Evaluation of indigenous medicinal plants with potential antidepressant effects

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LIST OF OUTPUTS

Conference contribution

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Bonokwane MB, Lekhooa M, Struwig M and Aremu AO. *In vitro* antidepressant screening and phenolic content of three plants used traditionally to manage depression. 48th Annual South African Association of Botanists Conference. 17-20 January 2023. Hosted by the University of Limpopo, South Africa.

Research publication

Bonokwane MB, Lekhooa M, Struwig M and Aremu AO (2022) Antidepressant effects of South African plants: An appraisal of ethnobotanical surveys, ethnopharmacological and phytochemical studies. Frontiers in Pharmacology 13:895286. doi: 10.3389/fphar.2022.895286. (Published 29 June 2022).

ABSTRACT

Major depressive disorder (MDD) is a common mental health condition that represents one of the foremost causes of disease burden worldwide, including in South Africa. The search for safe and potent natural-based treatment for depression is receiving renewed interest given the numerous side-effects associated with existing antidepressant drugs. In South Africa, the use of plants to manage depression is fairly documented among different ethnic groups.

The aim of this study was to review South African medicinal plants used traditionally to manage depression-like ailments, to estimate the total phenolic content and evaluate the *in vitro* antidepressant-like effects of these plants. A literature review of existing ethnobotanical, ethnopharmacological and phytochemical studies was conducted from which *Artemisia afra* Jacq. ex Willd., *Adenia gummifera* (Harv.) Harms and *Olea woodiana* Knobl. were selected for evaluation. The plant materials were extracted using three solvents of increasing polarities (hexane, acetone and water) to evaluate the effects of solvent polarity on extraction yield, total phenolic content and biological activity. The total phenolic content of 12 extracts was estimated using the Folin-Ciocalteu's method. Thereafter, the antidepressant potential of each extract was evaluated using the serotonin transporter (SERT) and the adenosine A_1R & $A_{2A}R$ radioligand binding assays.

The systematic review of twenty eligible ethnobotanical publications identified 186 indigenous South African plants from 63 families used traditionally to manage depression and related ailments. Only 27 of these plants were previously screened for antidepressant activity using various *in vitro* and *in vivo* tests. Phytochemical investigation on 9 plants revealed 24 compounds with antidepressant-like effects. A significant portion (≈85%) of the 186 plants with ethnobotanical records still requires pharmacological studies to assess their potential antidepressant-like effects. In this study, water and acetone extracts exhibited a higher phenolic content than the hexane extracts, suggesting that total phenolic content levels increased significantly with increasing polarity of the solvents. The acetone extract from *A. afra* leaf exhibited significant affinity to the adenosine A_1 receptor, indicating the potential antidepressant-like effects of this plant, however, this needs to be confirmed in an animal model of depression. None of the other extracts showed any significant affinity in any of the assays conducted.

This study serves as an essential step towards the authentication of the antidepressant-like effects of indigenous medicinal plants and their phytochemicals.

Keywords: Depression, phytotherapy, phenolics, Asteraceae, antidepressants, ethnobotany, psychoactive plants, indigenous knowledge

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$$y = mx+c$$

CHAPTER 1: STUDY INTRODUCTION

1.1 Background

Depression is one of the most widespread and extremely comorbid psychiatric illnesses associated with biochemical, cognitive, behavioural and psychological changes that bring about a negative emotional experience (Saki *et al.*, 2014). It is estimated that at least 1 in 5 people is affected by a mood disorder at least once in their lifetime (Stafford *et al.*, 2009). The monoamine-deficiency theory briefly states that the basis of the pathophysiology of depression is the reduction of the neurotransmitters such as serotonin, dopamine or norepinephrine, which leads to a deficiency of monoaminergic activity in the central nervous system (CNS) and that depression can be treated by drugs that increase these neurotransmitters, thus increasing this activity (Schildkraut, 1965). In South Africa, the use of herbal medicine is common amongst patients with mood disorders, including depression (Sarris *et al.*, 2011). For centuries, psychotropic plants and complementary medicines have been used traditionally to treat mental health disorders and continue to be used in modern societies to treat psychiatric conditions such as depression, epilepsy and other CNS ailments (Casteleijn *et al.*, 2019; Stafford, 2009; Stafford *et al.*, 2007). These plants can induce pharmacological effects such as stimulant, sedative/narcotic, hallucinogenic, euphoria, and change in consciousness. Moreover, their psychoactive effects may cause changes in emotion, perception and/or cognition (Alrashedy & Molina, 2016; Khan *et al.*, 2018; Martins & Brijesh, 2018).

Research into psychotropic plants that may influence the CNS has thrived, with plenty of pre-clinical *in vitro* and *in vivo* evaluations validating the fact that herbal therapies possess an array of biopsychological effects on humans and some animals (Sarris, 2018; Sarris *et al.*, 2011; Wang *et al.*, 2019). The importance of herbal medicine in the treatment of depression has become more established over the previous decade, with a noteworthy development in the scientific understanding of medicinal plants and phytotherapeutic preparations, e.g., *Hypericum perforatum* L. and *Piper methysticum* G. Forst. possess reputable clinical evidence validating the antidepressant activity of these plants (Lee & Bae, 2017; Sarris, 2018). Over 40 South African plants are used traditionally to treat depression-like ailments, and these include *Agapanthus campanulatus* F.M.Leight., *Boophone disticha* Herb., *Mondia whitei* Skeels and *Xysmalobium undulatum* (L.) W.T.Aiton (Pedersen *et al.*, 2008; Stafford *et al.*, 2008). The traditional medicinal usage of *Hypericum perforatum* include treatment of mild depression and it represents an acceptable substitute to conventional antidepressants (Nielsen *et al.*, 2004). Pharmacological studies on *Schinus molle* L., commonly known as peperboom, have reported that it possesses

properties such as sedative and anti-depressive activity (Machado *et al.*, 2007; Taylor *et al.*, 2016).

A key goal of antidepressant research is to find novel therapeutic agents that can advance preceding antidepressants with a speedier onset of antidepressant activity, significant efficacy and lessened side effects (Sandager *et al.*, 2005). Most herbal medicines, including psychotropic plants, often pose fewer risks and side effects compared to conventional antidepressants (Bazrafshan *et al.*, 2020; Khan *et al.*, 2018). Investigating phytochemicals in plants for the effect they have on receptors involved in mental disorders might aid in improving our understanding of these plants (Nielsen *et al.*, 2004). Additionally, the discovery of these phytochemicals may lead to better, newer, and more effective therapeutic agents that possess fewer side effects in the treatment of depression. This evaluation will investigate indigenous South African medicinal plants and their potential use in the treatment of depression, taking a closer look at the traditional use, biochemistry and pharmacology of documented plants.

1.2 Research problem

Depression is among the top three sources of disease burden globally and in South Africa (Mungai & Bayat, 2019; Nglazi *et al.*, 2016). In 2013, the estimated global prevalence of depression increased significantly by 53% from 1990 to reach over 253 million cases (Nglazi *et al.*, 2016). The unpredictable nature of COVID-19 coupled with the loss of personal freedom, fear of infection and growing financial losses had a negative psychological impact on people and the public health measures implemented are deemed a threat to physical and mental well-being (Bueno-Notivol *et al.*, 2021; Huremović, 2019), leading to an increase in the risks of psychopathology such as depression. The global estimated pooled prevalence of depression through the COVID-19 pandemic was 25% in 2020 (Bueno-Notivol *et al.*, 2021). In comparison to the 3.44% pooled prevalence of depression in the general population observed for the year 2017, the global prevalence of depression was seven times higher in 2020 (Bueno-Notivol *et al.*, 2021). It is assumed that depression will be deemed the global disease burden by 2030 based on the serious limitation of existing methods of treatment due to the effectiveness, success and safety of therapy using conventional antidepressants (Abbas *et al.*, 2015; Mungai & Bayat, 2019). Current treatment with conventional antidepressants possesses a high level of homogeneity and other limitations such as recurrence of depressive symptoms (Fathinezhad *et al.*, 2019), relapses (Rajput, 2011), drug discontinuation due to adverse events (Gao *et al.*, 2011), delayed onset of antidepressant effects (Martins & Brijesh, 2018) and nonresponse in other patients. The therapeutic lag observed between the relative immediate effects of antidepressants in increasing monoamine levels in the synapse and the several weeks required to notice any clinical evidence

of the alleviation of depressive symptoms continues to compromise the efficacy of conventional antidepressant agents (Popa-Velea *et al.*, 2015). This has led to an increased need to investigate the potential of medicinal plants as an alternative and/or co-therapy in the treatment of depression and related symptoms.

The biological and cultural diversity of Africa is of importance to its people, and they have for a long period of time been dependent on ancient medicinal systems (Mothibe & Sibanda, 2019; Stafford *et al.*, 2005; Stafford *et al.*, 2009). However, these ancient medicinal systems, together with their traditions, have been poorly documented and there is a need for further evaluation to establish the safety and effectiveness of medicinal plant extracts (Stafford *et al.*, 2008). Although research in the area of herbal psychopharmacology has increased significantly over the past decades, a comprehensive review investigating the application of herbal medicine on models of depression is still scanty (Sarris *et al.*, 2011). Most of the indigenous plants used traditionally to treat depression and related ailments in South Africa remain understudied and pharmacological studies are required to authenticate the acclaimed antidepressant potential of these plants.

1.2.1 Aim and objectives

1.2.1.1 Research aim

The aim of this study is to review ethnobotanical, ethnopharmacological and phytochemical studies on South African medicinal plants used to manage depression-like ailments, to estimate the total phenolic content and evaluate the *in vitro* antidepressant binding affinity of these plants.

1.2.1.2 Research objectives

- To conduct a literature review of South African medicinal plants used against depression
- To estimate the total phenolic content of some medicinal plants
- To evaluate the *in vitro* antidepressant potential of some plants using the SERT binding assay, the adenosine A_1 & A_{2A} receptors radioligand binding assays

1.3 Study design

The current study was divided into 3 phases, namely the literature review, phytochemical analysis and *in vitro* antidepressant binding assays (**Figure 1.1**). The systematic literature review was

conducted to identify medicinal plants used by South African traditional healers to manage depression and related ailments. Phytochemical analysis was done to estimate the total phenolics present in each of the extracts, followed by *in vitro* binding assays to assess their antidepressant potential using the SERT binding assay and the adenosine A_1R & $A_{2A}R$ radioligand binding assay.

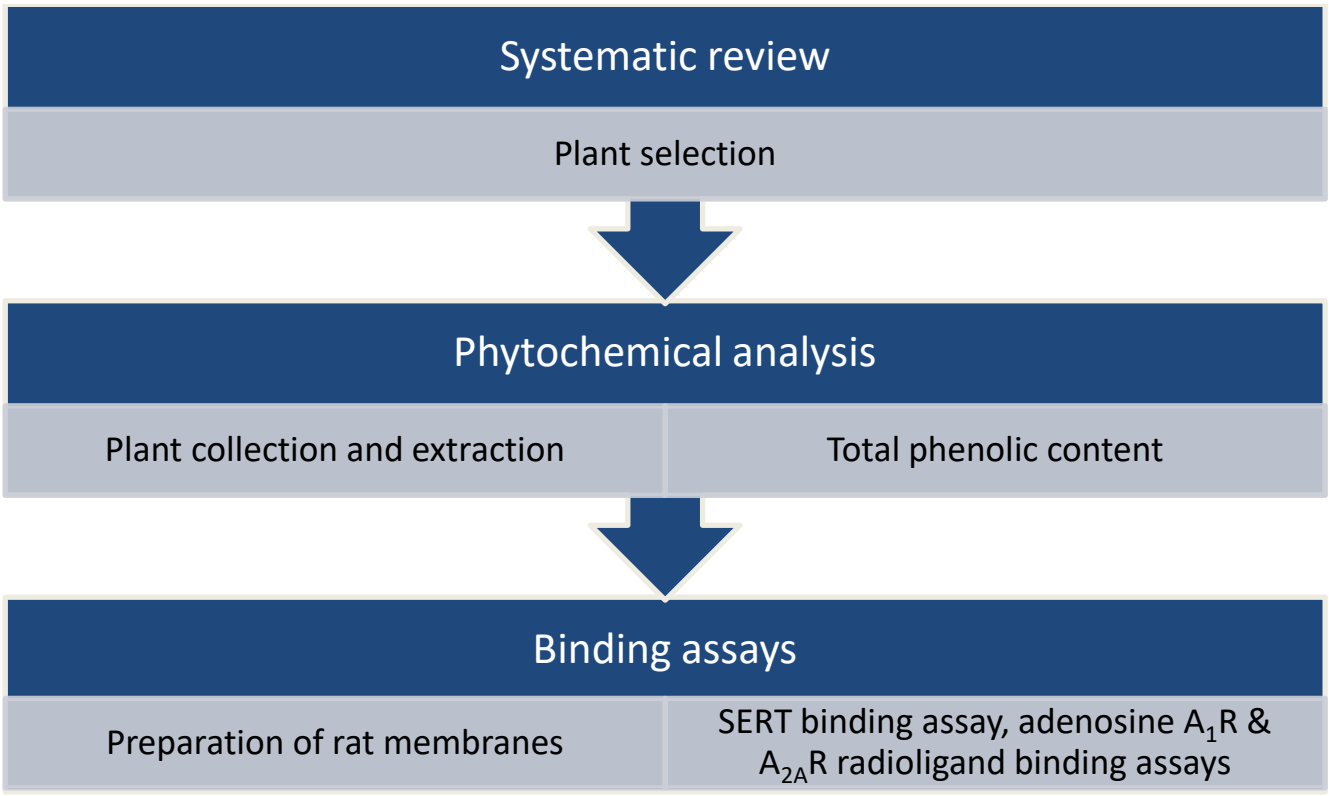


Figure 1.1: **Graphical representation of the study design for the evaluation of indigenous medicinal plants with potential antidepressant effects**

1.4 Ethical considerations

Ethical training and clearance for this study were obtained from the North-West University Animal Care, Health and Safety Research Ethics Committee (NWU-AnimCareREC) based at the Faculty of Health Sciences. Ethical clearance for category 0 projects using animal vertebrates or higher invertebrates for research, but without ethical implications, was applied for and approved by the NWU-AnimCareREC (ethics number-NWU-00758-22-A5; **Appendix 1**). A plant collection permit was obtained from the North West Department of Economic Development, Environment, Conservation and Tourism, Nature Conservation Permit Office (Application ID 38027; **Appendix**

2). The rat brains used for *in vitro* analysis in this study were obtained from redundant Sprague Dawley rats provided by the NWU-PCDDP Vivarium (**Appendix 3**).

1.5 Overview of chapters in dissertation

Chapter 1: This chapter forms the introduction to the current study. Background information on the topic was included in this chapter, together with the problem statement, aim, objectives and study design.

Chapter 2: The systematic literature review includes an in-depth appraisal of the South African medicinal plants used to treat depression, pharmacological assessments conducted into their effectiveness as antidepressants and phytochemical studies reporting the bioactive constituents present in these plants.

Chapter 3: This chapter describes the materials and methods employed in this study. The methodological framework is described for the *in vitro* antidepressant binding assays and phytochemical analysis of the selected plants. The final results are also reported and discussed in this chapter.

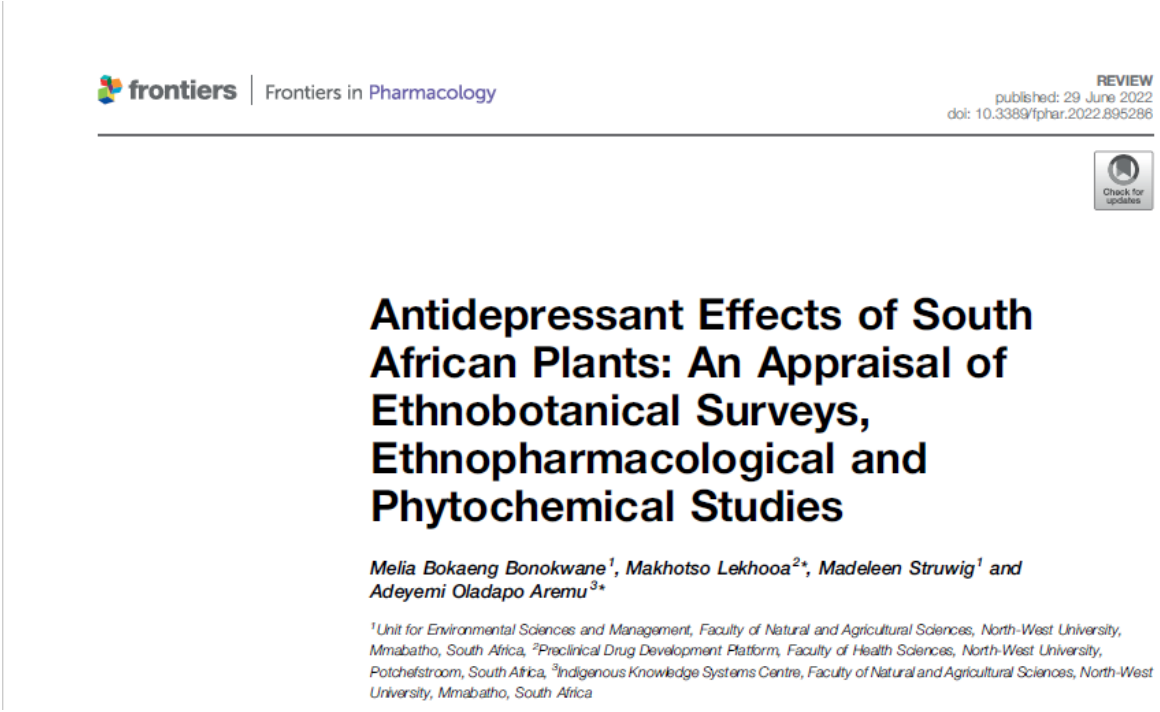
Chapter 4: The conclusion to this study and recommendations for future studies are included in this chapter.

Annexures: This section contains all the supplementary materials not included in the main dissertation. This includes supplementary tables and figures, the plant collection permit, ethics approval of the study and photos of medicinal plants used in this study.

CHAPTER 2: LITERATURE REVIEW

Preface

The contents of this systematic literature review have been published as a review article by Frontiers in Pharmacology, Ethnopharmacology section.



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Antidepressant Effects of South African Plants: An Appraisal of Ethnobotanical Surveys, Ethnopharmacological and Phytochemical Studies

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Abstract

Globally, the search for safe and potent natural-based treatment for depression is receiving renewed interest given the numerous side-effects associated with many existing drugs. In South Africa, the use of plants to manage depression and related symptoms is fairly documented among different ethnic groups. In the current study, existing ethnobotanical, ethnopharmacological and phytochemical studies on South African medicinal plants used to manage depression were reviewed. Electronic databases were accessed for scientific literature that meets the inclusion criteria. Plants with ethnobotanical evidence were subjected to a further pharmacological review to establish the extent (if any) of their effectiveness as antidepressants. Critical assessment resulted in 20 eligible ethnobotanical records that were included in the systematic review, which generated an inventory of 186 plants from 63 plant families as treatment remedy for depression and related ailments. Due to the cultural differences observed in the definition of depression, or lack of definition in some cultures, most plants are reported to treat a wide range of atypical

symptoms related to depression. *Boophone disticha*, *Leonotis leonurus* and *Mentha longifolia* were identified as the three most popular plants, with over 8 mentions each from the ethnobotanical records. The dominant families were Asteraceae (24), Fabaceae (16), Amaryllidaceae (10), and Apocynaceae (10) which accounted for about 32% of the 186 plants. Only 27 (≈14.5%) of these plants have been screened for antidepressant activity using *in vitro* and *in vivo* models for depression. *Agapanthus campanulatus*, *Boophone disticha*, *Hypericum perforatum*, *Mondia whitei* and *Xysmalobium undulatum*, represent the most extensively explored plants. Phytochemical investigation on 9 out of the 27 plants revealed 24 compounds with antidepressant-like effects. Some of these included buphanidrine and buphanamine which were isolated from the leaves of *Boophone disticha*, Δ^9- tetrahydrocannabinol, cannabidiol and cannabichromene obtained from the buds of *Cannabis sativa* and carnosic acid, rosmarinic acid and salvigenin from *Rosmarinus officinalis, Boophone disticha* and *Rosmarinus officinalis* had the highest number of isolated phytochemicals and evaluated for their antidepressant effects. A significant portion (≈85%) of 186 plants with ethnobotanical records still require pharmacological studies to assess their potential antidepressant-like effects. This review remains a valuable reference material that may guide future ethnobotanical surveys to ensure their robustness and validity as well as a database to identify promising plants to screen for pharmacology efficacy.

2.1 Introduction

Clinical depression represents one of the most prevalent and highly comorbid psychiatric conditions that causes a negative emotional experience associated with pathophysiological changes (Saki *et al.*, 2014). In this review, we will differentiate between clinical depression (MDD) and the ailments/symptomology described as depression amongst communities (MDD-com). MDD is a mental health disorder characterized by a persistent depressed mood and loss of interest in activities once enjoyed, causing noticeably significant changes in the patient's life (Mungai & Bayat, 2019). MDD brings about physiological, behavioural and psychological symptoms initiated through stressful life situations that are difficult to overcome (Fathinezhad *et al.*, 2019; Martins & Brijesh, 2018). Symptoms of MDD may include a depressed mood, pervasive lack of energy, a change in sleeping patterns and psychomotor activity, changes in appetite and/or weight, cognitive disturbances that make it difficult to think, concentrate, or make decisions, feeling worthless, guilt, and repeated thoughts of death and suicidal attempts or idealization. (American Psychiatric Association, 2000; World Health Organisation, 2017). MDD is caused by imbalances with certain neurotransmitters in the central nervous system (Munir *et al.*, 2020). According to Schildkraut (1965), the basis of the pathophysiology of MDD is the deficiency of monoaminergic activity in the brain, which leads to the depletion of serotonin, dopamine and

norepinephrine neurotransmitters. The noradrenaline and serotonin systems play an important role in stress response, the regulation of emotions and the neurobiology of depression (Ressler & Nemeroff, 2000). Antidepressants are the current MDD treatment focusing on improving monoamine activity, however, they are associated with adverse side effects and a delayed response time (Popa-Velea *et al.*, 2015; Saki *et al.*, 2014), leading to poor clinical outcomes.

MDD is one of the three leading causes of disease burden in South Africa and globally (Nglazi *et al.*, 2016). The WHO ranked it the largest contributor to global disability, with an estimated 300 million people suffering from MDD globally in 2015 (World Health Organisation, 2017). It is assumed that by 2030, MDD will be considered the global disease burden based on serious limitation on existing treatment methods owed to the therapeutic success, safety and efficacy of available antidepressants (Abbas *et al.*, 2015). According to the South African Stress and Health (SASH) study, MDD is among the mental disorders with the highest lifetime prevalence in South Africa (Herman *et al.*, 2009). MDD is more prevalent in females than in males (World Health Organisation, 2017). The correlation between hormonal changes in women and an increased prevalence of depression suggests that fluctuations in hormones during puberty, menstruation, after pregnancy and around menopause may trigger depression in women (Albert, 2015). In most developing countries, there is limited access to treatment for mental disorders, especially in primary health care settings (Ferrari *et al.*, 2013; Stafford *et al.*, 2008). As a result, the communities have often used plants to alleviate some of the symptomology associated with depression or feeling unwell. Following the development of the WHO Traditional Medicine Strategy 2014-2023 and due to the legal acknowledgement of traditional healers, the South African government, as a member state of the WHO, developed legislation and policies to facilitate the institutionalization of traditional medicine in South Africa (Mothibe & Sibanda, 2019).

MDD-com includes mental health related problems that could be classified as depression based on similar clinical symptoms and is perceived by African traditional healers as caused by ancestors or bewitchment (Sorsdahl *et al.*, 2009). When interviewed on the understanding of depression, one participant (traditional healer) from a South African study conducted by Starkowitz (2013) stated that “traditionally, there is not something like depression”, while another participant replied, “we don’t have the word depression”. Those with some degree of understanding of the concept defined MDD-com as “an experience of ‘feeling sick’ inward and feeling down in your heart while the outside flesh will be feeling fine”, and “an experience that makes a person feel like lying down and covering themselves with a blanket” (Starkowitz, 2013). Several states, such as ‘being put down’ by ancestors, experiencing headaches and migraines,

being possessed by evil spirits, mourning, sorrow and being inflicted with curses often resemble a depressed and hopeless state (Stafford *et al.*, 2008). Multiple somatic complaints such as headaches and fatigue represent the most common presentations of MDD-com countries like Zimbabwe, (Todd *et al.*, 1999). Wei *et al.* (2016) reported that the prevalence rate of depression in patients with headaches was 19.7%, and that about 15.2% of the patients had a headache due to somatic symptoms of depression (cause of headache) and 10.9% attributed their primary headache as a comorbidity of depression. Moreover, depression increased the risk of tension-type headache in study participants following laboratory stress (Janke *et al.*, 2004). This overlap between depression and headache provides evidence of a link between medicinal plants used traditionally for headaches and potential antidepressant properties. Appropriate utilization of disease categories and classification allow for cross-culture comparisons (Staub *et al.*, 2015). Therefore, the emic perception and categorization of diseases (which usually comes from within a culture) has to be understood for the development of a culturally appropriate classification system (Heinrich *et al.*, 2009).

The use of traditional medicine is widespread in South Africa, where an estimated 70 to 84% of the black population consult traditional healers at some point (Koen *et al.*, 2003; Robertson, 2006; Stafford *et al.*, 2008). In addition, more people often utilize traditional healers than medical practitioners for their primary health care (Robertson, 2006). This can be attributed to preferences, the increased demand for herbal products in preventative health care and general well-being, and the easy availability of the cheaper, individualized and culturally appropriate traditional healthcare system (Mander, 1998; Viljoen *et al.*, 2019). The use of commercially processed herbal preparations as part of traditional medicine, and the sale thereof, has increased over the last decade (Ndhlala *et al.*, 2011). The use of herbal medicine such as psychotropic plant extracts is common amongst patients with mood-like and anxiety-like disorders, including MDD (Sarris *et al.*, 2011). *Boophone disticha* Herb. is one of the most popular medicinal plants in South Africa where bulb infusions of this plant are used to treat headaches and mental diseases (Hutchings *et al.*, 1996; Sobiecki, 2002). *Sceletium tortuosum* (L.) N.E.Br. was used in prehistoric times as a mood-altering substance, and the plant is chewed or drank traditionally for depression and stress (Hutchings *et al.*, 1996; Sobiecki, 2002; Van Wyk & Gericke, 2000; Van Wyk *et al.*, 1997). Infusions made from leaf, fruit and leaf decoctions of *Schinus molle* L. are used as antidepressants (Bhat & Jacobs, 1995). Several medicinal herbs have been approved by regulatory authorities and are being used to treat mental disorders, such as depression (Fathinezhad *et al.*, 2019). *Hypericum perforatum* L. is being used to treat mild depression and represents an acceptable alternative to conventional synthetic antidepressants (Nielsen *et al.*, 2004), while *Sceletium tortuosum* has been developed into a commercial product, called Zembrin™, sold for mood elevation (Stafford *et al.*, 2008).

Despite the anecdotal and long-term use of medicinal plants for the treatment of MDD and related ailments in South Africa, an in-depth and up-to-date appraisal on this subject is lacking. Moreover, the need to bridge the cross-cultural gap observed in the definition of depression by exploring the regional, cultural and/or traditional concepts of depression as understood by South African traditional healers cannot be overemphasized. This review focuses on the ethnobotanical studies on South African plants with potential antidepressant effects, their pharmacological (*in vitro* and *in vivo*) screening and phytochemical assessment. This study is envisaged to provide an in-depth and current state of knowledge in the search for South African plants with antidepressant potential and identify critical gaps for future research direction in the on-going global search for safe and efficient antidepressants.

2.2 Literature search strategy

A web-based systematic literature search was conducted from March to November 2021 to identify ethnobotanical information on medicinal plants used traditionally in South Africa to treat MDD and MDD-com. The systematic review was conducted according to the PRISMA guidelines for reporting systematic reviews and meta-analysis (Moher *et al.*, 2009; Moher *et al.*, 2015). Electronic databases such as the Web of Science, MEDLINE (PubMed), ScienceDirect and Google Scholar were searched for published and unpublished scientific literature, including journal articles, books, theses, and dissertations, on South African medicinal plants used locally to treat clinical depression and related ailments. These databases were searched using keywords/phrases such as South African medicinal plants, antidepressant effects of medicinal plants, ethnobotany of South Africa, indigenous plant use, medicinal plant use, South African psychoactive plants, Zulu medicinal plants. In addition, literature was retrieved from the library of the North-West University (NWU), South Africa. All medicinal plants identified by this search were subject to a further literature review to establish the extent (if any) of pharmacological research conducted into the efficacy of the plants as antidepressants. The electronic databases mentioned above were used to search for pharmacological studies providing supporting evidence of the antidepressant-like effects for each plant species, both *in vitro* and *in vivo*. To filter these studies, search terms “antidepressant effect of plants” and other keywords relating to depression and specific monoamines involved, together with plant species names, were used.

2.2.1 Eligibility criteria

The screening of all search results involved reviewing the title and abstract of articles and identifying and selecting eligible publications, downloading identified research articles, and critically assessing the articles on how they met the inclusion criteria.

Inclusion criteria

For a research article to be included in the review it must:

- Be a published ethnobotanical survey reporting potential antidepressant effects of medicinal plants
- Indicate the traditional use of medicinal plants for depression and related ailments (e.g., headache, sorrow, mourning, nervousness, stress, tension, mental illness known as 'spirits', alcoholism, insomnia, insanity, feeling like crying) in South Africa
- Be published or made available on the internet during the research period (i.e., up to November 30, 2021)

For ethno-pharmacological studies

- Preparation prepared from the plant extracts must be investigated against serotonin transporter (SERT), dopamine transporter (DAT) and noradrenalin transporter (NAT) receptors *in vitro* and measure behavioral markers of depression *in vivo*

Exclusion criteria

Research articles were excluded from the review based on the following:

- Ethnobotanical review articles (literature or systematic)
- Focus on natural resources (other than plants) used for depression
- Ethnobotanical surveys that are not focusing on South Africa
- Have limited data (e.g., missing scientific plant names) on the medicinal use of plants for depression

2.3 Data collection

Relevant data on the antidepressant-like effects of South African medicinal plants were extracted to Excel spreadsheets following the pre-defined criteria. Bibliographies from accessed articles, together with their citations, were downloaded and saved on an online reference manager (EndNote). Missing information from some articles (e.g., local names, life form of the plant, and misspelt scientific names) and in cases of research papers lacking geographic locations of the study, the data was retrieved through direct web searching (Google). Scientific names of plants,

families, local names, life form of plants, method of preparation of the plant-based medicine, route of administration and short notes were collected for each plant from each article. Where possible, data on the ethnic group that uses the plants traditionally were collected. Plant species and family were validated in references to The World Flora Online (http://theworlflora.online) and PlantZAfrica (http://pza.sanbi.org/) while the local names were confirmed using PlantZAfrica (http://pza.sanbi.org/). Any botanical synonyms or unaccepted names were updated to the recent updated plant nomenclature and are reported as such in this review. The plants listed in this review are arranged in alphabetical order based on plant families and scientific names.

2.4 Results

2.4.1 Literature search results

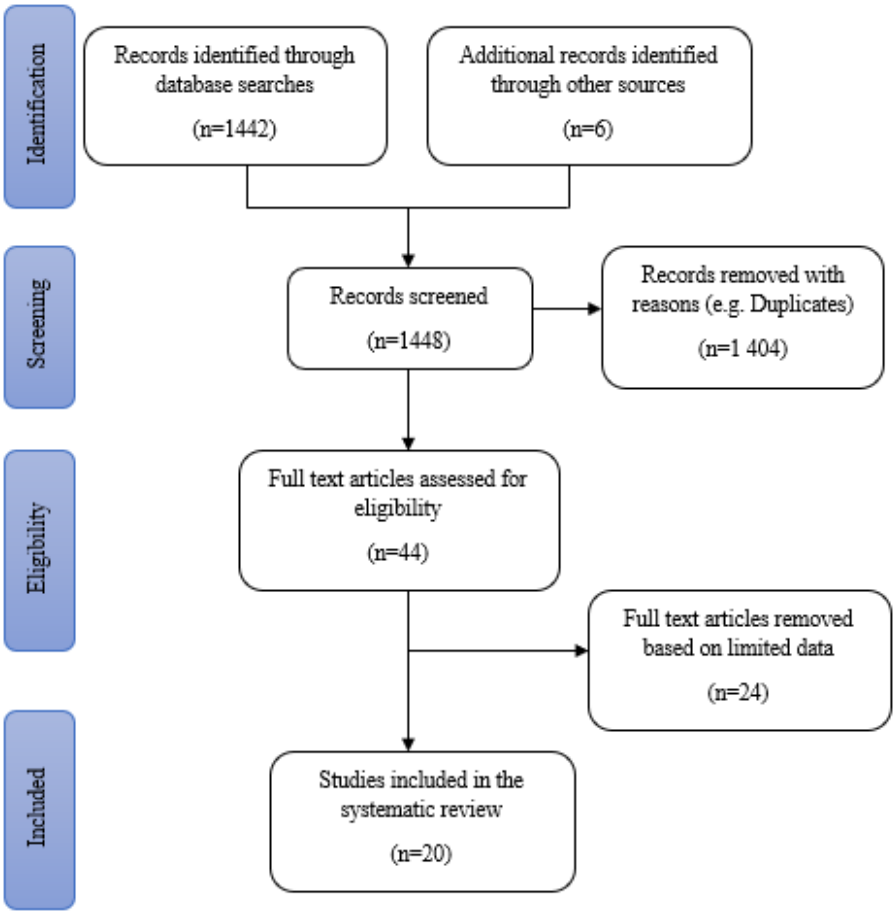


Figure 2.1: Search strategy results for the identification of studies included in the systematic review

A total of 1442 publication records were identified from online database searches. Additional data were obtained from 6 literature sources (books) retrieved from the NWU library, making a total of

1448 records that were screened. After the removal of duplicates, 44 articles and books were assessed for eligibility and explored relative to the inclusion criteria. The full text of 44 studies was reviewed in detail and 24 studies were removed due to limited data and other reasons stated in the exclusion criteria. Finally, 20 studies were reviewed for the documentation of the potential antidepressant-like effects of medicinal plants **(Figure 2.1)**. The literature comprises a total of 15 area specific ethnobotanical surveys (75%) and 5 plant inventories (25%). These studies covered 6 out of the 9 (approximately 67%) provinces in South Africa, with multiple studies being conducted in KwaZulu-Natal and Western Cape (both 4 studies). The most common method of data collection across the eligible articles was semi-structured and structured interviews (40% of the literature). Most of the literature reviewed covers a wide range of medicinal uses for each plant. However, for the purpose of this review, only the plant medicinal uses related to depression and associated symptoms were listed in the generated plant inventory.

The operational definition of MDD was inconsistent across most of the ethnobotanical literature reviewed. Most of the literature did not define MDD but a broad range of traditional medicinal uses in their surveys, rather than medicinal uses specific to MDD. Since most medicinal uses reported in the literature are indigenous knowledge gathered from South African traditional healers, records of the potential antidepressant effects of medicinal plants are listed as MDD-com, which entails ailments based largely on somatic symptoms such as headaches, fatigue, or spirits. According to Mosotho *et al.* (2008), there has been the misconception, until recently, that developing countries are relatively free of psychiatric problems, such as MDD, which are encountered in industrialized nations. Mosotho *et al.* (2008) also highlighted that MDD may be easily misdiagnosed since physical complaints, such as headaches, are not always recognized as a manifestation of depression. This lack of inconsistency between western and indigenous disease classification makes the evaluation of medicinal plant use in a western scientific setting more difficult (Staub *et al.*, 2015; Weckerle *et al.*, 2018).

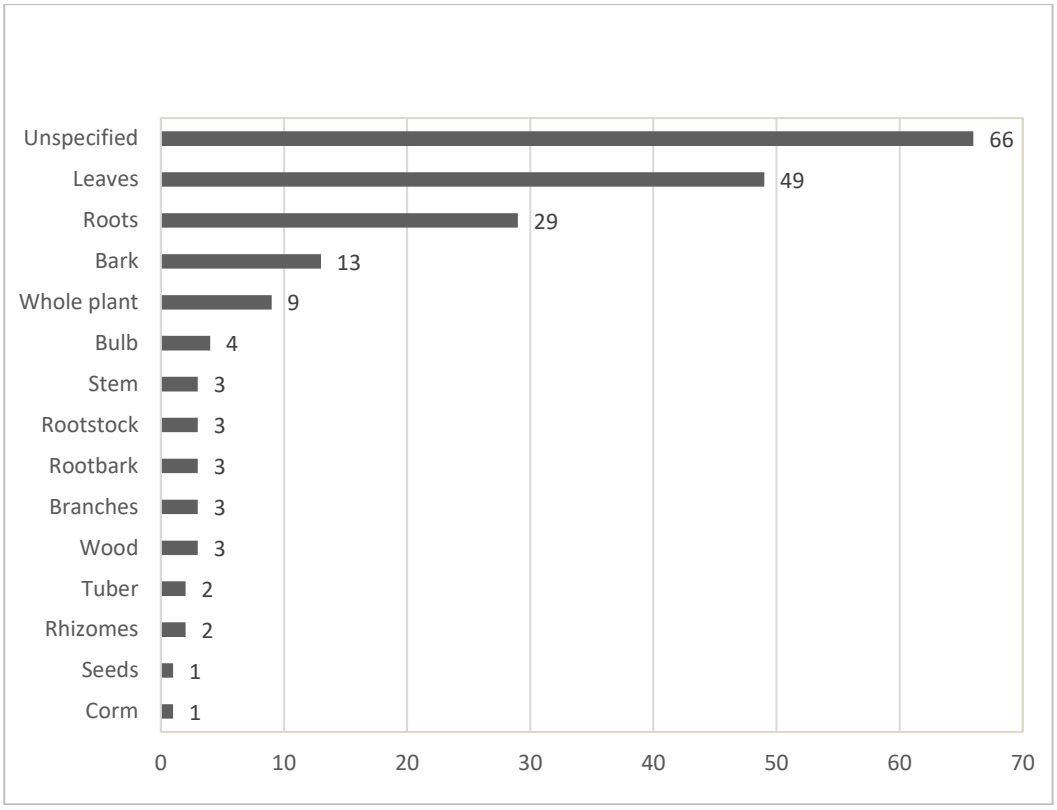


Figure 2.2: **The frequency of medicinal plant parts used in South Africa for the traditional treatment of depression and related ailments**

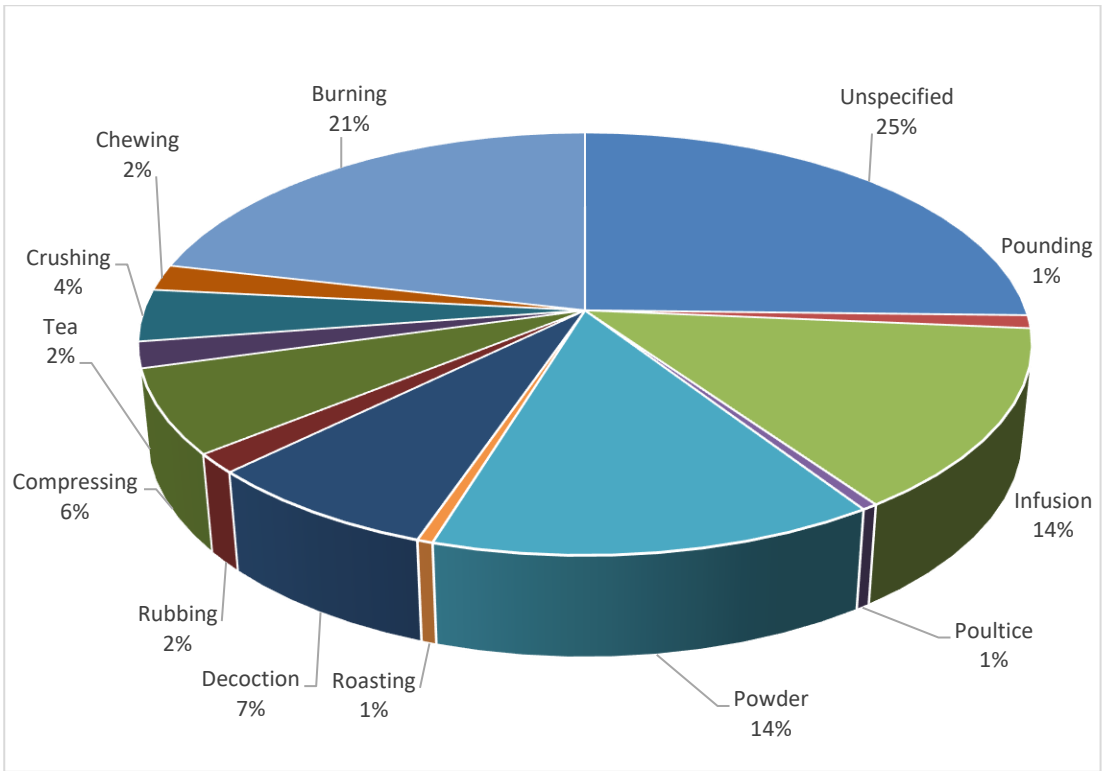


Figure 2.3: **Different methods of preparation of medicinal plants (*n* = 215). Several of the 186 medicinal plants recorded had >1 method used for preparation**

The absence of methodological framework was evident in one of the reviewed study (Moteetee *et al.*, 2019). A number of concerns were observed with the naming of the listed plants which are often encountered in published articles (Rivera *et al.*, 2014). About 50% (10) of the articles listed at least one scientific name incorrectly either by misspelling the scientific name, recording an incorrect or ambiguous scientific name or by using non-standard author abbreviation (Hulley & Van Wyk, 2019; Mogale *et al.*, 2015; Van Wyk & Gericke, 2000; Van Wyk *et al.*, 1997; Van Wyk *et al.*, 2008). In approximately 65% of the reviewed studies, there was no evidence of the identity of the medicinal plant recorded as the authors failed to provide details of the voucher specimen of the plants. Failure to provide sufficient details on the identification of the voucher specimen often makes the validation of the plant species difficult (Weckerle *et al.*, 2018). Although some authors specified the plant parts used for the herbal preparation for managing depression and related ailments, this information was absent in many cases (**Figure 2.2**). Likewise, a similar concern was evident with the method used for preparing the plants (**Figure 2.3**). These concerns are important information required for the generation of plant inventory to ensure the integrity of data from ethnobotanical surveys.

Table 2.1: An overview of ethnobotanical literature documenting South African medicinal plants with potential antidepressant effects. KZN = KwaZulu-Natal

Reference	Province	Area/region	Title/focus of the study	Ethnic group	No. of plant species	No. of plant families	Voucher specimen deposited	Characteristics of participants	Methodological framework (data collection and analysis, techniques)
Van Wyk and Gericke (2000)	Southern Africa	Unspecified	An inventory of useful plants of Southern Africa	Unspecified	12	9	Unspecified	Unspecified	Ethnobotanical book
Van Wyk *et al.* (2008)	Eastern Cape and Western Cape	South-eastern Karoo, Graaff-Reinet and Murraysburg regions	An ethnobotanical survey of medicinal plants used in the southeastern Karoo	Xhosa, Khoikhoi and San	8	5	Unspecified	Local experts	Ethnobotanical field studies
Nortje and van Wyk (2015)	Northern Cape	Kamiesberg, Namaqualand	An ethnobotanical survey of medicinal plants of the Kamiesberg, Namaqualand, South Africa	Khoisan	12	11	Yes	Local inhabitants	Semi-structured and structured interviews
Hutchings (1989)	KZN	Unspecified	A survey and analysis of traditional medicinal plants as used by the Zulu, Xhosa and Sotho	Zulu, Xhosa and Sotho	40	22	Unspecified	Unspecified	Ethnobotanical book
Hutchings *et al.* (1996)	KZN	Unspecified	An inventory of Zulu medicinal plants	Zulu	54	31	Unspecified	Traditional healers	Ethnobotanical book

Van Wyk *et al.* (1997)	Unspecified	South Africa	Inventory of medicinal plants of South Africa	Unspecified	27	19	Unspecified	Unspecified	Ethnobotanical book
Sobiecki (2002)	Unspecified	Unspecified	A preliminary inventory of plants used for psychoactive purposes in southern African healing traditions	Unspecified	40	26	Unspecified	Traditional healers	Interviews
Corrigan *et al.* (2011)	KZN	KwaNibela Peninsula, St Lucia	An ethnobotanical survey of plants used in the KwaNibela Peninsula, St Lucia, South Africa	Zulu and Swati	2	2	Unspecified	Community members and traditional knowledge experts	Ethnobotanical field studies
Bhat and Jacobs (1995)	Eastern Cape	Transkei	An ethnobotanical survey of traditional herbal medicine used in Transkei	Xhosa	3	3	Yes	Elderly villagers, traditional doctors and herbalists	Interviews
Stafford (2009)	KZN and Western Cape	Unspecified parts of KZN and Western Cape	Ethnobotanical literature survey of South African medicinal plants with central nervous system related activity and use	Zulu	34	23	Unspecified	Unspecified	Ethnobotanical studies
Moffett (2016)	Lesotho and Free State	Unspecified	An ethnobotanical survey of medicinal plants used by the Basotho	Sotho	45	23	Unspecified	Traditional healers	Interviews
Venter and Venter (2016)	Unspecified	Unspecified	A list of useful South African indigenous tress	Unspecified	11	8	Unspecified	Unspecified	Ethnobotanical book

De Beer and Van Wyk (2011)	Northern Cape	Agter–Hantam	An ethnobotanical survey of the Agter–Hantam, Northern Cape province, South Africa	Khoi–San	7	6	Yes	Traditional healers and local people of Khoi–San descent	Interviews
Hulley and Van Wyk (2019)	Western Cape	Western Little Karoo/Kannaland (Barrydale, Zoar, Calitzdorp and Vanwyksdorp)	Quantitative medicinal ethnobotany of Kannaland (Western Little Karoo), South Africa	Khoi-San	21	16	Yes	Children (13 to 19y/o), adults (20 to 59y/o) and senior citizens (60y/o and above) of Khoi descent	Quantitative ethnobotanical survey
Mogale *et al.* (2019)	Limpopo	Central Sekhukhuneland (Frisgewaght at Phokwane, Ga-Moretsele/Tsehlwaneng near Jane Furse and Ga-Sekhele near Schoonoord)	The ethnobotany of Sekhukhuneland and the plants used by rural Bapedi people	Pedi	3	2	Unspecified	Twenty-seven local inhabitants	Ethnobotanical surveys and interviews
Moteetee *et al.* (2019)	Lesotho and the Free State	Unspecified	The ethnobotany of the Basotho of Lesotho and the Free State province of South Africa (South Sotho)	Sotho	17	13	Unspecified	Unspecified	Unspecified
Philander (2011)	Western Cape	Unspecified	An ethnobotany of Western Cape Rasta bush medicine	Khoi-San, Rastafari	18	15	Yes	Bush doctors	Ethnobotanical survey, interview

Mongalo and Makhafola (2018)	Limpopo	Blouberg area	Ethnobotanical knowledge of the lay people of Blouberg area (Pedi tribe), Limpopo province, South Africa	Pedi	2	2	Yes	Traditional healers and medicinal plant sellers	Ethnobotanical survey, questionnaires
Thring and Weitz (2006)	Western Cape	Bredasdorp/Elim region of the Southern Overberg	Medicinal plant use in the Bredasdorp/Elim region of the Southern Overberg in the Western Cape province of South Africa	Coloured population	10	5	Yes	Elderly people	Interviews, questionnaires
Mhlongo and Van Wyk (2019)	KZN	Amandawe	Zulu medicinal ethnobotany: new records from the Amandawe area of KwaZulu-Natal, South Africa	Zulu	2	2	Unspecified	Community members	Ethnobotanical survey

Table 2.2: Examples of popular medicinal plants used against depression and related ailments in South Africa based on multiple mentions and the presence of pharmacological evidence. Species and family names for each plant species were validated in references to The Plant List (www.theplantlist.org), The World Flora Online (http://theworlflora.online) and PlantZAfrica (http://pza.sanbi.org/) and the local names were confirmed using PlantZAfrica (http://pza.sanbi.org/)

Plant family	Scientific name [synonyms]	Local name	Life form	Plant parts used	Method of preparation, route of administration and/or short notes	References
Aizoaceae	*Sceletium tortuosum* (L.) N.E. Br [*Mesembryanthemum tortuosum* L.]	Kanna (E); Kougoed (A); Kanna (K)	Herb	Whole plant	Used as a psychoactive substance; Emetics made from leaves in boiling water are administered for the fearful dreams; Leaves used to treat headache; Whole plant chewed or drunk for depression and anxiety disorders; Past use as a mood-altering substance from prehistoric times; The dried plant material is prepared traditionally and chewed, smoked, or powdered and inhaled as a snuff; Whole plant used to elevate mood and reduce anxiety and stress	Hutchings *et al.* (1996); Nortje and van Wyk (2015); Philander (2011); Sobiecki (2002); Van Wyk and Gericke (2000); van Wyk *et al.* (1997)
Amaryllidaceae	*Agapanthus campanulatus* F.M. Leight. [*A. campanulatus* subsp. *patens* (F.M. Leight.) F.M. Leight.]	Bell agapanthus (E); Bloulelie (A); Ubani (Z); Leta-la-phofu (S); Ugebeleweni (X)	Herb	Unspecified	Unspecified; Unspecified parts used by the Sotho to treat people with “spirit”, which is a type of mental disturbance; Unspecified	Moffett (2016); Sobiecki (2002); Stafford (2009)
	Boophone disticha (L.f.) Herb. [*Amaryllis disticha* L.f., *Brunsvigia disticha* (L.f.) Sweet, *B. toxicaria* (L.f. ex Aiton) Herb.]	Cape poison bulb, sore eye flower (E); Gifbol, seeroogblom (A); Leshoma (S); Incwadi (X); Incotho (Z)	Herb	Bulb	Used as emetics and snuffed or inhaled medicines; Bulb decoctions are administered by mouth to adults suffering from headaches; Unspecified; Unspecified; Bulb infusions are drunk to induce hallucinations and to treat mental diseases; Unspecified; Bulbs are used to treat headache; Weak decoctions of bulb scales administered by mouth or as enemas for headache	Hutchings (1989); Hutchings *et al.* (1996); Moffett (2016); Philander (2011); Sobiecki (2002); Stafford (2009); Van Wyk and Gericke (2000); van Wyk *et al.* (1997)

Plant family	Scientific name [synonyms]	Local name	Life form	Plant parts used	Method of preparation, route of administration and/or short notes	References
	Haemathus coccineus L. [*H. latifolius* Salisb.]	March flower, paintbrush lily (E); Bergajuin, bloedblom (A); Uzaneke (Z)	Herb	Roots	Boiled root decoctions are taken as emetics	Hutchings *et al.* (1996)
	Scadoxus puniceus (L.) Friis & Nordal [*Haemanthus puniceus* L,, *H. rouperi* auct. *H. superbus* Baker]	Paintbrush lily (E); Rooikwas (A); Umgola (Z)	Shrub	Bulbs	Bulbs are used for headaches; Unspecified	Hutchings *et al.* (1996); van Wyk *et al.* (1997)
Anacardiaceae	*Schinus molle* L. [*S. angustifolia* Sessé & Moc., *S. huigan* Molina, *S. molle* var. *molle*, *S. occidentalis* Sessé & Moc.]	False pepper tree (E); Peperboom (A)	Tree	Stem, leaves	Infusions made from leaves and fruits and leaf decoctions are used as antidepressants; Unspecified part pressed on the head for headache; Leaves used as compress to treat headache; Fresh leaves placed on a cloth with vinegar and wrapped on the head for headache	Bhat and Jacobs (1995); Hulley and Van Wyk (2019); Nortje and van Wyk (2015); Van Wyk *et al.* (2008)
Apiaceae	*Alepidea amatymbica* Eckl. & Zeyh. [*A. amatymbica* var *amatymbica* Eckl. & Zeyh., *A. amatymbica* var. *cordata* Eckl. & Zeyh., *A. aquatica* Kuntze, *Eryngium amathymbicum* (Eckl. & Zeyh.) Koso-Pol]	Giant alepidea (E); Kalmoes (A); Ikhathazo (Z); Iqwili (X); Lesoko (S)	Herb	Rhizome; roots	Dry rhizome and roots are smoked, or powdered and taken as a snuff to help prevent nervousness; Dry rhizomes are smoked or powdered and taken as snuff for mild sedation and vivid dreams; Fresh rhizomes are chewed, or decoctions are made from dried product. Also administered as snuff or burnt and inhaled. Smoke from roots used as a mild sedative	Sobiecki (2002); Van Wyk and Gericke (2000); van Wyk *et al.* (1997)
	Centella asiatica (L.) Urb. [*C. asiatica* var. a*siatica*, *C. asiatica* var. *crista* Makino, *C. hirtella* Nannf.]	Indian pennywort (E); Inyongwane (X); Varkoortjies (A)	Herb	Leaves	Finely ground leaves used as snuff; Dried, powdered leaf used as a snuff, which produces a calming, sedative effect; Possesses anti-inflammatory, tranquilizing and age-related neuroprotective effects	Sobiecki (2002); Van Wyk and Gericke (2000); van Wyk *et al.* (1997)

Plant family	Scientific name [synonyms]	Local name	Life form	Plant parts used	Method of preparation, route of administration and/or short notes	References
	Heteromorpha trifoliata (H.L.Wendl.) Eckl. & Zeyh. [*Bupleurum trifoliatum* H. L. Wendl. & Bartl.]	Parsley tree (E); Mkatlala (S); Umbangandlala (Z)	Tree	Leaves	Emetics and snuffed or inhaled medicines; Leaf decoctions are administered for mental and nervous diseases e.g. smoked for headaches; The Sotho administer leaf decoctions for mental and nervous diseases, and Xhosa administer warm leaf infusions for similar purposes	Hutchings (1989); Hutchings *et al.* (1996); Sobiecki (2002)
Apocynaceae	*Gomphocarpus fruticosus* (L.) W. T. Aiton [*Asclecias fruticosa* L., *G. fruticosus* subsp. *decipiens* (N.E.Br) Goyder & Nicholas, *G. fruticosus* subsp. *flavidus* (N.E.Br) Goyder & Nicholas, *G. fruticosus* subsp. *rostratus* (N.E.Br) Goyder & Nicholas]	Milkweed (E); Tontelbos (A); Lebejana (S); Umsinga-lwesalukazi (Z)	Herb	Whole plant	Emetics and snuffed or inhaled medicines; Dried aerial parts used as snuff; Leaves are taken orally as headache treatment; Roots used as snuff to treat headache; Snuff made from powdered leaves used as a sedative; Unspecified; Snuff made from powdered leaves is used as a sedative; Snuff from powdered leaves is used as a sedative	Hutchings (1989); Moffett (2016); Mogale *et al.* (2019); Nortje and van Wyk (2015); Stafford (2009); Van Wyk and Gericke (2000); van Wyk *et al.* (1997)
	Hoodia gordonii (Masson) Sweet ex Decne. [*Scytanthus gordonii* (Masson) Hook., *Stapelia gordonii* Masson]	Bushman's hat, Hoodia (E); Bitterghaap (A); Khobab (K)	Shrub	Unspecified	Unspecified	van Wyk *et al.* (1997)
	Mondia whitei (Hook.f.) Skeels [*Chlorocodon whitei* Hook. f., *C. whiteii* Hook. f.]	White's ginger (E); Umondi (Z)	Herb	Roots	Root infusions used to treat stress and tension in adults; Unspecified	Sobiecki (2002); Stafford (2009)

Plant family	Scientific name [synonyms]	Local name	Life form	Plant parts used	Method of preparation, route of administration and/or short notes	References
	Xysmalobium undulatum (L.) W.T.Aiton [*Asclepias ciliata* Murray ex Decne*., A. leucotrica* Schltr*., A. undulata* L., *Gomphorcarpus undulatus* (L.) Schltr.]	Milk bush (E); Bitterhout/melkbos (A); Iyeza (X); Ishinga (Z); Leshokoa (S)	Herb	Roots	Emetics and snuffed or inhaled medicines; Unspecified; Used as decongestant and for headache; Roots contain several glycosides with weak activity as central nervous system depressant and antidepressant; Unspecified; Powdered root used as snuff	Hutchings (1989); Hutchings *et al.* (1996); Moffett (2016); Sobiecki (2002); Stafford (2009); van Wyk *et al.* (1997)
Asparagaceae	*Bowiea volubilis* Harv. [*Ophiobostryx volubilis* (Harv.) Skeels, *Schizobasopsis volubilis* (Harv.) J.F.Macbr.]	Climbing onion (E); Knolklimop (A); Ugibisisila, iguleni, (Z); Umgaqana (X)	Herb	Bulb	Emetics and snuffed or inhaled medicines; Infusions made from crushed bulbs are used as protective washes when travelling; Bulb used to treat sore eyes and headache; Unspecified	Hutchings (1989); Hutchings *et al.* (1996); Philander (2011); van Wyk *et al.* (1997)
Asteraceae	*Afroaster hispida* (Thunb.) J.C.Manning & Goldblatt [*Aster bakerianus* Burtt Davy ex C.A.Sm., *A. asper* (Less.) Schönland, *A. bakerianus* subsp. *albiflorus* W.Lippert]	Baker's wild aster (E); Udlutshana (Z); Umthekisana (X); Phoa (S)	Herb	Roots	Emetics and snuffed or inhaled medicines; Ground roots are taken as snuff for headaches; Dried, powdered roots taken as snuff or decoctions taken orally for headache**;** Dried, powdered roots taken as snuff	Hutchings (1989); Hutchings *et al.* (1996); Moffett (2016); van Wyk *et al.* (1997)
	Artemisia afra Jacq. ex Willd. [*A. tenuifolia* Moench]	African wormwood (E); Wilde-als (A); Umhlonyane (X); Mhlonyane (Z); Lengana (B)	Shrub	Leaves	Leaves used in the treatment of headache and anxiety; Infusions or steam from crushed leaves are commonly inhaled for headaches and colds; Unspecified; Tea made from leaves used to treat headache; Unspecified	Hulley and Van Wyk (2019); Hutchings *et al.* (1996); Stafford (2009); Thring and Weitz (2006); van Wyk *et al.* (1997)
	Artemisia dracunculus L. [*A. dracunculoides* Pursh]	True tarragon, biting dragon (E)	Herb	Unspecified	Unspecified	Stafford (2009)

Plant family	Scientific name [synonyms]	Local name	Life form	Plant parts used	Method of preparation, route of administration and/or short notes	References
	Pluchea scabrida DC. [*Conyza scabrida* (DC.) DC. Ex Miq]	Oven bush (E); Bakbos (A); Mokotedi-wa-thaba (S)	Shrub	Leaves	Unspecified parts used to treat headache; In Transkei, ground leaves are snuffed for headaches; Roots are used to treat depression; Leaves are placed on cloth with vinegar/brandy and wrapped around head for headache; Unspecified; Leaves placed in cloth with vinegar/brandy and wrapped around the head to treat headache	Hulley and Van Wyk (2019); Hutchings *et al.* (1996); Mogale *et al.* (2019); Moteetee *et al.* (2019); Thring and Weitz (2006); van Wyk *et al.* (1997); Van Wyk *et al.* (2008)
	Tarchonanthus camphoratus L. *[T. camphoratus* var. *camphoratus, T. abyssinicus* Sch.Bip.]	Camphor bush (E); Kankerbos (A); Igqeba-elimhlophe (Z); Sefahla (S)	Tree	Branches; leaves	Sotho's use smoke from burning green branches as an inhalant for headaches; Infusions of leaves and twigs used to treat headache; Branches are burnt, and smoke inhaled for the relief of headache	Hutchings *et al.* (1996); Moffett (2016); Venter and Venter (2016)
Cannabaceae	*Cannabis sativa* L.	Marijuana (E); Dagga (A); Umnya (X); Matekwane (S); Nsangu (Z)	Herb	Whole plant	Used in the treatment of depressive mental conditions; Whole plant is used to treat "Vaal sick" and excessive headache; Smoked to induce well-being, relaxation, sociability and/or spirituality; Administered orally, intravenously or by topical application for treatment of depression and other conditions	Hutchings *et al.* (1996); Mongalo and Makhafola (2018); Van Wyk and Gericke (2000); van Wyk *et al.* (1997)
Capparaceae	*Capparis tomentosa* Lam. [*C. alexandrae* Chiov., *C. biloba* Hutch. & Dalziel, *C. floribunda* Wight]	Woolly caper bush (E); Wollerige(A); Imfihlo (X); Umabusane (Z)	Shrub	Roots	Emetics and snuffed or inhaled medicines; Roots are burnt to form a powder that is rubbed into scarifications for the relief of headache; The Zulu use unspecified parts to treat madness; Powdered, burnt roots rubbed into skin for headache	Hutchings (1989); Hutchings *et al.* (1996); Sobiecki (2002); van Wyk *et al.* (1997)

Plant family	Scientific name [synonyms]	Local name	Life form	Plant parts used	Method of preparation, route of administration and/or short notes	References
	Maerua angolensis DC. [*M. angolensis* subsp. *angolensis*]	Bead-bean tree, bead-pod tree (E); Knoppiesboontjieboom (A); Umenwayo (Z); Mogogwane (S); Mutamba-na-mme (V)	Tree	Leaves	Steam from leaves inhaled to treat headache	Venter and Venter (2016)
Euphorbiaceae	*Synadenium cupulare* L.C. Wheeler	Dead-man's tree(E); Gifboom(A); Umbulele(Z)	Tree	Leaves	Emetics and snuffed or inhaled medicines; Leaves are broken up and inhaled to relieve headaches; Leaves are used as medicine for headache	Hutchings (1989); Hutchings *et al.* (1996); Van Wyk and Gericke (2000)
Fabaceae	*Albizia adianthifolia* (Schum.) W. Wight [*A. adianthifolia* var. *adianthifolia* Schum.) W.Wight, *Mimosa adianthifolia* Schum.]	Flat- crown albizia (E); Platkroon (A); Umgadankawu (Z); Umhlandlothi (X)	Tree	Bark	Taken as snuff; Powdered bark is taken as a snuff for headaches; Powdered bark used as snuff; Bark is powdered and used as snuff for the relief of headache	Corrigan *et al.* (2011); Hutchings *et al.* (1996); van Wyk *et al.* (1997); Venter and Venter (2016)
	Tephrosia capensis (Jacq.) Pers.	Cape Tephrosia (E); Pelodimaroba (S)	Shrub	Roots	Emetics and snuffed or inhaled medicines; Dried powdered roots are used as snuff to relieve headaches; Dried roots snuffed for headache; Dried powdered roots are used as a snuff for headaches and plant decoctions for nervousness	Hutchings (1989); Hutchings *et al.* (1996); Moffett (2016); Sobiecki (2002)
Hypericaceae	*Hypericum perforatum* L. [*H. vulgare* Lam., *H. perforatum* var. *petiolatum* Peterm.	Saint John's wort (E); Johanneskruid (A)	Shrub	Whole plant	Popular in the West and in South Africa for treating mild depression, anxiety and sleep disorders; Powdered extracts used as antidepressants	Sobiecki (2002); van Wyk *et al.* (1997)

Plant family	Scientific name [synonyms]	Local name	Life form	Plant parts used	Method of preparation, route of administration and/or short notes	References
	Hypericum revolutum Vahl [*H. kalmianum* Vahl, *H. revolutum* subsp. *revolutum*]	Curry bush, forest primrose (E); Kerriebos (A)	Shrub	Unspecified	Unspecified	Stafford (2009)
Hypoxidaceae	*Hypoxis hemerocallidea* Fisch., C.A. Mey. & Avé-Lall. [*H. elata* Hook. f., *H. obconica* Nel, *H. patula* Nel, *H. rooperi* T. Moore, *H. rooperi var. forbesii* Baker	Star flower, yellow star (E); Sterblom (A); Inkomfe (Z); Lotsane (S)	Shrub	Corm	Emetics and snuffed or inhaled medicines; Corm infusions are given as emetics for mental disorders; Used as charm to cure headache and for anxiety and depression; Medicinal plant used for headache; Corm infusions are used for insanity; Infusions of corms and leaves used as emetics	Hutchings (1989); Hutchings *et al.* (1996); Moffett (2016); Moteetee *et al.* (2019); Sobiecki (2002); van Wyk *et al.* (1997)
Lamiaceae	*Ballota hirsuta* Benth. [*B. africana* Colmeiro, *B. cinerea* (Desr.) Briq.]	Cape horehound (E); Katterkruie (A)	Shrub	Leaves	Leaf infusions used for treating headache; Compresses on head to treat headache; Treats headaches; Used to treat headaches; Infusions used to treat headache; Drank to treat headache	Hulley and Van Wyk (2019); Nortje and van Wyk (2015); Philander (2011); Thring and Weitz (2006); van Wyk *et al.* (1997); Van Wyk *et al.* (2008)
	Leonotis leonurus (L.) R.Br. [*Leonurus africanus* Mill., *Leonurus grandiflorus* Moench, *Leonurus superbus* Medik., *Phlomis leonurus* L., *P. speciosa* Salisb.]	Lion's ear, wild dagga (E); Wildedagga (A); Imvovo (X); Umcwili (Z)	Shrub	Whole plant	Emetics and snuffed or inhaled medicines; Cold water infusions from leaves are inhaled to relieve feverish headaches; Unspecified; Leaves are smoked for epilepsy and partial paralysis; Unspecified parts used for headache; Decoctions of flowers, stems and leaves are used to treat headache; Decoctions taken for headache	Hutchings (1989); Hutchings *et al.* (1996); Philander (2011); Sobiecki (2002); Stafford (2009); Thring and Weitz (2006); Van Wyk and Gericke (2000); van Wyk *et al.* (1997)

Plant family	Scientific name [synonyms]	Local name	Life form	Plant parts used	Method of preparation, route of administration and/or short notes	References
	Mentha longifolia (L.) L. [*M. longifolia* (L.) Huds.]	Wild mint (E); Kruisement (A); Bohatsu (S); Umfuthana lomhlhanga (Z)	Herb	Leaves	Leaf infusion drank as tea and warm compress of leaves used for headache; Unspecified part compressed on the head for headache; Emetics and snuffed or inhaled medicines**;** Sotho's sometimes plug their nose with crushed leaves and bind with a cloth for the relief of headaches; Used medicinally to treat headache; Unspecified parts used to treat headache; Crushed leaf infusions or decoctions drank for headache; Leaf infusion mixed with *kruisement* in tea for headache and general malaise	De Beer and Van Wyk (2011); Hulley and Van Wyk (2019); Hutchings (1989); Hutchings *et al.* (1996); Philander (2011); Thring and Weitz (2006); van Wyk *et al.* (1997); Van Wyk *et al.* (2008)
	Mentha spicata L. [*Mentha crispata* Schrad. ex Willd.]	Spearmint, garden mint (E)	Herb	Leaves	Leaf infusion taken as tea to treat headache and colds	Van Wyk *et al.* (2008)
	Rosmarinus officinalis L. [*R. communis* Noronha, *R. communis* var. *communis*]	Rosemary (E)	Shrub	Unspecified	Used medicinally to treat headache	Philander (2011)
Lauraceae	*Cinnamomum camphora* (L.) J. Presl [*Camphora* (L.) H. Karst., *Laurus camphora* L.]	Camphor laurel, camphor tree (E)	Tree	Unspecified	Details not disclosed	Stafford (2009)
	Ocotea bullata (Burch.) E. Meyer in Drege [*Laurus bullata* Burch., *Oreodaphne bullata* (Burch.) Nees]	Black stinkwood (E); Stinkhout (A); Unukani (X,Z)	Tree	Bark	Emetics and snuffed or inhaled medicines; Bark used as snuff, inhaled to treat headache; South Africans use unspecified parts as an emetic for emotional and nervous disorders; Finely ground bark used as snuff for headache	Hutchings (1989); Hutchings *et al.* (1996); Sobiecki (2002); van Wyk *et al.* (1997)
Meliaceae	*Ekebergia capensis* Sparrm. [*E. mildbraedii* Harms*, E. ruppeliana* (Fresen.) A. Rich., *E. senegalensis* Fuss]	Cape ash (E); Essenhout (A); Mmidibidi (S); Umnyamatsi (SS)	Tree	Leaves and roots	The Vha-Venda use leaves and bark in emetics and for headache; Leaves are pounded in cold water and the solution is extracted and inhaled to treat mental problems; Roots used to treat headache; Root decoction taken orally to relieve headache	Hutchings *et al.* (1996); Sobiecki (2002); van Wyk *et al.* (1997); Venter and Venter (2016)
	Melia azedarach L.	Chinaberry, persian lilac (E)	Tree	Leaves	Infusions made from a handful of leaves in half a cup of water are taken for abdominal pains	Hutchings *et al.* (1996)

Plant family	Scientific name [synonyms]	Local name	Life form	Plant parts used	Method of preparation, route of administration and/or short notes	References
Myricaceae	*Morella serrata* (Lam.) Killick [*Myrica serrata* Lam.]	Mountain Waxberry (E); Berg-wasbessie (A); Ulethu (Z); Umaluleka (X); Maleleka (S)	Shrub	Rootbark	Emetics and snuffed or inhaled medicines; Rootbark decoctions are taken for headaches; Rootbark used for headache	Hutchings (1989); Hutchings *et al.* (1996); Moffett (2016)
Oleaceae	*Olea europaea* subsp. *cuspidata* (Wall. & G.Don) Cif. [*O. europaea* subsp. a*fricana* (Mill.) P.S.Green Kew Bull., *O. chrysophylla* Lam., *O. kilimandscharica* Knobl.]	Olive tree, wild olive (E); Olienhout (A); Umnquma (Z, X); Motlhware (B); Mutlhwari (V)	Tree	Leaves	Unspecified; Infusions of dry leaves used to treat headache; Medicinal plant used for headache; Unspecified	Hutchings *et al.* (1996); Moffett (2016); Moteetee *et al.* (2019); Stafford (2009)
Passifloraceae	*Adenia gummifera* (Harv.) Harms [*Modecca gummifera* Harv., *A. rhodesica* Suess., *A. gummifera* var. *gummifera*]	Snake-climber, monkey rope (E); Slangklimop (A); Impinda (Z)	Shrub	Roots	Root is used to make tonic, taken orally as stimulant for seediness or depression; Infusions made from roots in boiling water are administered as emetic tonics or stimulants for seediness or depression; Unspecified parts used to treat depression	Corrigan *et al.* (2011); Philander (2011); Sobiecki (2002)
Peraceae	*Clutia pulchella* L. [*C. cotinifolia* Salisb*., C. pulchella* var. *genuina* Müll.Arg., *C. pulchella* var. *pulchella*, *C. gapinii* Pax]	Common lightning bush (E); Gewone bliksembos (A); Podimolwetse (S); Umsimpane (X); Umembesa (Z)	Shrub	Unspecified	Used as emetics and snuffed or inhaled medicines; Used to treat headaches; Medicinal plant used for headache	Hutchings (1989); Moffett (2016); Moteetee *et al.* (2019)

Plant family	Scientific name [synonyms]	Local name	Life form	Plant parts used	Method of preparation, route of administration and/or short notes	References
Phyllanthaceae	*Pseudophyllanthus ovalis* (E.Mey. ex Sond.) Voronts. & Petra Hoffm. [*Andrachne ovalis* (E. Mey. ex Sond.) Müll. Arg., *Savia ovalis* (E.Mey. ex Sond.) Pax & K.Hoffm.]	False lightning bush (E)	Shrub	Roots	Used as emetics and snuffed or inhaled medicines; Burnt roots are sniffed for headache; Root emetics taken to relieve morning stress and body aches and burned roots snuffed for headache	Hutchings (1989); Hutchings *et al.* (1996); Philander (2011)
Poaceae	*Cymbopogon nardus* (L.) Rendle [*C. virgatus* Stapf ex Bor, *C. validus* (Stapf) Stapf ex Burtt Davy, *Sorghum nardus* (L.) Kuntze]	Tamboekiegras (A); Isicunge/ isiqunga (Z)	Grass	Shoot, roots	Used to revitalise the nerves of moody people, Zulu use the roots and shoots to strengthen the nervous system	Sobiecki (2002)
Polygalaceae	*Securidaca longipedunculata* Fresen. [*Elsota longipendunculata* (Fresen.) Kuntze, *S. longipendunculata* var. *longipendunculata*]	Violet tree, fibre tree (E); Rooipeultjie (A); Mmaba (S); Iphuphuma (Z); Mpesu (V)	Tree	Roots; wood	Root kernel is used to treat headache; Powdered root/wood rubbed on forehead for headache	Mongalo and Makhafola (2018); van Wyk *et al.* (1997)
Polygonaceae	*Rumex sagittatus* Thunb. [*R. scandens* Burch., *Acetosa sagittata* Johnson & Briggs]	Climbing dock (E); Ranksuring (A); Umdende (Z); Tshitamba-tshedzi (V); Bodilaboboholo (S)	Herb	Rootstock	Emetics and snuffed or inhaled medicines; Powdered rootstock used by the Sotho as a snuff for headaches; Powdered rootstock used as snuff for headache; Used medicinally to treat headache	Hutchings (1989); Hutchings *et al.* (1996); Moffett (2016); Moteetee *et al.* (2019)
Pteridaceae	*Adiantum capillus-veneris* L. [*A. capillus-veneris* var. *capillus-veneris* L., *A. capillus-veneris* f.	Southern maidenhair fern (E)	Herb	Leaves	Used as emetics and snuffed or inhaled medicines; Dried leaves are smoked for head and chest colds	Hutchings (1989); Hutchings *et al.* (1996)

Plant family	Scientific name [synonyms]	Local name	Life form	Plant parts used	Method of preparation, route of administration and/or short notes	References
	dissectum (M. Martens & Galeotti) Ching]					
Ranunculaceae	*Ranunculus multifidus* Forssk. [*R. striatus* Hochst. ex A. Rich., *R. udus Freyn*.]	Common buttercup (E); Botterblom, kankerblare (A); Isijojokazana (Z); Hlapi (S)	Herb	Unspecified	Emetics and snuffed or inhaled medicines; Burning plant inhaled by the Sotho people to relieve headache; Smoke is inhaled to relieve headache	Hutchings (1989); Hutchings *et al.* (1996); Moffett (2016)
Rhamnaceae	*Ziziphus mucronata* Willd. [*Z. madecassus* H. Pierrier, *Z. mucronata* subsp. *mucronata*]	Buffalo thorn (E); Blinkblaar-wag-'n-bietjie (A); Umphafa (Z); Mongalo (S)	Tree	Leaves; bark	Unspecified; Powdered leaf and bark in water is taken as an emetic	van Wyk *et al.* (1997); Venter and Venter (2016)
Rutaceae	*Ptaeroxylon obliquum* (Thunb.) Radlk. [*P. utile* Eckl. & Zeyh., *Rhus obliqua* Thunb.]	Sneezewood tree (E); Nieshout (A); Umthathi (X)	Tree	Bark and wood	Emetics and snuffed or inhaled medicines; Xhosas use powdered bark traditionally as a snuff and medically to relieve headaches; Used medicinally to treat headache; Powdered bark used as snuff; Powdered wood used as snuff; Bark and wood used to make snuff to treat headache	Hutchings (1989); Hutchings *et al.* (1996); Philander (2011); Sobiecki (2002); van Wyk *et al.* (1997); Venter and Venter (2016)
Salicaceae	*Salix mucronata* Thunb. [*S. subserrata* Willd.]	Cape Willow (E); Kaapse Wilger (A); Mogokare (S); Umnyezane (Z); Munengeledzi (V)	Tree	Leaves, roots	Leaves are compressed on the head to treat headache; Leaf compress used for headache; Used medicinally to treat headache; Decoctions or infusions used for headache; Root decoction used to treat headache	Hulley and Van Wyk (2019); Nortje and van Wyk (2015); Philander (2011); van Wyk *et al.* (1997); Venter and Venter (2016)

Plant family	Scientific name [synonyms]	Local name	Life form	Plant parts used	Method of preparation, route of administration and/or short notes	References
Solanaceae	*Datura ferox* L. [*D. laevis* Bertol. *D*. *quercifolia* Kunth]	Long-spined thorn apple (E); Groot stinkblaar (A)	Shrub	Unspecified	Unspecified	Stafford (2009)
	Datura metel L. [*D. metel* var. *dentata* Schltdl. & Cham., *D. metel* var. *fastuosa* (L.) Saff.]	Angel's trumpet (E)	Shrub	Unspecified	Emetics and snuffed or inhaled medicines; Unspecified parts smoked for the relief of headache; Unspecified	Hutchings (1989); Hutchings *et al.* (1996); Sobiecki (2002)
	Datura stramonium L. [*D. stramonium* var. *canescens* Roxb., *D. stramonium* var. *chalybaea* W.D.J.Koch]	Common thorn apple(E); Malpitte(A); Ijoyi, umhlabavutha (X); Iloyi (Z)	Shrub	Leaves	Unspecified part compressed on the head to relieve headache; Emetics and snuffed or inhaled medicines; Unspecified parts smoked for the relief of headache; The Venda use the leaves to treat insanity. Healers inhale powdered roots and leaves as snuff for divinatory purposes; Unspecified; Dried and powdered leaves used as consciousness-altering snuff by diviners; Leaves used to treat headache	Hulley and Van Wyk (2019); Hutchings (1989); Sobiecki (2002); Stafford (2009); Thring and Weitz (2006); Van Wyk and Gericke (2000); van Wyk *et al.* (1997)
	Nicotiana glauca Graham [*N. glauca lateritia* Lillo]	Tree tobacco (E)	Shrub	Leaves	Compressed on the head for headache (external use only); Leaf compress used to treat headache; Leaves are warmed and put on the head to relieve headache; Fresh leaves applied to the head as a poultice for headache	Hulley and Van Wyk (2019); Nortje and van Wyk (2015); Van Wyk and Gericke (2000); Van Wyk *et al.* (2008)

2.4.2 South African medicinal plants with potential antidepressant effects

Error! Reference source not found. presents an overview of the key characteristics of each of the e thnobotanical studies reviewed. Included studies were conducted and published during the period from 1989 to 2019. A total of 8 studies were conducted in the past 7 years. In this review, 186 medicinal plants from 63 plant families have been listed as being used in South Africa to treat MDD and MDD-com. The life forms for the plants included shrubs (40%), trees (21%), herbs (38%) and grasses (1%). **Table 2.2** represents an overview of 54 medicinal plants that comprised of popular plants based on multiple mentions (37) from ethnobotanical studies and 27 plants with pharmacological evidence for depression. About 20% of the recorded plants (i.e., *Adenia gummifera* (Harv.) Harms, *Albizia adianthifolia* (Schum.) W. Wight, *Ballota hirsuta* Benth., *Boophone disticha*, *Gomphocarpus fruticosus* (L.) W.T.Aiton, *Mentha longifolia* (L.) L. and *Leonotis leonurus* (L.) R.Br.) were categorized as the most popular based on a relatively high number of mentions in the ethnobotanical surveys reviewed. The three most popular plants, with over 8 mentions each from ethnobotanical surveys, included *Boophone disticha*, *Leonotis leonurus* and *Mentha longifolia*. Several other plants, such as *Agapanthus campanulatus, Cannabis sativa* L., *Heteromorpha trifoliata* (H. L. Wendl.) Eckl. & Zeyh., *Sceletium tortuosum*, *Schinus molle* and *Xysmalobium undulatum*, have been used traditionally in South Africa to treat MDD and MDD-com (Hutchings *et al.*, 1996; Van Wyk & Gericke, 2000; Van Wyk *et al.*, 1997).

Boophone disticha is an important Southern Africa medicinal bulb and is popular in South Africa where it is used as herbal medicine by traditional healers to induce hallucinations and as a medication for mental disorders (Hutchings *et al.*, 1996; Neergaard *et al.*, 2009; Stafford *et al.*, 2008). Bulb infusions of this plant are drank by South African traditional healers and patients (Neergaard *et al.*, 2009), and weak decoctions made from the bulb scales are taken by mouth or as enemas for headache (Hutchings *et al.*, 1996; Van Wyk *et al.*, 1997). *Sceletium tortuosum* has more recently attracted attention for its long history of use in traditional medicine and its possible use in promoting well-being and treating depression and/or stress (Harvey *et al.*, 2011; Sobiecki, 2002). It was likely to have been used in prehistoric times by hunter-gatherers and pastoralists as a mood-altering substance (Van Wyk & Gericke, 2000). The whole plant is chewed or drank traditionally as a psychoactive substance or medicine to elevate mood, reduce stress and treat anxiety and depression disorders (Hutchings *et al.*, 1996; Sobiecki, 2002; Van Wyk *et al.*, 1997). *Xysmalobium undulatum* has a long history of therapeutic use in South African traditional medicine (Helmstädter, 2015). The roots of this plant contain glycosides with weak central nervous system depressants and the extracts have indicated antidepressant activity (Hutchings *et al.*, 1996). Powdered roots are used as snuff or inhaled medicine and as emetics (Hutchings,

1989; Van Wyk *et al.*, 1997). Unspecified parts of *Agapanthus campanulatus* are used by the Sotho to treat people with “spirit”, which is a type of mental disturbance (Sobiecki, 2002). Infusions made from leaves, fruits, and leaf decoctions of *Schinus molle* are used traditionally as antidepressants (Bhat & Jacobs, 1995). Fresh leaves are placed on a cloth with vinegar and wrapped as a compress on the head to treat headaches (Hulley & Van Wyk, 2019; Nortje & van Wyk, 2015; Van Wyk *et al.*, 2008). *Cannabis sativa* is widely used in traditional African medicine for both recreational and medicinal purposes. (El-Alfy *et al.*, 2010; Hutchings *et al.*, 1996). The whole plant is used to treat “Vaal sick” and excessive headache (Mongalo & Makhafola, 2018), smoked to induce well-being, relaxation, sociability and/or spirituality and administered orally, intravenously or by topical application for treatment of depression and other conditions (Van Wyk & Gericke, 2000; Van Wyk *et al.*, 1997). Decoctions from the leaves of *Heteromorpha trifoliata* are administered traditionally for mental and nervous diseases and smoked for headaches (Hutchings *et al.*, 1996). The Sotho and Xhosa administer leaf decoctions and infusions for the same purpose (Hutchings *et al.*, 1996; Sobiecki, 2002). The continuous reliance on traditional medicine has led to an extensive indigenous knowledge and expertise within local communities, and documentation thereof, from which herbal product development can be initiated (Fennell *et al.*, 2004; Ndhlala *et al.*, 2011).

2.4.3 Plant parts used, methods of preparation and routes of administration

Based on the reviewed ethnobotanical literature, different plant parts were used in the preparation of medicinal plants used to treat MDD and MDD-com. Plant parts used for the preparations are specified in most studies, with leaves (49 times) being the most predominantly used plant part **(Figure 2.2**). However, about 35% (66) of the plants did not specify the plant parts used in the preparation of plant medicines. Fourteen different plant parts (e.g., leaves, bark, roots, seeds, bulbs and the stem) are used for the preparation of herbal medicines in various studies **(Figure 2.2)**. Recorded plants were prepared using various preparation methods, including decoction, infusion, burning, compressing, crushing, pounding, tea and powder. The most frequently used method of preparation was burning, which accounted for approximately 21% of the plant medicines recorded **(Figure 2.3)**. This method involves burning medicinal plant material and inhaling the smoke or smoking the dried plant material. The second most frequently used methods of preparation are infusion and powder, both used 28 times each. Recorded routes of administration included oral (drinking, chewing), nasal (snuffing, inhaling, steaming) and topical (applied on the skin, wrapped around the head). The most recorded route of administration is the nasal route which includes plants that are powdered and sniffed, and plants that are burnt and their smoke inhaled.

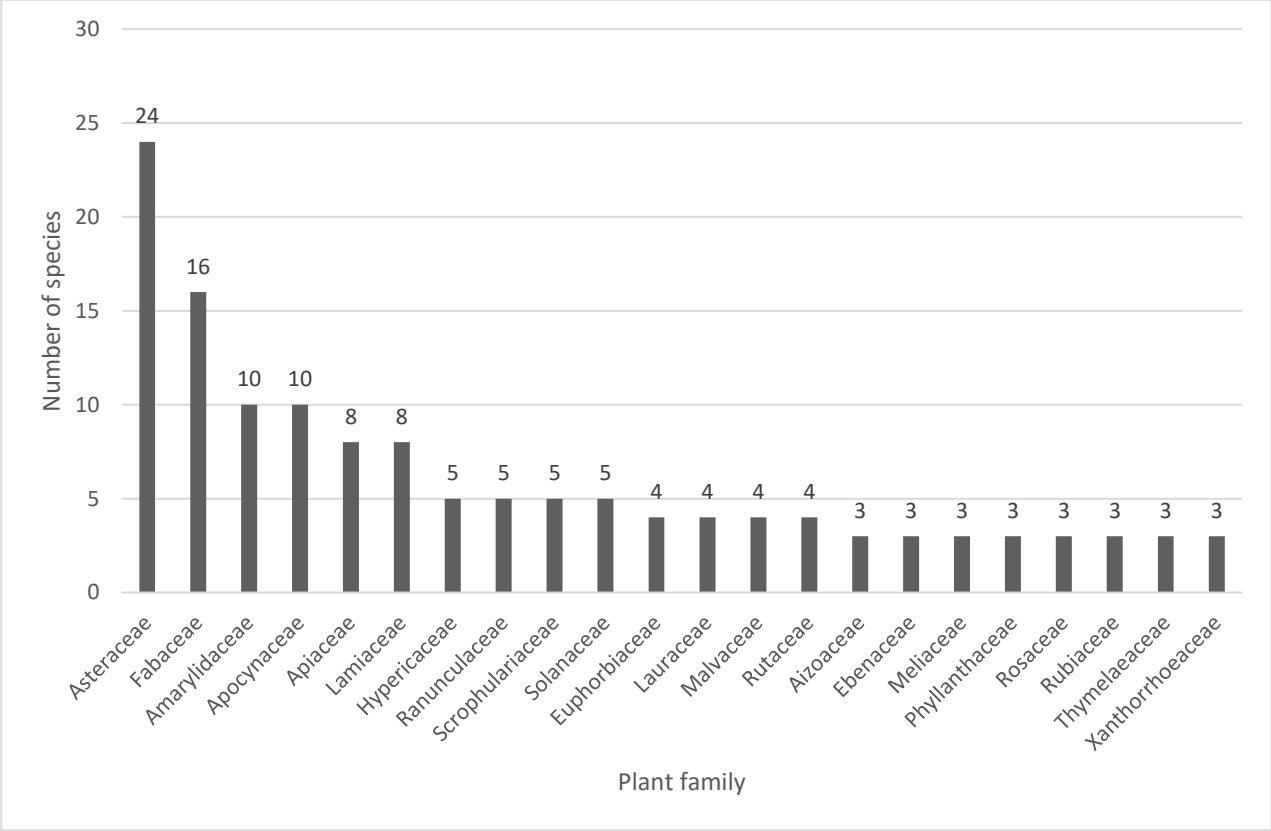


Figure 2.4: **Twenty-two plant families with three or more plant species recorded for the treatment of depression. The remaining 41 families had only one or two plants**

2.4.4 Plant families used for depression and related ailments

A total of 63 plant families are being used in South Africa to treat MDD and MDD-com **(Figure 2.4)**. Twenty-two of the plant families recorded represented more than 2 plant species used traditionally against MDD. The remaining 41 plant families represented only one or two plant species. Plant families with 3 or more plant species recorded for the treatment of depression and related ailments were shown in **Figure 2.4**. Several plant families contain relatively higher numbers of species with potential antidepressant effects than others. Plant families with the highest numbers of plant species recorded included Asteraceae (24) and Fabaceae (16), Amaryllidaceae (10), and Apocynaceae (10).

The Asteraceae (Compositae) as a plant family is known to have therapeutic applications, and has a long history in traditional medicine (Rolnik & Olas, 2021). Members of the Asteraceae are commonly used to treat various diseases since ancient times (Panda & Luyten, 2018), including

the observation in the current study **(Figure 2.4)**. Asteraceae are known to produce a large quantity of terpenoids, such as hemiterpenes, sesquiterpenes, diterpenes, monoterpene and polyterpenes, and flavonoids, with common flavonols, quercetin and kaempferol and flavones apigenin and luteolin being widely distributed (Hutchings *et al.*, 1996; Sülsen *et al.*, 2017). Trigo *et al.* (2003) isolated and identified pyrrolizidine alkaloids of the senecionine, platyphylline, rosmarinine, senkirkine subgroups and triangularine group from the inflorescences of 14 *Senecio* species (Asteraceae), one of the largest genera of flowering plants distributed worldwide. Sarker *et al.* (2001) investigated a methanolic extract from the seeds of *Centaurea cyanus* L. (Asteraceae) using preparative RP-HPLC analysis and afforded 4 alkaloids of the indole variety: moschamine, *cis*-moschamine, centcyamine and *cis*-centcyamine. Existing pharmacological studies have reported the antidepressant-like activity of *Artemisia dracunculus* L. (Asteraceae) in animal models of depression (Ilkhanizadeh *et al.*, 2021; Jahani *et al.*, 2019). Although these studies did not isolate or identify any phytochemicals in their experiments, several phenolic compounds, such as, syringic acid, vanillic acid, chlorogenic acid, ferulic acid, caffeic acid, quercetin and luteolin are present in the plant material of *A. dracunculus (Mumivand et al., 2017)*. The presence of these alkaloids may be rationale behind the extensive use of plants in this family for the alleviation of depressive symptoms in traditional medicine. Despite this plant family being the most mentioned in ethnobotanical surveys, a few of the plants recorded have been investigated pharmacologically using models of depression to validate the recorded traditional uses.

Fabaceae often produces indole variety alkaloids, such as N-methyltryptamine, N-methyltryptophan, and choline, able to mimic the structure of the neurotransmitter serotonin, thereby antagonizing its action with resulting neuroprotective effects (Hutchings *et al.*, 1996). Although no specific phytochemicals were isolated, aqueous extract of *Albizia adianthifolia* W.Wight (Fabaceae) leaves increased swimming time and decreased immobility time in the forced swimming test conducted using male Wistar rats (Beppe *et al.*, 2015). The antidepressant-like effect of this plant may be attributed to the potential presence of the indole alkaloids (Hutchings *et al.*, 1996). The similarity in structure of indole alkaloids to neurotransmitters including serotonin has led to the prediction of the potential neurological and antidepressant effects of several medicinal plants and their active phytochemicals (Hamid *et al.*, 2017). Further investigations are required to identify these alkaloids from medicinal plants belonging to the Fabaceae and explore their antidepressant-like effects.

Amaryllidaceae plants are extensively used traditionally for central nervous system activation, with uses such as for the treatment of depression, epilepsy and other mental disorders (Nair & Van Staden, 2014; Stafford *et al.*, 2008). Their pharmacological efficacy can be attributed to the

presence of unique alkaloids previously isolated from several medicinal plants belonging to this plant family (Elgorashi, 2019; Nair *et al.*, 2013). Amaryllidaceae is one of the 20 most important alkaloid containing plant families (Koutová *et al.*, 2020). Plants from the Amaryllidaceae produce isoquinoline alkaloids classified into unique structurally diverse groups, with three major structural-types galanthamine, lycorine and crinine (Francesc *et al.*, 1997; Nair & Van Staden, 2013). The minor series of these alkaloids include tazettine, homolycorine, and montanine (Nair & Van Staden, 2013; Nair *et al.*, 2013). Previous studies of *Boophone disticha* (Amaryllidaceae), a popular plant in South African traditional medicine, led to the identification of buphanamine, buphanisine, buphanidrine, distichamine and crinine and a confirmation of its antidepressant effects (Neergaard *et al.*, 2009; Nielsen *et al.*, 2004; Sandager *et al.*, 2005). Raghoo *et al.* (2021) identified aromatic, lycorine or crinine type Amaryllidaceae alkaloids from the bulb of *Ammocharis coranica* Herb. (Elisha *et al.*, 2013; Koorbanally *et al.*, 2000a). Bay-Smidt *et al.* (2011) explored the tribe Haemantheae for potential target species for the discovery of serotonin reuptake transport protein inhibitors. From the study, lycorine, homolycorine and montanine type alkaloids were isolated from *Haemanthus hirsutus* Baker, the extract of *H. sanguineus* Jacq. yielded only montanine type alkaloids and *H. coccineus* L. yielded montanine type and crinine type alkaloids, all with antidepressant effects. Furthermore, the alkaloid-rich extracts *Haemanthus coccineus*, *H. montanus* Baker and *H. sanguineus* yielded two isoquinoline montanine type Amaryllidaceae alkaloids, montanine and coccinine, both with antidepressant effects (Stafford *et al.*, 2013). This serves as evidence that the plant family Amaryllidaceae is of significance and importance in phytochemical-based antidepressant drug discovery.

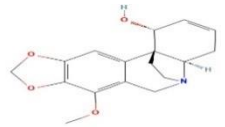
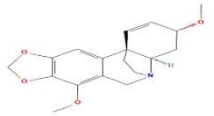
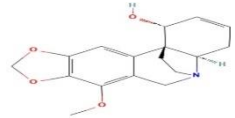
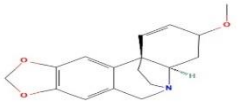
Apocynaceae as a plant family is an important and popular source of a number of drugs including simple indoles, carbolines, steroidal amines, isomeric quinindolines and quinindoles and miscellaneous type alkaloids (Dey *et al.*, 2017; Raffauf & Flagler, 1960). Members of the Apocynaceae contain alkaloid ibogaine, which is used as a psychedelic drug for the treatment of substance addiction (Dey *et al.*, 2017; Koenig & Hilber, 2015), ajmalicine, an alkaloid used as an antihypertensive drug used to treat high blood pressure (Wink & Roberts, 1998), and alstonine, an antipsychotic picralima alkaloid which prevents hyperlocomotion, memory deficit and social interaction deficit through antipsychosis mediated by 5-HT2A/C receptors (Dey *et al.*, 2017). Members of the Apocynaceae often produce a vast range of indolic alkaloids, including tryptophan, and harman type alkaloids that are psychoactive (Trease & Evans, 1983). *Mondia whitei* (Hook.f.) Skeels commonly known as White's ginger, is a popular South African plant used in folk medicine to treat diseases of the nervous system and it has demonstrated antidepressant properties under *in vitro* conditions (Aremu *et al.*, 2011; Egebjerg *et al.*, 2006; Grabarczyk *et al.*, 2015; Neergaard *et al.*, 2010; Pedersen *et al.*, 2008). Koorbanally *et al.* (2000b) reported the isolation and identification of the previously identified chemical compounds 2-hydroxy-4-

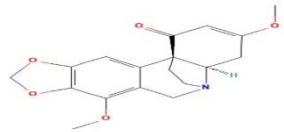
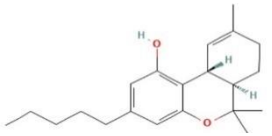
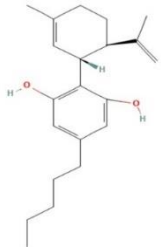
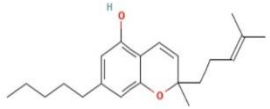
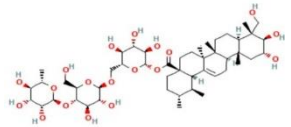
methoxybenzaldehyde and reported the presence of Isovanillin from a methylene chloride extract of *M. whitei*. Neergaard *et al.* (2010) isolated a monoterpene lactone (−)-loliolide from *M. whitei* leaves and tested it for *in-vitro* for its affinity to SERT. The extracts showed good displacement of [3H]-citalopram in the SERT binding assay. In a binding assay, extracts of *M. whitei* had exhibited affinity to SERT (Nielsen *et al.*, 2004). Ethanolic extracts have further exhibited antidepressant activity in a functional SERT inhibition assay and had an antidepressant-like effect in 2 *in vivo* models of depression (Pedersen *et al.*, 2008). Authors suggested that the *in vitro* serotonin transporter affinity exhibited by the plant extracts was due to the lactone (−)-loliolide.

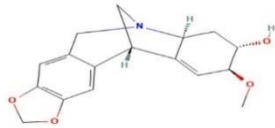
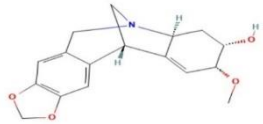
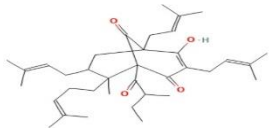
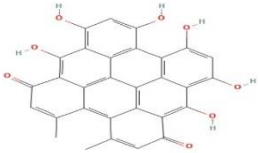
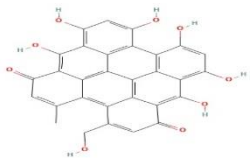
Two members of the Solanaceae have demonstrated antidepressant-like effects in *in vitro* and *in vivo* investigations that evaluated the potential antidepressant effects of medicinal plants. An aqueous extract from *Datura ferox* L. seeds exhibited high affinity in the SERT binding assay (Nielsen *et al.*, 2004), while extracts from the fresh leaves of *Datura stramonium* L. exhibited antidepressant-like effects in the forced swimming test and open field test conducted by Devi *et al.* (2012). Other families representing medicinal plant species with pharmacological evidence against MDD are Aizoaceae (*Sceletium tortuosum*), Cannabaceae (*Cannabis sativa*), Hypericaceae (*Hypericum perforatum*), Pteridaceae (*Adiantum capillus-veneris* L.), Lauraceae (*Cinnamomum camphora* (L.) J.Presl.), Poaceae (*Cymbopogon nardus* (L.) Rendl.), Capparaceae (*Maerua angolensis* DC.), Meliaceae (*Melia azedarach* L.), Polygalaceae (*Securidaca longepedunculata* Fresen), Rhamnaceae (*Ziziphus mucronate* Willd.) and Anarcadiaceae (*Schinus molle*) (**Figure 2.4**).

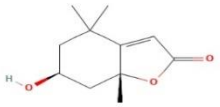
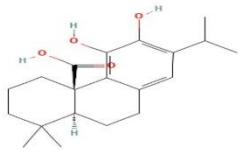
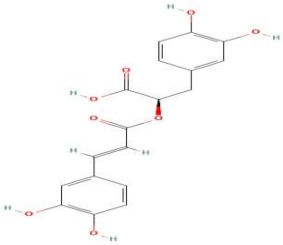
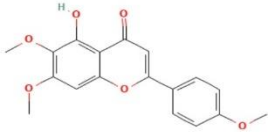
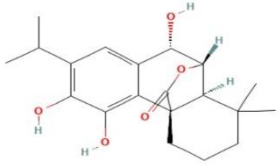
To allow for easy identification and selection of potential medicinal plants for pharmacological investigation, the relationship between plant families with potential antidepressant effects and the various phytochemicals present in plants belonging to these families remain pertinent. This was achieved by looking closely at the phytochemical profiles of medicinal plants belonging to the four plant families with the highest number of plants recorded in this review. Based on the fact that plants in the same family may have similar phytochemical profiles, it is hypothesized that recognizing plant families of potential (based on the presence of anti-depressive phytochemicals) could lead to the discovery of potential medicinal plants within those families for pharmacological investigations using models of MDD. This approach may provide insight into the potential presence of novel phytochemical compounds of antidepressant value not previously isolated from medicinal plants and stimulate alternative antidepressant drug discovery.

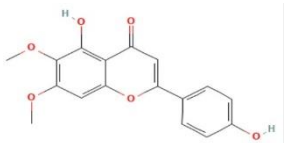
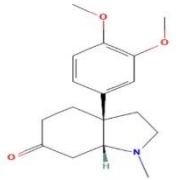
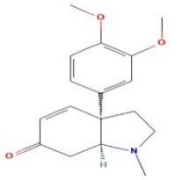
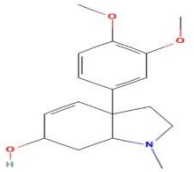
Table 2.3: Phytochemicals isolated and chemical structures of compounds from plants with antidepressant-like effects. TLC- Thin layer chromatography, VLC- Vacuum liquid chromatography, HPLC- High-performance liquid chromatography, GC/MS- Gas chromatography-mass spectrometry

Scientific name	Reference	Plant part	Method of extraction	Methods for the isolation and identification	Compound	Molecular structure
Boophone disticha	Sandager *et al.* (2005)	Leaves	Vacuum filtration	Bioassay-guided fractionation on VLC and preparative TLC	Buphanamine	
					Buphanadrine	
	Neergaard *et al.* (2009)	Bulbs	Liquid–liquid partitioning	HPLC–UV separation	Buphanamine	
					Buphanisine	

					Distichamine	
Cannabis sativa	El-Alfy *et al.* (2010)	Buds	Column chromatography	Preparative C18 HPLC	Δ^9-tetrahydrocannabinol (Δ^9-THC)	
					Cannabidiol (CBD)	
					Cannabichromene (CBC)	
Centella asiatica	Kalshetty *et al.* (2012)	Leaves	Vacuum filtration	HPLC	Asiaticoside	

Cymbopogon nardus	Victoria *et al.* (2014)	Unspecified	Reduced pressure distillation	GC/MS	(R)-Citronellal	
Haemathus coccineus	Stafford *et al.* (2013)	Bulb scales	Maceration	Column chromatography and TLC profile	Montanine	
					Coccinine	
Hypericum perforatum	Tian *et al.* (2014)	Unspecified	Unspecified	Unspecified	Adhyperforin	
	Öztürk *et al.* (1996)	Aerial parts	Maceration	HPLC methods	Hypericin	
					Pseudohypericin	

Mondia whitei	Neergaard *et al.* (2010)	Leaves	Liquid–liquid partitioning	Vacuum liquid chromatography & Preparative HPLC	Loliolide	
Rosmarinus officinalis	Sasaki *et al.* (2013)	Leaves	Maceration	HPLC analysis	Carnosic acid	
					Rosmarinic acid	
	Abdelhalim *et al.* (2015)	Whole plant	Infusion	Column chromatography and preparative thin layer chromatography	Salvigenin	
					Rosmanol	

					Cirsimaritin	
Sceletium tortuosum	Loria *et al.* (2014)	Leaves	Reduced pressure distillation	HPLC fingerprinting analysis	Mesembrine	
	Harvey *et al.* (2011)	Above-ground parts	Maceration	Filtration and column chromatography	Mesembrenone	
					Mesembrenol	

2.4.5 Phytochemicals with potential antidepressant effects

The reported ethnobotanical uses of the 186 medicinal plants were based on indigenous knowledge and expertise from traditional healers and knowledgeable community members. Many of these plants contain phytochemicals with psychoactive and pharmacological effects ranging from sedation, stimulation to euphoria and hallucinations (Sobiecki, 2002). Moreover, their effects lead to altering of perception, emotion and cognition, and changes in consciousness (Alrashedy & Molina, 2016; Khan *et al.*, 2018; Martins & Brijesh, 2018). Only 27 (14.5%) of the recorded plants have been investigated pharmacologically for depression while only 9 were phytochemically characterised to identify phytochemicals with antidepressant-like effects. A total of 24 phytochemicals with antidepressant-like effects have been isolated and identified **(Table 2.3).** The plant parts from which the phytochemicals were isolated, the methods of extraction used, the methods of isolation and identification as well as the molecular structures of the compound were identified**.** These plants were represented by 12 pharmacological studies previously conducted to investigate their *in vitro* and *in vivo* antidepressant-like effects. Medicinal plants with the highest number of phytochemicals identified are *Boophone disticha* and *Rosmarinus officinalis* L., each with 5 phytochemicals isolated, identified and their antidepressant effects investigated in various models of depression (Abdelhalim *et al.*, 2015; Neergaard *et al.*, 2009; Sandager *et al.*, 2005; Sasaki *et al.*, 2013). Phytochemical studies on *Rosmarinus officinalis* led to the identification of carnosic acid, rosmarinic acid (Sasaki *et al.*, 2013), salvigenin, rosmanol and cirsimaritin (Abdelhalim *et al.*, 2015), with proven antidepressant effects in the force swim test (Abdelhalim *et al.*, 2015; Machado *et al.*, 2009), TST (Abdelhalim *et al.*, 2015; Machado *et al.*, 2009; Sasaki *et al.*, 2013; Sasaki *et al.*, 2021) and OFT (Machado *et al.*, 2009). The mood elevating properties of extract *Sceletium. tortuosum*, Zembrin™, are due to the presence of mesembrine, a phytochemical with potent selective serotonin (5-HT) re-uptake activity (Harvey *et al.*, 2011; Van Wyk & Gericke, 2000).

The most frequently used solvent for extraction of plant material was ethanol, being used in 6 out of the 12 studies (50%), while the most popular method of extraction was maceration, which was used in four of the studies (33.3%). Various methods were used to isolate and identify phytochemicals from plant extracts, and these include HPLC–UV separation, bioassay-guided fractionation, vacuum liquid chromatography, gas chromatography, column chromatography and preparative thin layer chromatography. Using bioassay-guided fractionation on VLC and preparative TLC, Sandager *et al.* (2005) isolated buphanidrine and buphanamine from the leaves of *Boophone disticha* and tested them for their affinity to the serotonin transporter (SERT) protein. These phytochemicals inhibited affinity to the SERT in the rat brain. In addition, Neergaard *et al.*

(2009) isolated buphanamine, buphanisine, crinine, buphanidrine and distichamine by repeated preparative HPLC and tested the activity of these compounds in a SERT binding and a functional SERT inhibition assay. Buphanamine, buphanidrine and distichamine showed high activity in SERT binding assay, whereas buphanidrine and distichamine showed activity in the functional SERT inhibition assay. El-Alfy *et al.* (2010) used preparative C_{18} HPLC to isolate Δ^9-tetrahydrocannabinol (Δ^9-THC), cannabidiol (CBD) and cannabichromene (CBC) from the buds of *Cannabis sativa*, the three phytochemicals that showed antidepressant-like effects in two animal models of depression (the force swimming test and the tail suspension test) conducted in the same study. Other phytochemicals isolated from indigenous plant extracts in similar studies, such as (R)-Citronellal, asiaticoside, adhyperforin, hypericin, loliolide and carnosic acid, have been investigated in various models of depression, where they showed positive antidepressant-like results (Kalshetty *et al.*, 2012; Neergaard *et al.*, 2010; Öztürk *et al.*, 1996; Sasaki *et al.*, 2021; Victoria *et al.*, 2014). The use of phytochemicals in MDD therapy is reported to decreases the risk of some severe disorders, including cardiovascular, autoimmune and neurodegenerative diseases (Lee & Bae, 2017).

2.4.6 Ethnopharmacological investigations

For the analysis of pharmacological literature, we critically assessed the experimental approaches used, following methods described by Heinrich *et al.* (2020). Briefly, the correct identification of plant material under study, appropriate methodology, models, and controls were the focus in the identification of pharmacological antidepressant studies included in this review. For a pharmacological study to be included in this review, it had to include a validated source of material, standard methodology for herbal antidepressant assays and access to full text articles written in the English language. Based on the inclusion criteria for this review, a total of 27 medicinal plants were investigated pharmacologically for their antidepressant activity. However, the majority of these plants were screened for antidepressant-like effects despite the absence of any ethnobotanical records indicating their use for such purposes in South African traditional medicine. Plants such as *Agapanthus campanulatus*, *Haemathus coccineus, Scadoxus puniceus*, and *Mondia whitei* have been confirmed to possess antidepressant effects, however, they are documented ethnobotanically to be administered in the traditional treatment of mental disorders, insanity, headache, nervous disorder, used as tranquillizers, snuff and sedatives (**Table 2.2**). Out of the 186 medicinal plants recorded in this review, only 18 plants (9.7% of the total recorded plants) were recorded with indications for managing depression in ethnobotanical surveys. This has led to a paucity of pharmacological evidence on the antidepressant-like effects of South African medicinal plants.

Several biological assays have been used to investigate the antidepressant-like effects of South African medicinal plants, including *in vitro* biological assays (Harvey *et al.*, 2011; Neergaard *et al.*, 2009; Nielsen *et al.*, 2004; Sandager *et al.*, 2005; Stafford *et al.*, 2013) and *in vivo* assays conducted using rodent models of depression (Ahmadpoor *et al.*, 2019; El-Alfy *et al.*, 2010; Machado *et al.*, 2009; Machado *et al.*, 2007; Rabiei & Setorki, 2019). The mechanism of action of most psychoactive compounds involves endocrine modulation of specific molecules in the CNS, modification of multiple biological effects on reuptake and/or receptor binding of various monoamines and interacting with neuronal receptors (Alrashedy & Molina, 2016; Saki *et al.*, 2014). Medicinal plants and their bioactive compounds produce antidepressant therapeutic effects via interaction with serotonergic systems (SERT), noradrenergic (NAT) and dopaminergic (DAT) receptors (Machado *et al.*, 2007; Stafford *et al.*, 2008). Therefore, screening medicinal plant extracts for the effects they have on these neurotransmitters remains relevant for exploring plants used in traditional medicine for treating depression. Furthermore, the forced swimming test (FST) and tail suspension test (TST) represent the most widely used and well-established paradigm for screening medicinal plants for antidepressant activity using animals (Pedersen *et al.*, 2008).

Table 2.4: Different *in vitro* assays utilized for investigating the antidepressant potential of indigenous medicinal plants

Plant species	Reference	Plant part	Solvent used	Serotonin transporter (SERT) binding assay	SERT inhibition assay	Dopamine transporter (DAT) inhibition assay	Noradrenalin transporter (NAT) inhibition assay
Agapanthus campanulatus	Pedersen *et al.* (2008)	Unspecified	Ethanol	Extracts inhibited the binding of [3H]-citalopram with IC_{50} value of 4.9±1.3 mg dry extract/ml	The extract inhibited SERT significantly with IC_{50} = 99.4 µg/ml	Extract exhibited potent inhibition of NAT, with IC_{50} = 84.9 µg/ml	
	Nielsen *et al.* (2004)	Leaves; flowers	Ethanol and water	Aqueous extracts of leaves and flowers from *A. campanulatus* had more than 60% transport protein bound [^{3}H]citalopram at the three highest concentrations.	-	-	-
Boophone disticha	Pedersen *et al.* (2008)	Unspecified	Ethanol	Extracts inhibited the binding of [3H]-citalopram with IC_{50} = 0.5±1.5 mg/ml	The extract inhibited SERT with IC_{50} = 423.8 µg/ml	Extract inhibited DAT, with IC_{50} = 93.5 µg/ml	Extract had potent inhibition of NAT, with IC_{50} = 77.3 µg/ml
	Sandager *et al.* (2005)	Leaves	Ethanol	Buphanamine and buphanadrine showed affinity to the SERT	-	-	-
	Nielsen *et al.* (2004)	Leaves; bulbs	Ethanol and water	Extracts displaced more than 50% of the transport protein bound [^{3}H]citalopram at the three highest concentrations	-	-	-
	Neergaard *et al.* (2009)	Bulbs	Ethanol	Buphanamine, buphanidrine and distichamine were the most active (IC_{50} = 55±4 µM (Ki = 23 µM), 63±9 µM (Ki = 26 µM) and 65± 7 µM (Ki = 27 µM), respectively).	Buphanidrine and distichamine showed activity in the functional assay.	-	-

Datura ferox	Nielsen *et al.* (2004)	Seeds	Ethanol and water	Extract had 80% transport protein bound [3H]citalopram at the three highest concentrations	-	-	-
Haemathus coccineus	Stafford *et al.* (2013)	Bulbs	Methanol	Extracts had considerable affinity for the SERT, with IC_{50} = 2 µg/ml	-	-	-
Hypericum perforatum	Tian *et al.* (2014)	Unspecified	Unspecified	Adhyperforin had strong binding affinity to the hSERT with Ki value = 18.7567.76 mg/ml	Adhyperforin potently blocked the uptake of 5-HT, with IC_{50} = 4.14±0.29 mg/ml	Adhyperforin potently blocked the uptake of DA, with IC_{50} = 0.89±0.07 mg/ml	Adhyperforin potently blocked the uptake of NE, with IC_{50} = 2.64±0.35 mg/ml
	Fiebich *et al.* (2011)	Aerial parts	Ethanol	*Hypericum* extracts (500 µg/ml) inhibited serotonin uptake by greater than 80%	-	-	-
Mondia whitei	Pedersen *et al.* (2008)	Unspecified	Ethanol	Extracts inhibited the binding of [3H]-citalopram with IC_{50} value of 2.2±1.4 mg dry extract/ml	The extract inhibited SERT with IC_{50} = 283 µg/ml	No significant effect on this transporter	No significant effect on this transporter
	Neergaard *et al.* (2010)	Leaves	Ethanol	Fractions containing (−)-loliolide showed good displacement of [3H]-citalopram from the SERT	-	-	-
Scadoxus puniceus	Bay-Smidt *et al.* (2011)	Bulbs	Methanol	Showed affinity to the SERT protein with IC_{50} >50	-	-	-
Sceletium tortuosum	Harvey *et al.* (2011)	Above - ground parts	70% ethanol, 30% water	Extract had a marked effect (>80% inhibition of binding) at the 5-HT transporter binding site	No significant effect on this transporter.	No significant effect on this transporter	No significant effect on this transporter

	Coetzee *et al.* (2016)	Unspecified	Unspecified	-	In astrocytes, extract had comparable effects to citalopram. In GT1-7 cells, similar effects to citalopram, but came by slower	-	-
Xysmalobium undulatum	Pedersen *et al.* (2008)	Unspecified	Ethanol	Extracts inhibited the binding of [3H]-citalopram with IC_{50} = 1.1±2.3 mg dry extract/ml	No significant effect on this transporter	No significant effect on this transporter	No significant effect on this transporter
	Nielsen *et al.* (2004)	All parts	Ethanol and water	Extract had more than 50% of the transport protein bound [^{3}H]citalopram at the three highest concentrations	-	-	-

2.4.6.1 *In vitro* biological assays (SERT, DAT and NAT screening assays)

In vitro assays utilized for the investigation of potential antidepressant effects of medicinal plants include the SERT (serotonin transporter) protein binding assay, the functional SERT uptake inhibition assay, the functional DAT (dopamine transporter) uptake inhibition assay and the functional NAT (noradrenalin transporter) uptake inhibition assay. The SERT binding assay includes, briefly, mixing a dilution of the extract with [3H] citalopram and rat brain tissue suspension, replacing the extract with paroxetine for positive control and with a buffer for negative control. All samples are then incubated for 2 h and filtered under a vacuum before radioactivity is measured by liquid scintillation (Nielsen *et al.*, 2004). For the functional inhibition of SERT, DAT and NAT, human SERT, NAT and DAT clones transfected in COS-7 cells are incubated for 30 min in PBSCM containing 50 nM [^{3}H]-5-HT (SERT assay) or 50 nM [^{3}H]-dopamine (NAT and DAT assays) and increasing concentrations of extracts. The amount of accumulated [^{3}H]-5-HT or [^{3}H]-dopamine is determined by solubilizing cells in scintillant, followed by direct counting of plates. Specific uptake is calculated by subtracting uptake values from control values (Pedersen *et al.*, 2008).

Nine plants including *Agapanthus campanulatus*, *Boophone disticha*, *Datura ferox*, *Hypericum perforatum* and *Mondia whitei* were investigated for their antidepressant activity using *in vitro* models of depression. The summary of these studies, including the plant part used, solvents used, *in vitro* model used, assay conducted, and an overview of the findings are presented in **Table. 2.4**. Most of the *in vitro* investigations on the potential antidepressant effects of medicinal plants were conducted using the SERT binding assay (**Table 2.4**). The pharmacological screening of medicinal plants for antidepressant effects has yielded several plants with noteworthy antidepressant activity. Ethanol extracts from the leaves of *Agapanthus campanulatus, Boophone disticha* and *Mondia whitei* exhibited antidepressant activity *in vitro* (**Table 2.4**). Methanol and ethanol extracts from the bulbs of *Boophone disticha, Haemathus coccineus* and *Scadoxus puniceus* showed affinity for the SERT protein *in vitro*. Most of these active leaf and bulb extracts were extracted using ethanol as a solvent. Generally, ethanol extracts were observed to have the most significant antidepressant activity. Pharmacological studies on *Boophone disticha* revealed that ethanolic extracts from this plant possess affinity to the SERT protein, and functional inhibition of SERT, DAT and NAT (Neergaard *et al.*, 2009; Nielsen *et al.*, 2004; Pedersen *et al.*, 2008; Sandager *et al.*, 2005). Powdered extracts of *Hypericum perforatum* are used traditionally as antidepressants and represent an acceptable alternative to conventional synthetic antidepressants (Nielsen *et al.*, 2004; Van Wyk *et al.*, 1997). *In vitro* pharmacological investigations on *H. perforatum* reported that this plant possesses affinity to SERT, and it inhibited

SERT, NAT and DAT (Fiebich *et al.*, 2011; Tian *et al.*, 2014). The observed antidepressant pharmacological activities of *H. perforatum* appear to be attributed to adhyperforin, hypericin and pseudohypericin, previously isolated from dry extracts, with antidepressant activities as effective as conventional antidepressants desipramine and trimipramine (Öztürk *et al.*, 1996; Tian *et al.*, 2014). An ethanol extract from *Sceletium tortuosum*, with alkaloids mesembrine, mesembrenone and mesembrenol, has been shown to inhibit serotonin uptake (Harvey *et al.*, 2011).

Table 2.5: Different *in vivo* assays utilized for investigating the antidepressant potential of indigenous medicinal plants

Plant species	References	Plant part	Solvent used	Forced swimming test (FST)	Tail suspension test (TST)	Open-field test (OFT)
Adiantum capillus-veneris	Ahmadpoor *et al.* (2019)	Whole plant	Ethanol	Plant extract (100, 200, and 400 mg/kg) significantly decreased the immobility time	-	-
	Rabiei and Setorki (2019)	Whole plant	Ethanol	Extract (200 mg/kg) significantly reduced immobility time duration while doses of 50 and 100 mg/kg had no significant effect on immobility	-	-
Agapanthus campanulatus	Pedersen *et al.* (2008)	Unspecified	Ethanol	Extract exhibited antidepressant-like effects at doses 250 and 500mg/kg and yielded 74.4% & 62.3% relative immobility, respectively.	No significant effects.	No effect on the spontaneous activity of mice or rats.
	Pedersen *et al.* (2008)	Unspecified	Ethanol	Extract exhibited no significant effects.	-	-
Albizia adianthifolia	Aderibigbe (2018)	Leaves	Ethanol	Extract significantly reduced immobility time at doses 1.25 mg/kg (40.8 ± 13.1) and 2.50 mg/kg (42.4 ± 9.7) compared to control (170.0 ± 10.1) [$p < 0.05$]	Extract significantly reduced immobility time at dose1.25 mg/kg (85.2 ±8.9) compared to control (142.6 ± 3.9) [$p < 0.05$].	-
	Beppe *et al.* (2015)	Leaves	Distilled water	Both doses exhibited significant effects evidenced by the swimming time ($F(3,36) = 21.09$, $p < 0.0001$) and the immobility time ($F(3,36) = 78.59$, $p < 0.0001$)	-	-

Artemisia dracunculus	Jahani *et al.* (2019)	Aerial parts	Ethanol	At doses 100, 200, and 400 mg/kg, ethanolic extract decreased immobility time (162.30 ± 6.87, 161.60 ± 5.54, and 153.60 ± 6.87 sec, respectively)	Extract at the doses of 100 and 200 mg/kg decreased immobility time (124.00 ± 6.58, 117.20 ± 2.50 sec, respectively). No significant effect at dose 400 mg/kg	Extract decreased immobility time in Swiss mice treated with 100 mg/kg
	Ilkhanizadeh *et al.* (2021)	Whole plants	Ethanol	Extract (50 mg/kg) significantly reduced depression induced immobility time in comparison to OVX group ($P < 0.05$)	Extract (50 mg/kg) significantly reduced depression induced immobility time ($P < 0.05$)	Extract at doses 25 and 50 mg/kg significantly increased the number of crossing in OFT test.
Boophone disticha	Pedersen *et al.* (2008)	Unspecified	Ethanol	Extract exhibited an antidepressant-like activity at doses 250 and 500 mg/kg and yielded 84.9% & 83.3% relative immobility, respectively	At a dose of 125 mg/kg, extract significantly exhibited an antidepressant-like activity	No effect on the spontaneous activity of mice or rats
	Pedersen *et al.* (2008)	Unspecified	Ethanol	Extract exhibited an antidepressant-like activity at dose 250 mg/kg and yielded 74.2% relative immobility	-	-
Cannabis sativa	Zanelate *et al.* (2010)	Unspecified	Unspecified	CBD treatment reduced immobility time ($F_{6,59} = 3.89$, $P < 0.01$) at dose 30 mg/kg	-	-
	El-Alfy *et al.* (2010)	Buds	Hexane & water	Δ9-THC showed significant overall reduction in immobility time ($F_{[3,35]} = 8.32$; $p = 0.0003$). Δ8-THC had no significant effect on immobility time ($F_{[3,44]} = 2.14$; $p = 0.11$). CBD revealed a significant decrease in	Δ9-THC resulted in significant decrease in immobility time ($F_{[3,32]} = 3.29$; $p = 0.033$). CBC resulted in significant reduction in immobility time	-

				immobility time (F[3,42] = 3.89; p = 0.015)	(F[3,33] = 6.24; p = 0.002). CBD did not affect immobility time at any of the doses (F[3,33] = 0.59; p = 0.623)	
	Sales *et al.* (2018)	Unspecified	Unspecified	CBD at dose 10 mg/kg significantly reduced the immobility time in the FST (F3,25 = 6.104, p < 0.05)	-	No significant effect on the locomotor activity in the open-field test
Centella asiatica	Selvi *et al.* (2012)	Whole plant	Ethanol and water	The extract at both doses exhibited significant reduction in immobility time compared to control	-	-
	Ceremuga *et al.* (2015)	Unspecified	Unspecified	Asiatic acid (AA) from *Centella asiatica* had no significant effect on immobility time. Significant effect (1.8), (P = .001) in the faecal pellet output (FPO)	-	-
	Kalshetty *et al.* (2012)	Leaves	Isopropyl alcohol	-	-	INDCA (3, 10 or 30 mg/kg) significantly reduced the ambulation scores (30.3%, 51.2% and 64.7%; P < 0.001) in the open filed test. INDCA (30 mg/kg) showed significant (P < 0.01) reduction of rearing score (12.8, reduction of 47.5%). INDCA (30 mg/kg) treatment reduced the grooming score by 33.3%

Cinnamomum camphora	Rabadia *et al.* (2013)	Bark	Ethanol	Rats showed significant decrease in their immobility times at all 3 doses (116.7 ± 6.146, 110.8 ± 12.54; p<0.05 and 101.7 ± 9.458; p<0.01), respectively.	Rats showed significant decrease in their immobility times at all 3 doses (133.3 ± 8.758, 128.3 ± 8.33; p < 0.05 and 122.5 ± 8.342; p < 0.01), respectively	-
Cymbopogon nardus	Victoria *et al.* (2014)	Unspecified	Ethanol	No significant effects in the mouse FST.	No significant effects on mouse TST	No significant effect on exploratory and locomotor activities of mice
Datura stramonium	Devi *et al.* (2012)	Leaves	Water	Extract caused a significant increase in immobility time (p < 0.05 for 20 mg/kg, p < 0.01 for 40 mg/kg).	-	The extract significantly decreased the locomotor activity when compared with the control. For 20mg/kg (p < 0.05) and for 40mg/kg (p < 0.001)
Hoodia gordonii	Citó *et al.* (2015)	Unspecified	Unspecified	Significantly reduced immobility time of animals after acute administration of *H. gordonii* at doses of 25 mg/kg and 50 mg/kg. Animals treated for 15 consecutive days at doses of 25 mg/kg and 50 mg/kg also reduced immobility time	-	Extract did not alter locomotor activity when either dose was used
Hypericum perforatum	Tian *et al.* (2014)	Unspecified	Unspecified	Adhyperforin (16 mg/kg) significantly decreased the immobility times of mice in the FST (p=0.049)	Adhyperforin (16 mg/kg) significantly reduced the immobility times of mice (p = 0.043)	Adhyperforin had no significant effect on spontaneous locomotor activity in mice at any dose tested

	Öztürk *et al.* (1996)	Aerial parts	50% ethyl alcohol	Dried plant extract from aerial parts of *Hypericum perforatum* decreased the swimming performance to a statistically significant extent ($p < 0.05$)		Dried plant extracts from the aerial parts of H.P. decreased the walking time to a statistically significant extent ($p < 0.05$)
	Ramalhete *et al.* (2016)	Aerial parts	Methanol	Extract showed no significant effects.	Extract showed non-significant effects like those of the control.	-
	Fiebich *et al.* (2011)	Aerial parts	Ethanol and water	*Hypericum* extract exerted a significant inhibitory effect on the Immobility of the rats at doses 180 and 360 mg/kg ($p < 0.01$)	-	-
Hypericum revolutum	Hailu and Engidawork (2014)	Leaves	Methanol	Extract significantly brought down immobility time at both 200 mg/kg (33.72%, $p < 0.05$) and 400 mg/kg (38.42%, $p < 0.01$)	Extract at doses of 200 (44%, $p < 0.01$) and 400 mg/kg (49%, $p < 0.01$) significantly reduced immobility time, 100 mg/kg did not show any significant change	Extract failed to produce any significant alteration in the parameters measured in the OFT
Maerua angolensis	Benneh *et al.* (2018)	Stem bark	Petroleum ether/ethyl acetate (50:50) mixture	MAE (1000mg/kg) significantly decreased the immobility time and increased swimming time ($F3, 38 = 10.33$, $p < 0.0001$) of mice.	MAE at 1000 mg/kg significantly decreased the immobility time ($F3\ 20 = 5.744$, $P = 0.0053$). Significant effect on duration at dose 300mg kg ($F3, 20 = 3.493$, $P = 0.0347$)	-

Melia azedarach	Ishaq (2016)	Flowers, twigs and roots	Methanol	15 mg/kg dose significantly (22.7%, $p < 0.005$) decreased immobility time.	-	-
Mentha spicata	Jedi *et al.* (2017)	Unspecified	Unspecified	MS essential oil (120 & 240 mg/kg) reduced immobility time in mice	MS essential oil at doses 120 7 240 mg/kg reduced immobility time in mice	-
Mondia whitei	Pedersen *et al.* (2008)	Unspecified	Ethanol	Extract exhibited no significant effects.	No significant effects.	No effect on the spontaneous activity of mice or rats
	Pedersen *et al.* (2008)	Unspecified	Ethanol	Extract exhibited an antidepressant-like activity at dose 250 mg/kg and yielded 69.9% relative immobility	-	-
Olea europaea subsp. *cuspidata*	Badr *et al.* (2020)	Unspecified	Unspecified	Immobility times were significantly reduced by Oleuropein at 8 mg/kg ($p < 0.01$), 16 mg/kg ($p < 0.001$) and 32 mg/kg ($p < 0.001$)	Oleuropein treatment at doses 8, 16 and 32 mg/kg significantly decreased the immobility time in TST	Immobility time significantly decreased with oleuropein at 8 mg/kg ($p < 0.01$), 16 mg/kg ($p < 0.001$) and 32 mg/kg ($p < 0.001$)
	Perveen *et al.* (2013)	Unspecified	Unspecified	Significant increase in struggling time ($P < 0.05$) following repeated administration of extra-virgin olive oil	-	-
	Tariq *et al.* (2021)	Fruit	Ethanol	Green and black olive extract significantly reduced the immobility period	Green and black olive extract treatment displayed less immobility time in TST	-

Rosmarinus officinalis	Machado *et al.* (2009)	Stems and leaves	Ethanol	The percent of reduction in the immobility time was 49.5% in FST with one-way ANOVA revealing a significant effect of the extract (100 mg/kg) in FST [F(4,31) = 6.24, Pb0.01]	The percentage of reduction in immobility time was 22.9%, 28.0% in TST, one-way ANOVA revealed a significant effect of the extract (10–100 mg/kg) in TST [F(4,29) = 6.80, Pb0.01]	Extract (1–300 mg/kg, p.o.) did not significantly alter the number of the rearings of mice in OFT
	Sasaki *et al.* (2013)	Leaves	Ethanol	-	Immobility times were decreased to 91.98 ± 12.06 s for 50 mg/kg and 66.81 ± 17.00 s for 100 mg/kg	-
	Sasaki *et al.* (2021)	Whole plant	Ethanol & citric acid crystal	-	While 100 mg/kg significantly decreased immobility time from day 2 onwards, 10 mg/kg only had decrease in immobility time at day 7	-
	Abdelhalim *et al.* (2015)	Whole plant	Petroleum ether, ethanol & ethyl acetate	Salvigenin, rosmanol and cirsimaritin (30 and 100 mg/kg) significantly decreased the immobility time in the FST as compared to the control (vehicle) ($p < 0.05$; $p < 0.001$)	Salvigenin, rosmanol and cirsimaritin (30 and 100 mg/kg) caused a significant decrease in the immobility time as compared to the vehicle control group ($p < 0.05$; $p < 0.01$)	-

Securidaca longipedunculata	Adebiyi *et al.* (2006)	Roots	Water	Aqueous extract significantly decreased duration of immobility (P < 0.05) at the dose of 400 mg/kg	-	-
Sceletium tortuosum	Loria *et al.* (2014)	Leaves	Methanol and chloroform	*S. tortuosum* extract significantly decreased floating time of rats	-	-
Schinus molle	Machado *et al.* (2007)	Stems, leaves	Hexane	-	Extract significantly decreased the immobility time at all doses. One-way ANOVA revealed a significant effect [F(4,25)=11.14, Pb0.01]	Extract at dose range 100–600 mg/kg had no significant effect on locomotor activity of mice as compared to control group. One-way ANOVA revealed [F(3,18) = 1.38, p = 0.27]
Xysmalobium undulatum	Pedersen *et al.* (2008)	Unspecified	Ethanol	Extract exhibited an antidepressant-like activity at doses of 250 and 500 mg/kg and yielded 77.6%67.9% relative immobility, respectively	No significant effects	No effect on the spontaneous activity of mice or rats
	Pedersen *et al.* (2008)	Unspecified		Extract exhibited no significant effects	-	-
Ziziphus mucronata	Wado *et al.* (2020)	Leaves	Methanol and water	Chronic treatment with ZM at both doses significantly reversed depressive behaviour ([F (5, 27) = 6.284; p < 0.0005] lowering in swimming time and [F (5, 27) = 10.44; p < 0.0001] increase in immobility time)	-	-

2.4.6.2 *In vivo* biological models (The FST, TST and OFT)

In vivo behavioural models such as the forced swimming test (FST), tail suspension test (TST) and the open-field test (OFT) were applied on *in vivo* models for the investigation of potential antidepressant effects of medicinal plants. The forced swimming test is the most widely used test for screening of antidepressants and it involves placing rats or mice individually in plastic cylinders containing a column of water with no possible escape for 6 min (Rabadia *et al.*, 2013). After allowing the rats to acclimatize for 2 minutes, immobility time (in seconds) is recorded in the last 4 minutes of the test (Ahmadpoor *et al.*, 2019). In the tail suspension test, rats or mice are individually hung by the tail using adhesive tape and attached to the edge of a tabletop hanging about 75 cm above the floor. The total duration of immobility (the absence of any limb or body movements) is then recorded manually during the 6-minute session (Pedersen *et al.*, 2008). Differences can be noted between the FST and the TST, the response to drugs in both tests and the apparent increased sensitivity of the TST (Machado *et al.*, 2009). The locomotor activity test involves recording the spontaneous activity of animals treated with plant extracts in photoresistor actometers. Mice or rats are individually placed in actometers illuminated by 2 light beams for the recording of light beam interruptions and the number of light beam crossings are counted (Pedersen *et al.*, 2008). Imipramine is used for positive control and Tween 80:water (1:10) was used as the negative control in all three *in vivo* studies (Pedersen *et al.*, 2008).

A total of 24 plants, including *Adiantum capillus-veneris*, *Agapanthus campanulatus*, *Albizia adianthifolia*, *Artemisia dracunculus*, *Boophone disticha* and *Cannabis sativa* have been investigated in *in vivo* studies using rodent models of depression. **Table 2.5** summarizes the details of these studies, including the plant part used, the solvent used, the animal model and an overview of the findings. Most of the *in vivo* studies were conducted using the rodent forced swimming test (**Table 2.5**). Ethanol extracts from *Albizia adianthifolia, Rosmarinus officinalis, Sceletium tortuosum* and *Ziziphus mucronata* exhibited antidepressant activity *in vivo* (**Table 2.5**). Ethanolic extracts from *Boophone disticha* exhibited antidepressant-like effects in the FST and TST (Pedersen *et al.*, 2008). Previously isolated from *B. disticha*, buphanamine and buphanadrine structurally have the benzo-1,3-dioxole moiety in common with SSRI paroxetine, which could explain the observed anti-depressant-like properties (Neergaard *et al.*, 2009; Sandager *et al.*, 2005; Stafford *et al.*, 2013), and further, the traditional use of this plant to treat mental disorders (Hutchings *et al.*, 1996). *Cannabis sativa* cannabinoids Δ^9-THC, CBD and CBC induced significant antidepressant-like effects in animal models of depression (FST &TST) (El-Alfy *et al.*, 2010; Zanelate *et al.*, 2010). A study into CBD-induced antidepressant-like effects in

the FST revealed that the antidepressant effects depend on levels of serotonin, but not noradrenaline, in the CNS (Sales *et al.*, 2018; Zanelate *et al.*, 2010). This serves as rationale for the traditional use of *C. sativa* to treat depressive mental conditions (Hutchings *et al.*, 1996; Van Wyk *et al.*, 1997). Oleuropein, which is considered the most active phenolic active ingredient of *Olea europaea* subsp. *cuspidata*, significantly reduced levels of serotonin and dopamine and exhibited antidepressant-like effects in the FST, TST and OFT (Badr *et al.*, 2020).

The most extensively studied plants included *Agapanthus campanulatus*, *Boophone disticha*, *Hypericum perforatum*, *Mondia whitei* and *Xysmalobium undulatum*. These plants were investigated in all the bioassays included in this study. Based on the plant parts screened, eleven plants demonstrated antidepressant activity in leaf extracts (*Agapanthus campanulatus, Boophone disticha, Mondia whitei, Albizia adianthifolia, Centella asiatica, Rosmarinus officinalis, Sceletium tortuosum, Hypericum revolutum, Datura stramonium, Schinus molle* and *Ziziphus mucronata*). These studies serve as a scientific rationale for the utilization of the reviewed pharmacological assays in the investigation of medicinal plants of potential with no *in vivo* or *in vitro* pharmacological evidence. Furthermore, this pharmacological review provides insight into the plant parts and solvents used in the preparation of anti-depressive plant extracts for pharmacological investigation.

Table 2.6: Toxicity, side effects and safety indication of South African medicinal plants used traditionally to manage depression and related ailments. SOD- Superoxide dismutase, GPX- Glutathione peroxidase, CAT- Catalase, GSH- Glutathione, MDA- Malondialdehyde, ROS- Reactive oxygen species

Plant species	Toxicity	Side effects	Safety indication	References
Agapanthus campanulatus	No reported toxicity	Gastrointestinal tract and kidney problems		Ndhlala *et al.* (2013)
Albizia adianthifolia	No reported toxicity.	No reported side effects.	Leaf extracts do not induce neurotoxicity and this effect could be related to its antioxidant activity (increased activities of SOD, CAT, GSH level and the decreased levels of protein carbonyl and MDA	Beppe *et al.* (2015); Hutchings *et al.* (1996); Van Wyk *et al.* (1997)
Artemisia dracunculus	No reported toxicity.	No reported side effects.	Possible mechanism for anxiolytic and antidepressant effects reported to be the anti-oxidant activity of tarragon. Extract reduced the serum MDA while elevated SOD and GPX levels and has protective effect against ROS production and oxidative stress	Ilkhanizadeh *et al.* (2021); Khosravi *et al.* (2017); Mumivand *et al.* (2017)
Boophone disticha	Bulbs are reported to have caused both acute and fatal poisoning in human beings, following medicinal administration	Symptoms of non-fatal toxicity include dryness of the mouth and increased thirst, nausea, vomiting, impaired vision, and variable emotional reactions followed by stupor and sleep for about an hour	Several human deaths have been reported due to extremely toxic alkaloids. Internal use is dangerous and should be avoided.	Hutchings *et al.* (1996); Ndhlala *et al.* (2011); Van Wyk and Gericke (2000); Van Wyk *et al.* (1997)
Cannabis sativa	Toxic effects of prolonged use include lassitude, indifference, lack of productive activity, insomnia, headaches, nystagmus, increased susceptibility to infections,	Side effects include increased heart rate, palpitations, orthostatic hypotension, acute panic reactions, mental confusion, depression and paranoia. Acute toxic psychosis in some users.	The mouse tetrad assay determined that Δ9-THC at 2.5 mg/kg dose in both the FST and TST does not cause any impairment of locomotor activity, change in body temperature or catalepsy.	El-Alfy *et al.* (2010); Hutchings *et al.* (1996)

	gastrointestinal disturbances, sexual impotence and personality changes.			
Centella asiatica	No reported toxicity	No reported side effects		Kalshetty *et al.* (2012); Van Wyk and Gericke (2000)
Cinnamomum camphora	Death from respiratory causes is rare however use as an inhalant for children, or in large amounts should be avoided as it may cause systemic toxicity. Seeds contain cytotoxic proteins camphorin and cinnamomin	Large doses of this plant cause nausea and vomiting while high doses produce epileptiform convulsions	Small doses warm and soothe the epigastric region	Hutchings *et al.* (1996); Rabadia *et al.* (2013); Van Wyk *et al.* (1997)
Cymbopogon nardus	No reported toxicity	No reported side effects	The treatment of mice with (R)-Citronellal did not cause death of any animals and demonstrated lack of toxicological effects	Victoria *et al.* (2014)
Datura ferox	All plant parts are toxic	Pupil dilation, convulsion, tremor and appetite depression. Toxicity in livestock causes reduction in body weight, hypersalivation and an altered gait.		Kovatsis *et al.* (1993)
Datura stramonium	Poisoning from cooked leaves as reported in KZN. Ingestion of the seeds produces similar effects to *Cannabis* poisoning in children. Studies have shown it has potential to cause damage to the ultrastructure of the brain cells.	Severe mental confusion, hallucinations, insomnia, increased heart rate and decreased saliva due to atropine, tropane, and hyoscine alkaloids. Blurred vision, suppressed salivation, vasodilation, hallucinations and delirium	There exists dangers of harmful side effects and self-medication without medical advice is not recommended.	Devi *et al.* (2012); Hutchings *et al.* (1996); Ndhlala *et al.* (2013); Van Wyk *et al.* (1997)
Haemanthus coccineus	All members of genus *Haemanthus* are considered capable of causing dermatitis.	Side effects have not been reported		Bay-Smidt *et al.* (2011); Stafford *et al.* (2013)

Hoodia gordonii	Has anorectic effects (produces loss of appetite)	Experiments resulted in decrease in food consumption and body mass of rats and an increase in ATP in the hypothalamus		Van Wyk *et al.* (1997)
Hypericum perforatum	No reported toxicity associated with internal use	Photosensitivity may occur in fair-skinned users, especially under fairly strong sunshine		Öztürk *et al.* (1996); Ramalhete *et al.* (2016); Tian *et al.* (2014); Van Wyk *et al.* (1997)
Maerua angolensis	Seizure induction potential in mice.	Physical signs such as tonic and/or clonic convulsions were observed during the Irwin test		Benneh *et al.* (2018)
Melia azedarach	Significant poisoning may occur after large amounts of the fruit have been ingested. All plant parts are reported to be toxic. Fatal poisoning from the fruit and bark.	Anuria, severe stomatitis and violent and sanguineous vomiting, nausea, and diarrhoea, followed by, mental confusion and stupor, respiratory problems, convulsions and partial to complete paralysis	Gastric lavage, egg whites and milk for shock treatment and symptomatic measures are recommended treat poisoning	Hutchings *et al.* (1996)
Mondia whitei	No reported toxicity	No reported side effects	*Mondia* is reasonably expected to be safe when prepared and used according to traditional practices.	Aremu *et al.* (2011); Koorbanally *et al.* (2000b); Neergaard *et al.* (2010)
Olea europaea subsp. cuspidata	No reported toxicity	Gastric symptoms may occur due to an irritant effect on the mucosa. Therefore, plant medicine should always be taken after meals.	Significantly decreased the antioxidant pool of rat's brain	Hutchings *et al.* (1996); Tariq *et al.* (2021)
Rosmarinus officinalis	Dose ranges 50-200 mg/kg of Salvigenin, rosmanol and cirsimaritin did not produce any toxicity effects	No reported side effects	Protect neuronal cells against corticosterone-induced toxicity. Extract improved cell viability 30% in vitro.	Abdelhalim *et al.* (2015); Sasaki *et al.* (2013)

Scadoxus puniceus	Human deaths and poisoning have been reported.	Symptoms of non-fatal poisoning from the bulbs or leaves include visual disturbances, dizziness and CNS excitation or depression. Hypotension and convulsion		Ndhlala *et al.* (2013); Van Wyk *et al.* (1997)
Sceletium tortuosum.	No toxicity reported	Intoxicating doses can cause euphoria	No severe adverse effects have been documented	Gericke and Viljoen (2008); Harvey *et al.* (2011); Van Wyk *et al.* (1997)
Securidaca longipedunculata	Securinine is toxic. Overdoses are potentially lethal and suicidal use has been documented.			Van Wyk and Gericke (2000); Van Wyk *et al.* (1997)
Xysmalobium undulatum	Low toxicity	Severe gastrointestinal irritation from protracted poisoning		Van Wyk and Gericke (2000); Van Wyk *et al.* (1997)
Ziziphus mucronate	Low toxicity. Fruit is toxic but is considered edible and is used for porridge. Extracts from leaves have shown potential genotoxic activity.			Hutchings *et al.* (1996); Van Wyk *et al.* (1997)

2.4.7 Toxicity and safety of South African plants used for managing depression

South Africa has a rich diversity of medicinal plants, however, this rich flora includes several plants with the potential to poison humans (Nair & Van Staden, 2013; Ndhlala *et al.*, 2013). As highlighted in **Table 2.6**, there are safety concerns for some of the South African plants with anti-depressant potential. Among the listed plants, *Boophone disticha, Haemanthus coccineus* and *Scadoxus puniceus* have the isoquinoline alkaloids as the toxic compound characterized by symptoms such as dizziness (Ndhlala *et al.*, 2013). In South Africa, traditional medicine prescription and use were not regulated in the past due to the dangers of misadministration, and the potential long-term genotoxic effects that follow the prolonged use of self-prescribed popular herbal medicines(Fennell *et al.*, 2004). Poisonous plants can lead to serious poisoning when ingested or irritation and/or discomfort after contact with the skin (Hutchings *et al.*, 1996; Van Wyk *et al.*, 1997). Adverse effects may include hallucinations, sedation, irrational behaviour and more seriously coma and death (Van Wyk *et al.*, 1997). Toxic substances from medicinal plants can affect important human organs while some are able to affect functional systems of the body, like the central nervous system (CNS), and interfere with the nerve function coordination of the body (Ndhlala *et al.*, 2013). A comprehensive review on the ethnopharmacology and toxicology of alkaloids of the Amaryllidaceae of South Africa has been published (Nair & Van Staden, 2013). *Boophone disticha* is amongst the first recorded toxic plants to have caused fatalities due to poisoning after consumption by Sotho, Xhosa and Zulu people from South Africa or when its bulbs were used as arrow poisons by the Khoi and San, and is the most widely studied for its toxic effects (Hutchings *et al.*, 1996; Nair & Van Staden, 2013). Many health complications have resulted from the toxic use of this plant (Sobiecki, 2002). Symptoms of non-fatal administration of this plant include an unsteady gait, dryness of the mouth and increased thirst, nausea, vomiting, impaired vision and variable emotional reactions followed by stupor and sleep for about an hour (Hutchings *et al.*, 1996). The prolonged use of *Cannabis sativa* has toxic effects that include indifference, insomnia, lassitude, headaches, increased susceptibility to infections, sexual impotence, gastrointestinal disturbances, and personality changes (Hutchings *et al.*, 1996). Side effects of regular use include heart palpitations, orthostatic hypotension, acute panic reactions, mental confusion, depression paranoia and acute toxic psychosis in some users (Hutchings *et al.*, 1996). The seeds of *Cinnamomum camphora*, commonly known as camphor bush, contain cytotoxic proteins camphorin and cinnamomin that cause systemic toxicity when used as an inhalant for children, or in large amounts (Hutchings *et al.*, 1996; Van Wyk *et al.*, 1997).

2.5 Conclusion

South Africa has a rich diversity of plants that have been recorded for the treatment of MDD. The systematic review of 20 ethnobotanical records led to the documentation of 186 medicinal plants from 63 families used locally for the treatment of MDD and MDD-com. Most of the plants were from Asteraceae, Fabaceae, Amaryllidaceae and Apocynaceae. These families have demonstrated rationale for their extensive use in traditional medicine given the presence of unique bioactive phytochemicals with antidepressant-like properties in plants belonging to these families. A large number of the literature reviewed listed local uses of medicinal plants as somatic symptoms described as mental health related problems, such as headache, that could be classified as depression based on similar clinical symptoms. The overlap and comorbidity observed between depression and headache in studies where experiments were conducted on humans provide evidence of a link between medicinal plants used traditionally for headache and the potential antidepressant effects of those plants. This link can be seen with plants such as *Boophone disticha*, *Scadoxus puniceus*, *Maerua angolensis* and *Mentha spicata*, which are listed in ethnobotanical literature as traditional medicine for headaches, but also exhibited antidepressant-like effects in pharmacological studies reviewed in this paper. Moreover, *Sceletium tortuosum*, *Xysmalobium undulatum*, *Cannabis sativa*, *Schinus molle* and *Hypoxis hemerocallidea* represent some of the plants with reported ethnopharmacological uses for both depression and headache and have been validated for their antidepressant-like activity, suggesting that medicinal plants used ethnopharmacologically for headache may have some degree of antidepressant-like effects worth investigating.

Despite the extensive use of medicinal plants for MDD-com in traditional medicine, pharmacological evidence validating the antidepressant action of most plants and the search for phytochemicals of anti-depressive novelty are limited. Only 27 plants (14.5%) have been investigated for their antidepressant-like activity in *in vitro* and *in vivo* models of depression. *Agapanthus campanulatus*, *Boophone disticha*, *Hypericum perforatum*, *Mondia whitei* and *Xysmalobium undulatum*, were the most extensively pharmacologically studied based on investigation in all the models of depression reviewed in this paper. A total of nine plants were investigated in four *in vitro* models of depression, while 23 plants were investigated using *in vivo* models of depression. Nine out of the 27 plants, including *Boophone disticha*, *Mondia whitei*, *Cannabis sativa*, *Hypericum perforatum* and *Rosmarinus officinalis* underwent a further phytochemical investigation to identify the bioactive phytochemicals responsible for their antidepressant-like effects. This led to the identification and isolation of 24 phytochemicals, including buphanidrine, buphanamine, distichamine, cannabidiol, asiaticoside, adhyperforin, hypericin and loliolide, of anti-depressive value. On the basis of the pharmacological and

phytochemical data reviewed, medicinal plant extracts may contain more novel phytochemicals that can improve antidepressant therapy with a more rapid onset of antidepressant action and minimized side effects. This review provides a useful foundation for the investigation of pharmacological antidepressant-like effects of those plants used traditionally but without pharmacological validation. Furthermore, it will stimulate the development of new pharmacotherapies derived from plant extracts for use in the clinical treatment of depression. Future research of the antidepressant effects of South African medicinal plants should be explored and validated using additional models of depression to discover plant species and novel compounds effective at treating MDD. Simultaneous evaluation of the ethnopharmacology and phytochemistry of medicinal plants used traditionally for depression provides a useful framework for the selection of potential candidate species for drug discovery based on traditional use and the presence of phytochemicals with antidepressant effects. Overall, Asteraceae, Fabaceae, Amaryllidaceae and Apocynaceae may hold plant species with phytochemicals capable of effectively treating MDD and its associated symptoms.

CHAPTER 3: DETERMINATION OF PHENOLIC CONTENT AND *IN VITRO* BINDING AFFINITY TO SERT AND ADENOSINE $A_{1/2A}$ RECEPTORS

3.1 Introduction

Despite the availability of multiple antidepressant drug classes, current treatment with conventional antidepressants has shown poor patient compliance and lower remission due to adverse side effects, delayed antidepressant activity, and recurrence of the disease (Fajemiroye *et al.*, 2016; Fathinezhad *et al.*, 2019; Gao *et al.*, 2011; Popa-Velea *et al.*, 2015). This serves as rationale for the investigation and identification of alternative therapeutics that have a potential to improve existing antidepressant drug classes with a more rapid antidepressant action, significant efficacy and minimized side effects (Lee & Bae, 2017; Machado *et al.*, 2007; Sandager *et al.*, 2005). Ethnobotanical reports have identified plants used by South African traditional healers to treat mental disorders, their long-term anecdotal use necessitates further investigation in order to better understand their potential pharmacological perspective as they constitute a well-known and understandable source of botanical material for the investigation of drugs that are therapeutically effective (Fajemiroye *et al.*, 2016; Nielsen *et al.*, 2004). Furthermore, psychotropic plants contain phytochemicals, which evolved as allelochemicals but target specific neuronal receptors when ingested or inhaled (Alrashedy & Molina, 2016). Studies have demonstrated that the antidepressant effects of polyphenols found in plants (e.g. rosmanol from *Rosmarinus officinalis*) are produced by interactions with the serotonergic, noradrenergic, and dopaminergic receptors (Machado *et al.*, 2009; Sasaki *et al.*, 2013).

Physiologically, the monoamine deficiency theory has been suggested to play a vital role in the pathophysiology of depression and other mood disorders, such as anxiety (Song *et al.*, 2021). In the recent years, the serotonin transporter (SERT) is targeted for clinical antidepressants of the selective serotonin reuptake inhibitor (SSRI) type (Jäger *et al.*, 2013). The mechanism of action of the SSRI is to bind to its site on the neuronal serotonin transporter (SSRI site), decreasing transporter affinity for serotonin, thus resulting in inhibition of serotonin binding to the transporter (Stahl, 1998). Serotonin receptors have been used as potential therapeutic targets for novel antidepressant drug development (Martins & Brijesh, 2018). Previous studies have demonstrated that some medicinal plants indigenous to South Africa and their active constituents (including buphanamine and buphanadrine from *Boophone disticha* Herb., loliolide from *Mondia whitei* (Hook.f.) Skeels and rosmanol from *Rosmarinus officinalis* L.) exert antidepressant effects through interaction with the serotonergic system (Machado *et al.*, 2009; Machado *et al.*, 2007; Neergaard *et al.*, 2010; Neergaard *et al.*, 2009). According to Nielsen *et al.* (2004), the antidepressant effects of plants could be due to the SERT affinity of bioactive compounds present

in the plants extracts and screening plants for affinity to SERT represents a way to evaluate the antidepressant potential of these plants.

The impact of depression on the activity of the adenosinergic system has been investigated previously (Bruns *et al.*, 1987; Coelho *et al.*, 2014; Kaster *et al.*, 2004). Moreover, adenosinergic drugs commercially available in the market are known to be safer (Gomes *et al.*, 2021). Research findings have demonstrated that caffeine, which represents one of the most widely used psychoactive substance, exerts its antidepressant effects through adenosine A_{2A} receptors antagonism (El Yacoubi *et al.*, 2001; Kaster *et al.*, 2015). Studies have suggested that the pharmacological blockade of the adenosine A_{2A} receptors produces antidepressant-like effects in some animal models of depression (Batalha *et al.*, 2013; El Yacoubi *et al.*, 2001; Yamada *et al.*, 2014). Furthermore, it is known to reverse stress-induced hippocampal deficits resulting from maternal separation (Batalha *et al.*, 2013). Adenosine A_{2A} receptor antagonists, which are drugs that cause the blockade or inactivation of the receptors, possess antidepressant-like effects likely mediated by an increased dopaminergic transmission (El Yacoubi *et al.*, 2001). The antidepressant action of adenosine is also associated with enhanced A_1 receptors signalling brought about by the activation of A_1 receptors (De Mendonça *et al.*, 2000; Serchov *et al.*, 2015). At the presynaptic level, the activation of the A_1 receptor decreases serotonin, dopamine and acetylcholine release, leading to synaptic transmission inhibition (Proctor & Dunwiddie, 1987). The antidepressant effects evoked by the activation of adenosine A_1 receptors was shown previously in a line of transgenic mice where the upregulation or activation of A_1 receptors led to notable acute and chronic resilience to depressive-like behavior in numerous tests, while A_1 receptor blockade displayed an increased susceptibility to depressive-like ailments (Serchov *et al.*, 2015). The A_1 receptor plays a pivotal role in the regulation of synaptic plasticity and transmission, with its activation having the ability to attenuate long-term depression (De Mendonça *et al.*, 1997). Adenosine receptors may represent a new target for the discovery of novel anti-depressants, with adenosine A_1 receptor agonists and A_{2A} receptor antagonists emerging as candidate therapeutic agents (El Yacoubi *et al.*, 2001; Kaster *et al.*, 2015; Serchov *et al.*, 2015). This is mainly due to the ability of adenosine and its subtypes to produce antidepressant-like effects through controlling aberrant plasticity, synaptotoxicity and neuroplasticity (Batalha *et al.*, 2013; Gomes *et al.*, 2021).

In this study, *Artemisia afra* Jacq. ex Willd., *Adenia gummifera* (Harv.) Harms and *Olea woodiana* Knobl., which have been used traditionally to treat mental disorders that could be described as depression in South Africa (Hutchings, 1989; Sobiecki, 2002; Stafford *et al.*, 2009), were selected for evaluation. The total phenolic content of the aqueous, acetone and hexane extracts of three plants was estimated, followed by the investigation of antidepressant effects using binding affinity

to SERT and $A_{1/2A}$ to assess the reported indigenous knowledge. The binding affinity of the extracts was evaluated using the SERT binding assay, which represents one of the methods of investigating the antidepressant activity of medicinal plants (Nielsen *et al.*, 2004), and the adenosine A_1 and A_{2A} receptors radioligand binding assay, a newer, alternative and novel approach employed in the search for effective antidepressants (El Yacoubi *et al.*, 2001).

3.2 Materials and methods

3.2.1 Plant materials

3.2.1.1 Plant selection from systematic review

Using a combination of ethnobotanical and ethnopharmacological data from the literature review (**Chapter 2**), *Artemisia afra* Jacq. ex Willd., *Adenia gummifera* (Harv.) Harms and *Olea woodiana* Knobl. were selected for phenolic content estimation and binding affinity studies to investigate their potential antidepressant-like effects. Briefly, the three plants (**Table 3.1**) were selected from the combination of most recorded and/or pharmacologically evaluated plant families with phytochemicals previously investigated for antidepressant effects (**Chapter 2, Table 2.2**). Additionally, plants were selected based on the presence of phytochemicals isolated from the plants and plant families, and ethnobotanical notes specifying traditional medicinal use for ailments that could be described as depression (**Chapter 2, Table 2.2**).

Table 3.1: Plant family, species, part used, and voucher specimen of indigenous medicinal plants collected for phytochemical analysis and *in vitro* antidepressant binding in this study

Plant family	Scientific name	Plant parts investigated	Voucher specimen
Asteraceae	*Artemisia afra* Jacq. ex Willd.	Leaf	NU0093805
Oleaceae	*Olea woodiana* Knobl.	Leaf and stem	NU0093803
Passifloraceae	*Adenia gummifera* (Harv.) Harms	Stem	NU0093804

A. afra belongs to Asteraceae with the most recorded plants used traditionally for depression-like ailments and pharmacological studies on antidepressant effects of this family (Ilkhanizadeh *et al.*,

2021; Jahani *et al.*, 2019). Furthermore, *A*. *afra* represents one of the plants with ethnobotanical usage for depression-like ailments but with no pharmacological studies validating its antidepressant effects (Hulley & Van Wyk, 2019; Hutchings *et al.*, 1996; Van Wyk *et al.*, 1997). *A*. *gummifera* is recorded in ethnobotany as being used locally as antidepressants (Corrigan *et al.*, 2011; Philander, 2011; Sobiecki, 2002). Moreover, this plant belongs to Passifloraceae with antidepressant phytochemicals previously isolated from plants of this family, such as quercetin and rutin from *Passiflora* species (Ozarowski & Karpińnski, 2021). *O*. *woodiana* belongs to Oleaceae, a plant family with evaluated antidepressant effects in previous studies (Perveen *et al.*, 2013; Tariq *et al.*, 2021). This family has phytochemicals such as oleuropein, found in olive oil, known to have antidepressant effects (Badr *et al.*, 2020). Plant families were looked at based on the similarities seen in phytochemical profiles of plants belonging to the same family. Due to seasonal changes and the unavailability of some parts during the collection period, only the stems and leaves of the plants were collected for investigation.

3.2.1.2 Plant materials collection and voucher specimen

Artemisia afra (leaf), *Adenia gummifera* (stem) and *Olea woodiana* (leaf and stem) were collected from the Botanical Garden of the University of Kwa-Zulu Natal (UKZN), South Africa from April to June 2022. Voucher specimens were prepared and housed at the UKZN Botanical Garden. Initially, the intention was to collect all plant materials from the North West province. However, due to unforeseen circumstances relating to seasonal changes, some plants were not available during collection and had to be sourced from botanical gardens.

3.2.1.3 Preparation of plant extracts

Plant extracts preparation was done following a method previously described by Nielsen *et al.* (2004). As is in the current study, the aforementioned study screened the antidepressant-like effects of indigenous South African plants used traditionally to manage depression. Briefly, freshly collected plant materials were rinsed thoroughly with water and dried in an oven at 50°C for a maximum of 2 days. The leaf of *O. woodiana* was separated from the stem and all plants including *A*. *afra* and *A*. *gummifera* were separately pulverized into a fine powder using a blender. The pulverized plant materials were weighed before and after sieving and kept in a dry place until ultrasonic extraction. Dried, pulverized plant materials from *A. afra* leaf, *A. gummifera* stem, *O woodiana* leaf and *O. woodiana* stem were extracted once using either water, acetone or hexane for 60 min in an ultrasonic bath (temperature: 40 °C) using ratio 1:10, weight/volume (Jäger *et al.*,

2013; Nielsen *et al.*, 2004; Pedersen *et al.*, 2008). Following filtration under vacuum through Whatman No. 1 filter papers, the aqueous filtrates were frozen at −80 °C and dried in a freeze dryer for up to 4 days, while acetone and hexane filtrates were evaporated to dryness under reduced pressure at 40 °C using a rotary evaporator. The dry extracts were weighed and the percentage extraction yield for each extract was calculated. Plant extracts were stored at 2 - 8 °C until analysis. Percentage extraction yield was calculated using the formula:

$$\text{Extraction yield (\%)} = \frac{\text{weight of final dry extract}}{\text{weight of dry plant}} * 100$$

3.2.2 Phytochemical analysis: Total phenolic content

The total phenolic content of the plant extracts was estimated using the Folin-Ciocalteu method (Makkar, 2000). A total of 12 extracts from three plants (*A. afra* leaf, *A. gummifera* stem, *O woodiana* leaf and *O. woodiana* stem) were prepared using either water, acetone or hexane and analysed using gallic acid as a standard. To prepare the stock solution, 0.5 g of dry gallic acid was dissolved in 10 ml of methanol to create stock solution 1. The solution was transferred into a 100 ml volumetric flask and diluted to volume with up to 100 ml of water to get a 5 mg/ml gallic acid solution to create stock solution 2. For gallic acid calibration standards,1, 2, 3, 5 and 10 ml of gallic acid stock solution were diluted in distilled water up to 100 ml to make the concentration of 50, 100, 150, 250 and 500 µg/ml, respectively. A blank that does not contain any amount of gallic acid was included using a reagent blank with a solvent (50% methanol). To ensure method validity and dependability, a gallic acid calibration curve was plotted after subtracting the blank , with increasing concentration of calibration standards concentration. Methanol was used to dissolve the plant extracts at a concentration of 20 mg/ml. For the analysis,100 µl of the plant extract solutions/gallic acid standards/blank was transferred into reaction tubes before it was mixed with 250 µl of Folin-Ciocalteu reagent, 450 µl of distilled water and 1250 µl of $NaCO_3$ (2%) solution. The reaction mixture was sonicated and incubated in the dark for 40 min at room temperature and centrifuged at 16,000×*g* for 10 min at 40 °C. The contents of the reagent tubes were transferred into 96-well microplate and absorbance was measured at 725 nm. The estimation of total phenolic content was done using three technical replicates.

3.2.3 *In vitro* antidepressant binding assays

3.2.3.1 Preparation of rat membrane

Twenty rats were dissected in order to obtain either rat **whole brains** for the [^{3}H]-citalopram binding assay and the adenosine A_1 receptor radioligand binding assay, or rat **striata**l membranes for the adenosine A_{2A} receptor radioligand binding assay (**Figure 3.1A & B**). Whole brains from Sprague–Dawley rats were snap frozen with liquid nitrogen and stored at -80 °C. Membrane preparation was carried out following previously described methods (Jäger *et al.*, 2013; Van der Walt & Terre'Blanche, 2015). Briefly, on the day of preparation, the tissue was thawed, weighed and homogenized for 30 s (striata) or 90 s (whole brain) with a Polytron homogenizer in 10 volumes of ice-cold 50 mM Tris buffer (5 mM TRIS base, 20 mM EDTA, 150 mM NaCl, pH7.5) (**Figure 3.1C**). The resulting homogenate was centrifuged at 20,000×*g* for 10 min at 40 ^oC and the homogenized tissue pellet was washed with 1:10 (w/v) of the same Tris buffer (**Figure 3.1D & E**). The supernatant was discarded, and the pellet was re-suspended in a buffer (5 mM TRIS

base & 5 mM EDTA, pH 7.5), left for 20 min to react, followed by centrifugation at 20,000×*g* for 10 min at 40 °C. The supernatant was discarded, and the pellet was suspended in 1:10 (w/v) buffer (Tris base 50 mM; NaCl 120 mM; KCl 5 mM; pH 7.5) and centrifuged at 20,000×*g* for 10 min at 40 °C. The supernatant was discarded, and the protein pellet was suspended in 120 ml of the same buffer and then centrifuged at 16,000×*g* for 10 min (**Figure 3.1F**). The pellets were aliquoted into microcentrifuge tubes and stored at -80 ºC until analysis (**Figure 3.1G**).

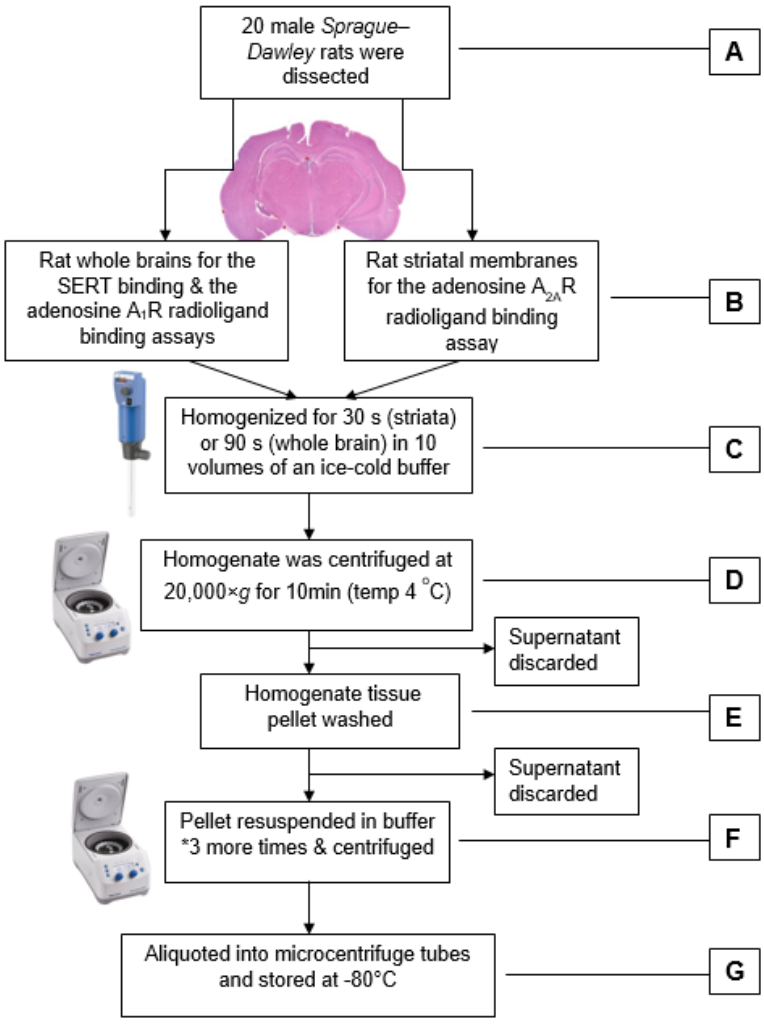


Figure 3.1: Preparation of Sprague–Dawley rat brain membranes for *in vitro* antidepressant binding assays

3.2.3.2 SERT binding assay

The citalopram binding assay was done following previously described methods (Jäger *et al.*, 2013; Pedersen *et al.*, 2008). DMSO was used to dissolve the dry plant extracts to obtain a stock concentration of 10 mg/ml and tested at concentrations of 0.1, 0.01 and 0.001 mg/ml. Plant extract solutions (10 µl) were mixed with 100 µl of [^{3}H]-citalopram (0.7 nM) and 890 µl of tissue suspension. For determining unspecific binding, paroxetine (1.5 µM) was mixed with 100 µl [^{3}H]-citalopram and 890 *µ*l rat whole brain membrane. The total binding of [^{3}H]-citalopram was determined with a DMSO buffer blank by mixing 10 µl DMSO with 100 µl [^{3}H]-citalopram and 890 µl rat whole brain membrane. All samples were incubated for 2 h at room temperature. After incubation, an ice-cold DMSO buffer (5 ml) was added to the samples and the solution was poured directly onto glass fibre filters (Advantec GC50) under vacuum, and immediately washed once with an ice-cold DMSO buffer (5 ml). After 24 h, the amount of radioactivity on the filters was determined by liquid scintillation. Specific binding was calculated as total binding minus unspecific binding. All experiments were done in duplicate. The first phase of the assay included screening extracts for affinity to the SERT protein using stock solution concentrations of 10, 1 and 0.1 mg/ml to obtain final concentrations of 0.1, 0.01 and 0.001 mg/ml. For the second phase, the activity of the extracts during the screening process was used to calculate the IC_{50} values for extracts that exhibited affinity to SERT in this study.

3.2.3.3 Adenosine A_1 & A_{2A} receptors radioligand binding assays

The A_1 receptor radioligand binding assay used rat whole brain membranes (expressing the A_1 AR) and 1,3-[^{3}H]-dipropyl-8-cyclopentylxanthine ([^{3}H]DPCPX) as radioligand, while the A_{2A} receptor radioligand binding assay used rat striatal membranes (expressing the A_{2A} AR) and 5'-N-ethylcarboxamido[^{3}H]adenosine ([^{3}H]NECA) as radioligand (Bruns *et al.,* 1987; Bruns *et al.,* 1986). DMSO was used to dissolve the dry plant extracts to obtain a stock concentration of 10mg/ml and tested at concentrations of 0.1, 0.01 and 0.001 mg/ml.

Each incubation of the A_1R radioligand binding assay contained plant extract solutions (10 µl), [^{3}H]DPCPX (radioligand solution, 100 µl) (0.1 nM), rat whole brain membranes (120 µg) and 0.1 units/ml adenosine deaminase (membrane suspension, 890 µl). For the determination of non-specific binding, N^6-cyclopentyladenosine (CPA) was used, and DMSO was used to determine the total binding of the extracts to adenosine A_1 receptors.

Each incubation of the A_{2A}R radioligand binding assay contained plant extract solutions (10 µl), [^{3}H]NECA (radioligand solution, 100 µl) (4 nM), rat striatal membranes (120 µg), 0.2 units/ml

adenosine deaminase (membrane suspension, 790 μl) and 50 nM CPA (100 µl). The adenosine A_{2A} receptor binding studies were determined in the presence of CPA to minimize the binding of [^{3}H]NECA to adenosine A_1 receptors as this radioligand solution is non-selective.

Both were incubated for 30 min, vortexed for 6 s, and incubated for another 30 min. Incubations were terminated by filtration through Whatman filters on a Brandel 48 R cell harvester, followed by rapid washing of the filters three times with 3 ml ice-cold Tris buffer. 5 ml of scintillation fluid was added, and the filters were counted using a liquid scintillation analyser. All experiments were done in duplicate. The first phase of the assays included the screening of extracts for affinity to the adenosine A_1 and A_{2A} receptors using stock solution concentrations of 10, 1 and 0.1 mg/ml to obtain final concentrations of 0.1, 0.01 and 0.001 mg/ml. For the second phase, the activity of the extracts during the screening process was used to calculate the IC_{50} values for extracts that exhibited affinity to A_1 and/or A_{2A} in this study.

3.2.4 Data and statistical analysis

3.2.4.1 Total phenolic content

The total phenolic content of the samples was calculated at gallic acid equivalents GAE/g of dry plant material based on gallic acid standard curve. The calibration curve of gallic acid was plotted and linearity was obtained using a blank and increasing concentrations of gallic acid (50, 100, 150, 250 and 500 µg/ml). The total phenolic content of plant extracts was determined using formula:

$$y = mx+c$$

The standard curve regression equation (y = mx +c) where y = absorbance at 725 nm and x = total phenolic content in the extracts, and the regression coefficient (R^2) were obtained using Microsoft Excel 2007. Spectrophotometric absorbance (725 nm) values obtained for each extract were substituted into the equation in order to find 'x', the unknown concentration of gallic acid in the extracts (µg/ml). The results for total phenolic content of the extracts were expressed as mg of gallic acid equivalent weight (GAE)/g of dry plant material mass. The x values (µg/ml) were converted to mg/ml (divided by 1000), then converted to mg (GAE)/g using formula $\frac{x}{a} \times 1000$, where 'x' is total phenolic content (mg/ml) and 'a' is concentration of sample in the extract solution (mg/ml). Resulting values were used to plot the total phenolic content (mg/g gallic acid equivalents) graphs of the selected plants using the IBM SPSS statistical software (version 27). The separation of means test for total phenolic content of the extracts was done by one-way

ANOVA applying Tukey's test using the IBM SPSS data analysis software. Pairs with a $p \leq 0.05$ were considered significantly different.

3.2.4.2 *In vitro* antidepressant binding assays

The results of the SERT binding assay were analysed with MS Excel and the IBM SPSS statistical software (version 27). In the first phase of the SERT binding assay, total binding of the radioligand to receptors minus non-specific binding was used to determine the specific binding of the radioligand to the receptors (zero point) using the formula below:

Specific binding of radioligand at target receptors = Total binding -Non-specific binding

Specific binding of the radioligand in the presence of the test compound was determined using the formula:

$$\text{Test compound's specific binding (\%)} = \frac{\text{Test compound's total binding-Non-specific binding}}{\text{zero point's specific binding}} \times 100$$

Extracts with affinity to SERT had to exhibit a dose-dependent inhibition with less than 50% of the specific binding of the radioligand used. For the second phase of the SERT binding assay, IC_{50} values were obtained by plotting the specific binding of the radioligand against the log value of the concentration on a dose-response curve.

The results of the adenosine A_1 & A_{2A} receptors radioligand binding assays were analysed with MS Excel and the IBM SPSS statistical software (version 27). In the first phase of the assays, nonspecific binding was defined as binding in the presence of N^6-cyclopentyladenosine (CPA), and specific binding as total binding minus nonspecific binding. For the second phase, IC_{50} values were calculated by weighted non-linear least-squares curve-fitting to the logistic equation. For the calculation of the inhibition constant (K_i) values by non-linear regression analysis, the IC_{50} values of the test and reference samples were calculated according to the Cheng–Prusoff equation (Yung-Chi & Prusoff, 1973) and K_d values of 0.36 nM for [^{3}H]DPCPX at rat whole brain membranes (Bruns *et al.*, 1987), while 15.3 nM for [^{3}H]NECA at rat striata membranes (Bruns *et al.*, 1986) were used. Extracts with affinity to A_1 or A_{2A} had to exhibit a dose-dependent inhibition with less than 50% of the specific binding of the radioligand used. All incubations were conducted in duplicate, and the K_i values were expressed as mean ± standard error of mean (SEM).

Cheng-Prusoff equation for the A_1 radioligand binding assay using [^{3}H]DPCPX as radioligand:

$$K_i = \frac{IC_{50}}{1+\frac{[RL]}{K_d}}$$

Cheng-Prusoff equation for the A_{2A} radioligand binding assay using [^{3}H]NECA as radioligand:

$$K_i = \frac{IC_{50}}{1+\frac{[RL]}{K_d}+\frac{[CPA]}{K_c}}$$

3.3 Results and discussion

3.3.1 Percentage extraction yield

Table 3.2 shows the extraction yield in percentages of extracts from the leaf of *Artemisia afra,* the stem of *Adenia gummifera*, and the leaf and stem of *Olea woodiana*. The extracts were obtained by the ultrasonic-assisted method using three solvents of increasing polarities, non-polar (n-hexane), medium polar (acetone) and polar (water), to evaluate the suitability of the solvents for extraction and to ensure extraction of a wide range of phytochemicals with different polarities (Nawaz *et al.*, 2020; Ngouana *et al.*, 2021). To study the effects of solvent polarity on the percentage extraction yield of the selected plants, the effects of three solvents on the extraction yield percentage of each plant and across all plants was investigated in this study.

Table 3.2: Percentage extraction yield of three medicinal plants using water, acetone and hexane as solvents

Solvent used and polarity	Plant species	Plant part	Yield after drying (g)	Percentage yield (%)
Water (polar)	*Artemisia afra* Jacq. ex Willd.	Leaf	3.73	**18.65**
	Adenia gummifera (Harv.) Harms	Stem	2.42	8.07
	Olea woodiana Knobl.	Leaf	6.05	6.05
		Stem	2.83	7.08
Acetone (medium polar)	*Artemisia afra* Jacq. ex Willd.	Leaf	0.83	4.14
	Adenia gummifera (Harv.) Harms	Stem	1.02	3.40
	Olea woodiana Knobl.	Leaf	0.65	1.30
		Stem	0.76	1.90
Hexane (non-polar)	*Artemisia afra* Jacq. ex Willd.	Leaf	0.24	1.19
	Adenia gummifera (Harv.) Harms	Stem	1.35	4.51
	Olea woodiana Knobl.	Leaf	2.33	4.65
		Stem	2.27	5.69

3.3.1.1 Effect of solvent polarity on extraction yield of *A. afra*

The aqueous extract from the leaf of *A. afra* (highlighted in blue) had the highest yield of 18.65%, while the hexane extract was the lowest at 1.19% (**Table 3.2**). The acetone extract of *A. afra* leaf

had a yield of 4.14%. The order of extraction yield of different solvents with *A*. *afra* leaf was water > acetone > hexane, suggesting that for *A*. *afra*, extraction yield was directly proportional to the polarity of the solvent used. These results agree with extractions done on *Sphaeranthus indicus* Linn. (Asteraceae), where the highest extraction yield was obtained from leaf aqueous extract (9.25%), while the lowest extraction yield was obtained from hexane extract of the root (0.4%) (Tandon & Gupta, 2020). The extraction of 14 different species of Asteraceae using aqua-methanol and aqua-acetone (20:80 v/v) as solvents revealed the highest extraction yield in the aqua-methanol extract of *A*. *houstonianum* (66.14%) and the lowest in the aqua-acetone extract of *X*. *strumerium* (Rawat & Rao, 2018), supporting the suggestion that maximum extraction from Asteraceae species is achieved using solvents of high polarity, such as water and methanol.

3.3.1.2 Effect of solvent polarity on extraction yield of *A. gummifera*

Aqueous extract from the stem of *A*. *gummifera* (highlighted in green) had a higher yield (8.07%) than the acetone and hexane extracts. However, for this plant, the hexane extract had a higher yield of 4.51% compared to the acetone extract with a 3.40% extraction yield (**Table 3.2**). This trend is comparable to results obtained with the extraction of three species belonging to the Passifloraceae family (*Passiflora quadrangularis, P*. *maliformis, and P*. *edulis*) where methanol, a polar solvent, had the maximum effectiveness in the extraction of therapeutic compounds, followed by acetone, then petroleum ether which had the lowest polarity index (Shiamala *et al.*, 2014). In another investigation of Passifloraceae species (*Passiflora edulis*, *P*. *incarnata* and *P*. *ligularis*), the methanol (polar) extract of *P*. *incarnata* exhibited the highest extraction yield (16%), while the dichloromethane (non-polar) extract gave the lowest extraction yield of 4.79% (Marroquín *et al.*, 2012). This suggests the efficiency of polar solvents such as methanol and water in the maximum extraction of plant material from *A*. *gummifera* and other Passifloraceae species.

3.3.1.3 Effect of solvent polarity on extraction yield of *O. woodiana*

For *O. woodiana*, the stem aqueous extract had the highest yield of 7.08%, followed by the hexane extract (5.69%). The acetone extract from the stem of *O. woodiana* had the lowest extraction yield of 1.90%. The aqueous extract from the leaf of *O. woodiana* exhibited the highest extraction yield (6.05%), followed by the hexane extract (4.65%), then the acetone (1.30%) with the lowest extraction yield (**Table 3.2**). Generally, the stem of *O. woodiana* had a higher extraction yield than the leaf of this plant. The order of the extraction yield of *O. woodiana* leaf and stem

extracts on the basis of solvents used is water > hexane > acetone. Similarities are observed with the previous extraction of *Olea ferruginea* Royle (Oleaceae) where the stem extracts exhibited a higher extraction yield percentage than the leaf extracts, with the highest extraction yield in the aqueous extracts than the ones extracted with hexane, chloroform and alcohol, which have a lower polarity index than water (Rafique *et al.*, 2021). The extraction of *Olea europaea* subsp. *africana* (Mill) P.S. Green (Oleaceae) leaf revealed the highest extraction yield in the aqueous extracts (158.2 mg), followed by methanol (109.1 mg), while the lowest yield was observed with the hexane (Masoko & Mamabolo, 2019). These results suggest the suitability of water in extracting plant material from *Olea* species, and a higher extraction yield in the leaf than the stem of *O*. *woodiana.*

3.3.1.4 Effect of solvent polarity on extraction yield across all plants

Previous studies have analysed the effects of solvents such as ethanol, methanol, n-hexane, petroleum ether, ethyl acetate, water and acetone on the extraction yield, phytochemical content and biological activities of plant extracts (Nawaz *et al.*, 2020; Thouri *et al.*, 2017; Wakeel *et al.*, 2019). The polarity of the solvents used in this study, from most polar to least, is as follows: water > acetone > hexane. The extraction yield of all the plant material ranges from 1.19% to 18.65% of the dry weight, with the highest being extracted with a high quantity of a more polar solvent. Generally, the aqueous extracts of all plants exhibited a high extraction yield than the acetone and hexane extracts (**Table 3.2**). In this study, the percentage extraction yield largely depends on the polarity of the solvent used to extract the plant material. The high extraction yield observed in the aqueous extracts indicates that there are more water soluble and polar components in the plant material extracted compared to the non-polar components (Nawaz *et al.*, 2020). Based on the plant parts used, *O. woodiana* stem extracts collectively exhibited a higher extraction yield percentage than the leaf extracts of this plant. However, the opposite was observed with the other two plants where *A. afra* leaf extracts had a higher extraction yield percentage than *A. gummifera* stem extracts, suggesting little correlation between the extraction yield percentage and plant parts used. The order of the sum of the extraction yield obtained for each plant (presented as a sum % of 3 extracts from each plant) was *A. afra* leaves (23.98) > *A. gummifera* stem (15.98%) > *O. woodiana* stem (14.67%) > *O. woodiana* leaves (12%).

3.3.2 Total phenolic content

The total phenolic content of the aqueous, acetone and hexane extracts of *Artemisia afra* (leaf), *Adenia gummifera* (stem), *Olea woodiana* (leaf and stem) was estimated by the Folin-Ciocalteu's method using gallic acid as a standard. The final concentration of phenolic compounds in the extracts was extrapolated with a standard curve with the regression equation: y = 0.0027x - 0.1051, R^2 = 0.959, where y = absorbance at 725 nm and x = total phenolic content in the extracts (**Appendix 5**). Total phenolic content was estimated at gallic acid equivalents GAE/g of dry plant material based on gallic acid standard curve (50, 100, 150, 250 and 500 μg/ml) (**Appendix 5**). The estimation of total phenolic content revealed a wide variation in the total phenolic compounds in the extracts, ranging from 3.52 to 66.64 mg/g GAE (**Table 3.3**).

3.3.2.1 The effects of solvent polarity on the total phenolic content of *A. afra*

For *A. afra* leaf, the acetone extract exhibited the highest phenolic content (38.03 mg/g GAE), followed by the aqueous extract (33.64 mg/g GAE) (**Figure 3.2**, **Table 3.3**). The hexane extract from this plant exhibited the lowest phenolic content (4.91 mg/g GAE) (**Figure 3.2**, **Table. 3.3**). Statistical analysis by one-way ANOVA revealed differences that were statistically significant in the total phenolic content of the water and acetone extracts (p = 0.034), the water and hexane extracts (p = 0.002) and acetone and hexane extracts (0.001) of *A*. *afra*. In a study done by Kane *et al.* (2019), the ethanolic extract from *A. afra* leaf exhibited the highest total phenolic content, putting it over the aqueous extract, while the hexane extract exhibited the lowest total phenolic content. Both acetone (medium polar) and ethanol (polar solvent) have a lower polarity index than water, which is also a polar solvent, suggesting the suitability of polar and medium polar solvents such as acetone and ethanol in the complete extraction of phenolic compounds from *A. afra* leaf extracts (Kane *et al.*, 2019). This is comparable to a study on *Sphaeranthus indicus* from the Asteraceae family where total phenolic content was the highest in polar fractions of leaf methanol extract and aqueous extracts (Tandon & Gupta, 2020). Preliminary phytochemical analysis revealed that phenolic quinones, flavonoids, saponins, alkaloids, steroids and tannins are present in the leaf extract from *A. afra* (Kane *et al.*, 2019; Yimam & Desalew, 2022). Species from the *Artemisia* genus, such as *Artemisia dracunculus* L. (Asteraceae), have been reported to have phenolic compounds (e.g. quercetin), as well as antidepressant effects mediated by possible interaction with serotoninergic systems (Behbahani *et al.*, 2017; Ilkhanizadeh *et al.*, 2021; Jahani *et al.*, 2019).

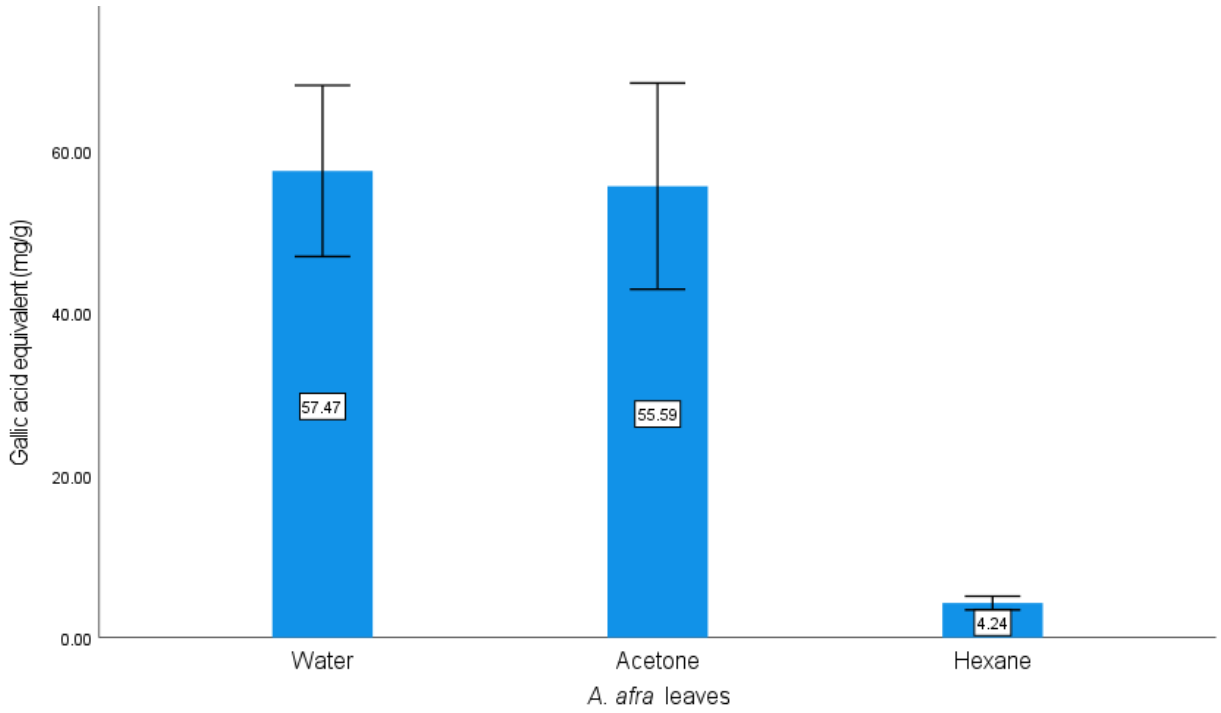


Figure 3.2: **The effects of solvent polarity on the total phenolic content (mg/g gallic acid equivalents) of *Artemisia afra* using water, acetone and hexane as solvents. Values are presented as mean ± SEM (n = 3). *P* values ≤ 0.05 were considered significantly different.**

3.3.2.2 The effects of solvent polarity on the total phenolic content of *A. gummifera*

The aqueous extract from the stem of *A. gummifera* exhibited the highest phenolic content of 37.80 mg/g GAE, followed by the acetone extract (22.25 mg/g GAE) (**Figure 3.3**, **Table 3.3**). The hexane extract of *A. gummifera* exhibited the lowest phenolic content of 3.65 mg/g GAE (**Figure 3.3**, **Table 3.3**). Statistical analysis by one-way ANOVA revealed statistically significant differences in the total phenolic content of the water and acetone extracts (p = 0.038), the acetone and hexane extracts (0.027) and the water and hexane extracts (p = 0.000) of *A. gummifera*. For this plant, the most effective solvent in the extraction of phenolic compounds was water, followed by acetone, then hexane (**Figure 3.3**), suggesting a strong correlation between higher quantities of phenolic compounds and increasing polarity of the solvent. A study on *Passiflora quadrangularis, P. maliformis, and P. edulis* from the Passifloraceae family revealed that for both the stems and leaves of these plants, methanol (polar solvent) extracted maximum total phenolic content, while petroleum ether (non-polar solvent) showed to be the least effective at extracting phenolic compounds (Shiamala *et al.*, 2014). These results obtained in the above-mentioned study agree with the current study and suggest that more polar solvents achieve the highest yield

of phenolic compounds in Passifloraceae species. Polyphenolic compounds such as quercetin, apigenin, rutin and luteolin have been reported to be present in *Passiflora* spp. (Passifloraceae) (Ozarowski & Karpińnski, 2021). Previously, phenolic compounds were found to be present in the stem of *A. gummifera* (Adedapo *et al.*, 2008; Maroyi, 2020). The presence of phenolic acids such as *p*-coumaric, sinapic, and caffeic acids has been reported in the aqueous extracts of *Adenia viridiflora* Craib. (Warewat *et al.*, 2021).

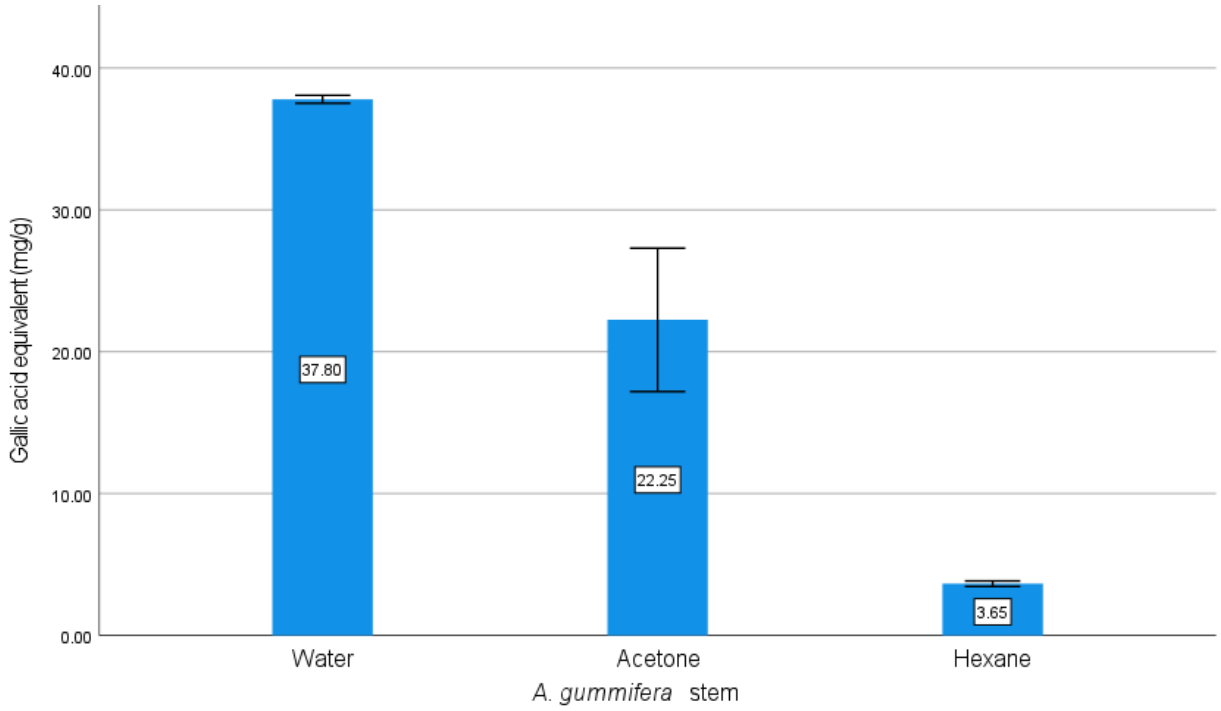


Figure 3.3: **The effects of solvent polarity on the total phenolic content (mg/g gallic acid equivalents) of *Adenia gummifera* using water, acetone and hexane as solvents. Values are presented as mean ± SEM (n = 3). *P* values ≤ 0.05 were considered significantly different.**

3.3.2.3 The effects of solvent polarity on the total phenolic content of *O. woodiana*

For *O. woodiana* stem, the aqueous and acetone extracts exhibited the highest phenolic content (both 66.64 mg/g GAE), while the hexane extract had the lowest total phenolics yield (3.52 mg/g GAE) (**Figure 3.4**, **Table 3.3**). The aqueous extract from the leaf of *O. woodiana* exhibited the highest phenolic content of 48.30 mg/g GAE, followed by the acetone extract with 44.55 mg/g GAE (**Figure 3.4**, **Table 3.3**). The hexane extract exhibited the lowest total phenolic content of 4.97 mg/g GAE (**Figure 3.4**, **Table 3.3**). Statistical analysis by one-way ANOVA revealed statistically significant differences in the total phenolic content of the acetone and hexane extracts

(p = 0.001) and the water and hexane extracts (p = 0.000) from the leaf of *O*. *woodiana*. For the stem extracts of this plant, significant differences were observed in the acetone and hexane extracts (p = 0.000) and the water and hexane extracts (p = 0.000). No significant differences were observed with the water and acetone extracts from both the stem and the leaf of *O*. *woodiana*. For this plant, the aqueous and acetone extracts had a higher phenolic content than the hexane extracts, while the stem extracts generally exhibited a higher phenolic content than the leaf extracts. This suggests that phenolic compounds are present in both the leaf and stem of this plant in varying quantities. The stem and leaf of *Olea ferruginea* Royle, a member of the Oleaceae family, have been reported to possess biologically active compounds such as alkaloids, saponins, flavonoids, minimizing sugars, cardiac glycosides, tannins and terpenoids (Rafique *et al.*, 2021). Although phenolic compounds were not investigated in the above-mentioned study, phytochemical analysis revealed the presence of the phytochemicals at higher concentrations in alcohol and aqueous extracts of *O. ferruginea* (Rafique et al., 2021). Phytochemical analysis of extracts from chetoui *Olea europaeae* variety revealed the highest amounts of phenolic compounds in the methanol-water fractions decreasing with the polarity of the solvents in the order methanol, ethyl acetate and hexane (Khlif *et al.*, 2015). The results of the current study agree with the ones obtained in the above-mentioned studies, suggesting the suitability of more polar solvents for extracting phenolic compounds from *Olea* species. Previous studies have indicated that phenolic acids such as oleic acid and caffeic acid are the major bioactive compounds found in olive oil obtained from the leaves of *Olea europaea* L. (Perveen *et al.*, 2013; Zhang *et al.*, 2022). Oleuropein, a phenolic compound with validated antidepressant effects was found to be present in the extracts of *O. ferruginea* (Rafique et al., 2021). A previous study revealed that oleuropein has been considered a promising plant-based compound to alleviate symptoms of depression through normalizing levels of biogenic amines, inhibiting lipid peroxidation and maintaining reduced glutathione (Badr *et al.*, 2020).

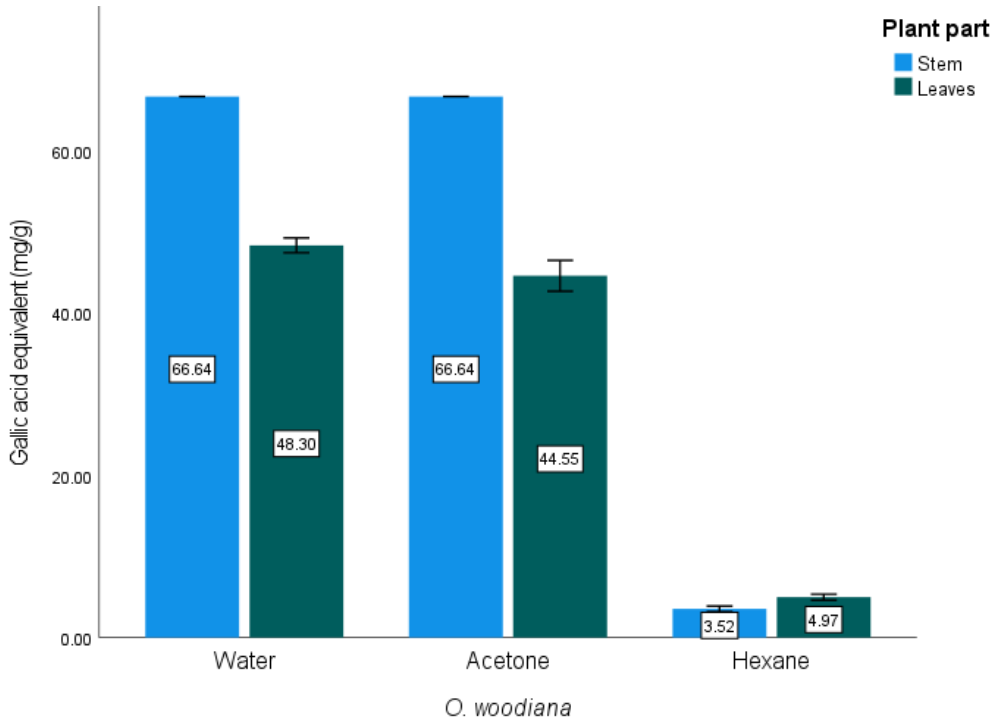


Figure 3.4: **The effects of solvent polarity on the total phenolic content (mg/g gallic acid equivalents) of *Olea woodiana* using water, acetone and hexane as solvents. Values are presented as mean ± SEM (n = 3). *P* values ≤ 0.05 were considered significantly different.**

3.3.2.4 Effects of water, acetone and hexane extraction on phenolic content

Observations from the current study indicate that the highest total phenolic content was observed in aqueous and acetone extracts of *O. woodiana* (stem) extracts (both equivalent to 66.64 mg/g GAE) (**Table 3.3**). The hexane extract from the stem of *O. woodiana* exhibited the lowest (3.52 mg/g GAE) total phenolic content in this study (**Table 3.3**). In this study, water was the most effective in extracting phenolic compounds as the aqueous extracts of all plants showed a comparatively higher phenolic content than the acetone and hexane extracts. The order of different solvents in effectively extracting total phenolics was water (46.6 mg/g GAE) > acetone (42.87 mg/g GAE) > hexane (4.26 mg/g GAE) represented as the mean of the total phenolic content of all extracts obtained using the solvent. These results agree with previous studies where extraction yield percentage and phytochemical content of plants were found to be significantly dependant on increasing polarity of the solvent (Iloki-Assanga *et al.*, 2015; Nawaz *et al.*, 2020; Wakeel *et al.*, 2019). According to Thouri *et al.* (2017), the level of extracted polyphenols from plant extracts may be significantly impacted by the type of extraction solvent used and its polarity. Polarities of polyphenolic compounds range from polar to non-polar, therefore complete extraction is obtained when using polar solvents with better efficiency of solvation due to interactions

between the polar sites of the compounds and the polar solvent (Liu *et al.*, 2007). The complete extraction of phenolic compounds is usually achieved using solvents with a high polarity index, such as water and methanol (Iloki-Assanga *et al.*, 2015). Previous studies have elucidated that water extracts contain a higher phenolic content than the acetone and hexane extract due to the high polarity of water (Nawaz *et al.*, 2020; Thouri *et al.*, 2017). This could explain the high quantity of total phenolics in the aqueous extracts and the low quantity of total phenolic contents in hexane extracts, which has the lowest polarity index.

Table 3.3: Total phenolic content of *Artemisia afra* Jacq. ex Willd., *Adenia gummifera* (Harv.) Harms and *Olea woodiana* Knobl. as mg/g gallic acid equivalents (GAE)

Solvent used and polarity	Plant species	Plant part analysed	Phenolic content (mg/g GAE)
Water (polar)	*Artemisia afra* Jacq. ex Willd.	Leaf	33.64
	Adenia gummifera (Harv.) Harms	Stem	37.80
	Olea woodiana Knobl.	Leaf	48.30
		Stem	66.64
Acetone (medium polar)	*Artemisia afra* Jacq. ex Willd.	Leaf	38.03
	Adenia gummifera (Harv.) Harms	Stem	22.25
	Olea woodiana Knobl.	Leaf	44.55
		Stem	66.64
Hexane (non-polar)	*Artemisia afra* Jacq. ex Willd.	Leaf	4.91
	Adenia gummifera (Harv.) Harms	Stem	3.65
	Olea woodiana Knobl.	Leaf	4.97
		Stem	3.52

Previous studies have shown that natural phenolic compounds possess reputable neuroprotective activity and therapeutic value in various mental disorders, including depression (Pathak *et al.*, 2013; Szwajgier *et al.*, 2017). Rosmanol, a common polyphenol found in *Rosmarinus officinalis,* exhibited significant antidepressant activity in the tail suspension test and the forced swimming test (Abdelhalim *et al.*, 2015). Oleuropein, the most bioactive phenolic compound of *Olea europaea* subsp. *cuspidata* that is found in olive oil, caused significant reduction in levels of serotonin and dopamine and decreased immobility time in the tail suspension test, forced swimming test and the open field test, exhibiting antidepressant-like effects (Badr *et al.*, 2020). The antidepressant effects of *Artemisia dracunculus* L. have been previously associated with the presence of quercetin, a well-known phenolic phytochemical with antidepressant effects, in the extracts of this plant (Jahani *et al.*, 2019; Mumivand *et al.*, 2017).

Quercetin, also found in *Hypericum perforatum* L. (St. John's wort), a popular South African plant with reputable antidepressant effects, possesses antidepressant effects brought about by the inhibition of MAO activity in the brain, which could further prevent the breakdown of neurotransmitter (Clarke & Ramsay, 2011). The ability of phenolic compounds to alter different neurotransmitter systems and exhibit antidepressant effects in animal models of depression has led to researchers exploring their usefulness in treating major depression (Pathak *et al.*, 2013), and is rationale for the estimation of total phenolic content of plant extracts in the current study.

3.3.3 *In vitro* antidepressant binding assays

3.3.3.1 Adenosine A_1 & A_{2A} receptors radioligand binding assays

Phase 1: Screening of extracts for affinity to $A_{1\&2A}$ receptors

Table 3.4 illustrates the binding affinity of the aqueous, acetone and hexane extracts of 3 medicinal plants to the adenosine A_1 receptor. Since no extract showed any significant affinity to the A_{2A} receptor, the binding results for the A_{2A} receptor radioligand binding assay are tabulated in **Appendix 6**. The results are represented as specific binding of the radioligand in the presence of the test compound (%) as the mean of 2 samples (duplicates). The solubility issue observed with the aqueous, acetone and hexane extracts from the stem of *A. gummifera* and the hexane extract from the leaf of *A. afra* may have led to the reduction in bioavailability in this study.

Table 3.4: Screening 3 medicinal plants for affinity to the adenosine A_1 receptor

Plant	Plant part analysed	Extraction solvent	Specific binding (%)		
			rA_1 vs 0.1 nM [^{3}H]DPCPX		
			mg/ml		
			0.001	**0.01**	**0.1**
***Artemisia afra* Jacq. ex Willd.**	Leaf	**Water**	**34**	**37**	**38**
		Acetone	95	65	**17**
		Hexane	105	103	77
***Adenia gummifera* (Harv.) Harms**	Stem	Water	106	107	108
		Acetone	97	95	82
		Hexane	86	94	87
***Olea woodiana* Knobl.**	Leaf	Water	98	100	101
		Acetone	81	89	**43**
		Hexane	105	111	87
	Stem	Water	105	109	95
		Acetone	105	88	51
		Hexane	106	105	77
		K_i ± SEM (nM)			
		DPCPX	0.5 ± 0.1		

Inhibition constant (K_i, mg/ml or nM) value is represented as the mean ± standard error of the mean (SEM), n = 3 samples.

Specific binding (%) of the radioligand is represented as the mean, n = 2 samples.

rA_1: rat whole brain membranes expressing adenosine A_1 receptor.

Concentration used were adopted from previous A_1 receptor radioligand binding studied (Bruns *et al.*, 1986; Bruns *et al.*, 1987).

Phase 2: Specific binding of the extracts

Only one extract exhibited significant affinity to the adenosine A_1 receptor (*A. afra* leaf acetone extract) with a specific binding of 17 % at the highest concentration of 0.1 mg/ml (K_i value= 0.0256 ± 0.0076 mg/ml) (red font in **Table 3.4**). The IC_{50} values for the acetone extract from the leaves of *A*. *afra* were obtained by plotting the specific binding of the radioligand against the log value of the concentration on a dose-response curve (**Appendix 7**).

The aqueous extract from the leaf of *A. afra* exhibited considerable affinity to the adenosine A_1 receptor with a specific binding of 34, 37 & 38% at concentrations of 0.001, 0.01 and 0.1 mg/ml, respectively (blue font in **Table 3.4**). *Artemisia afra* is a popular herbal medicine in South Africa used traditionally to treat headaches and anxiety (du Toit & van der Kooy, 2019; Hulley & Van Wyk, 2019; Hutchings *et al.*, 1996). Antidepressant phytochemicals such as phenolic compounds quercetin and luteolin were previously isolated from genus *Artemisia* (Mumivand *et al.*, 2017). It is possible that the observed affinity of *A. afra* results from the presence of phenolic compounds in the leaf of this plant. Despite the affinity exhibited by the acetone extract and the considerable affinity by the aqueous extract of this plant, the hexane extract did not show any promising results in this study. This could be attributed to the solubility issue previously observed in the preparation of the hexane extract and/or the ability of the high polarity of water and acetone as solvents to draw a higher variety of phytochemical constituents with antidepressant-like effects than the non-polar n-hexane (Jamuna *et al.*, 2014).

The acetone extract from the leaf of *O. woodiana* also exhibited promising affinity to A_1 with a specific binding of 43% at the highest tested concentration of 0.1 mg/ml (**Table 3.4**). These plants warrant further investigation using more *in vitro* models of depression at higher concentrations of the extracts.

None of the extracts exhibited affinity towards the adenosine A_{2A} receptor in this study (**Appendix 6**). It is possible that no binding affinity observed in the A_{2A} receptors radioligand binding assay is due to the plants having different mechanisms of antidepressant action other than binding affinity to adenosine A_{2A} receptors (Nielsen *et al.*, 2004).

The A_1 receptor plays a pivotal role in mediating symptoms of depression and may represent a novel target for future antidepressant drug development (Bruns *et al.*, 1987; Gomes *et al.*, 2021; Van Calker *et al.*, 2019). The involvement of this receptor in alleviating mood disorders such as depression has been previously studied (Bruns *et al.*, 1987; Kaster *et al.*, 2004). The antidepressant effects of adenosine are dependent on the activation of receptors A_1 through a mechanism of action similar to that of existing non-pharmacological therapies such as plants,

moreover, this receptor is proposed to be of physiological importance in the neuromodulatory activity of adenosine (Gomes *et al.*, 2021; Ribeiro *et al.*, 2003).

3.3.3.2 SERT binding assay

Phase 1: Screening of extracts for affinity to the SERT protein

None of the extracts screened showed any significant affinity to the SERT protein at the 3 tested concentrations, therefore, the binding results for the SERT binding assay are tabulated in **Appendix 6**.

Phase 2: Specific binding of the extracts

The aqueous extract from the leaf of *A. afra* and hexane extract from the leaves of *O. woodiana* exhibited promising results as they were bound to SERT more than the radioligand [^{3}H] citalopram (**Appendix 6**). These warrant more *in vitro* investigations at higher concentrations of the extracts. The antidepressant activity of bioactive phytochemical constituents has been associated with the serotonin reuptake inhibition activity of bioactive chemicals present in the plant extracts (Stafford *et al.*, 2008). The blockage of the serotonin binding site leads to the prevention of reuptake of serotonin released into the synaptic space back into the cell, thus increasing the level of serotonin in the synaptic cleft (Rang *et al.*, 2007). The increase in serotonin levels leads to the alleviation of depressive symptoms in patients (Jäger *et al.*, 2013). Screening plant extracts for the serotonin transporter protein affinity is an effective way to evaluate their potential use as antidepressants and a means towards the development of newer and more effective antidepressant therapeutic agents (Nielsen *et al.*, 2004). The low activity observed with the SERT binding results may suggest that the plants exert antidepressant effects through mechanisms of actions other than the blockade of the serotonin binding site (Nielsen *et al.*, 2004).

In this study, only *A. afra* exhibited significant binding affinity to the adenosine A_1 receptor in one of the assays employed. Based on the plant parts investigated, affinity was observed in the leaf, rather than the stem extracts of the plants. The most significant binding affinity was observed in the extract obtained using acetone as a solvent, suggesting the suitability of acetone to efficiently extract plants for antidepressant pharmacological studies. Based on these results, we can speculate that the *A. afra* acetone extract is an ideal candidate for further evaluation using animal models of depression.

3.4 Conclusion

The calculated percentage extraction yield revealed that the aqueous extracts of all plants generally exhibited a comparatively higher extraction yield than the less polar extracts (acetone and hexane), while the hexane extract had the lowest extraction yield. This observation shows that the yield of extraction was largely dependent on the polarity of the solvent used. Moreover, it is an indication of the presence of more water soluble and polar components in the plant material extracted compared to the non-polar components.

The estimation of total phenolics revealed the highest phenolic content in the aqueous and acetone extracts of all plants, while the hexane extracts had the lowest phenolic content. The order of the different solvents in effectively extracting total phenolics was water > acetone > hexane, represented as the mean total phenolic content of each solvent. Based on these results, water was the most effective solvent for the extraction of total phenolic compounds. Total phenolic content levels of the extracts increased significantly with increasing polarity of the solvents, with the *A*. *afra* extracts being the only exception as the acetone extract had a higher phenolic content than the aqueous extract. In this study, water and acetone extracts exhibited a higher phenolic content due to the high polarity of the solvents, while the hexane extract had the lowest phenolic content, as reported previously.

Only one extract exhibited significant affinity in the adenosine A_1R radioligand binding assay. Some extracts that showed considerable affinity in the adenosine A_1R radioligand binding assay and SERT binding assay warrant further investigation at higher concentrations. None of the extracts screened showed affinity in the adenosine $A_{2A}R$ radioligand binding assay. This evaluation represents the first step in the pharmacological authentication of plants used traditionally to manage depression. On the basis of various hypotheses of the pathophysiology of depression as reported in literature, no general conclusions are made on the antidepressant effects of the plants as they might have varying mechanisms of antidepressant action than the ones investigated in this study.

CHAPTER 4: GENERAL CONCLUSION AND RECOMMENDATIONS

4.1 Study outcome and general conclusion

In South Africa, the use of plants to manage depression and related symptoms is evident among different ethnic groups. Many indigenous plants represent an acceptable alternative to conventional antidepressants, with many phytochemicals presenting to be novel antidepressant therapeutic agents. A systematic review of 20 eligible ethnobotanical literature generated an inventory of 186 plants from 63 plant families recorded as treatment remedy for depression and related ailments (**Chapter 2**). A total of 54 plants were recorded as the most popular based on multiple mentions in ethnobotany and the presence of pharmacological studies authenticating the antidepressant-like effects of these plants. The dominant families, Asteraceae, Fabaceae, Amaryllidaceae, and Apocynaceae, accounted for about 32% of the 186 recorded plants. Only 27 plants (≈15%) have been screened for antidepressant activity using various models of depression. Phytochemical investigation on 9 out of the 27 plants led to the identification of 24 compounds with antidepressant-like effects. These include buphanidrine and buphanamine from the leaves of *Boophone disticha*, Δ9- tetrahydrocannabinol, cannabidiol and cannabichromene from the buds of *Cannabis sativa* and carnosic acid, rosmarinic acid and salvigenin from *Rosmarinus officinalis*. Despite South Africa's rich diversity of medicinal plants, it is noted that this biodiversity comprises several plant species with the potential to poison humans. There are safety concerns and lack of scientific knowledge for some of the South African medicinal plants with anti-depressant potential and there is a need for toxicity studies to assess the safety of these plants as antidepressants. A significant portion (≈85%) of 186 plants recorded in ethnobotany still require pharmacological studies to assess their potential antidepressant-like effects.

Phytochemical analysis of the selected plants focused on the estimation of phenolic content while their antidepressant potential was evaluated using the SERT binding assay and the adenosine A_1 & A_{2a} receptors radioligand binding assays. Based on ethnobotanical and ethnopharmacological data from the literature review, *Artemisia afra* (Asteraceae), *Adenia gummifera* (Passifloraceae) and *Olea woodiana* (Oleaceae) were selected for phytochemical analysis and *in vitro* antidepressant studies using binding assays. Plant extracts were obtained by the ultrasonic-assisted method of extraction using three solvents of increasing polarities (hexane, acetone & water). The highest extraction yield was observed in extracts where a more polar solvent (water) was used, while the lowest was observed in extracts obtained using the least polar solvent (hexane). The estimation of total phenolic contents revealed a wide variation in the total phenolic content of the extracts investigated in this study. Generally, water and acetone extracted a higher

phenolics content than the extracts obtained using hexane. These results agree with several studies where extraction yield and phenolic content of plants was higher in extracts obtained using solvents with a higher polarity than the non-polar ones, suggesting that the extractability of phenolic compounds is significantly dependent on solvent polarity and the solubility of phenolic compounds in the solvent. *In vitro* antidepressant studies revealed only one extract with significant affinity to the adenosine A_1 receptor (*A. afra* leaves in acetone), putting the plant forward as a potential subject for further pharmacological testing. None of the other extracts showed significant affinity in the assays employed, however, two extracts exhibited promising results in the adenosine A_1 receptor radioligand binding assay, while two other extracts were bound to the SERT protein more than the radioligand, exhibiting promising results in this study. Based on the dose-dependent response observed in the binding results, these plants warrant further investigation at higher concentrations of the extracts.

A. *afra, A*. *gummifera* and *O*. *woodiana* represent South African medicinal plants with promising antidepressant activity in ethnobotanical literature. These plants have been previously used locally against depression and this study serves as a foundation for the investigation into their efficacy as plant-based antidepressants. *A*. *afra* (Asteraceae), one of the most popular medicinal plants, could have reputable *in vitro* antidepressant-like effects mediated by its affinity to the adenosine A_1 receptors. Asteraceae represents one of the most popular plant families utilized traditionally to manage depression, and an important source of novel therapeutic agents for plant-based antidepressant drug discovery. To conclude, the aim of this study has been achieved through a systematic review of ethnobotanical literature that identified 186 plants used to manage depression, the estimation of total phenolic content and evaluation of binding affinity of three selected plants to authenticate their neuroprotective effects. Based on the results obtained in this study, South African medicinal plants may possess reputable antidepressant activity attributed to the phenolic content of these plants. Moreover, medicinal plants may hold more novel phytochemicals, such as flavonoids, alkaloids, tannins and saponins, with antidepressant medicinal value. This study serves as a critical step towards the validation of the pharmacological antidepressant-like effects of indigenous medicinal plants used traditionally for depression without pharmacological authentication.

4.2 Study limitations

- For this study, only the stems and leaves of the selected plants were available for collection and analysis due to limitations relating to seasonal change.

- The extraction of plant material was done using only three solvents (hexane, acetone, and water) of increasing polarity rather than more common solvents used in the comparison of solvent effectiveness based on polarity.
- Initially, three phytochemicals (total phenolics, total flavonoids and condensed tannins) were to be analyzed in this study. However, due to time constraints and the unavailability of reagents (standards) needed for the estimation of total flavonoids and condensed tannins, only total phenolics of the selected plants were investigated in this study.
- Due to the limitations of time, only the estimation of total phenolic compounds was done without further separation, identification, and quantification of the present phenolic compounds.
- The estimation of total phenolic compounds was done using three technical replicates where the absorbance of one sample was read three time to get 3 absorption values, rather than in triplicates.
- Only three *in vitro* models of depression were employed for the screening of plants for binding affinity, rather than a combination of *in vitro* and *in vivo* models of depression to achieve an in-depth evaluation into the antidepressant potential of the selected plants.

4.3 Recommendations and future studies

- The simultaneous evaluation of the ethnopharmacology and phytochemistry of medicinal plants used traditionally for depression to drive drug discovery based on traditional knowledge and the presence of phytochemicals with antidepressant effects.
- Future research on the antidepressant effects of medicinal plants from popular plant families such as Asteraceae, Fabaceae, Amaryllidaceae and Apocynaceae, which may hold plant species with antidepressant activity and phytochemicals capable of effectively treating depression and its associated symptoms.
- Collection of plant material during peak seasons of plant material availability is recommended to avoid the issues of certain plant parts or species being unavailable during some periods of the year (e.g. winter season).
- Extraction of plant material for affinity studies and phytochemical analysis using more solvent of increasing polarities (hexane, petroleum ether, acetone, dichloromethane, water, and methanol) to better compare the effects of increasing polarity on extraction yield, phytochemical content, and antidepressant activity of the plant extracts.
- Future research and efficacy assessment of the antidepressant effects of South African medicinal plants using additional *in vitro* models (e.g., monoamine oxidase inhibition,

serotonin transporter (SERT), noradrenalin transporter (NAT) and dopamine transporter (DAT) uptake inhibition assays) and *in vivo* models of depression (e.g., forced swimming test, tail suspension test, open-field test) to discover the in-depth antidepressant potential of these plants.

- Based on the dose-dependent antidepressant activity observed in this study, further *in vitro* antidepressant investigations on selected plants at higher concentrations than those used in this study are required with the hypothesis that higher concentrations will yield more significant results.
- Based on the significant binding affinity observed with the acetone extract from *A*. *afra* leaves, further pharmacological studies using additional models of depression are required for a thorough investigation of this plant as potential herbal antidepressant medicine.
- Further phytochemical analysis of the selected plants, where extracts are screened for other antidepressant phytochemicals such as total flavonoids, total terpenoids and condensed tannins, and the separation, identification and quantification of the known and unknown active compounds using liquid chromatography-mass spectrometry (LC/MS) to identify novel compounds effective at treating depression.

References

Abbas, G., Rauf, K. & Mahmood, W. 2015. Saponins: the phytochemical with an emerging potential for curing clinical depression. *Natural Product Research*, 29(4):302-307. 10.1080/14786419.2014.942661

Abdelhalim, A., Karim, N., Chebib, M., Aburjai, T., Khan, I., Johnston, G.A. & Hanrahan, J. 2015. Antidepressant, anxiolytic and antinociceptive activities of constituents from *Rosmarinus officinalis*. *Journal of Pharmacy and Pharmaceutical Sciences*, 18(4):448-459. 10.18433/j3pw38

Adebiyi, R.A., Elsa, A.T., Agaie, B.M. & Etuk, E.U. 2006. Antinociceptive and antidepressant like effects of *Securidaca longepedunculata* root extract in mice. *Journal of Ethnopharmacology*, 107(2):234-239. 10.1016/j.jep.2006.03.017

Adedapo, A.A., Jimoh, F.O., Afolayan, A.J. & Masika, P.J. 2008. Antioxidant activities and phenolic contents of the methanol extracts of the stems of *Acokanthera oppositifolia* and *Adenia gummifera*. *BMC Complementary and Alternative Medicine*, 8(1):54. 10.1186/1472-6882-8-54

Aderibigbe, A.O. 2018. Antidepressant activity of ethanol extract of *Albizia adianthifolia* (Schumach) W. F. Wight leaf in mice. *African Journal of Medicine and Medical Sciences*, 47(2),

Ahmadpoor, J., Chahardahcheric, S.V. & Setorki, M. 2019. The protective effect of hydroalcoholic extract of the Southern Maidenhair Fern (*Adiantum capillus-veneris*) on the depression and anxiety caused by chronic stress in adult male mice: an experimental randomized study. *Iranian Red Crescent Medical Journal*, In Press, 10.5812/ircmj.86750

Albert, P.R. 2015. Why is depression more prevalent in women? *Journal of Psychiatry and Neuroscience*, 40(4):219-221. 10.1503/jpn.150205

Alrashedy, N.A. & Molina, J. 2016. The ethnobotany of psychoactive plant use: a phylogenetic perspective. *Peer-Reviewed Journal*, 4:2546. 10.7717/peerj.2546

American Psychiatric Association. 2000. *Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR)*.

Aremu, A.O., Cheesman, L., Finnie, J.F. & Van Staden, J. 2011. *Mondia whitei* (Apocynaceae): A review of its biological activities, conservation strategies and economic potential. *South African Journal of Botany*, 77(4):960-971. 10.1016/j.sajb.2011.06.010

Badr, A.M., Attia, H.A. & Al-Rasheed, N. 2020. Oleuropein reverses repeated corticosterone-induced depressive-like behavior in mice: Evidence of modulating effect on biogenic amines. *Scientific Reports*, 10(1):3336. 10.1038/s41598-020-60026-1

Batalha, V.L., Pego, J.M., Fontinha, B.M., Costenla, A.R., Valadas, J.S., Baqi, Y., Radjainia, H., Müller, C.E., Sebastião, A.M. & Lopes, L.V. 2013. Adenosine A(2A) receptor blockade reverts hippocampal stress-induced deficits and restores corticosterone circadian oscillation. *Molecular Psychiatry*, 18(3):320-331. 10.1038/mp.2012.8

Bay-Smidt, M.G.K., Jäger, A., Krydsfeldt, K., Meerow, A., Stafford, G., Staden, J. & Rønsted, N. 2011. Phylogenetic selection of target species in Amaryllidaceae tribe Haemantheae for acetylcholinesterase inhibition and affinity to the serotonin reuptake transport protein. *South African Journal of Botany*, 77:175-183. 10.1016/j.sajb.2010.07.016

Bazrafshan, M.R., Jokar, M., Shokrpour, N. & Delam, H. 2020. The effect of lavender herbal tea on the anxiety and depression of the elderly: A randomized clinical trial. *Complementary Therapies in Medicine*, 50:102393. 10.1016/j.ctim.2020.102393

Behbahani, B.A., Shahidi, F., Yazdi, F.T., Mortazavi, S.A. & Mohebbi, M. 2017. Antioxidant activity and antimicrobial effect of tarragon (*Artemisia dracunculus*) extract and chemical composition of its essential oil. *Food Measure*, 11:847-863. 10.1007/s11694-016-9456-3

Benneh, C.K., Biney, R.P., Adongo, D.W., Mante, P.K., Ampadu, F.A., Tandoh, A., Jato, J. & Woode, E. 2018. Anxiolytic and antidepressant effects of *Maerua angolensis* DC. stem bark extract in mice. *Depression Research and Treatment*, 2018:1537371. 10.1155/2018/1537371

Beppe, G.J., Dongmo, A.B., Foyet, H.S., Dimo, T., Mihasan, M. & Hritcu, L. 2015. The aqueous extract of *Albizia adianthifolia* leaves attenuates 6-hydroxydopamine-induced anxiety, depression and oxidative stress in rat amygdala. *BMC Complementary and Alternative Medicine*, 15(1):374. 10.1186/s12906-015-0912-0

Bhat, R.B. & Jacobs, T.V. 1995. Traditional herbal medicine in Transkei. *Journal of Ethnopharmacology*, 48(1):7-12. 10.1016/0378-8741(95)01276-J

Bruns, R.F., Lu, G.H. & Pugsley, T.A. 1986. Characterization of the A2 adenosine receptor labeled by [3H]NECA in rat striatal membranes. *Molecular Pharmacology*, 29(4):331-346.

Bruns, R.F., Fergus, J.H., Badger, E.W., Bristol, J.A., Santay, L.A., Hartman, J.D., Hays, S.J. & Huang, C.C. 1987. Binding of the A1-selective adenosine antagonist 8-cyclopentyl-1,3-dipropylxanthine to rat brain membranes. *Naunyn-Schmiedeberg's Archives of Pharmacology*, 335(1):59-63. 10.1007/bf00165037

Bueno-Notivol, J., Gracia-García, P., Olaya, B., Lasheras, I., López-Antón, R. & Santabárbara, J. 2021. Prevalence of depression during the COVID-19 outbreak: A meta-analysis of community-based studies. *International Journal of Clinical Health and Psychology*, 21(1):100-196. 10.1016/j.ijchp.2020.07.007

Casteleijn, D., Steel, A., Bowman, D., Lauche, R. & Wardle, J. 2019. A naturalistic study of herbal medicine for self-reported depression and/or anxiety a protocol. *Integrative Medicine Research*, 8(2):123-128. 10.1016/j.imr.2019.04.007

Ceremuga, T.E., Valdivieso, D., Kenner, C., Lucia, A., Lathrop, K., Stailey, O., Bailey, H., Criss, J., Linton, J., Fried, J., Taylor, A., Padron, G. & Johnson, A.D. 2015. Evaluation of the anxiolytic and antidepressant effects of asiatic acid, a compound from *Gotu kola* or *Centella asiatica*, in the male Sprague Dawley rat. *American Association of Nurse Anesthesiology Journal*, 83(2):91-98.

Citó, M.C.O., Silva, M.I.G., Santos, L.K.X., Fernandes, M.L., Melo, F.H.C., Aguiar, J.A.C., Lopes, I.S., Sousa, P.B., Vasconcelos, S.M.M., Macedo, D.S. & Sousa, F.C.F. 2015. Antidepressant-like effect of *Hoodia gordonii* in a forced swimming test in mice: evidence for involvement of the monoaminergic system. *Brazilian Journal of Medical and Biological Research*, 48(1): 57-64. 10.1590/1414-431X20144215

Clarke, S.E.D. & Ramsay, R.R. 2011. Dietary inhibitors of monoamine oxidase. *Journal of Neural Transmission*, 118(1):1031-1041. 10.1007/s00702-010-0537-x

Coelho, J.E., Alves, P., Canas, P.M., Valadas, J.S., Shmidt, T., Batalha, V.L., Ferreira, D.G., Ribeiro, J.A., Bader, M., Cunha, R.A., do Couto, F.S. & Lopes, L.V. 2014. Overexpression of adenosine A_{2A} receptors in rats: effects on depression, locomotion, and anxiety. *Frontiers in Psychiatry*, 5:67. 10.3389/fpsyt.2014.00067

Coetzee, D.D., López, V. & Smith, C. 2016. High-mesembrine *Sceletium extract* (Trimesemine™) is a monoamine releasing agent, rather than only a selective serotonin reuptake inhibitor. *Journal of Ethnopharmacology*, 177:111-116. 10.1016/j.jep.2015.11.034

Corrigan, B.M., Van Wyk, B.E., Geldenhuys, C.J. & Jardine, J.M. 2011. Ethnobotanical plant uses in the KwaNibela Peninsula, St Lucia, South Africa. *South African Journal of Botany*, 77(2):346-359. 10.1016/j.sajb.2010.09.017

De Beer, J.J.J. & Van Wyk, B.E. 2011. An ethnobotanical survey of the Agter–Hantam, Northern Cape province, South Africa. *South African Journal of Botany*, 77(3):741-754. 10.1016/j.sajb.2011.03.013

De Mendonça, A., Sebastia˜o, A.M. & Ribeiro, J.A. 2000. Adenosine: does it have a neuroprotective role after all? *Brain Research Reviews*, 33(2-3):258–274. 10.1016/S0165-0173(00)00033-3

De Mendonça, A., Almeida, T., Bashir, Z.I. & Ribeiro, J.A. 1997. Endogenous adenosine attenuates long-term depression and depotentiation in the CA1 region of the rat hippocampus. *Neuropharmacology*, 36:161–167. 10.1016/S0028-3908(96)00173-6

Devi, M.R., Bawari, M., Paul, S.B. & Sharma, G.D. 2012. Characterization of the toxic effects induced by *Datura stramonium* L. leaves on mice: a behavioural, biochemical and ultrastructural approach. *Asian Journal of Pharmaceutical and Clinical Research*, 5(3),

Dey, A., Mukherjee, A. & Chaudhury, M. 2017. Chapter 10 - Alkaloids from Apocynaceae: origin, pharmacotherapeutic properties, and structure-activity studies. In: Atta ur, R., ed. *Studies in Natural Products Chemistry*. 52: Elsevier. pp. 373-488.

du Toit, A. & van der Kooy, F. 2019. Artemisia afra, a controversial herbal remedy or a treasure trove of new drugs? *Journal of Ethnopharmacology*, 244:112127. 10.1016/j.jep.2019.112127

Egebjerg, M., Weng, A., Sert, A., Stafford, G., Staden, J., Nielsen, M. & Jäger, A. 2006. Pharmacological studies on *Xysmalobium undulatum* and *Mondia whitei* – Two South African plants with in vitro SSRI activity. *Planta Medica*, 72(14), 10.1055/s-2006-949923

El-Alfy, A.T., Ivey, K., Robinson, K., Ahmed, S., Radwan, M., Slade, D., Khan, I., ElSohly, M. & Ross, S. 2010. Antidepressant-like effect of Δ9-tetrahydrocannabinol and other cannabinoids isolated from *Cannabis sativa* L. *Pharmacology Biochemistry and Behavior*, 95(4):434-442. 10.1016/j.pbb.2010.03.004

El Yacoubi, M., Ledent, C., Parmentier, M., Bertorelli, R., Ongini, E., Costentin, J. & Vaugeois, J.M. 2001. Adenosine A2A receptor antagonists are potential antidepressants: evidence based on pharmacology and A2A receptor knockout mice. *British Journal of Pharmacology*, 134(1):68-77. 10.1038/sj.bjp.0704240

Elgorashi, E. 2019. Phytochemistry and pharmacology of the Family Amaryllidaceae: an overview of research at RCPGD. *Natural Product Communications*, 14:1934578X1987292. 10.1177/1934578X19872929

Elisha, I.L., Elgorashi, E.E., Hussein, A.A., Duncan, G. & Eloff, J.N. 2013. Acetylcholinesterase inhibitory effects of the bulb of *Ammocharis coranica* (Amaryllidaceae) and its active constituent lycorine. *South African Journal of Botany*, 85:44-47. 10.1016/j.sajb.2012.11.008

Fajemiroye, J.O., da Silva, D.M., de Oliveira, D.R. & Costa, E.A. 2016. Treatment of anxiety and depression: medicinal plants in retrospect. *Fundamental & Clinical Pharmacology*, 30(3):198-215. 10.1111/fcp.12186

Fathinezhad, Z., Sewell, R.D.E., Lorigooini, Z. & Rafieian-Kopaei, M. 2019. Depression and treatment with effective herbs. *Current Pharmaceutical Design*, 25(6):738-745. 10.2174/1381612825666190402105803

Fennell, C.W., Lindsey, K.L., McGaw, L.J., Sparg, S.G., Stafford, G.I., Elgorashi, E.E., Grace, O.M. & van Staden, J. 2004. Assessing African medicinal plants for efficacy and safety: pharmacological screening and toxicology. *Journal of Ethnopharmacology*, 94(2-3):205-217. 10.1016/j.jep.2004.05.012

Ferrari, A.J., Charlson, F.J., Norman, R.E., Patten, S.B., Freedman, G., Murray, C.J., Vos, T. & Whiteford, H.A. 2013. Burden of depressive disorders by country, sex, age, and year: findings from the global burden of disease study 2010. *Public Library of Science Medicine*, 10(11):e1001547. 10.1371/journal.pmed.1001547

Fiebich, B.L., Knörle, R., Appel, K., Kammler, T. & Weiss, G. 2011. Pharmacological studies in an herbal drug combination of St. John's Wort (*Hypericum perforatum*) and passion flower (*Passiflora incarnata*): *In vitro* and *in vivo* evidence of synergy between *Hypericum* and

Passiflora in antidepressant pharmacological models. *Fitoterapia*, 82(3):474-480. 10.1016/j.fitote.2010.12.006

Francesc, V., Bastida, J., Codina, C., Nair, J. & Campbell, W. 1997. Alkaloids of the South African Amaryllidaceae: A Review. *Recent Research Developments in Phytochemistry*, 1:131-172.

Gao, K., Kemp, D.E., Fein, E., Wang, Z., Fang, Y., Ganocy, S.J. & Calabrese, J.R. 2011. Number needed to treat to harm for discontinuation due to adverse events in the treatment of bipolar depression, major depressive disorder, and generalized anxiety disorder with atypical antipsychotics. *Journal of Clinical Psychiatry*, 72(8):1063-1071. 10.4088/JCP.09r05535gre

Gericke, N. & Viljoen, A.M. 2008. *Sceletium*-A review update. *Journal of Ethnopharmacology*, 119(3):653-663.

Gomes, J.I., Farinha-Ferreira, M., Rei, N., Gonçalves-Ribeiro, J., Ribeiro, J.A., Sebastião, A.M. & Vaz, S.H. 2021. Of adenosine and the blues: The adenosinergic system in the pathophysiology and treatment of major depressive disorder. *Pharmacological Research*, 163:105363. 10.1016/j.phrs.2020.105363

Grabarczyk, M., Wińska, K., Mączka, W., Potaniec, B. & Anioł, M. 2015. Loliolide - the most ubiquitous lactone. *Folia Biologica et Oecologica*, 11:1-8. 10.1515/fobio-2015-0001

Hailu, A. & Engidawork, E. 2014. Screening of the antidepressant-like activity of two *Hypericum* species found in Ethiopia. *Ethiopian Pharmaceutical Journal*, 30, 10.4314/epj.v30i1.3

Hamid, H.A., Ramli, A.N.M. & Yusoff, M.M. 2017. Indole alkaloids from plants as potential leads for antidepressant drugs: a mini review. *Frontiers in Pharmacology*, 8, 10.3389/fphar.2017.00096

Harvey, A.L., Young, L.C., Viljoen, A.M. & Gericke, N.P. 2011. Pharmacological actions of the South African medicinal and functional food plant *Sceletium tortuosum* and its principal alkaloids. *Journal of Ethnopharmacology*, 137(3):1124-1129. 10.1016/j.jep.2011.07.035

Heinrich, M., Edwards, S., Moerman, D.E. & Leonti, M. 2009. Ethnopharmacological field studies: a critical assessment of their conceptual basis and methods. *Journal of Ethnopharmacology*, 124(1),

Heinrich, M., Appendinob, G., Efferthc, T., Fürst, R., Izzoe, a.a., Kayserf, O., Pezzutog, J.M. & Viljoen, A. 2020. Best practice in research – overcoming common challenges in phytopharmacological research. *Journal of Ethnopharmacology*, 246:1-8.

Helmstädter, A. 2015. *Xysmalobium undulatum* (Uzara) research– how everything began. *Journal of Ethnopharmacology*, 164:385-387. 10.1016/j.jep.2014.12.022

Herman, A.A., Stein, D.J., Seedat, S., Heeringa, S.G., Moomal, H. & Williams, D.R. 2009. The South African stress and health (SASH) study: 12-month and lifetime prevalence of common mental disorders. *South African Medical Journal*, 99(5):339-344.

Hulley, I.M. & Van Wyk, B.E. 2019. Quantitative medicinal ethnobotany of Kannaland (western Little Karoo, South Africa): Non-homogeneity amongst villages. *South African Journal of Botany*, 122:225-265. 10.1016/j.sajb.2018.03.014

Huremović, D. 2019. Social Distancing, Quarantine, and Isolation. In. pp. 85-94.

Hutchings, A. 1989. A survey and analysis of traditional medicinal plants as used by the Zulu; Xhosa and Sotho. *Bothalia*, 19(1), 10.4102/abc.v19i1.947

Hutchings, A., Scott, A.H., Lewis, G. & B., C.A. 1996. *Zulu Medicinal Plants. An inventory.* Pietermaritzburg, South Africa: University of Natal Press

Ilkhanizadeh, A., Asghari, A., Hassanpour, S. & Safi, S. 2021. Anti-depressant effect of *Artemisia dracunculus* extract is mediated via GABAergic and serotoninergic systems in

ovariectomized mice. *Journal of Basic and Clinical Pathophysiology*, 9(2):32-41. 10.22070/jbcp.2021.15060.1148

Iloki-Assanga, S.B., Lewis-Luján, L.M., Lara-Espinoza, C.L., Gil-Salido, A.A., Fernandez-Angulo, D., Rubio-Pino, J.L. & Haines, D.D. 2015. Solvent effects on phytochemical constituent profiles and antioxidant activities, using four different extraction formulations for analysis of Bucida buceras L. and Phoradendron californicum. *BMC Research Notes*, 8(1):396. 10.1186/s13104-015-1388-1

Ishaq, H. 2016. Anxiolytic and antidepressant activity of different methanolic extracts of *Melia azedarach* Linn. *Pakistan Journal of Pharmaceutical Sciences*, 29(5):1649-1655.

Jäger, A.K., Gauguin, B., Andersen, J., Adsersen, A. & Gudiksen, L. 2013. Screening of plants used in Danish folk medicine to treat depression and anxiety for affinity to the serotonin transporter and inhibition of MAO-A. *Journal of Ethnopharmacology*, 145(3):822-825. 10.1016/j.jep.2012.12.021

Jahani, R., Khaledyan, D., Jahani, A., Jamshidi, E., Kamalinejad, M., Khoramjouy, M. & Faizi, M. 2019. Evaluation and comparison of the antidepressant-like activity of *Artemisia dranunculus* and *Stachys lavandulifolia* ethanolic extracts: an *in vivo*s tudy. *Research in Pharmaceutical Sciences*, 14(6):544-553.

Jamuna, S., Subramaniam, P. & Krishnamoorthy, K. 2014. Phytochemical analysis and evaluation of leaf and root parts of the medicinal herb, *Hypochaeris radicata* L. for *in vitro* antioxidant activities. *Asian Pacific Journal of Tropical Biomedicine*, 4:359-367. 10.12980/APJTB.4.2014C1030

Janke, E.A., Holroyd, K.A. & Romanek, K. 2004. Depression increases onset of tension-type headache following laboratory stress. *Pain*, 111(3):230-238.

Jedi, B., Saeid, A.M. & Mousavi, S.E. 2017. The antidepressant-like effects of *Mentha spicata* essential oil in animal models of depression in male mice. *Journal of Fasa University of Medical Sciences*,

Kalshetty, P., Aswar, U., Bodhankar, S., Sinnathambi, A., Mohan, V. & Thakurdesai, P. 2012. Antidepressant effects of standardized extract of *Centella asiatica* L in olfactory bulbectomy model. *Biomedicine & Aging Pathology*, 2(2):48-53. 10.1016/j.biomag.2012.03.005

Kane, N.F., Kyama, M.C., Nganga, J.K., Hassanali, A., Diallo, M. & Kimani, F.T. 2019. Comparison of phytochemical profiles and antimalarial activities of *Artemisia afra* plant collected from five countries in Africa. *South African Journal of Botany*, 125:126-133. 10.1016/j.sajb.2019.07.001

Kaster, M.P., Rosa, A.O., Rosso, M.M., Goulart, E.C., Santos, A.R.S. & Rodrigues, A.L.S. 2004. Adenosine administration produces an antidepressant-like effect in mice: evidence for the involvement of A1 and A2A receptors. *Neuroscience Letters*, 355(1):21-24. 10.1016/j.neulet.2003.10.040

Kaster, M.P., Machado, N.J., Silva, H.B., Nunes, A., Ardais, A.P., Santana, M., Baqi, Y., Müller, C.E., Rodrigues, A.L., Porciúncula, L.O., Chen, J.F., Tomé Â, R., Agostinho, P., Canas, P.M. & Cunha, R.A. 2015. Caffeine acts through neuronal adenosine A_{2A} receptors to prevent mood and memory dysfunction triggered by chronic stress. *Proceedings of the National Academy of Sciences of the United States of America*, 112(25):7833-7838. 10.1073/pnas.1423088112

Khan, H., Perviz, S., Sureda, A., Nabavi, S.M. & Tejada, S. 2018. Current standing of plant derived flavonoids as an antidepressant. *Food and Chemical Toxicology*, 119:176-188. 10.1016/j.fct.2018.04.052

Khlif, I., Jellali, K., Michel, T., Halabalaki, M., Skaltsounis, A.L. & Allouche, N. 2015. Characteristics, phytochemical analysis and biological activities of extracts from Tunisian chetoui *Olea europaea* variety. *Journal of Chemistry*, 2015, 10.1155/2015/418731

Khosravi, H., Rahnema, M. & Asle, R.M. 2017. Anxiolytic and antidepressant effects of tarragon (Artemisia dracunculus L.) hydroalcoholic extract in male rats exposed to chronic restraint stress. 4(1):1-8.

Koen, L., Niehaus, D., Muller, J. & Laurent, C. 2003. Use of traditional treatment methods in a Xhosa schizophrenia population. *South African Medical Journal*, 93(433),

Koenig, X. & Hilber, K. 2015. The anti-addiction drug ibogaine and the heart: a delicate relation. *Molecules*, 20(2):2208-2228. 10.3390/molecules20022208

Koorbanally, N., Mulholland, D.A. & Crouch, N. 2000a. Alkaloids and triterpenoids from *Ammocharis coranica* (Amaryllidaceae). *Phytochemistry*, 54(1):93-97. 10.1016/S0031-9422(00)00039-X

Koorbanally, N.A., Mulholland, D.A. & Crouch, N.R. 2000b. Isolation of Isovanillin from aromatic roots of the medicinal African Liane, *Mondia whitei*. *Journal of Herbs, Spices & Medicinal Plants*, 7(3):37-43. 10.1300/J044v07n03_05

Koutová, D., Maafi, N., Havelek, R., Opletal, L., Blunden, G., Rezácˇová, M. & Cahlíková, L. 2020. Chemical and biological aspects of montanine-type alkaloids isolated from plants of the Amaryllidaceae family. *Molecules*, 25:1-19. 10.3390/molecules25102337

Kovatsis, A., Flaskos, J., Nikolaidis, E., Kotsaki-Kovatsi, V.P., Papaioannou, N. & Tsafaris, F. 1993. Toxicity study of the main alkaloids of Datura ferox in broilers. *Food and Chemical Toxicology*, 31(11):841-845. 10.1016/0278-6915(93)90222-k.

Lee, G. & Bae, H. 2017. Therapeutic effects of phytochemicals and medicinal herbs on depression. *BioMed Research International*, 2017:6596241. 10.1155/2017/6596241

Liu, X., Dong, M., X., C., Jiang, M., Lv, X. & Yan, G. 2007. Antioxidant activity and phenolics of an endophytic Xylaria sp. From Ginkgo biloba. *Food and Chemical Toxicology*, 105(2):548-554.

Loria, M.J., Ali, Z., Abe, N., Sufka, K.J. & Khan, I.A. 2014. Effects of *Sceletium tortuosum* in rats. *Journal of Ethnopharmacology*, 155(1):731-735. 10.1016/j.jep.2014.06.007

Machado, D.G., Bettio, L.E.B., Cunha, M.P., Capra, J.C., Dalmarco, J.B., Pizzolatti, M.G. & Rodrigues, A.L.S. 2009. Antidepressant-like effect of the extract of *Rosmarinus officinalis* in mice: Involvement of the monoaminergic system. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 33(4):642-650. 10.1016/j.pnpbp.2009.03.004

Machado, D.G., Kaster, M.P., Binfaré, R.W., Dias, M., Santos, A.R.S., Pizzolatti, M.G., Brighente, I.M.C. & Rodrigues, A.L.S. 2007. Antidepressant-like effect of the extract from leaves of *Schinus molle* L. in mice: Evidence for the involvement of the monoaminergic system. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 31(2):421-428. 10.1016/j.pnpbp.2006.11.004

Makkar, H. 2000. Quantification of tannins in tree foliage: A laboratory manual for the FAO/IAEA co-ordinated research project on 'Use of nuclear and related techniques to develop simple tannin assays for predicting and improving the safety and efficiency of feeding ruminants of tanniniferous tree foliage. Joint FAO/IAEA of Nuclear Techniques in Food and Agriculture. Animal Production and Health Sub-programme. *FAO/IAEA Working Document, Vienna*:3-5.

Mander, M. 1998. *Marketing of Indigenous Medicinal Plants in South Africa: A case study in Kwazulu-Natal*. KwaZulu-Natal, South Africa:

Maroyi, A. 2020. Evaluation of medicinal uses, phytochemistry, and biological activities of *Adenia gummifera* (Harv.) Harms *Journal of Pharmacy and Nutrition Sciences*, 10(5):280-286.

Marroquín, M.N., Cruz, S.M. & Cáceres, A. 2012. Antioxidant activity and phenolic compounds in three species of Passifloraceae (*Passiflora edulis*, *P. incarnata*, *P. ligularis*) from Guatemala. *Acta Horticulturae*, 964:93-98. 10.17660/ActaHortic.2012.964.11

Martins, J. & Brijesh, S. 2018. Phytochemistry and pharmacology of anti-depressant medicinal plants: A review. *Biomedicine & Pharmacotherapy*, 104:343-365. 10.1016/j.biopha.2018.05.044

Masoko, P. & Mamabolo, S.K. 2019. Phytochemical and biological investigations of *Olea europaea* subspecies *africana* leaves. *Journal of Pharmaceutical Sciences and Research*, 11(8):3020-3029.

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

Moffett, R.O. 2016. *Basotho Medicinal Plants / Meriana ya Dimela tsa Basotho*. Translated by Ramotshela, L. Bloemfontein, South Africa SUN MeDIA Bloemfontein

Mogale, M.M.P., Van Wyk, B.E. & Raimondo, D.C. 2015. Ethnobotany of the Bapedi. *South African Journal of Botany*, 98:191. 10.1016/j.sajb.2015.03.088

Mogale, M.M.P., Raimondo, D.C. & VanWyk, B.E. 2019. The ethnobotany of Central Sekhukhuneland, South Africa. *South African Journal of Botany*, 122:90-119. 10.1016/j.sajb.2019.01.001

Moher, D., Liberati, A., Tetzlaff, J. & Altman, D.G. 2009. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *British Medical Journal*, 339:b2535. 10.1136/bmj.b2535

Moher, D., Shamseer, L., Clarke, M., Ghersi, D., Liberati, A., Petticrew, M., Shekelle, P., Stewart, L.A. & Group, P.-P. 2015. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Systematic Reviews Journal*, 4(1):1-9.

Mongalo, N.I. & Makhafola. 2018. Ethnobotanical knowledge of the lay people of Blouberg area (Pedi tribe), Limpopo Province, South Africa. *Journal of Ethnobiology and Ethnomedicine*, 14:46,

Mosotho, N.L., Louw, D.A., Calitz, F.J. & Esterhuyse, K.G. 2008. Depression among Sesotho speakers in Mangaung, South Africa. *African Journal of Psychiatry*, 11(1):35-43. 10.4314/ajpsy.v11i1.30253

Moteetee, A., Moffett, R.O. & Seleteng-Kose, L. 2019. A review of the ethnobotany of the Basotho of Lesotho and the Free State province of South Africa (South Sotho). *South African Journal of Botany*, 122:21-56. 10.1016/j.sajb.2017.12.012

Mothibe, M. & Sibanda, M. 2019. African Traditional Medicine: South African Perspective. In: Cengiz, M., ed. *Traditional and Complementary Medicine*.

Mumivand, H., Babalar, M., Tabrizi, L., Craker, L.E., Shokrpour, M. & Hadian, J. 2017. Antioxidant properties and principal phenolic phytochemicals of Iranian tarragon (*Artemisia dracunculus* L.) accessions. *Horticultural and Environvironmental Biotechnology*, 58:414-422.

Mungai, K. & Bayat, A. 2019. An overview of trends in depressive symptoms in South Africa. *South African Journal of Psychology*, 49(4):518–535. 10.1177/0081246318823580

Munir, S., Shahid, A., Aslam, B., Ashfaq, U.A., Akash, M.S.H., Ali, M.A., Almatroudi, A., Allemailem, K.S., Rajoka, M.S.R. & Khurshid, M. 2020. The therapeutic prospects of naturally occurring and synthetic indole alkaloids for depression and anxiety disorders. *Evidence Based Complementary and Alternative Medicine*, 2020:8836983. 10.1155/2020/8836983

Nair, J.J. & Van Staden, J. 2013. Pharmacological and toxicological insights to the South African Amaryllidaceae. *Food and Chemical Toxicology*, 62(1):262-275. 10.1016/j.fct.2013.08.042

Nair, J.J. & Van Staden, J. 2014. Traditional usage, phytochemistry and pharmacology of the South African medicinal plant *Boophone disticha* (L.f. Herb. (Amaryllidaceae). *Journal of Ethnopharmacology*, 151:12-26.

Nair, J.J., Bastida, J., Codina, C., Viladomat, F. & van Staden, J. 2013. Alkaloids of the South African Amaryllidaceae: a review. *Natural Product Communications*, 8(9):1335-1350.

Nawaz, H., Shad, M., Rehman, N., Andaleeb, H. & Ullah, N. 2020. Effect of solvent polarity on extraction yield and antioxidant properties of phytochemicals from bean (*Phaseolus vulgaris*) seeds. *Brazilian Journal of Pharmaceutical Sciences*, 56, 10.1590/s2175-97902019000417129

Ndhlala, A.R., Stafford, G.I., Finnie, J.F. & Van Staden, J. 2011. Commercial herbal preparations in KwaZulu-Natal, South Africa: The urban face of traditional medicine. *South African Journal of Botany*, 77(4):830-843. 10.1016/j.sajb.2011.09.002

Ndhlala, A.R., Ncube, B., Okem, A., Mulaudzi, R.B. & Van Staden, J. 2013. Toxicology of some important medicinal plants in southern Africa. *Food and Chemical Toxicology*, 62:609-621. 10.1016/j.fct.2013.09.027

Neergaard, J.S., Rasmussen, H.B., Stafford, G.I., Van Staden, J. & Jager, A.K. 2010. Serotonin transporter affinity of (-)-loliolide, a monoterpene lactone from *Mondia whitei*. *South African Journal of Botany*, 76(3):593-596. 10.1016/j.sajb.2010.02.091

Neergaard, J.S., Andersen, J., Pedersen, M.E., Stafford, G.I., Staden, J.V. & Jäger, A.K. 2009. Alkaloids from *Boophone disticha* with affinity to the serotonin transporter. *South African Journal of Botany*, 75(2):371-374. 10.1016/j.sajb.2009.02.173

Nglazi, M.D., Joubert, J.D., Stein, D.J., Lund, C., Wiysonge, C.S., Vos, T., Pillay-van Wyk, V., Roomaney, R.A., Muhwava, L.S. & Bradshaw, D. 2016. Epidemiology of major depressive disorder in South Africa (1997-2015): a systematic review protocol. *British Medical Journal Open*, 6(7):e011749. 10.1136/bmjopen-2016-011749

Ngouana, V., Zeuko'o Menkem, E., Youmbi, D.Y., Yimgang, L.V., Toghueo, R.M.K. & Boyom, F.F. 2021. Serial exhaustive extraction revealed antimicrobial and antioxidant properties of *Platycerium stemaria* (Beauv) Desv. *BioMed Research International*, 2021:1584141. 10.1155/2021/1584141

Nielsen, N.D., Sandager, M., Stafford, G.I., van Staden, J. & Jager, A.K. 2004. Screening of indigenous plants from South Africa for affinity to the serotonin reuptake transport protein. *Journal of Ethnopharmacology*, 94(1):159-163. 10.1016/j.jep.2004.05.013

Nortje, J.M. & van Wyk, B.E. 2015. Medicinal plants of the Kamiesberg, Namaqualand, South Africa. *Journal of Ethnopharmacology*, 171:205-222. 10.1016/j.jep.2015.04.049

Ozarowski, M. & Karpiński, T.M. 2021. Extracts and flavonoids of Passiflora species as promising anti-inflammatory and antioxidant substances. *Current Pharmaceutical Design*, 27(22):23. 10.2174/1381612826666205261500113

Öztürk, Y., Aydin, S., Beis, R., Başer, K.H.C. & Berberoğlu, H. 1996. Effects of *Hypericum perforatum* L. and *Hypericum calycinum* L. extracts on the central nervous system in mice. *Phytomedicine*, 3(2):139-146. 10.1016/S0944-7113(96)80027-4

Panda, S.K. & Luyten, W. 2018. Antiparasitic activity in Asteraceae with special attention to ethnobotanical use by the tribes of Odisha, India. *Parasite*, 25(10),

Pathak, L., Agrawal, Y. & Dhir, A. 2013. Natural polyphenols in the management of major depression. *Expert Opinion on Investigational Drugs*, 22(7):863-880. 10.1517/13543784.2013.794783

Pedersen, M.E., Szewczyk, B., Stachowicz, K., Wieronska, J., Andersen, J., Stafford, G.I., van Staden, J., Pilc, A. & Jager, A.K. 2008. Effects of South African traditional medicine in animal models for depression. *Journal of Ethnopharmacology*, 119(3):542-548. 10.1016/j.jep.2008.08.030

Perveen, T., Hashmi, B.M., Haider, S., Tabassum, S., Saleem, S. & Siddiqui, M.A. 2013. Role of monoaminergic system in the etiology of olive oil induced antidepressant and anxiolytic effects

in rats. *International Scholarly Research Network Pharmacology*, 2013:615685. 10.1155/2013/615685

Philander, A.L. 2011. An ethnobotany of Western Cape Rasta bush medicine. *Journal of Ethnopharmacology*, 138(2):578-594. 10.1016/j.jep.2011.10.004

Popa-Velea, O., Gheorghe, I.R., Truţescu, C.I. & Purcărea, V.L. 2015. Current challenges and pitfalls in the pharmacological treatment of depression. *Journal of Medicine and Life*, 8(2):181-186.

Proctor, W.R. & Dunwiddie, T.V. 1987. Pre- and postsynaptic actions of adenosine in the *in vitro* rat hippocampus. *Brain Research*, 426(1):187–190. 10.1016/0006-8993(87)90441-0

Rabadia, J., Satish, S., Ramanjaneyulu, J. & B., N.V. 2013. An investigation of anti-depressant activity of *Cinnamomum camphora* oil in experimental mice. *Asian Journal of Biomedical and Pharmaceutical Sciences*, 3(20):44-48.

Rabiei, Z. & Setorki, M. 2019. Effect of ethanol *Adiantum capillus-veneris* extract in experimental models of anxiety and depression. *Brazilian Journal of Pharmaceutical Sciences*, 55:9.

Raffauf, R.F. & Flagler, M.B. 1960. Alkaloids of the Apocynaceae. *Economic Botany*, 14(1):37-55. 10.1007/BF02859365

Rafique, R., Khan, Z., Iltaf, S., Riaz, A., Ali, S., Shaukat, M., Shaukat, H., Sindhu, M., Sindhu, M. & Habib, A. 2021. Phytochemical analysis and FT-IR of leaf and stem extracts of *Olea ferruginea* Royle. *Pakistan Journal of Science*, 73:656-674.

Raghoo, M., Crouch, N.R. & Koorbanally, N.A. 2021. A novel and unusual tetramethoxylated crinine alkaloid from *Ammocharis coranica* (Amaryllidaceae). *South African Journal of Botany*, 137:451-454. 10.1016/j.sajb.2020.11.012

Rajput, M. 2011. Herbal antidepressants. *International Journal of Pharmaceutical Frontier Research*, 1:159.

Ramalhete, N., Machado, A., Serrano, R., Gomes, E.T., Mota-Filipe, H. & Silva, O. 2016. Comparative study on the *in vivo* antidepressant activities of the Portuguese *Hypericum foliosum*, *Hypericum androsaemum* and *Hypericum perforatum* medicinal plants. *Industrial Crops and Products*, 82:29-36. 10.1016/j.indcrop.2015.12.014

Rang, H.P., Dale, M.M., Ritter, J.M. & Moore, P.K. 2007. *Pharmacology, sixth ed*. Churchill Livingstone:

Rawat, D. & Rao, P.B. 2018. Elucidation of phytochemicals and antioxidant potential of some selected plant species of family Asteraceae. *International Journal of Science and Research*, 7(426):1001-1007. 10.21275/ART20203469

Ressler, K.J. & Nemeroff, C.B. 2000. Role of serotonergic and nonadrenergic systems in the pathophysiology of depression and anxiety disorders. *Depression and anxiety*, 12:2-19.

Ribeiro, J.A., Sebastia˜o, A.M. & de Mendonc¸a, A. 2003. Adenosine receptors in the nervous system: pathophysiological implications. *Progress in Neurobiology*, 68:377-392.

Rivera, D., Allkin, R., Obón, C., Alcaraz, F., Verpoorte, R. & Heinrich, M. 2014. What is in a name? The need for accurate scientific nomenclature for plants. *Journal of Ethnopharmacology*, 152(393-402),

Robertson, B.A. 2006. Does the evidence support collaboration between psychiatry and traditional healers? Findings from three South African studies. *South African Psychiatry*, 9:87-90. 10.4314/ajpsy.v9i2.30210

Rolnik, A. & Olas, B. 2021. The plants of the Asteraceae family as agents in the protection of human health. *International Journal of Molecular Sciences*, 22(3009),

Saki, K., Bahmani, M. & Rafieian-Kopaei, M. 2014. The effect of most important medicinal plants on two important psychiatric disorders (anxiety and depression)-a review. *Asian Pacific Journal of Tropical Medicine*, 7:34-42. 10.1016/S1995-7645(14)60201-7

Sales, A.J., Crestani, C.C., Guimarães, F.S. & Joca, S.R.L. 2018. Antidepressant-like effect induced by cannabidiol is dependent on brain serotonin levels. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 86:255-261. https://doi.org/10.1016/j.pnpbp.2018.06.002

Sandager, M., Nielsen, N.D., Stafford, G.I., van Staden, J. & Jager, A.K. 2005. Alkaloids from *Boophone disticha* with affinity to the serotonin transporter in rat brain. *Journal of Ethnopharmacology*, 98(3):367-370. 10.1016/j.jep.2005.01.037

Sarker, S.D., Laird, A., Nahar, L., Kumarasamy, Y. & Jaspars, M. 2001. Indole alkaloids from the seeds of *Centaurea cyanus* (Asteraceae). *Phytochemistry*, 57(8):1273-1276. 10.1016/S0031-9422(01)00084-X

Sarris, J. 2018. Herbal medicines in the treatment of psychiatric disorders: 10-year updated review. *Phytotherapy Research*, 32(7):1147-1162. 10.1002/ptr.6055

Sarris, J., Panossian, A., Schweitzer, I., Stough, C. & Scholey, A. 2011. Herbal medicine for depression, anxiety and insomnia: A review of psychopharmacology and clinical evidence. *European Neuropsychopharmacology*, 21(12):841-860. doi: 10.1016/j.euroneuro.2011.04.002

Sasaki, K., El Omri, A., Kondo, S., Han, J. & Isoda, H. 2013. *Rosmarinus officinalis* polyphenols produce anti-depressant like effect through monoaminergic and cholinergic functions modulation. *Behavioural Brain Research*, 238:86-94. 10.1016/j.bbr.2012.10.010

Sasaki, K., Ferdousi, F., Fukumitsu, S., Kuwata, H. & Isoda, H. 2021. Antidepressant- and anxiolytic-like activities of *Rosmarinus officinalis* extract in rodent models: Involvement of oxytocinergic system. *Biomedicine & Pharmacotherapy*, 144:112291. 10.1016/j.biopha.2021.112291

Schildkraut, J.J. 1965. The catecholamine hypothesis of affective disorders: a review of supporting evidence. *American Journal of Psychiatry*, 122(5):509-522. 10.1176/ajp.122.5.509

Selvi, P.T., Kumar, M.S., Rajesh, R. & Kathiravan, T. 2012. Antidepressant activity of ethanolic extract of leaves of *Centella asiatica*. Linn by *In vivo* methods. *Asian Journal of Research in Pharmaceutical Sciences*, 2(2):76-79.

Serchov, T., Clement, H.W., Schwarz, M.K., Iasevoli, F., Tosh, D.K., Idzko, M., Jacobson, K.A., de Bartolomeis, A., Normann, C., Biber, K. & van Calker, D. 2015. Increased signaling via adenosine a1 receptors, sleep deprivation, imipramine, and ketamine inhibit depressive-like behavior via induction of Homer1a. . *Neuron* 87(3):549–562. 10.1016/j.neuron.2015.07.010

Shiamala, D.R., Japar, S.B. & Muta, H.Z. 2014. Assessment of total phenolic, antioxidant, and antibacterial activities of *Passiflora* species. *The Scientific World Journal*, 10.1155/2014/167309

Sobiecki, J.F. 2002. A preliminary inventory of plants used for psychoactive purposes in southern African healing traditions. *Transactions of the Royal Society of South Africa*, 57(1-2):1-24. 10.1080/00359190209520523

Song, Y., Seo, S., Lamichhane, S., Seo, J., Hong, J.T., Cha, H.J. & Yun, J. 2021. Limonene has anti-anxiety activity via adenosine A2A receptor-mediated regulation of dopaminergic and GABAergic neuronal function in the striatum. *Phytomedicine*, 83:153474. 10.1016/j.phymed.2021.153474

Sorsdahl, K., Stein, D.J., Grimsrud, A., Seedat, S., Flisher, A.J., Williams, D.R. & Myer, L. 2009. Traditional healers in the treatment of common mental disorders in South Africa. *Journal of Nervous and Mental Dissease*, 197(6):434-441. 10.1097/NMD.0b013e3181a61dbc

Stafford, G.I. 2009. *Southern African plants used to treat central nervous system related disorders.* Pietermaritzburg , South Africa: University of KwaZulu-Natal.

Stafford, G.I., Jäger, A.K. & van Staden, J. 2005. Activity of traditional South African sedative and potentially CNS-acting plants in the GABA-benzodiazepine receptor assay. *Journal of Ethnopharmacology*, 100(1):210-215. 10.1016/j.jep.2005.04.004

Stafford, G.I., Jäger, A.K. & Van Staden, J. 2009. African psychoactive plants. In: Juliani, H.R. & Simon, J., eds*. African Natural Plant Products: New Discoveries and Challenges in Chemistry and Quality*. pp. 323-346.

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

Stafford, G.I., Pedersen, M.E., van Staden, J. & Jager, A.K. 2008. Review on plants with CNS-effects used in traditional South African medicine against mental diseases. *Journal of Ethnopharmacology*, 119(3):513-537. 10.1016/j.jep.2008.08.010

Stafford, G.I., Birer, C., Brodin, B., Christensen, S.B., Eriksson, A.H., Jäger, A.K. & Rønsted, N. 2013. Serotonin transporter protein (SERT) and P-glycoprotein (P-gp) binding activity of montanine and coccinine from three species of *Haemanthus* L. (Amaryllidaceae). *South African Journal of Botany*, 88:101-106.

Stahl, S.M. 1998. Mechanism of action of serotonin selective reuptake inhibitors: Serotonin receptors and pathways mediate therapeutic effects and side effects. *Journal of Affective Disorders*, 51(3):215-235. 10.1016/S0165-0327(98)00221-3

Starkowitz, M. 2013. *African traditional healers' understanding of depression as a mental illness: implications for social work practice.* Pretoria: University of Pretoria. (Mini-dissertation).

Staub, P.O., Geck, M.S., Weckerle, C.S., Casu, L. & Leonti, M. 2015. Classifying diseases and remedies in ethnomedicine and ethnopharmacology. *Journal of Ethnopharmacology*, 174:514-519. 10.1016/j.jep.2015.08.051

Sülsen, V.P., Lizarraga, E., Mamadalieva, N.Z. & Lago, J.H.D. 2017. Potential of terpenoids and flavonoids from Asteraceae as anti-inflammatory, antitumor, and antiparasitic agents. *Evidence-Based Complementary and Alternative Medicine*, 2017,

Szwajgier, D., Borowiec, K. & Pustelniak, K. 2017. The neuroprotective effects of phenolic acids: Molecular mechanism of action. *Nutrients*, 9(477), 10.3390/nu9050477

Tandon, D. & Gupta, A.K. 2020. Comparative assessment of antimicrobial and antioxidant activity between whole plant and parts of *Sphaeranthus indicus* Linn. (Asteraceae). *Clinical Phytoscience*, 6(23):1-15. 10.1186/s40816-020-00172-1

Tariq, U., Butt, M.S., Pasha, I. & Faisal, M.N. 2021. Neuroprotective effects of *Olea europaea* L. fruit extract against cigarette smoke-induced depressive-like behaviors in Sprague-Dawley rats. *Journal of Food Biochemistry*, 45(12):e14014. 10.1111/jfbc.14014

Taylor, A., Oyedeji, O., Aremu, O., Oyemitan, I., Gwebu, E.T., Oyedeji, A. & Nkeh-Chungag, B. 2016. Assessment of the analgesic, anti-inflammatory and sedative effects of the dichloromethanol extract of *Schinus molle*. *European Review for Medical and Pharmacological Sciences*, 20:372-380.

Thouri, A., Chahdoura, H., El Arem, A., Omri Hichri, A., Ben Hassin, R. & Achour, L. 2017. Effect of solvents extraction on phytochemical components and biological activities of Tunisian date seeds (var. Korkobbi and Arechti). *BMC Complement Alternative Medicine*, 17(1):248. 10.1186/s12906-017-1751-y

Thring, T.S.A. & Weitz, F.M. 2006. Medicinal plant use in the Bredasdorp/Elim region of the Southern Overberg in the Western Cape province of South Africa. *Journal of Ethnopharmacology*, 103:261–275. 10.1016/j.jep.2005.08.013

Tian, J., Zhang, F., Cheng, J., Guo, S., Liu, P. & Wang, H. 2014. Antidepressant-like activity of adhyperforin, a novel constituent of *Hypericum perforatum* L. *Scientific Reports*, 4(5632):6. 10.1038/srep05632

Todd, C., Patel, V., Simunyu, E., Gwanzura, F., Acuda, W. & Winston, M. 1999. The onset of common mental disorders in primary care attenders in Harare, Zimbabwe. *Psychology Medicine*, 29:97-104.

Trease, G.E. & Evans, W.C. 1983. Pharmacognosy. 4:343-383.

Trigo, J.R., Leal, I.R., Matzenbacher, N.I. & Lewinsohn, T.M. 2003. Chemotaxonomic value of pyrrolizidine alkaloids in southern Brazil Senecio (Senecioneae: Asteraceae). *Biochemical Systematics and Ecology*, 31(9):1011-1022. 10.1016/S0305-1978(03)00038-3

Van Calker, D., Biber, K., Domschke, K. & Serchov, T. 2019. The role of adenosine receptors in mood and anxiety disorders. *Journal of Neurochemistry*, 151(1):11-27. 10.1111/jnc.14841

Van der Walt, M.M. & Terre'Blanche, G. 2015. 1,3,7-Triethyl-substituted xanthines—possess nanomolar affinity for the adenosine A1 receptor. *Bioorganic & Medicinal Chemistry*, 23(20):6641-6649.

Van Wyk, B.E. & Gericke, N. 2000. *People's Plants: A revised version*. Pretoria, South Africa Briza Publication

Van Wyk, B.E., van Oudtshoorn, B. & Gericke, N. 1997. *Medicinal Plants of South Africa* Pretoria, South Africa Briza Publications

Van Wyk, B.E., de Wet, H. & Van Heerden, F.R. 2008. An ethnobotanical survey of medicinal plants in the southeastern Karoo, South Africa. *South African Journal of Botany*, 74(4):696-704. 10.1016/j.sajb.2008.05.001

Venter, F. & Venter, J.-A. 2016. *Making the most of indigenous trees*. 3rd Vol. Pretoria, South Africa: Briza Publications

Victoria, F.N., Anversa, R., Penteado, F., Castro, M., Lenardão, E.J. & Savegnago, L. 2014. Antioxidant and antidepressant-like activities of semi-synthetic α phenyl selenocitronellal. *European Journal of Pharmacology*, 782:131-138.

Viljoen, A., Sandasi, M. & Vermaak, I. 2019. The role of the South African Journal of Botany as a vehicle to promote medicinal plant research– A bibliometric appraisal. *South African Journal of Botany*, 122:3-10. 10.1016/j.sajb.2018.07.024

Wado, E.K., Kubicki, M., Ngatanko, A.H.H., Léa Blondelle, K.D., Jorelle linda, D., Roland, R.N., Balbine, K., Lamshoeft, M., Assongalem, A.E. & Foyet, H.S. 2020. Anxiolytic and antidepressant effects of *Ziziphus mucronata* hydromethanolic extract in male rats exposed to unpredictable chronic mild stress: Possible mechanisms of actions. *Journal of Ethnopharmacology*, 260:112987. 10.1016/j.jep.2020.112987

Wakeel, A., Jan, S.A., Ullah, I., Shinwari, Z.K. & Xu, M. 2019. Solvent polarity mediates phytochemical yield and antioxidant capacity of Isatis tinctoria. *Peer-Reviewed Journal*, 7:e7857. 10.7717/peerj.7857

Wang, Y., Shi, Y.-h., Xu, Z., Fu, H., Zeng, H. & Zheng, G.-q. 2019. Efficacy and safety of Chinese herbal medicine for depression: A systematic review and meta-analysis of randomized controlled trials. *Journal of Psychiatric Research*, 117:74-91. https://doi.org/10.1016/j.jpsychires.2019.07.003

Warewat, W., Nattira, O.-N., Chaowanee, C., Piya, T., Somsri, C. & Uthaiwan, S. 2021. *In vitro* phytotherapeutic properties of aqueous extracted *Adenia viridiflora* Craib. towards civilization diseases. *Molecules*, 24(1082), 10.3390/molecules26041082

Weckerle, C.S., de Boer, H.J., Puri, R.K., van Andel, T., Bussmann, R.W. & Leonti, M. 2018. Recommended standards for conducting and reporting ethnopharmacological field studies. *Journal of Ethnopharmacology*, 210:125-132.

Wei, C.B., Jia, J.P., Wang, F., Zhou, A.H., Zuo, X.M. & B., C.C. 2016. Overlap between headache, depression, and anxiety in general neurological clinics: A cross-sectional study. *Chinese Medical Journal*, 129(12):1394-1399. 10.4103/0366-6999.183410

Wink, M. & Roberts, M.W. 1998. *Alkaloids: biochemistry, ecology, and medicinal application*. New York: Plenum Press.

World Health Organisation. 2017. *Depression and other common mental disorders: Global health estimates*.

Yamada, K., Kobayashi, M. & Kanda, T. 2014. Chapter Fifteen -involvement of adenosine A_{2A} receptors in depression and anxiety. In: Mori, A., ed. *International Review of Neurobiology*. 119: Academic Press. pp. 373-393.

Yimam, B.B. & Desalew, A. 2022. Phytochemical screening, antibacterial effect, and essential oil extract from the leaf of *Artemisia afra* against selected pathogens. *Advances in Microbiology*, 12(7):386-397. 10.4236/aim.2022.127028

Yung-Chi, C. & Prusoff, W.H. 1973. Relationship between the inhibition constant (ki) and the concentration of inhibitor which causes 50 per cent inhibition (i50) of an enzymatic reaction. *Biochemical Pharmacology*, 22(23):3099-3108.

Zanelate, T.V., Biojone, C., Moreira, F.A., Guimarães, F.S. & Joca, S.R.L. 2010. Antidepressant-like effects of cannabidiol in mice: possible involment of 5-HT_{1A} receptors. *British Journal of Pharmacology*, 159(1):122-128. 10.1111/j.1476-5381.2009.00521.x

Zhang, C., Xin, X., Zhang, J., Zhu, S., Niu, E., Zhou, Z. & Liu, D. 2022. Comparative evaluation of the phytochemical profiles and antioxidant potentials of Olive leaves from 32 cultivars grown in China. *Molecules*, 27(4):1292. 10.3390/molecules27041292

APPENDICES

Appendix 1: Ethics approval letter of the study

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Tel: 056 010 9696
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North-West University Animal Care, Health and Safety Research Ethics Committee (NWU-AnimCareREC)

Tel: 018 299-1206
Email: Ethics-AnimCare@nwu.ac.za (for animal studies)

8 July 2022

ETHICS APPROVAL LETTER OF STUDY

Based on approval by the North-West University Animal Care, Health and Safety Research Ethics Committee (NWU-AnimCareREC) on 08/07/2022, the NWU-AnimCareREC hereby approves your study as indicated below. This implies that the NWU-AnimCareREC grants its permission that, provided the general conditions specified below are met and pending any other authorisation that may be necessary, the study may be initiated, using the ethics number below.

Study title: Evaluation of South African medicinal plants with potential antidepressant effects
Principal Investigator/Study Supervisor/Researcher: Dr M Lekhooa
Student: MB Bonokwane - 28032758

Ethics number: N W U - 0 0 7 5 8 - 2 2 - A 1
Institution Study Number Year Status
Status: S = Submission; R = Re-Submission; P = Provisional Authorisation; A = Authorisation

Application Type: Single study
Commencement date: 08/07/2022
Expiry date: 31/07/2023
Risk: Category 0

Approval of the study is provided for a year, after which continuation of the study is dependent on receipt and review of an annual monitoring report and the concomitant issuing of a letter of continuation. A monitoring report is due at the end of July annually until completion of the study.

General conditions:

While this ethics approval is subject to all declarations, undertakings and agreements incorporated and signed in the application form, the following general terms and conditions will apply:

- *The principal investigator/study supervisor/researcher must report in the prescribed format to the NWU-AnimCareREC:*
  - *annually on the monitoring of the study, whereby a letter of continuation will be provided annually, and upon completion of the study; and*
  - *without any delay in case of any adverse event or incident (or any matter that interrupts sound ethical principles) during the course of the study.*
- *The approval applies strictly to the proposal as stipulated in the application form. Should any amendments to the proposal be deemed necessary during the course of the study, the principal investigator/study supervisor/researcher must apply for approval of these amendments at the NWU-AnimCareREC, prior to implementation. Should there be any deviations from the study proposal without the necessary approval of such amendments, the ethics approval is immediately and automatically forfeited.*
- *Annually a number of studies may be randomly selected for active monitoring.*

- *The date of approval indicates the first date that the study may be started.*
- *In the interest of ethical responsibility, the NWU-AnimCareREC reserves the right to:*
  - *request access to any information or data at any time during the course or after completion of the study;*
  - *to ask further questions, seek additional information, require further modification or monitor the conduct of your research or the informed consent process;*
  - *withdraw or postpone approval if:*
    - *any unethical principles or practices of the study are revealed or suspected;*
    - *It becomes apparent that any relevant information was withheld from the NWU-AnimCareREC or that information has been false or misrepresented;*
    - *submission of the annual monitoring report, the required amendments, or reporting of adverse events or incidents was not done in a timely manner and accurately; and/or*
    - *new institutional rules, national legislation or international conventions deem it necessary.*
- *NWU-AnimCareREC can be contacted for further information via Ethics-AnimCare@nwu.ac.za or 018 299 1206*

Special conditions of the research approval due to the COVID-19 pandemic:

Please note: Due to the nature of the study i.e. (laboratory work involving the *in vitro* analysis of the anti-depressant effects of specific medicinal plants in rodent brains), this study will be able to proceed during the current alert level, following receipt of the approval letter. No additional COVID-19 restrictions have been placed on the study except that the researcher must ensure that before proceeding with the study that all research team members have reviewed the North-West University COVID-19 Occupational Health and Safety Standard Operating Procedure.

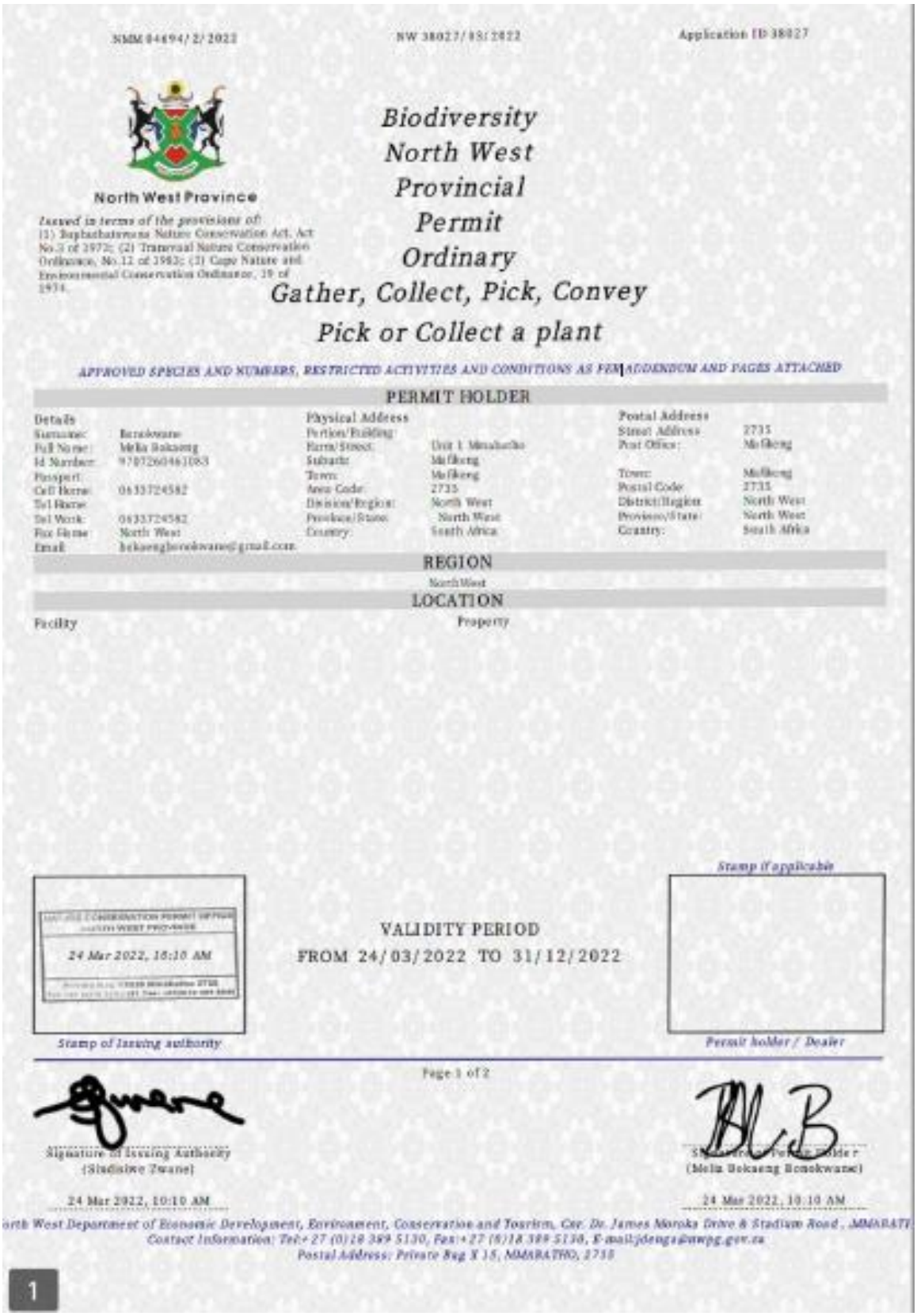
NWU-AnimCareREC would like to remain at your service and wishes you well with your study. Please do not hesitate to contact the NWU-AnimCareREC for any further enquiries or requests for assistance.

Yours sincerely,

Digitally signed by
Christiaan B Brink (Tiaan)
Date: 2022.07.11
10:02:02 +02'00'

Chairperson: NWU-AnimCareREC

Appendix 2: Plant collection permit



NMM 04494/2/2022 NW 38027/03/2022 Application ID 38027

North West Province

Issued in terms of the provisions of:
(1) Bophuthatswana Nature Conservation Act, Act No.3 of 1973; (2) Transvaal Nature Conservation Ordinance, No.12 of 1983; (3) Cape Nature and Environmental Conservation Ordinance, 19 of 1974.

Biodiversity
North West
Provincial
Permit
Ordinary

Gather, Collect, Pick, Convey

Pick or Collect a plant

APPROVED SPECIES AND NUMBERS, RESTRICTED ACTIVITIES AND CONDITIONS AS PER ADDENDUM AND PAGES ATTACHED

PERMIT HOLDER

Details		Physical Address		Postal Address	
Surname:	Bereakwane	Portion/Building:		Street Address:	2735
Full Name:	Melia Bokaeng	Farm/Street:	Unit 1 Mmabatho	Post Office:	Mafikeng
Id Number:	9707260461083	Suburb:	Mafikeng		
Passport:		Town:	Mafikeng	Town:	Mafikeng
Cell Home:	0633724582	Area Code:	2735	Postal Code:	2735
Tel Home:		Division/Region:	North West	District/Region:	North West
Tel Work:	0633724582	Province/State:	North West	Province/State:	North West
Fax Home:	North West	Country:	South Africa	Country:	South Africa
Email:	bokaengbereakwane@gmail.com				

REGION

North West

LOCATION

Facility Property

Stamp if applicable

24 Mar 2022, 10:10 AM

Stamp of Issuing authority

VALIDITY PERIOD
FROM 24/03/2022 TO 31/12/2022

Permit holder / Dealer

Page 1 of 2

Signature of Issuing Authority
(Sindisiwe Zwane)

24 Mar 2022, 10:10 AM

(Melia Bokaeng Bereakwane)

24 Mar 2022, 10:10 AM

North West Department of Economic Development, Environment, Conservation and Tourism, Cnr. Dr. James Moroka Drive & Stadium Road, MMABATHO
Contact Information: Tel: + 27 (0)18 389 5130, Fax: + 27 (0)18 389 5130, E-mail: jdeng@nwpg.gov.za
Postal Address: Private Bag X 15, MMABATHO, 2735

1

ADDENDUM

SPECIES INFORMATION

Scientific Name	Common Name	Number	Gender	Description/Markings
Boophane disticha	Cape poison bulb	1	Unknown	None
Datura stramonium	Common thorn apple	1	Both (Male and/or Female)	None
Olea europaea subsp. africana (Mill.) (P.S. Green)	Wild olive	1	Both (Male and/or Female)	None
Bowiea volubilis subsp. Volubilis (Bowiea volubilis)	"kroeldraad"	1	Both (Male and/or Female)	None
Agapanthus spp.	All species	1	Both (Male and/or Female)	None

ACTIVITIES

Activity Name
Gather
Collect
Pick
Convey

STANDARD CONDITIONS

GENERAL CONDITIONS - ALL PERMITS

1) This permit, unless otherwise stated, is only valid within the boundaries of the North West Province (hereafter named "the Province") and then specifically as specified on the permit. 2) This permit is valid only : - a) for the specific species, sex and numbers as specified on this permit. b) for the specific activity / activities authorised. c) for the specified methods or instruments authorised. d) for the specific property / locality as specified. e) for the specific day, time or period stipulated. 3) This permit is only deemed valid : - a) in the original format and with the content as issued by the Issuing Authority. b) once it has been printed and the signature of the permit holder has been endorsed thereon in ink. 4) the Issuing Authority reserves the right to amend, withhold, withdraw or cancel any permit at any time. 5) This permit is not transferable to any individual, natural person, juristic person or any other legal entity. 6) Any alterations or attempt thereto, whether electronically or in any other way, shall immediately render it invalid. 7) This permit shall lapse and be deemed invalid when it is altered, lost or destroyed and no copy thereof shall be issued. 8) This permit does not grant the permit holder automatic access to any Protected Area, National Park, Provincial Nature Reserve, tribal areas or privately owned land and : - a) the permit holder must beforehand obtain all other relevant written permissions, documents, rights and licences. b) the permit holder must comply with any other / further conditions or restrictions that the manager / landowner may stipulate at their discretion. 9) The permit holder must at all times while performing any restricted activity authorised by this permit, have the permit and all other relevant documentation in their possession and without delay make it available upon request by any authorised person. 10) An authorised person must also be allowed access onto the property at any reasonable time for any inspection needed and can remain on such property as long as it is needed to do the inspection. 11) The permit holder must immediately after completion of any activity authorised by this permit, record the required particulars in the space provided therefore or on the annexure or document attached hereto or in the prescribed register related to the permit. 12) The permit holder must return the original signed permit to the Issuing Authority within (14) fourteen days: - a) after performing or completing the authorised restricted activity, or b) after the date of expiry thereof whichever happens first, and c) if applicable furnish the Issuing Authority with a prescribed written feedback report on the results of every activity conducted. 13) The permit holder must retain a copy of the permit together with all other relevant written permissions, documents, rights and licenses for a period of at least (2) two years from date of issue or for as long as the permit holder is in possession of the animal, plant or derivative, whichever period is the longer. 14) If applicable, the permit holder shall apply for the renewal of the permit to the Issuing Authority, on the appropriate application form, at least (3) three months prior to the expiry date thereof. 15) This permit, during the period of validity thereof, is also subject to: - a) all applicable norms and standards in existence at the time of issuance. b) the provisions of any law in force, in respect of the specific species, activity, method or instrument to which this permit applies. 16) It is the permit holder's responsibility to obtain the correct information on any other legislation, specification, requirement or charges thereto that may be applicable or are required by any other Issuing Authority / Organisation / Institute, relating to this permit. 17) By signing this permit, the permit holder declares that they are aware of the fact that : - a) any transgression or failure to render the required reports can lead to criminal prosecution and also jeopardise any future applications by or in the name of the permit holder. b) if the permit holder contravenes or fails to comply with any permit condition or requirement, they shall be guilty of an offence. 18) The prescribed fees paid to the Issuing Authority for the issue of this permit shall not be refunded.

HARVESTING - PICK PROTECTED PLANT

1) The permit holder must ensure that : - a) only plants which shall be destroyed or damaged as a result of development are collected. b) plants collected in terms of this permit are not sold, bartered or given away. c) the written permission of the owner or occupier of land is obtained before any plant is harvested / picked.

TRANSPORT - FLORA - LIFE / DEAD / DERIVATIVE)

1) The permit holder must : - a) ensure that the dead material does not leave the property before it is inspected, weighed and certified by an official of the Issuing Authority. b) apply for any additional import, export or transport permits where applicable

Signature of Issuing Authority
(Sindiwe Zwane)
24 Mar 2022, 10:10 AM

Signature of Permit Holder
(Melia Bokaeng Bonokwane)
24 Mar 2022, 10:10 AM

North West Department of Economic Development, Environment, Conservation and Tourism, Cnr. Dr. James Moroka Drive & Stadium Road, MMABATHO
Contact Information: Tel:+ 27 (0)18 389 5130, Fax:+ 27 (0)18 389 5130, E-mail:jdenga@nwpg.gov.za
Postal Address: Private Bag X 15, MMABATHO, 2735

Appendix 3: Provision of redundant rats by the PCDDP Vivarium

sanas G0019 aaalac PCDDP DST/NWU Preclinical Drug Development Platform

Dr 'Makhotso Lekhooa **Preclinical Drug Development Platform** **North-West University**	Dr Nico Minnaar Vivarium Manager PCDDP Building G10 North-West University Tel: +2718 285-2902 Fax: +2718 285-2233 Email: 28877535@nwu.ac.za

10 May 2022

Dear Dr Lekhooa

Redundant Sprague Dawley Rats at the Vivarium

Your request for sampling brains and blood from redundant animals refers.

Due to variation in litter size amongst breeding pairs of Sprague Dawley rats and continual breeding for stock replacement, production of surplus animals is inevitable. We are pleased to comply with your request (subjected to prior approval of NWU-AnimCareREC) to provide rat brain tissue when it becomes available as it ensures optimal use of surplus laboratory animals.

Yours sincerely

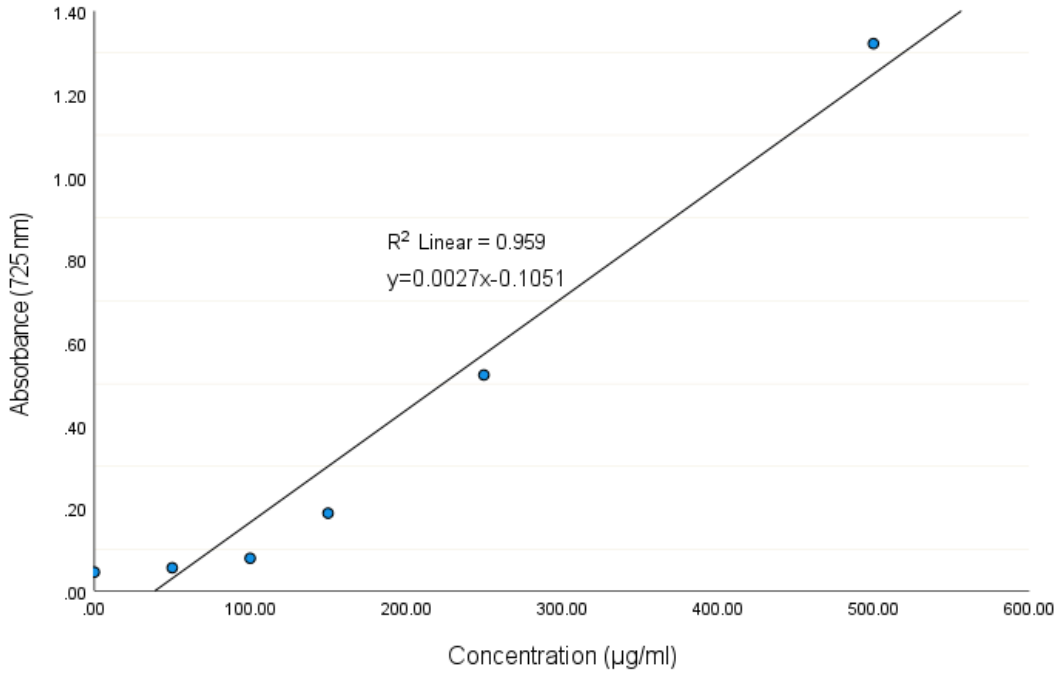
Dr Nico Minnaar
Manager: PCDDP Vivarium

NWU® PCDDP DST/NWU Preclinical Drug Development Platform science & technology

Appendix 4: Three medicinal plants selected for phytochemical analysis and *in vitro* neuroprotective screening in this study

***Artemisia afra* Jacq. ex Willd.**	***Adenia gummifera* (Harv.) Harms**	***Olea woodiana* Knobl.)**
 https://lifestyle.co.za/artemisia-afra/	 http://www.biodiversityexplorer.info/plants/passifloraceae/adenia_gummifera.htm	 https://spain.inaturalist.org/taxa/57145-Olea

Appendix 5: Calibration curve of gallic acid standard using concentrations ranging from 0-500 µg/ml



Appendix 6: Screening three medicinal plants for affinity to the serotonin transporter (SERT) protein and adenosine A_{2A} receptor

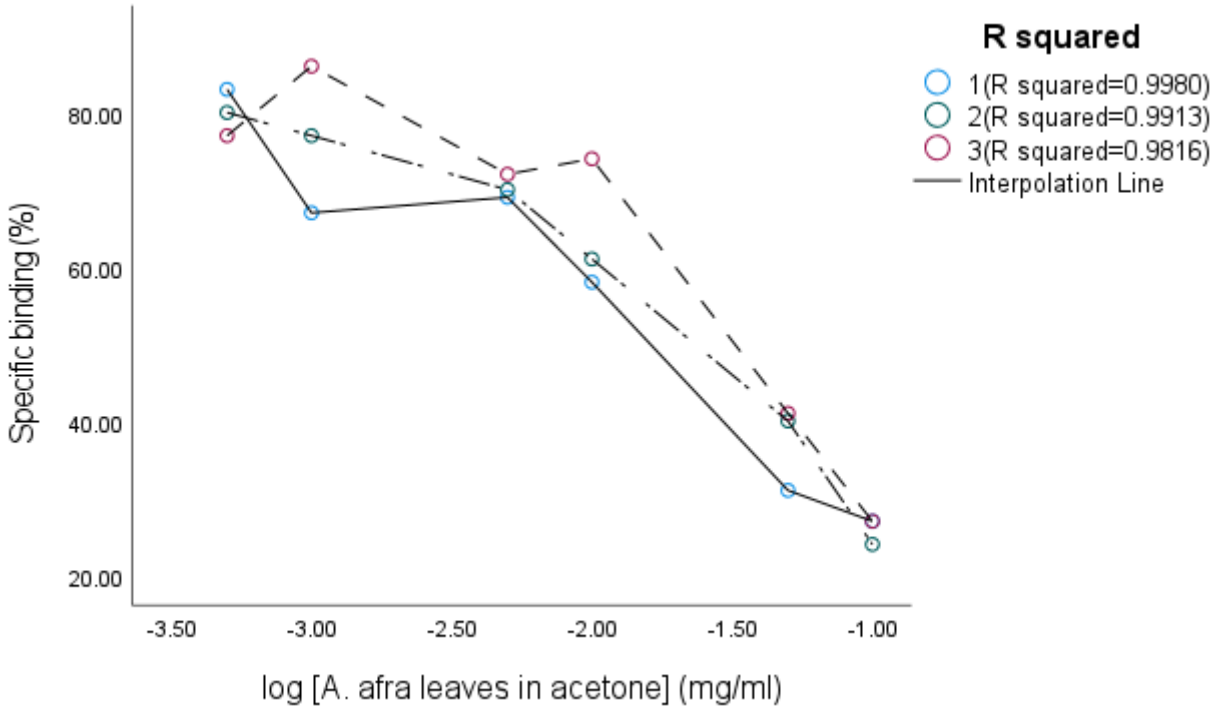
Plant	Plant part analysed	Extraction solvent	Specific binding (%) (K_i ± SEM (mg/ml)					
			rSERT vs 0.7 nM [^{3}H]Citalopram			**rA_{2A} vs 4 nM [^{3}H]NECA**		
			mg/ml					
			0.001	**0.01**	**0.1**	**0.001**	**0.01**	**0.1**
A. afra	Leaves	Water	43	62	56	98	64	67
		Acetone	73	80	97	85	112	63
		Hexane	102	70	89	101	98	107
A. gummifera	Stem	Water	93	102	121	91	102	84
		Acetone	82	87	104	133	107	120
		Hexane	81	81	79	114	124	123
O. woodiana	Leaves	Water	44	74	83	91	99	102
		Acetone	75	83	81	120	116	121
		Hexane	54	54	57	131	127	112
	Stem	Water	49	73	62	107	99	80
		Acetone	100	121	158	134	121	106
		Hexane	95	74	100	118	116	129
			K_i ± SEM (nM)					
		Citalopram	1.2 ± 0.6					
		Istradefylline					3.0 ± 0.9	

Inhibition constant (K_i, mg/ml or nM) value is represented as the mean ± standard error of the mean (SEM), n = 3 samples.

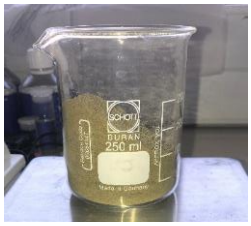
Specific binding (%) of the radioligand is represented as the mean, n = 2 samples.

rSERT: rat whole brain membranes expressing serotonin reuptake transport protein.

Appendix 7: The binding curves of *Artemisia afra* Jacq. ex Willd. leaves in acetone using [^{3}H]DPCPX as radioligand in rat whole brain membranes expressing adenosine A_1 receptors



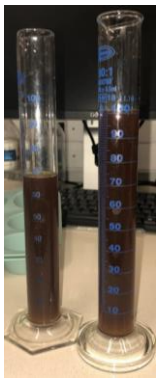
Appendix 8: Plant extraction process



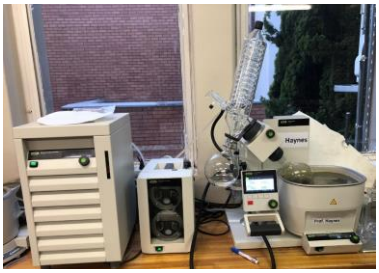
Plant material was dried, ground into a fine powder and weighed

Extracted once with water, acetone or hexane

Solutions were filtered under vacuum



All extracts were measured using volumetric flasks



Acetone and hexane extracts were evaporated to dryness using a rotary evaporator

Aqueous extracts were frozen at -80 °C and dried in a freeze dryer for 4 days