198.09	93.47	291.56
6	6.00	215.55	188.84	404.39
7	7.00	236.31	112.60	348.91
8	4.00	212.32	117.97	330.29
9	3.00	202.08	106.36	308.44
10	9.00	140.23	162.91	303.14
11	3.00	29.29	438.75	468.04
12	9.00	152.99	106.08	259.07
13	5.00	103.19	156.60	259.79
14	3.00	98.43	163.83	262.26
15	1.00	240.65	304.01	544.66
16	2.00	200.11	273.70	473.81
17	6.00	173.54	59.04	232.58
18	5.00	150.40	110.64	261.04
19	6.00	229.65	92.92	322.57
20	2.00	101.60	139.57	241.17
21	4.00	195.69	121.31	317.00
22	6.00	159.69	145.31	305.00
23	5.00	267.62	232.14	499.76
24	6.00	157.54	167.09	324.63
25	5.00	148.29	24.73	173.02
26	6.00	143.98	61.92	205.90
27	8.00	226.64	56.56	283.20
28	7.00	211.06	90.22	301.28
29	6.00	170.49	222.79	393.28
30	6.00	202.63	39.95	242.58
31	2.00	112.61	416.43	529.04
32	9.00	230.69	215.28	445.97
33	4.00	169.33	172.64	314.97
34	7.00	107.79	174.79	282.58
35	6.00	120.69	160.79	281.48
36	9.00	309.87	134.21	444.08
37	4.00	106.63	299.66	406.29
38	3.00	131.53	59.19	190.72

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	SFMPoT	STMPoT	SFEPoT	STENPoT
1	11.00	83.29	3.00	32.85
2	15.00	146.29	6.00	81.89
3	16.00	87.43	7.00	58.84
4	15.00	124.44	7.00	82.32
5	8.00	103.56	3.00	63.94
6	11.00	103.83	5.00	50.81
7	13.00	111.61	5.00	64.15
8	9.00	90.90	4.00	102.15
9	6.00	99.20	3.00	48.89
10	16.00	130.83	7.00	63.59
11	4.00	45.03	1.00	.00
12	17.00	164.23	9.00	21.48
13	13.00	153.43	7.00	85.44
14	6.00	97.24	3.00	73.56
15	1.00	55.73	.00	.00
16	4.00	57.13	2.00	19.62
17	16.00	210.34	8.00	49.64
18	10.00	165.84	5.00	74.71
19	12.00	163.35	5.00	45.75
20	5.00	123.30	3.00	52.22
21	7.00	104.77	3.00	55.66
22	10.00	101.05	4.00	97.14
23	8.00	62.09	3.00	9.04
24	11.00	144.54	5.00	53.22
25	10.00	104.81	5.00	219.71
26	12.00	99.69	6.00	105.68
27	16.00	164.28	8.00	91.88
28	10.00	141.18	3.00	108.08
29	12.00	129.06	5.00	18.07
30	12.00	240.74	5.00	46.30
31	3.00	60.87	1.00	.00
32	15.00	89.07	6.00	9.47
33	7.00	93.19	2.00	33.74
34	13.00	160.47	6.00	53.27
35	13.00	163.72	6.00	62.69
36	12.00	109.23	3.00	10.84
37	6.00	45.02	2.00	86.51
38	5.00	276.21	2.00	.00

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	STEFPoT	SCETTPoT	NFS1PoT	NTS1NPoT
1	202.97	235.82	3.00	28.77
2	127.08	208.97	9.00	41.66
3	94.58	153.42	3.00	60.03
4	121.86	204.18	3.00	22.62
5	141.18	205.12	1.00	.00
6	42.16	92.97	2.00	63.61
7	65.23	129.38	5.00	26.82
8	76.91	179.06	.00	.00
9	144.08	192.97	.00	.00
10	105.43	169.02	3.00	31.67
11	87.25	87.25	4.00	32.07
12	155.29	176.77	1.00	.00
13	101.60	187.04	2.00	45.79
14	166.62	240.18	3.00	21.65
15	.00	.00	2.00	28.94
16	44.73	64.35	1.00	19.03
17	107.75	157.39	4.00	20.29
18	98.93	173.64	4.00	19.53
19	68.54	114.29	2.00	18.47
20	189.58	241.80	.00	.00
21	122.71	178.37	1.00	45.18
22	97.07	194.21	4.00	74.05
23	29.47	38.51	4.00	33.71
24	77.78	131.00	4.00	21.32
25	102.76	322.47	2.00	20.22
26	188.71	294.39	1.00	53.73
27	60.80	152.68	2.00	59.77
28	49.04	157.12	1.00	12.13
29	59.85	77.92	2.00	4.70
30	116.88	163.18	2.00	43.92
31	10.29	10.29	1.00	.00
32	55.90	65.37	5.00	66.20
33	131.42	165.16	2.00	22.43
34	103.95	157.22	3.00	26.77
35	92.25	154.94	2.00	23.40
36	36.02	46.86	1.00	.00
37	62.57	149.08	1.00	10.47
38	138.96	138.96	.00	.00

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	NTS1FPoT	NCS1TTPoT	NFMPoT	NTMPoT
1	11.52	40.29	6.00	81.14
2	45.44	87.10	13.00	117.34
3	40.95	100.98	8.00	65.65
4	11.67	34.29	7.00	50.29
5	14.44	14.44	4.00	34.37
6	123.87	187.48	4.00	43.70
7	60.89	87.71	9.00	67.34
8	.00	.00	2.00	48.49
9	.00	.00	2.00	32.43
10	25.32	56.99	6.00	55.98
11	93.42	125.49	7.00	73.26
12	8.22	8.22	5.00	48.80
13	31.01	76.80	4.00	42.58
14	81.91	103.56	5.00	70.36
15	44.78	73.72	5.00	65.31
16	40.74	59.77	2.00	22.94
17	35.63	55.92	12.00	106.09
18	92.59	112.12	6.00	71.02
19	41.70	60.17	5.00	57.35
20	.00	.00	1.00	14.51
21	37.34	82.52	2.00	17.12
22	75.08	149.13	9.00	51.58
23	102.01	135.72	9.00	58.04
24	83.12	104.44	6.00	33.29
25	60.16	80.38	6.00	65.93
26	232.04	285.77	1.00	14.59
27	137.46	197.23	4.00	62.42
28	.00	12.13	4.00	40.86
29	27.73	32.43	6.00	66.23
30	19.80	63.72	6.00	89.44
31	313.17	131.17	1.00	15.40
32	34.03	100.23	11.00	75.61
33	67.13	89.56	4.00	29.02
34	71.37	98.14	6.00	90.19
35	95.49	118.89	7.00	82.23
36	2.06	2.06	2.00	23.86
37	259.33	269.80	1.00	30.67
38	.00	.00	1.00	42.73

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	NFS2PoT	NTS2NPoT	NTS2FPoT	NCS2TTPoT
1	3.00	103.51	75.38	178.89
2	4.00	92.00	3.73	95.73
3	4.00	101.30	32.33	133.63
4	7.00	82.32	121.86	204.18
5	3.00	216.69	34.88	251.57
6	2.00	58.51	10.86	69.37
7	4.00	132.77	12.45	145.22
8	2.00	91.90	159.94	251.84
9	2.00	85.46	182.57	268.03
10	3.00	149.87	37.52	187.39
11	3.00	54.10	47.48	101.58
12	4.00	35.03	208.41	243.44
13	2.00	85.65	95.36	181.01
14	2.00	96.78	34.76	131.54
15	2.00	105.22	56.14	161.36
16	1.00	134.75	82.83	217.58
17	7.00	102.16	35.92	138.08
18	2.00	68.32	48.77	117.09
19	3.00	93.86	109.00	202.86
20	1.00	168.98	116.76	285.74
21	1.00	109.10	91.42	200.52
22	5.00	94.74	4.83	99.57
23	4.00	81.14	25.64	106.78
24	3.00	85.08	55.48	140.56
25	3.00	73.03	80.90	153.93
26	.00	.00	.00	.00
27	2.00	32.80	8.18	40.98
28	3.00	134.25	113.11	247.36
29	4.00	152.86	48.79	201.65
30	3.00	103.98	43.15	147.15
31	.00	.00	.00	.00
32	6.00	79.86	44.54	124.40
33	2.00	81.13	101.01	182.14
34	3.00	65.95	45.86	111.81
35	4.00	37.23	61.87	99.10
36	1.00	168.82	105.80	274.62
37	.00	.00	.00	.00
38	1.00	136.11	121.51	257.62

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	TLCD1PrI	TLCD2PrI	TLCD3PrI	TLCD4PrI
1	34.31	33.17	34.62	33.64
2	38.87	37.02	38.57	36.95
3	34.21	34.14	34.74	35.71
4	39.74	35.43	37.01	30.59
5	43.09	35.05	40.00	30.74
6	42.13	37.47	38.50	37.14
7	31.57	43.83	29.48	31.07
8	43.57	49.07	42.09	40.16
9	39.87	38.26	41.09	41.58
10	37.73	44.90	49.92	45.57
11	38.18	38.14	32.67	43.78
12	39.82	36.33	38.10	36.75
13	33.76	37.26	38.23	30.23
14	38.13	45.21	40.22	32.45
15	39.52	47.53	46.69	45.56
16	40.55	36.72	38.98	36.25
17	34.75	33.51	42.93	35.76
18	38.64	36.62	34.84	33.34
19	32.68	37.74	40.21	35.37
20	32.92	38.74	38.51	33.44
21	44.32	33.73	33.39	30.42
22	42.77	40.95	45.26	34.81
23	39.16	45.45	51.55	44.34
24	41.87	53.87	47.02	46.04
25	56.55	63.51	47.90	40.10
26	33.18	29.44	29.41	33.43
27	53.29	50.26	46.03	48.16
28	36.58	32.63	32.54	36.51
29	34.38	34.42	32.60	34.40
30	46.88	32.35	28.97	33.13
31	41.49	47.94	50.36	52.47
32	43.71	41.75	40.75	40.28
33	47.08	49.75	45.14	43.84
34	31.50	30.76	27.74	33.60
35	41.72	47.28	48.45	45.48
36	58.99	59.37	53.25	49.27
37	28.80	37.53	40.36	32.32
38	50.05	49.66	45.93	45.25

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	AVG_TLC_Pr	SPD1Prl	SPD2Prl	SPD3Prl
1	33.94	28.00	43.00	11.00
2	37.85	5.00	57.00	82.00
3	34.70	20.00	30.00	14.00
4	35.69	8.00	31.00	7.00
5	37.22	44.00	27.00	84.00
6	38.81	26.00	53.00	44.00
7	33.99	34.00	38.00	24.00
8	43.72	4.00	50.00	89.00
9	40.20	40.00	79.00	91.00
10	44.53	63.00	91.00	93.00
11	38.19	24.00	15.00	57.00
12	37.75	71.00	85.00	90.00
13	34.87	53.00	40.00	66.00
14	39.00	12.00	53.00	83.00
15	44.83	70.00	81.00	89.00
16	38.13	46.00	62.00	55.00
17	36.74	97.00	65.00	88.00
18	35.86	94.00	71.00	67.00
19	36.50	2.00	2.00	3.00
20	35.90	65.00	67.00	94.00
21	35.47	.00	3.00	3.00
22	40.95	33.00	81.00	83.00
23	45.13	70.00	83.00	95.00
24	47.20	57.00	82.00	90.00
25	52.02	92.00	97.00	84.00
26	31.37	7.00	6.00	3.00
27	49.43	85.00	97.00	98.00
28	34.57	9.00	3.00	1.00
29	33.95	53.00	57.00	43.00
30	35.33	90.00	61.00	41.00
31	48.07	65.00	90.00	95.00
32	41.62	78.00	75.00	92.00
33	46.45	94.00	97.00	96.00
34	30.90	11.00	4.00	2.00
35	45.73	65.00	92.00	92.00
36	55.22	92.00	97.00	98.00
37	34.75	50.00	81.00	94.00
38	47.72	62.00	73.00	92.00

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	SPD4Prl	AVG_SP_Prl	TLCD1Pol	TLCD2Pol
1	28.00	27.50	43.41	26.52
2	77.00	55.25	35.05	27.47
3	29.00	23.25	34.28	27.00
4	11.00	14.25	31.07	27.67
5	12.00	41.75	39.29	28.15
6	56.00	44.75	35.86	27.67
7	22.00	29.50	39.00	22.33
8	83.00	56.50	74.14	41.15
9	94.00	76.00	38.94	29.50
10	93.00	85.00	55.51	31.21
11	26.00	30.50	36.66	22.26
12	89.00	83.75	47.51	44.27
13	61.00	55.00	47.39	31.03
14	96.00	61.00	35.85	35.11
15	79.00	79.75	72.59	38.05
16	22.00	46.25	55.37	37.18
17	86.00	84.00	44.85	36.12
18	48.00	70.00	33.95	20.47
19	2.00	2.25	40.09	33.51
20	91.00	79.25	62.02	29.21
21	5.00	2.75	37.88	24.14
22	6.00	50.75	47.79	36.92
23	88.00	84.00	72.58	51.80
24	88.00	79.25	74.32	41.35
25	74.00	86.75	37.65	38.39
26	3.00	4.75	34.05	33.28
27	97.00	94.25	45.98	53.26
28	1.00	3.50	24.12	28.13
29	38.00	47.75	31.27	30.72
30	55.00	61.75	26.96	28.33
31	95.00	86.25	22.29	31.13
32	36.00	70.25	32.97	39.30
33	96.00	95.75	40.22	47.86
34	3.00	5.00	35.34	23.71
35	94.00	85.75	49.42	54.52
36	92.00	94.75	39.01	45.94
37	92.00	79.25	47.62	49.28
38	84.00	77.75	39.67	56.64

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	TLCD3Pol	TLCD4Pol	AVG_TLC_Pol	SPD1Pol
1	40.20	33.13	35.82	65.00
2	30.21	31.19	30.98	71.00
3	34.94	35.46	32.92	61.00
4	30.56	30.28	29.90	4.00
5	37.14	36.41	35.25	91.00
6	25.61	34.63	30.94	80.00
7	28.49	31.98	30.45	73.00
8	41.49	53.10	52.47	95.00
9	40.70	32.02	35.29	95.00
10	54.91	51.26	48.22	89.00
11	34.68	35.87	32.37	84.00
12	43.32	37.39	43.12	97.00
13	40.59	33.40	38.10	92.00
14	33.36	44.69	37.25	92.00
15	42.61	46.29	49.89	97.00
16	34.22	43.73	42.63	94.00
17	44.16	40.30	41.36	96.00
18	28.97	35.21	29.65	90.00
19	35.65	41.61	37.72	60.00
20	36.24	46.94	43.60	95.00
21	30.67	38.39	32.77	95.00
22	41.48	44.51	42.68	97.00
23	45.14	51.29	55.20	98.00
24	46.32	41.93	50.98	98.00
25	31.57	36.03	35.91	63.00
26	29.46	32.98	32.44	69.00
27	48.72	57.44	51.35	94.00
28	28.44	30.58	27.82	2.00
29	33.05	39.89	33.73	44.00
30	32.05	23.24	27.65	84.00
31	29.75	32.26	28.86	8.00
32	40.21	44.42	39.22	60.00
33	38.91	40.67	41.92	83.00
34	34.05	27.86	30.24	3.00
35	48.17	40.10	48.05	95.00
36	42.65	37.52	41.28	91.00
37	34.68	38.45	42.51	97.00
38	31.32	42.57	42.55	94.00

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	SPD2Pol	SPD3Pol	SPD4Pol	AVG_SP_Pol
1	7.00	3.00	4.00	19.75
2	10.00	26.00	13.00	30.00
3	30.00	9.00	14.00	28.50
4	4.00	3.00	3.00	3.50
5	70.00	42.00	36.00	59.75
6	76.00	59.00	55.00	67.50
7	30.00	65.00	49.00	54.25
8	94.00	95.00	93.00	94.25
9	95.00	98.00	94.00	95.50
10	55.00	71.00	72.00	71.75
11	68.00	74.00	51.00	69.25
12	97.00	98.00	84.00	94.00
13	96.00	98.00	53.00	84.75
14	98.00	97.00	93.00	95.00
15	92.00	98.00	79.00	91.50
16	88.00	74.00	43.00	74.75
17	86.00	97.00	94.00	93.25
18	21.00	24.00	12.00	36.75
19	26.00	59.00	8.00	38.25
20	95.00	93.00	65.00	87.00
21	97.00	97.00	97.00	96.50
22	81.00	98.00	64.00	85.00
23	98.00	98.00	75.00	92.25
24	97.00	96.00	88.00	94.75
25	35.00	59.00	13.00	42.50
26	40.00	21.00	20.00	37.50
27	96.00	86.00	86.00	90.50
28	1.00	1.00	1.00	1.25
29	24.00	35.00	43.00	36.50
30	96.00	97.00	95.00	93.00
31	4.00	65.00	66.00	35.75
32	72.00	91.00	76.00	74.75
33	96.00	97.00	96.00	93.00
34	6.00	3.00	4.00	4.00
35	94.00	98.00	95.00	95.50
36	96.00	94.00	55.00	84.00
37	93.00	98.00	98.00	96.50
38	67.00	72.00	39.00	68.00

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	TLCD1PoT	TLCD2PoT	TLCD3PoT	TLCD4PoT
1	36.80	40.04	32.11	34.90
2	31.16	35.37	35.39	31.37
3	40.09	37.24	40.44	34.17
4	29.11	31.03	30.40	29.63
5	34.74	40.00	37.41	38.69
6	33.07	44.49	32.67	31.88
7	30.38	31.40	32.55	28.26
8	66.17	61.98	69.19	54.35
9	43.91	37.09	30.53	29.16
10	69.18	69.76	52.57	41.16
11	41.18	33.87	35.20	33.38
12	44.87	37.18	30.51	36.81
13	338.75	42.28	35.26	34.04
14	41.07	36.92	34.36	35.75
15	50.62	60.02	47.76	45.31
16	49.00	35.09	42.90	30.74
17	48.24	37.31	42.87	40.09
18	31.38	27.17	29.66	11.49
19	41.84	36.90	42.69	40.99
20	50.87	33.45	30.60	28.52
21	45.88	35.06	36.78	33.68
22	59.84	56.98	59.72	55.72
23	81.84	60.46	69.30	72.11
24	97.37	67.21	71.59	31.55
25	37.39	31.81	34.31	51.18
26	40.43	37.15	36.25	36.69
27	43.55	33.63	50.74	42.93
28	35.63	30.56	32.59	31.22
29	34.42	31.19	31.58	28.21
30	33.42	27.58	31.10	28.97
31	38.51	33.11	30.36	34.02
32	60.93	51.86	50.38	51.69
33	36.27	38.26	36.40	36.62
34	31.53	26.72	33.32	27.47
35	55.07	38.25	42.28	35.20
36	39.36	39.38	40.47	39.76
37	29.84	34.22	31.05	34.29
38	45.87	43.88	44.77	47.75

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	AVG_TLC_PoT	SPD1PoT	SPD2PoT	SPD3PoT
1	35.96	35.00	1.00	5.00
2	33.32	60.00	25.00	13.00
3	37.99	16.00	12.00	4.00
4	30.04	3.00	2.00	1.00
5	37.71	63.00	82.00	73.00
6	35.53	75.00	94.00	40.00
7	30.65	31.00	25.00	30.00
8	62.92	98.00	93.00	94.00
9	35.17	90.00	95.00	92.00
10	58.17	80.00	85.00	53.00
11	35.91	62.00	23.00	3.00
12	37.34	92.00	96.00	2.00
13	112.58	83.00	93.00	28.00
14	37.03	97.00	93.00	29.00
15	50.93	89.00	98.00	66.00
16	39.43	88.00	76.00	46.00
17	42.13	74.00	81.00	37.00
18	24.92	88.00	36.00	7.00
19	40.61	45.00	9.00	1.00
20	35.86	91.00	79.00	59.00
21	37.85	98.00	97.00	98.00
22	58.07	95.00	96.00	93.00
23	70.93	96.00	96.00	95.00
24	66.93	98.00	94.00	97.00
25	38.67	44.00	2.00	1.00
26	37.63	6.00	4.00	4.00
27	42.71	6.00	4.00	3.00
28	32.50	.00	.00	.00
29	31.35	42.00	15.00	4.00
30	30.27	8.00	2.00	2.00
31	34.00	48.00	45.00	9.00
32	53.71	82.00	72.00	65.00
33	36.89	11.00	2.00	2.00
34	29.76	2.00	1.00	1.00
35	42.70	2.00	1.00	1.00
36	39.74	1.00	.00	.00
37	32.35	11.00	2.00	2.00
38	45.57	2.00	.00	.00

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	SPD4PoT	AVG_SP_PoT	Bma	BV
1	3.00	11.00	2.36	2.73
2	12.00	27.50	2.13	3.23
3	4.00	9.00	2.08	3.56
4	.00	1.50	1.77	4.07
5	96.00	78.50	1.91	3.26
6	26.00	58.75	1.87	2.84
7	19.00	26.25	1.97	3.00
8	94.00	94.75	2.04	2.90
9	93.00	92.50	2.06	2.45
10	25.00	60.75	1.88	2.38
11	2.00	22.50	1.98	2.90
12	1.00	47.75	1.94	2.26
13	12.00	54.00	1.82	2.63
14	2.00	55.25	1.99	2.77
15	82.00	83.75	3.97	2.20
16	4.00	53.50	1.97	2.85
17	85.00	69.25	.94	1.43
18	86.00	54.25	1.79	2.42
19	1.00	14.00	1.66	.42
20	27.00	64.00	1.66	.81
21	97.00	97.50	1.83	1.83
22	97.00	95.25	1.85	1.85
23	97.00	96.00	2.20	2.20
24	23.00	78.00	1.93	1.93
25	1.00	12.00	1.86	2.26
26	8.00	5.50	1.90	2.66
27	.00	3.25	1.94	2.67
28	.00	.00	1.88	1.98
29	2.00	15.75	1.90	2.08
30	1.00	3.25	2.02	3.11
31	6.00	27.00	1.99	2.27
32	58.00	69.25	1.89	1.98
33	1.00	4.00	2.02	2.74
34	1.00	1.25	1.92	2.30
35	.00	1.00	1.97	2.35
36	.00	.25	2.01	1.76
37	2.00	4.25	1.96	1.72
38	.00	.50	1.87	1.48

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	APL	LW	SIH	HR
1	22.01	14.56	8.52	.0523
2	23.37	15.32	9.01	.0890
3	23.83	16.25	9.20	.0754
4	24.81	15.68	10.46	.1025
5	23.89	14.48	9.41	.0916
6	22.44	14.36	8.80	.1151
7	24.10	14.06	8.84	.0858
8	21.36	14.21	9.55	.0577
9	21.85	13.95	8.04	.4940
10	19.78	14.69	8.18	.1249
11	21.08	14.41	9.54	.0708
12	20.04	14.24	7.92	.0663
13	23.13	13.99	8.14	.0548
14	20.08	14.61	9.44	.0948
15	18.51	14.18	8.40	.0710
16	20.98	14.86	9.15	.0980
17	12.97	14.66	7.54	.0785
18	20.02	13.83	8.75	.1015
19	15.53	8.54	3.13	.0883
20	17.57	7.97	5.78	.0841
21	16.66	8.11	2.40	.0544
22	16.05	9.08	2.59	.1496
23	16.60	9.55	3.91	.0835
24	16.23	8.22	3.89	.0575
25	19.92	13.01	8.75	.0840
26	20.69	14.87	8.63	.0600
27	23.83	13.93	8.05	.0700
28	19.03	13.90	7.49	.0700
29	19.39	13.43	7.99	.0630
30	21.79	15.08	9.45	.1020
31	20.33	13.75	8.12	.0660
32	19.38	13.10	7.80	.1050
33	21.09	13.90	9.35	.0580
34	20.79	13.71	8.07	.0620
35	20.83	12.54	9.00	.0650
36	18.46	12.99	7.32	.0700
37	17.35	12.58	7.86	.1161
38	17.53	11.92	7.07	.0686

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	HL	PR	PL	SR
1	.1134	.0964	.1131	.0831
2	.1018	.0751	.1185	.0997
3	.0860	.0846	.1783	.0412
4	.0854	.0965	.0983	.0844
5	.0931	.1539	.1086	.0544
6	.0893	.0840	.1209	.0601
7	.0841	.0944	.0699	.0476
8	.1072	.1133	.1871	.0748
9	.0704	.1373	.0989	.0496
10	.0517	.0993	.0818	.0552
11	.0929	.1631	.1376	.0255
12	.0314	.1170	.1316	.0926
13	.1078	.0883	.1181	.0628
14	.0719	.1589	.2364	.0333
15	.0695	.1260	.0663	.0653
16	.0843	.1145	.1714	.0483
17	.0679	.1160	.1470	.0817
18	.0660	.0685	.0723	.0753
19	.0500	.0958	.0993	.0807
20	.0894	.1432	.1049	.0544
21	.0864	.1207	.1333	.0634
22	.0718	.1336	.1255	.0741
23	.0917	.1766	.1573	.0742
24	.0805	.1933	.1464	.0806
25	.0745	.1900	.1300	.0250
26	.0500	.1400	.1400	.0300
27	.0700	.1900	.1400	.1100
28	.0700	.1300	.1200	.0500
29	.0500	.1640	.1220	.0180
30	.0520	.1320	.0950	.0180
31	.0700	.2860	.1410	.0360
32	.0660	.1580	.1270	.0320
33	.0520	.1450	.0970	.0290
34	.0680	.1090	.1060	.0200
35	.0990	.1090	.1110	.0370
36	.0700	.1300	.1220	.0370
37	.0670	.0970	.1100	.0359
38	.0730	.1184	.1367	.0183

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	SL	DA_PFC	DA_VTA	ACh_PFC	ACh_Hippo
1	.0600	.269	.305	2.254	1.071
2	.0954	.266	.325	1.874	1.198
3	.0822	.265	.273	1.830	1.070
4	.0419	.306	.312	2.374	1.084
5	.0425	.384	.330	2.418	1.209
6	.0552	.278	.303	2.165	1.108
7	.0627	.276	.325	2.315	1.000
8	.0757	.390	.308	2.396	.989
9	.0692	.278	.331	2.245	1.249
10	.0575	.239	.271	2.339	1.252
11	.0700	.244	.290	2.284	.977
12	.0464	.253	.275	2.362	1.730
13	.0513	.274	.310	2.497	1.154
14	.0693	.256	.268	2.189	1.302
15	.0490	.235	.305	2.426	1.157
16	.0553	.298	.256	2.289	1.180
17	.0589	.258	.273	2.368	1.141
18	.0475	.263	.315	2.304	1.510
19	.0588	.276	.304	2.318	1.218
20	.0826	.292	.352	2.351	1.033
21	.1509	.265	.291	2.215	1.074
22	.7400	.350	.341	2.290	1.091
23	.0445	.387	.315	2.472	.905
24	.0513	.408	.333	2.344	.961
25	.0430	.293	.296	2.283	.916
26	.0200	.304	.297	2.531	1.370
27	.0400	.338	.332	2.279	1.030
28	.0300	.489	.394	2.507	1.125
29	.0200	.531	.369	2.589	1.281
30	.0180	.338	.324	2.466	1.099
31	.0250	.306	.317	2.572	1.349
32	.0280	.306	.319	2.619	1.243
33	.0308	.309	.330	2.655	1.507
34	.0490	.256	.291	2.502	1.062
35	.0380	.280	.253	2.536	.903
36	.0420	.269	.381	2.308	1.188
37	.0400	.301	.261	1.793	1.274
38	.0550	.238	.331	2.533	1.679

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	ID	IGROUP	TGROUP	WEIGHTAD1
39	39	2	5	204.00
40	40	2	5	201.00
41	41	2	6	189.00
42	42	1	3	148.00
43	43	1	2	157.00
44	44	1	2	157.00
45	45	1	3	144.00
46	46	1	1	124.00
47	47	1	1	139.00
48	48	1	2	168.00
49	49	1	2	146.00
50	50	1	3	142.00
51	51	1	3	157.00
52	52	2	4	139.00
53	53	2	4	162.00
54	54	2	4	188.00
55	55	2	4	174.00
56	56	2	4	165.00
57	57	2	5	180.00
58	58	2	5	174.00
59	59	2	5	198.00
60	60	2	5	212.00
61	61	2	5	217.00
62	62	2	6	213.00
63	63	2	6	190.00
64	64	2	6	193.00
65	65	2	6	200.00
66	66	2	6	200.00
67	.	.	.	.
68	.	.	.	.
69	.	.	.	.
70	.	.	.	.
71	.	.	.	.
72	.	.	.	.
73	.	.	.	.
74	.	.	.	.
75	.	.	.	.
76	.	.	.	.

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	WEIGHTAD7	AVG_ADW	WEIGHPBT1	WEIGHTID1
39	251.00	227.50	306.00	306.00
40	239.00	220.00	285.00	285.00
41	229.00	209.00	265.00	265.00
42	170.00	159.00	207.00	207.00
43	187.00	172.00	224.00	224.00
44	185.00	171.00	223.00	223.00
45	176.00	160.00	212.00	212.00
46	176.00	150.00	191.00	191.00
47	199.00	169.00	206.00	206.00
48	243.00	205.50	266.00	266.00
49	207.00	176.50	218.00	218.00
50	205.00	173.50	221.00	221.00
51	209.00	183.00	224.00	224.00
52	216.00	177.50	237.00	237.00
53	239.00	200.50	258.00	258.00
54	260.00	224.00	271.00	271.00
55	234.00	204.00	254.00	254.00
56	201.00	183.00	207.00	207.00
57	223.00	201.50	237.00	237.00
58	219.00	196.50	229.00	229.00
59	242.00	220.00	259.00	259.00
60	265.00	238.50	281.00	281.00
61	259.00	238.00	270.00	270.00
62	261.00	237.00	278.00	278.00
63	229.00	209.50	248.00	248.00
64	244.00	218.50	258.00	258.00
65	251.00	225.50	256.00	256.00
66	258.00	229.00	271.00	271.00
67	.	.	.	.
68	.	.	.	.
69	.	.	.	.
70	.	.	.	.
71	.	.	.	.
72	.	.	.	.
73	.	.	.	.
74	.	.	.	.
75	.	.	.	.
76	.	.	.	.

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	WEIGHTID5	AVG_IDW	WEIGHTPBT2	WEIGHTTD1
39	336.00	321.00	368.00	368.00
40	302.00	293.50	322.00	322.00
41	293.00	279.00	312.00	312.00
42	236.00	221.50	263.00	263.00
43	251.00	237.50	271.00	271.00
44	249.00	236.00	275.00	275.00
45	234.00	223.00	251.00	251.00
46	218.00	204.50	235.00	235.00
47	224.00	215.00	230.00	230.00
48	295.00	280.50	325.00	325.00
49	240.00	229.00	255.00	255.00
50	240.00	230.50	250.00	250.00
51	245.00	234.50	263.00	263.00
52	258.00	247.50	285.00	285.00
53	279.00	268.50	297.00	297.00
54	276.00	273.50	294.00	294.00
55	271.00	262.50	289.00	289.00
56	215.00	211.00	230.00	230.00
57	250.00	243.50	270.00	270.00
58	245.00	237.00	264.00	264.00
59	278.00	268.50	298.00	298.00
60	305.00	293.00	327.00	327.00
61	286.00	278.00	300.00	300.00
62	302.00	290.00	324.00	324.00
63	278.00	263.00	300.00	300.00
64	282.00	270.00	304.00	304.00
65	281.00	268.50	294.00	294.00
66	294.00	282.50	316.00	316.00
67	.	.	.	.
68	.	.	.	.
69	.	.	.	.
70	.	.	.	.
71	.	.	.	.
72	.	.	.	.
73	.	.	.	.
74	.	.	.	.
75	.	.	.	.
76	.	.	.	.

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	WEIGHTTD7	AVG_TW	WEIGHTPBT3	HFLPrl
39	374.00	371.00	403.00	2.00
40	335.00	328.50	357.00	.00
41	323.00	317.50	341.00	4.00
42	279.00	271.00	292.00	2.00
43	284.00	277.50	294.00	5.00
44	277.00	276.00	278.00	3.00
45	267.00	259.00	285.00	3.00
46	258.00	246.50	277.00	3.00
47	254.00	242.00	267.00	.00
48	347.00	336.00	366.00	3.00
49	261.00	258.00	274.00	5.00
50	272.00	261.00	289.00	3.00
51	276.00	269.50	292.00	.00
52	314.00	299.50	346.00	2.00
53	322.00	309.50	349.00	1.00
54	322.00	308.00	341.00	.00
55	312.00	300.50	331.00	.00
56	252.00	241.00	269.00	3.00
57	291.00	280.50	309.00	2.00
58	273.00	268.50	292.00	3.00
59	320.00	309.00	341.00	5.00
60	343.00	335.00	366.00	4.00
61	317.00	308.50	333.00	2.00
62	354.00	339.00	374.00	7.00
63	322.00	311.00	347.00	4.00
64	340.00	322.00	366.00	6.00
65	320.00	307.00	341.00	7.00
66	331.00	323.50	347.00	3.00
67	.	.	.	.
68	.	.	.	.
69	.	.	.	.
70	.	.	.	.
71	.	.	.	.
72	.	.	.	.
73	.	.	.	.
74	.	.	.	.
75	.	.	.	.
76	.	.	.	.

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	HTLPrI	HFMPrI	HTMPrI	HFRPrI
39	52.54	4.00	58.95	2.00
40	.00	1.00	46.05	1.00
41	58.93	8.00	102.62	4.00
42	188.89	4.00	72.98	1.00
43	93.47	9.00	58.20	5.00
44	49.49	6.00	38.76	4.00
45	29.43	8.00	129.97	5.00
46	172.16	6.00	65.73	3.00
47	.00	1.00	300.22	.00
48	48.48	8.00	112.53	5.00
49	121.27	9.00	57.97	4.00
50	87.02	6.00	70.51	3.00
51	.00	1.00	18.11	1.00
52	19.48	4.00	46.24	2.00
53	29.62	3.00	104.29	2.00
54	.00	2.00	79.07	2.00
55	.00	1.00	9.24	1.00
56	121.10	6.00	60.82	3.00
57	185.43	4.00	57.74	1.00
58	60.96	8.00	103.74	5.00
59	135.06	9.00	78.57	4.00
60	55.16	12.00	93.21	8.00
61	23.58	6.00	52.16	4.00
62	90.11	15.00	88.08	7.00
63	57.31	8.00	62.58	4.00
64	99.56	11.00	100.72	4.00
65	90.75	14.00	83.44	7.00
66	168.65	6.00	34.03	3.00
67	.	.	.	.
68	.	.	.	.
69	.	.	.	.
70	.	.	.	.
71	.	.	.	.
72	.	.	.	.
73	.	.	.	.
74	.	.	.	.
75	.	.	.	.
76	.	.	.	.

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	HTRPrl	SFS1Prl	STS1NPrl	STS1FPrl
39	188.87	5.00	359.31	148.18
40	254.36	.00	.00	.00
41	138.79	4.00	223.48	56.54
42	38.53	4.00	452.00	69.96
43	148.70	10.00	232.61	10.12
44	212.59	5.00	360.18	72.34
45	219.47	7.00	275.25	94.64
46	65.94	6.00	273.31	86.52
47	.00	2.00	243.93	92.59
48	139.22	7.00	235.61	170.74
49	120.89	9.00	254.26	69.95
50	142.78	4.00	199.93	87.51
51	282.23	1.00	265.97	309.39
52	234.78	3.00	173.07	23.73
53	166.45	2.00	173.59	10.86
54	221.39	4.00	256.12	210.91
55	291.10	2.00	46.76	.00
56	118.47	5.00	217.93	218.69
57	57.17	8.00	173.91	64.57
58	135.61	10.00	285.33	72.66
59	86.63	7.00	240.15	145.88
60	151.88	9.00	158.34	97.22
61	224.65	7.00	192.02	115.56
62	122.30	13.00	245.08	38.27
63	180.42	5.00	300.67	106.71
64	99.98	8.00	195.16	48.57
65	126.03	7.00	232.88	75.36
66	97.48	6.00	136.64	45.58
67	.	.	.	.
68	.	.	.	.
69	.	.	.	.
70	.	.	.	.
71	.	.	.	.
72	.	.	.	.
73	.	.	.	.
74	.	.	.	.
75	.	.	.	.
76	.	.	.	.

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	STS1TTPrl	SFMPrl	STMPrl	SFEPrl
39	507.49	5.00	57.68	2.00
40	.00	.00	.00	1.00
41	280.02	8.00	88.24	4.00
42	521.96	4.00	58.71	2.00
43	242.73	16.00	127.86	11.00
44	432.42	9.00	65.44	4.00
45	369.89	12.00	191.75	5.00
46	359.83	12.00	102.52	6.00
47	336.52	2.00	264.28	.00
48	406.35	10.00	122.22	4.00
49	324.21	15.00	143.27	6.00
50	287.51	9.00	100.03	4.00
51	575.36	1.00	24.90	.00
52	196.80	4.00	67.12	1.00
53	184.45	6.00	40.12	3.00
54	467.03	6.00	60.77	2.00
55	46.76	3.00	20.80	1.00
56	436.62	9.00	78.82	4.00
57	238.48	15.00	129.41	7.00
58	357.99	15.00	123.22	5.00
59	386.03	12.00	138.88	5.00
60	255.56	16.00	134.63	7.00
61	307.58	14.00	196.02	7.00
62	283.35	22.00	177.92	9.00
63	407.38	9.00	72.12	3.00
64	243.73	13.00	93.41	5.00
65	308.24	15.00	126.91	8.00
66	182.22	12.00	132.79	6.00
67	.	.	.	.
68	.	.	.	.
69	.	.	.	.
70	.	.	.	.
71	.	.	.	.
72	.	.	.	.
73	.	.	.	.
74	.	.	.	.
75	.	.	.	.
76	.	.	.	.

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	STENPrl	STEFPrl	STETTPrl	NFS1Prl
39	35.21	.00	35.21	1.00
40	365.79	234.39	600.14	.00
41	119.07	112.65	231.72	.00
42	19.67	.00	19.67	1.00
43	188.44	47.52	235.96	5.00
44	40.00	29.11	69.11	2.00
45	50.65	9.52	60.17	4.00
46	65.68	72.15	137.83	3.00
47	.00	.00	.00	2.00
48	31.51	41.21	72.72	3.00
49	43.88	88.83	132.71	1.00
50	62.59	150.18	212.77	2.00
51	.00	.00	.00	1.00
52	27.43	309.00	336.43	2.00
53	27.99	347.76	375.75	1.00
54	28.41	43.68	72.09	1.00
55	63.80	469.06	532.86	4.00
56	47.46	37.41	84.87	3.00
57	29.25	203.10	233.10	3.00
58	65.70	53.17	118.87	5.00
59	50.08	25.34	75.42	2.00
60	67.78	140.45	208.23	2.00
61	22.70	73.89	96.59	2.00
62	30.69	108.34	139.03	2.00
63	34.46	86.26	120.72	1.00
64	24.31	239.49	263.80	4.00
65	55.46	109.67	165.13	1.00
66	35.39	249.66	285.05	2.00
67	.	.	.	.
68	.	.	.	.
69	.	.	.	.
70	.	.	.	.
71	.	.	.	.
72	.	.	.	.
73	.	.	.	.
74	.	.	.	.
75	.	.	.	.
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Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	NTS1NPrI	NTS1FPrI	NFS1TTPrI	NFMPrI
39	15.26	30.27	45.53	2.00
40	.00	.00	.00	2.00
41	.00	.00	.00	4.00
42	233.15	67.36	300.51	.00
43	69.63	23.19	92.82	5.00
44	86.10	39.93	126.03	3.00
45	64.03	58.12	122.15	8.00
46	59.48	60.70	120.18	7.00
47	78.96	83.31	162.27	3.00
48	34.34	54.13	88.47	7.00
49	19.28	86.65	105.93	4.00
50	43.84	16.05	59.89	5.00
51	61.21	230.95	292.16	1.00
52	.00	16.22	16.22	4.00
53	27.45	15.36	42.81	5.00
54	22.34	83.28	105.62	2.00
55	140.39	54.62	195.01	6.00
56	25.58	154.16	179.74	5.00
57	52.06	149.43	201.49	6.00
58	8.19	98.27	106.46	10.00
59	10.08	100.27	110.35	4.00
60	72.57	30.43	103.00	4.00
61	42.11	109.17	151.28	5.00
62	15.86	6.83	22.69	6.00
63	.00	285.85	285.85	1.00
64	45.80	79.13	124.26	9.00
65	20.61	195.17	215.78	2.00
66	5.23	61.46	66.69	5.00
67	.	.	.	.
68	.	.	.	.
69	.	.	.	.
70	.	.	.	.
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72	.	.	.	.
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Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	NTMPrl	NFS2Prl	NTS2NPrl	NTS2FPrl
39	14.03	1.00	143.97	95.90
40	21.43	2.00	206.55	72.72
41	32.19	3.00	140.67	127.54
42	.00	.00	.00	.00
43	33.57	3.00	112.94	60.32
44	64.39	2.00	58.71	4.31
45	44.30	4.00	119.44	19.79
46	77.12	3.00	84.50	18.45
47	45.34	1.00	55.10	21.54
48	38.02	4.00	92.08	81.69
49	58.45	3.00	104.81	31.14
50	27.59	3.00	98.70	113.97
51	8.46	.00	.00	.00
52	38.14	2.00	90.53	155.45
53	46.25	4.00	132.20	79.76
54	161.04	1.00	33.74	.00
55	45.98	2.00	49.59	9.72
56	49.71	2.00	47.11	23.78
57	37.85	3.00	69.13	9.50
58	85.45	4.00	73.15	35.10
59	23.39	2.00	111.42	55.13
60	46.32	2.00	110.04	41.07
61	65.13	3.00	83.96	.00
62	45.09	4.00	142.64	89.77
63	14.54	.00	.00	.00
64	67.30	4.00	68.72	39.35
65	53.54	1.00	31.28	.00
66	44.31	3.00	128.86	60.43
67	.	.	.	.
68	.	.	.	.
69	.	.	.	.
70	.	.	.	.
71	.	.	.	.
72	.	.	.	.
73	.	.	.	.
74	.	.	.	.
75	.	.	.	.
76	.	.	.	.

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	NCS2TTPrI	HFLPoI	HTLPoI	HFMPoI
39	239.87	1.00	19.38	3.00
40	279.27	.00	.00	1.00
41	268.21	1.00	26.42	4.00
42	.00	6.00	69.81	12.00
43	173.26	2.00	48.51	7.00
44	63.02	4.00	181.44	5.00
45	139.23	2.00	34.64	6.00
46	102.95	3.00	117.87	6.00
47	76.64	4.00	89.63	7.00
48	173.77	.00	.00	2.00
49	135.95	3.00	87.70	6.00
50	212.67	1.00	29.84	3.00
51	.00	.00	.00	1.00
52	245.98	5.00	138.89	9.00
53	211.96	1.00	295.45	1.00
54	33.74	3.00	239.80	4.00
55	59.31	4.00	172.22	6.00
56	70.89	3.00	94.89	7.00
57	78.63	3.00	67.56	6.00
58	108.25	3.00	75.70	7.00
59	166.55	.00	.00	2.00
60	151.11	.00	.00	2.00
61	83.96	2.00	61.32	5.00
62	232.41	3.00	56.67	7.00
63	.00	3.00	81.24	6.00
64	108.07	3.00	119.15	7.00
65	31.28	3.00	83.53	6.00
66	189.29	6.00	97.69	10.00
67	.	.	.	.
68	.	.	.	.
69	.	.	.	.
70	.	.	.	.
71	.	.	.	.
72	.	.	.	.
73	.	.	.	.
74	.	.	.	.
75	.	.	.	.
76	.	.	.	.

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	HTMPol	HFRPol	HTRPol	SFS1Pol
39	53.14	2.00	228.98	5.00
40	34.75	1.00	265.62	8.00
41	86.60	2.00	187.35	5.00
42	87.36	6.00	143.11	10.00
43	102.60	4.00	149.17	8.00
44	66.54	3.00	56.64	8.00
45	39.72	4.00	226.03	3.00
46	105.55	3.00	76.90	8.00
47	68.37	3.00	142.37	3.00
48	33.51	2.00	266.91	.00
49	70.32	3.00	142.25	5.00
50	52.67	2.00	217.85	5.00
51	25.23	1.00	275.01	1.00
52	137.54	4.00	83.80	8.00
53	4.91	.00	.00	4.00
54	39.98	1.00	20.61	1.00
55	71.94	2.00	56.31	7.00
56	67.49	4.00	138.17	6.00
57	48.68	3.00	184.01	9.00
58	82.87	4.00	141.66	7.00
59	20.28	2.00	280.12	4.00
60	22.49	2.00	277.97	2.00
61	59.99	3.00	178.79	6.00
62	91.90	4.00	151.75	8.00
63	34.77	3.00	184.32	6.00
64	65.96	3.00	114.85	6.00
65	64.97	3.00	154.78	6.00
66	75.97	4.00	126.55	7.00
67	.	.	.	.
68	.	.	.	.
69	.	.	.	.
70	.	.	.	.
71	.	.	.	.
72	.	.	.	.
73	.	.	.	.
74	.	.	.	.
75	.	.	.	.
76	.	.	.	.

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	STS1NPol	STS1FPol	SCS1TTPol	SFMPol
39	56.15	210.76	266.91	9.00
40	143.39	201.17	344.56	13.00
41	60.40	208.64	269.04	9.00
42	358.04	88.37	446.41	16.00
43	208.39	49.03	257.42	15.00
44	231.22	175.42	406.64	13.00
45	48.79	13.67	62.46	6.00
46	264.92	100.93	365.85	15.00
47	125.51	64.12	189.63	6.00
48	.00	.00	.00	3.00
49	301.17	185.10	486.27	8.00
50	191.52	109.89	301.41	10.00
51	345.32	237.42	582.74	1.00
52	138.13	183.06	321.19	12.00
53	125.86	96.17	222.03	8.00
54	123.54	333.00	456.54	3.00
55	133.14	80.23	213.37	14.00
56	86.82	154.69	241.51	13.00
57	164.36	184.85	349.21	15.00
58	168.90	121.14	290.04	13.00
59	176.88	265.36	442.24	6.00
60	103.77	370.75	474.52	4.00
61	97.17	240.79	337.96	11.00
62	185.54	151.13	336.67	13.00
63	89.63	182.48	272.11	11.00
64	92.97	178.84	271.81	12.00
65	128.32	257.19	385.51	10.00
66	94.67	74.98	169.65	14.00
67	.	.	.	.
68	.	.	.	.
69	.	.	.	.
70	.	.	.	.
71	.	.	.	.
72	.	.	.	.
73	.	.	.	.
74	.	.	.	.
75	.	.	.	.
76	.	.	.	.

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	STMPol	SFEPol	STENPol	STEFPol
39	101.16	4.00	39.10	198.87
40	102.41	5.00	44.45	108.95
41	178.56	4.00	51.59	101.07
42	80.84	6.00	29.61	38.17
43	124.00	7.00	121.51	97.17
44	129.66	5.00	27.48	36.38
45	120.68	3.00	3.73	413.51
46	131.06	5.00	31.04	72.84
47	112.42	3.00	34.37	264.01
48	98.83	3.00	.00	502.53
49	70.69	3.00	12.59	30.69
50	82.16	5.00	80.82	135.83
51	17.53	.00	.00	.00
52	137.40	4.00	27.21	114.36
53	83.48	4.00	17.17	278.37
54	79.60	2.00	8.65	55.52
55	128.75	7.00	34.98	224.86
56	108.34	6.00	61.44	188.91
57	139.49	6.00	15.26	96.10
58	152.34	6.00	20.04	137.82
59	77.63	2.00	46.07	34.28
60	29.90	2.00	26.45	69.39
61	117.01	5.00	16.31	128.98
62	123.89	5.00	54.99	84.56
63	102.17	6.00	75.75	184.45
64	101.22	6.00	9.10	218.11
65	92.87	4.00	48.06	73.79
66	205.95	7.00	23.09	201.53
67	.	.	.	.
68	.	.	.	.
69	.	.	.	.
70	.	.	.	.
71	.	.	.	.
72	.	.	.	.
73	.	.	.	.
74	.	.	.	.
75	.	.	.	.
76	.	.	.	.

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	SCETTPol	NFS1Pol	NTS1NPol	NTS1FPol	NCS1TTPol
39	237.97	2.00	20.32	82.88	103.20
40	153.40	.00	.00	.00	.00
41	152.66	1.00	14.83	105.23	120.06
42	67.78	2.00	7.82	180.83	188.65
43	218.68	2.00	1.31	13.20	14.51
44	63.86	4.00	66.90	91.44	158.34
45	417.24	4.00	47.14	41.80	88.94
46	103.88	2.00	69.22	70.41	139.63
47	298.38	2.00	20.38	135.40	155.78
48	502.53	1.00	13.19	.00	13.19
49	43.28	.00	.00	.00	.00
50	216.65	3.00	44.21	163.90	208.11
51	.00	2.00	.00	276.70	276.70
52	141.57	4.00	26.31	25.53	51.84
53	295.54	2.00	15.64	43.49	59.13
54	64.17	1.00	8.08	258.72	266.80
55	259.84	2.00	7.70	36.18	43.88
56	250.35	4.00	20.88	166.94	187.82
57	111.36	4.00	46.23	62.52	108.75
58	157.86	2.00	.00	30.77	30.77
59	80.35	.00	.00	.00	.00
60	95.84	2.00	18.13	121.91	140.04
61	145.29	2.00	38.10	69.40	107.50
62	139.55	1.00	.00	20.93	20.93
63	260.20	2.00	22.66	38.98	61.64
64	227.21	2.00	14.92	4.95	19.87
65	121.85	.00	.00	.00	.00
66	224.62	2.00	4.71	48.04	52.75
67	.	.	.	.	.
68	.	.	.	.	.
69	.	.	.	.	.
70	.	.	.	.	.
71	.	.	.	.	.
72	.	.	.	.	.
73	.	.	.	.	.
74	.	.	.	.	.
75	.	.	.	.	.
76	.	.	.	.	.

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	NFMPol	NTMPol	NFS2Pol	NTS2NPol
39	5.00	46.95	3.00	51.82
40	3.00	21.01	3.00	93.12
41	3.00	46.35	2.00	27.60
42	5.00	49.11	3.00	48.18
43	7.00	71.32	5.00	97.40
44	5.00	95.98	2.00	40.49
45	6.00	86.29	3.00	61.17
46	5.00	45.69	3.00	95.05
47	3.00	15.17	1.00	84.61
48	4.00	63.49	3.00	125.86
49	2.00	21.06	2.00	129.08
50	5.00	35.97	2.00	43.66
51	2.00	24.08	.00	.00
52	11.00	77.91	6.00	72.55
53	6.00	44.83	4.00	68.90
54	1.00	33.59	.00	.00
55	7.00	164.42	5.00	31.33
56	7.00	34.89	3.00	37.92
57	7.00	56.87	4.00	59.19
58	6.00	39.80	4.00	145.93
59	1.00	15.27	1.00	34.49
60	3.00	28.94	1.00	50.43
61	4.00	53.74	2.00	45.52
62	2.00	9.61	1.00	138.04
63	6.00	48.34	4.00	66.69
64	6.00	55.94	3.00	58.73
65	1.00	5.96	1.00	14.56
66	7.00	68.02	4.00	36.43
67	.	.	.	.
68	.	.	.	.
69	.	.	.	.
70	.	.	.	.
71	.	.	.	.
72	.	.	.	.
73	.	.	.	.
74	.	.	.	.
75	.	.	.	.
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Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	NTS2FPol	NCS2TTPol	HFLPoT	HTLPoT
39	98.29	150.11	1.00	30.86
40	186.20	279.32	2.00	32.01
41	106.26	133.86	1.00	12.28
42	14.34	62.52	.00	.00
43	117.04	214.44	3.00	74.10
44	5.48	45.97	4.00	105.17
45	63.83	125.00	1.00	21.28
46	20.05	115.10	3.00	107.17
47	44.95	129.56	3.00	83.40
48	97.80	223.66	1.00	32.43
49	150.18	279.26	2.00	68.66
50	12.78	56.44	2.00	113.01
51	.00	.00	.00	.00
52	98.17	170.72	3.00	109.89
53	127.42	196.32	4.00	115.65
54	.00	.00	5.00	119.20
55	60.60	91.93	2.00	118.87
56	39.68	77.60	2.00	25.77
57	75.44	134.63	1.00	64.97
58	83.87	229.80	3.00	41.91
59	250.60	285.09	1.00	295.49
60	80.88	131.31	1.00	12.94
61	93.51	139.03	1.00	232.69
62	99.22	237.26	3.00	220.62
63	123.62	190.31	5.00	113.10
64	165.80	224.53	5.00	71.70
65	279.93	294.49	4.00	125.22
66	143.07	179.50	3.00	150.58
67	.	.	.	.
68	.	.	.	.
69	.	.	.	.
70	.	.	.	.
71	.	.	.	.
72	.	.	.	.
73	.	.	.	.
74	.	.	.	.
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76	.	.	.	.

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	HFMPoT	HTMPoT	HFRPoT	HTRPoT
39	3.00	25.58	2.00	243.44
40	5.00	41.95	3.00	226.27
41	3.00	29.59	2.00	258.55
42	2.00	31.02	2.00	269.46
43	7.00	47.70	4.00	178.46
44	7.00	54.97	3.00	140.16
45	5.00	41.69	3.00	237.39
46	7.00	63.92	3.00	129.19
47	7.00	74.82	4.00	142.14
48	3.00	34.88	2.00	233.09
49	5.00	53.22	3.00	178.45
50	5.00	66.95	2.00	120.57
51	1.00	29.30	1.00	271.18
52	8.00	66.26	5.00	124.18
53	7.00	43.69	3.00	140.96
54	8.00	64.39	3.00	116.71
55	5.00	30.29	3.00	151.15
56	6.00	56.03	4.00	218.44
57	3.00	36.70	2.00	198.65
58	6.00	105.62	3.00	152.79
59	1.00	4.93	.00	.00
60	2.00	12.94	1.00	274.52
61	2.00	22.23	1.00	45.49
62	5.00	33.65	2.00	46.09
63	9.00	63.84	4.00	123.16
64	12.00	92.84	7.00	135.71
65	7.00	43.82	3.00	131.24
66	6.00	28.84	3.00	120.88
67	.	.	.	.
68	.	.	.	.
69	.	.	.	.
70	.	.	.	.
71	.	.	.	.
72	.	.	.	.
73	.	.	.	.
74	.	.	.	.
75	.	.	.	.
76	.	.	.	.

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	SFS1PoT	STS1NPoT	STS1FPoT	SCS1TTPoT
39	6.00	141.06	202.74	343.80
40	10.00	239.21	175.12	414.33
41	6.00	258.91	73.61	332.52
42	1.00	87.55	17.58	105.13
43	7.00	172.47	148.22	320.69
44	9.00	238.72	189.01	427.73
45	5.00	99.10	103.30	202.40
46	6.00	233.59	173.80	407.39
47	3.00	192.34	73.00	265.34
48	.00	.00	.00	.00
49	3.00	211.65	169.22	380.87
50	3.00	132.11	84.35	216.46
51	.00	.00	.00	.00
52	7.00	160.85	140.21	301.06
53	9.00	113.07	155.42	268.49
54	5.00	247.46	156.51	403.97
55	4.00	215.03	107.25	322.28
56	6.00	100.33	216.96	317.29
57	6.00	97.86	252.39	350.25
58	5.00	256.32	231.64	487.96
59	5.00	145.97	234.79	380.76
60	5.00	118.75	70.78	189.53
61	5.00	124.77	74.43	199.20
62	4.00	162.29	113.96	276.25
63	8.00	208.50	248.73	457.23
64	7.00	107.66	201.90	309.56
65	6.00	171.87	164.94	336.81
66	10.00	126.03	111.87	237.90
67	.	.	.	.
68	.	.	.	.
69	.	.	.	.
70	.	.	.	.
71	.	.	.	.
72	.	.	.	.
73	.	.	.	.
74	.	.	.	.
75	.	.	.	.
76	.	.	.	.

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	SFMPoT	STMPoT	SFEPoT	STENPoT
39	10.00	113.85	3.00	33.07
40	14.00	98.90	4.00	33.47
41	11.00	105.29	5.00	94.06
42	2.00	23.09	1.00	41.67
43	12.00	92.27	5.00	84.35
44	13.00	99.28	5.00	24.95
45	10.00	109.61	4.00	116.38
46	11.00	114.49	4.00	35.88
47	7.00	134.13	3.00	88.80
48	2.00	17.19	2.00	19.28
49	5.00	141.13	2.00	30.69
50	6.00	135.70	2.00	74.70
51	1.00	5.65	1.00	36.16
52	12.00	99.12	5.00	67.24
53	17.00	158.48	8.00	54.21
54	8.00	97.87	3.00	23.45
55	9.00	85.35	5.00	69.64
56	12.00	119.41	6.00	37.29
57	10.00	111.55	4.00	5.76
58	9.00	73.51	3.00	20.35
59	8.00	49.36	3.00	25.69
60	10.00	88.92	5.00	43.90
61	10.00	80.12	5.00	61.99
62	6.00	97.29	3.00	38.09
63	13.00	84.32	5.00	21.72
64	14.00	138.75	6.00	33.47
65	11.00	113.95	5.00	73.85
66	20.00	125.91	10.00	38.81
67	.	.	.	.
68	.	.	.	.
69	.	.	.	.
70	.	.	.	.
71	.	.	.	.
72	.	.	.	.
73	.	.	.	.
74	.	.	.	.
75	.	.	.	.
76	.	.	.	.

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	STEFPoT	SCETTPoT	NFS1PoT	NTS1NPoT
39	109.57	142.64	3.00	87.02
40	53.47	86.94	4.00	16.71
41	65.34	159.40	3.00	87.10
42	430.39	472.06	.00	.00
43	103.80	188.15	2.00	55.46
44	47.97	72.92	4.00	48.01
45	171.30	287.68	2.00	21.88
46	46.34	82.22	2.00	46.56
47	111.96	200.76	2.00	61.87
48	583.25	602.53	2.00	79.55
49	48.16	78.85	1.00	23.27
50	189.66	264.36	2.00	20.75
51	558.56	594.72	.00	.00
52	132.68	199.92	3.00	21.43
53	119.53	173.74	3.00	17.99
54	74.95	98.40	1.00	.00
55	122.90	192.54	.00	.00
56	126.11	163.40	1.00	.00
57	132.58	138.34	3.00	66.71
58	18.36	38.71	2.00	32.93
59	144.40	170.09	.00	.00
60	321.78	365.68	2.00	13.89
61	258.96	320.95	3.00	43.92
62	207.10	245.19	1.00	.00
63	43.33	65.05	4.00	20.90
64	113.03	146.50	4.00	10.36
65	75.31	149.16	3.00	.00
66	194.21	233.02	3.00	30.22
67	.	.	.	.
68	.	.	.	.
69	.	.	.	.
70	.	.	.	.
71	.	.	.	.
72	.	.	.	.
73	.	.	.	.
74	.	.	.	.
75	.	.	.	.
76	.	.	.	.

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	NTS1FPoT	NCS1TTPoT	NFMPoT	NTMPoT
39	27.60	114.62	6.00	49.16
40	14.44	31.15	9.00	79.83
41	22.36	109.46	8.00	62.06
42	.00	.00	1.00	8.95
43	104.29	159.75	5.00	39.45
44	56.41	104.42	9.00	49.54
45	11.98	33.86	8.00	76.55
46	65.24	111.80	5.00	24.93
47	99.04	160.91	4.00	23.93
48	52.55	132.10	4.00	18.76
49	267.21	290.48	1.00	9.90
50	75.67	96.42	4.00	37.76
51	.00	.00	1.00	2.29
52	15.34	36.77	7.00	75.97
53	140.64	158.63	6.00	51.55
54	3.66	3.66	3.00	12.11
55	.00	.00	1.00	4.76
56	15.94	15.94	3.00	22.54
57	71.43	138.14	7.00	32.13
58	46.88	79.81	5.00	122.79
59	.00	.00	1.00	5.66
60	215.74	229.63	3.00	21.25
61	25.36	69.28	6.00	82.12
62	7.76	7.76	3.00	43.05
63	73.32	94.22	8.00	54.53
64	64.50	74.86	11.00	90.47
65	200.17	200.17	5.00	23.74
66	46.51	76.73	9.00	61.54
67	.	.	.	.
68	.	.	.	.
69	.	.	.	.
70	.	.	.	.
71	.	.	.	.
72	.	.	.	.
73	.	.	.	.
74	.	.	.	.
75	.	.	.	.
76	.	.	.	.

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	NFS2PoT	NTS2NPoT	NTS2FPoT	NCS2TTPoT
39	3.00	56.57	79.93	136.50
40	5.00	120.80	68.46	189.26
41	5.00	102.76	26.06	128.82
42	1.00	79.79	211.87	291.66
43	3.00	74.86	26.78	101.64
44	5.00	99.91	46.50	146.41
45	6.00	140.39	52.06	192.45
46	4.00	150.72	12.92	163.64
47	2.00	89.12	32.29	121.41
48	2.00	55.40	93.87	149.27
49	.00	.00	.00	.00
50	2.00	120.66	45.43	166.09
51	1.00	163.02	128.00	291.02
52	4.00	121.54	65.90	187.44
53	3.00	78.51	11.77	90.28
54	3.00	84.97	199.55	284.52
55	1.00	80.38	215.15	295.53
56	2.00	59.95	201.91	261.86
57	4.00	62.57	67.40	129.97
58	3.00	65.68	33.13	98.81
59	1.00	81.68	213.01	294.69
60	1.00	26.34	23.29	49.63
61	3.00	82.12	87.44	169.56
62	2.00	116.71	132.83	249.54
63	4.00	75.54	75.97	151.51
64	7.00	49.07	86.29	135.36
65	1.00	53.24	23.24	76.48
66	5.00	92.53	69.45	161.98
67	.	.	.	.
68	.	.	.	.
69	.	.	.	.
70	.	.	.	.
71	.	.	.	.
72	.	.	.	.
73	.	.	.	.
74	.	.	.	.
75	.	.	.	.
76	.	.	.	.

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	TLCD1PrI	TLCD2PrI	TLCD3PrI	TLCD4PrI
39	55.50	53.04	54.28	50.88
40	41.05	40.36	39.99	36.70
41	39.71	42.43	37.85	39.46
42	28.10	32.13	39.33	27.50
43	41.85	41.68	39.79	40.82
44	42.61	37.70	42.06	39.59
45	31.34	31.59	32.04	34.65
46	51.88	51.34	52.10	40.31
47	41.11	41.13	48.73	38.47
48	42.27	49.00	51.97	45.42
49	51.83	46.25	57.65	49.74
50	39.48	41.21	40.06	38.21
51	40.32	86.15	41.69	43.92
52	44.71	46.43	51.70	52.00
53	43.49	44.82	46.99	44.94
54	44.67	52.37	49.08	50.02
55	56.92	54.60	55.37	51.71
56	57.76	56.65	54.58	45.64
57	37.09	36.26	42.53	36.60
58	42.35	43.80	52.73	46.77
59	41.58	39.98	47.85	44.80
60	40.54	44.02	45.84	47.19
61	51.92	42.31	44.48	40.88
62	46.74	52.54	73.21	50.16
63	34.64	38.86	42.22	34.26
64	35.15	39.13	48.40	40.75
65	62.67	64.95	59.72	46.29
66	34.69	51.51	54.06	39.51
67	.	.	.	.
68	.	.	.	.
69	.	.	.	.
70	.	.	.	.
71	.	.	.	.
72	.	.	.	.
73	.	.	.	.
74	.	.	.	.
75	.	.	.	.
76	.	.	.	.

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	AVG_TLC_Pr	SPD1Prl	SPD2Prl	SPD3Prl
39	53.43	88.00	97.00	99.00
40	39.53	74.00	84.00	79.00
41	39.86	77.00	88.00	95.00
42	31.77	74.00	91.00	96.00
43	41.04	59.00	89.00	95.00
44	40.49	92.00	92.00	98.00
45	32.41	62.00	94.00	98.00
46	48.91	92.00	89.00	91.00
47	42.36	50.00	37.00	50.00
48	47.17	91.00	92.00	90.00
49	51.37	90.00	90.00	83.00
50	39.74	76.00	59.00	23.00
51	53.02	43.00	30.00	39.00
52	48.71	77.00	63.00	68.00
53	45.06	87.00	63.00	83.00
54	49.04	75.00	84.00	85.00
55	54.65	85.00	91.00	85.00
56	53.66	84.00	91.00	85.00
57	38.12	71.00	86.00	85.00
58	46.41	81.00	80.00	86.00
59	43.55	60.00	46.00	85.00
60	44.40	56.00	10.00	36.00
61	44.90	68.00	62.00	78.00
62	55.66	35.00	62.00	64.00
63	37.50	77.00	78.00	56.00
64	40.86	81.00	21.00	75.00
65	58.41	19.00	39.00	23.00
66	44.94	27.00	58.00	63.00
67	.	.	.	.
68	.	.	.	.
69	.	.	.	.
70	.	.	.	.
71	.	.	.	.
72	.	.	.	.
73	.	.	.	.
74	.	.	.	.
75	.	.	.	.
76	.	.	.	.

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	SPD4Prl	AVG_SP_Prl	TLCD1Pol	TLCD2Pol
39	85.00	92.25	44.70	49.75
40	81.00	79.50	34.64	35.32
41	85.00	86.25	36.04	33.04
42	81.00	85.50	44.64	26.44
43	88.00	82.75	40.37	49.52
44	93.00	93.75	69.97	45.05
45	97.00	87.75	30.96	34.13
46	89.00	90.25	39.98	33.55
47	43.00	45.00	27.31	30.07
48	90.00	90.75	36.44	38.97
49	92.00	88.75	33.50	35.05
50	11.00	42.25	96.95	33.06
51	68.00	45.00	24.27	28.77
52	42.00	62.50	30.80	33.85
53	45.00	69.50	42.17	40.71
54	82.00	81.50	135.82	35.24
55	90.00	87.75	36.89	38.64
56	78.00	84.50	33.39	36.03
57	86.00	82.00	29.46	30.10
58	73.00	80.00	35.28	32.93
59	49.00	60.00	36.29	35.53
60	18.00	30.00	33.13	33.72
61	80.00	72.00	34.03	29.72
62	56.00	54.25	38.46	48.91
63	61.00	68.00	26.91	27.47
64	30.00	51.75	31.27	31.83
65	69.00	37.50	126.50	39.10
66	73.00	55.25	34.72	39.61
67	.	.	.	.
68	.	.	.	.
69	.	.	.	.
70	.	.	.	.
71	.	.	.	.
72	.	.	.	.
73	.	.	.	.
74	.	.	.	.
75	.	.	.	.
76	.	.	.	.

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	TLCD3Pol	TLCD4Pol	AVG_TLC_Pol	SPD1Pol
39	52.53	48.09	48.77	98.00
40	26.91	31.88	32.19	91.00
41	36.12	36.12	35.33	93.00
42	28.10	28.79	31.99	95.00
43	44.03	46.34	45.07	94.00
44	38.77	31.99	46.45	74.00
45	31.50	30.71	31.83	91.00
46	34.95	32.73	35.30	4.00
47	25.46	28.60	27.86	2.00
48	37.78	40.15	38.34	60.00
49	35.21	35.31	34.77	.00
50	30.27	33.35	48.41	79.00
51	24.48	29.41	26.73	63.00
52	32.37	35.88	33.23	16.00
53	39.81	44.00	41.67	89.00
54	28.92	29.52	57.38	28.00
55	33.78	33.98	35.82	2.00
56	40.17	40.56	37.54	3.00
57	30.70	31.50	30.44	1.00
58	38.70	38.05	36.24	3.00
59	37.72	38.05	36.90	1.00
60	33.99	39.23	35.02	50.00
61	33.75	34.30	32.95	1.00
62	64.50	44.33	49.05	49.00
63	29.73	29.56	28.42	1.00
64	34.31	36.31	33.43	1.00
65	34.77	37.71	59.52	78.00
66	38.02	39.39	37.94	85.00
67	.	.	.	.
68	.	.	.	.
69	.	.	.	.
70	.	.	.	.
71	.	.	.	.
72	.	.	.	.
73	.	.	.	.
74	.	.	.	.
75	.	.	.	.
76	.	.	.	.

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	SPD2Pol	SPD3Pol	SPD4Pol	AVG_SP_Pol
39	97.00	95.00	94.00	96.00
40	76.00	85.00	75.00	81.75
41	78.00	97.00	83.00	87.75
42	94.00	62.00	36.00	71.75
43	96.00	97.00	85.00	93.00
44	96.00	97.00	84.00	87.75
45	96.00	97.00	96.00	95.00
46	5.00	5.00	4.00	4.50
47	2.00	1.00	1.00	1.50
48	22.00	13.00	5.00	25.00
49	.00	.00	.00	.00
50	2.00	2.00	.00	20.75
51	19.00	8.00	2.00	23.00
52	2.00	2.00	2.00	5.50
53	94.00	96.00	79.00	89.50
54	31.00	35.00	1.00	23.75
55	1.00	1.00	1.00	1.25
56	1.00	1.00	.00	1.25
57	1.00	.00	.00	.50
58	34.00	36.00	5.00	19.50
59	.00	.00	.00	.25
60	70.00	68.00	37.00	56.25
61	.00	.00	.00	.25
62	59.00	61.00	45.00	53.50
63	.00	.00	.00	.25
64	.00	.00	.00	.25
65	10.00	13.00	9.00	27.50
66	82.00	87.00	76.00	82.50
67	.	.	.	.
68	.	.	.	.
69	.	.	.	.
70	.	.	.	.
71	.	.	.	.
72	.	.	.	.
73	.	.	.	.
74	.	.	.	.
75	.	.	.	.
76	.	.	.	.

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	TLCD1PoT	TLCD2PoT	TLCD3PoT	TLCD4PoT
39	48.92	40.67	39.64	39.24
40	34.71	30.92	31.05	30.76
41	38.32	36.81	30.70	34.73
42	30.61	27.57	23.66	26.48
43	32.89	28.08	31.92	28.90
44	29.75	24.74	21.60	26.54
45	27.79	27.02	29.36	23.67
46	27.50	31.73	30.30	40.91
47	25.91	32.30	29.42	30.38
48	54.73	40.37	48.03	46.41
49	32.90	39.62	35.64	44.33
50	32.70	33.41	34.21	34.81
51	20.01	21.02	21.64	23.04
52	29.09	29.94	29.32	33.43
53	50.26	43.43	41.33	46.10
54	34.71	33.67	26.65	33.25
55	32.73	28.22	29.06	33.20
56	37.25	38.98	39.98	39.51
57	30.76	28.02	29.51	27.32
58	35.50	36.32	36.61	40.26
59	29.89	30.78	29.64	31.70
60	55.61	33.04	32.39	28.19
61	28.13	30.81	30.51	27.56
62	51.03	38.22	45.64	39.18
63	26.62	32.81	26.92	33.28
64	30.06	28.58	27.82	27.78
65	122.01	38.65	37.18	43.19
66	56.42	36.13	35.29	37.80
67	.	.	.	.
68	.	.	.	.
69	.	.	.	.
70	.	.	.	.
71	.	.	.	.
72	.	.	.	.
73	.	.	.	.
74	.	.	.	.
75	.	.	.	.
76	.	.	.	.

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	AVG_TLC_PoT	SPD1PoT	SPD2PoT	SPD3PoT
39	42.12	12.00	2.00	2.00
40	31.86	3.00	2.00	2.00
41	35.14	85.00	88.00	44.00
42	27.08	15.00	.00	.00
43	30.45	8.00	1.00	.00
44	25.66	13.00	21.00	8.00
45	26.96	67.00	92.00	65.00
46	32.61	6.00	5.00	37.00
47	29.50	2.00	2.00	2.00
48	47.39	97.00	88.00	96.00
49	38.12	.00	.00	.00
50	33.78	2.00	1.00	.00
51	21.43	4.00	.00	.00
52	30.45	3.00	1.00	2.00
53	45.28	92.00	84.00	77.00
54	32.07	1.00	.00	.00
55	30.80	3.00	.00	1.00
56	38.93	4.00	.00	.00
57	28.90	1.00	.00	.00
58	37.17	2.00	1.00	.00
59	30.50	1.00	.00	.00
60	37.31	97.00	90.00	80.00
61	29.25	1.00	.00	.00
62	43.52	70.00	46.00	30.00
63	29.91	2.00	.00	.00
64	28.56	1.00	.00	.00
65	60.26	80.00	46.00	26.00
66	41.41	90.00	87.00	91.00
67	.	.	.	.
68	.	.	.	.
69	.	.	.	.
70	.	.	.	.
71	.	.	.	.
72	.	.	.	.
73	.	.	.	.
74	.	.	.	.
75	.	.	.	.
76	.	.	.	.

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	SPD4PoT	AVG_SP_PoT	Bma	BV
39	2.00	4.50	1.94	1.70
40	.00	1.75	1.90	1.44
41	71.00	72.00	1.98	1.61
42	.00	3.75	1.91	1.49
43	1.00	2.50	1.79	1.65
44	5.00	11.75	1.95	1.60
45	9.00	58.25	1.75	1.57
46	2.00	12.50	1.62	3.47
47	1.00	1.75	1.68	2.93
48	92.00	93.25	2.08	2.95
49	.00	.00	1.85	1.89
50	.00	.75	1.76	3.24
51	.00	1.00	1.92	2.20
52	.00	1.50	1.78	1.64
53	93.00	86.50	1.88	1.92
54	.00	.25	1.97	1.74
55	.00	1.00	1.96	1.92
56	.00	1.00	1.70	1.75
57	.00	.25	1.89	2.17
58	.00	.75	1.71	2.17
59	.00	.25	1.99	2.03
60	10.00	69.25	1.98	2.22
61	.00	.25	1.96	2.22
62	24.00	42.50	1.97	2.00
63	.00	.50	2.01	1.95
64	.00	.25	1.92	2.04
65	7.00	39.75	2.01	1.93
66	85.00	88.25	1.94	1.82
67	.	.	.	.
68	.	.	.	.
69	.	.	.	.
70	.	.	.	.
71	.	.	.	.
72	.	.	.	.
73	.	.	.	.
74	.	.	.	.
75	.	.	.	.
76	.	.	.	.

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	APL	LW	SIH	HR
39	17.74	12.39	7.74	.0748
40	17.11	11.96	7.04	.0838
41	17.31	12.27	7.59	.0624
42	17.02	12.60	6.96	.0838
43	17.09	12.14	7.93	.0942
44	17.86	12.19	7.36	.0861
45	17.23	12.44	7.33	.0500
46	21.95	15.49	10.21	.0634
47	21.84	15.53	8.64	.0841
48	20.56	16.44	8.72	.0761
49	19.57	13.29	7.26	.0801
50	23.99	19.21	7.04	.0801
51	19.58	13.80	8.13	.0934
52	19.29	9.29	9.14	.0777
53	19.67	13.02	7.50	.0915
54	19.70	12.21	7.22	.0663
55	19.38	13.63	7.27	.0761
56	19.10	12.25	7.50	.0751
57	18.39	13.86	8.52	.0809
58	19.25	12.89	8.74	.0581
59	19.53	13.69	7.58	.0767
60	18.93	13.25	8.86	.0991
61	19.63	13.03	8.69	.0794
62	19.18	13.34	7.82	.0681
63	19.23	13.01	7.78	.0666
64	18.77	13.13	8.26	.0571
65	18.45	13.77	7.60	.0576
66	18.44	13.26	7.43	.0721
67	.	.	.	.
68	.	.	.	.
69	.	.	.	.
70	.	.	.	.
71	.	.	.	.
72	.	.	.	.
73	.	.	.	.
74	.	.	.	.
75	.	.	.	.
76	.	.	.	.

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	HL	PR	PL	SR
39	.0843	.1182	.1277	.0391
40	.0841	.1499	.1258	.0246
41	.0951	.1278	.1002	.0455
42	.0839	.1240	.1780	.0428
43	.0815	.1050	.1243	.0531
44	.1232	.1444	.1075	.0469
45	.0650	.1280	.1220	.0300
46	.0824	.0844	.0934	.0171
47	.0702	.0600	.0851	.0121
48	.0781	.1491	.1575	.0081
49	.0631	.1201	.1011	.0161
50	.0701	.0671	.0921	.0171
51	.0731	.1531	.1043	.0311
52	.0739	.1083	.1154	.0106
53	.0721	.1181	.1022	.0138
54	.0821	.1281	.1397	.0185
55	.0771	.0791	.0841	.0091
56	.0931	.0760	.0531	.0201
57	.0631	.1031	.1092	.0151
58	.0731	.1201	.0921	.0041
59	.0606	.0921	.0961	.0025
60	.0561	.1521	.1451	.0121
61	.0651	.1001	.0721	.0281
62	.0891	.0981	.0951	.0212
63	.0791	.1091	.0931	.0081
64	.0851	.0916	.0990	.0111
65	.0801	.1301	.1211	.0251
66	.0891	.1231	.1521	.0211
67	.	.	.	.
68	.	.	.	.
69	.	.	.	.
70	.	.	.	.
71	.	.	.	.
72	.	.	.	.
73	.	.	.	.
74	.	.	.	.
75	.	.	.	.
76	.	.	.	.

Animal Work Raw Data Set incl Biochemical tests 23-11-2023.sav

	SL	DA_PFC	DA_VTA	ACh_PFC	ACh_Hippo
39	.0476	.309	.299	2.444	1.126
40	.0463	.355	.281	2.538	.980
41	.0467	.355	.280	2.446	1.015
42	.0469	.340	.270	2.516	1.327
43	.0553	.325	.303	2.386	1.203
44	.0381	.405	.325	2.563	.903
45	.0430	.270	.269	2.389	1.123
46	.0111	.291	.306	2.172	1.220
47	.0138	.291	.320	2.601	1.193
48	.0131	.327	.311	2.445	1.090
49	.0151	.324	.345	2.560	1.346
50	.0241	.308	.319	2.444	1.232
51	.0259	.326	.354	2.390	1.116
52	.0154	.293	.291	1.668	.438
53	.0268	.288	.304	2.396	1.074
54	.0261	.323	.309	2.506	1.026
55	.0171	.344	.320	2.524	1.178
56	.0081	.339	.339	2.492	.975
57	.0191	.448	.297	2.457	.099
58	.0321	.460	.316	2.298	.843
59	.0131	.504	.344	2.543	.928
60	.0211	.481	.307	2.447	1.110
61	.0191	.509	.318	2.605	1.002
62	.0283	.423	.274	2.443	.772
63	.0207	.448	.327	2.421	.924
64	.0161	.492	.337	2.368	.895
65	.0301	.518	.304	2.086	.808
66	.0311	.520	.346	2.510	.932
67	.	.	.	.	.
68	.	.	.	.	.
69	.	.	.	.	.
70	.	.	.	.	.
71	.	.	.	.	.
72	.	.	.	.	.
73	.	.	.	.	.
74	.	.	.	.	.
75	.	.	.	.	.
76	.	.	.	.	.