PHYTOCHEMICAL ANALYSIS AND ACETYLCHOLINESTERASE INHIBITION ACTIVITY OF MEDICINAL PLANTS USED IN THE TRADITIONAL TREATMENT OF MENTAL DISORDERS IN KAVANGO EAST REGION OF NAMIBIA

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ABSTRACT

In Namibia, various plants are used in the treatment of various illnesses in traditional healing systems, however the majority of these plants have not been characterised in terms of chemical constituents, and therefore, their safety and efficacy are unknown. The present study focused on the phytochemical analysis and evaluation of the acetylcholinesterase (AChE) inhibition activity of the extracts of five medicinal plants (*Laggera decurrens, Ficus glumosa, Psendolachnostylis maprounifolia, Ochna pulchra and Ozoroa longipes*) used in the traditional treatment of mental disorders in Kavango east region. Extracts were prepared from the powdered roots and leaves of the plants using dichloromethane/methanol (50/50, %v/v) and ethanol. Extracts were screened for the presence of alkaloids, saponins, diterpenes and flavonoids using standard methods. Saponins, diterpenes and flavonoids were found to be present in all the extracts, except for the leaves of *O. longipes*, while no alkaloids were detected in any of the plant extracts. Diterpenes appeared to be the most abundant phytochemicals for most of the samples. Leaf extracts exhibited varying levels of AChE inhibition activity. The *L. decurrens* proved to be the most potent plant species with percentage inhibition ranging from 74–101% for the dichloromethane/methanol (DCM/MeOH) extract and 93–106% for the ethanol extract. The highest inhibition percentages were observed at concentrations of 200 and 100 µg/mL for the DCM/MeOH and ethanol extracts respectively. Compounds were be tentatively assigned to chemical classes based on their ultraviolet absorption spectra. Future studies should employ spectroscopic techniques such as mass spectroscopy (MS) and nuclear magnetic resonance (NMR) spectroscopy to identify the chemical compounds present in these extracts.

LIST OF PUBLICATION(S)/CONFERENCE(S) PROCEEDINGS

T. Nghilalulwa, M. Kalili and A. Cheikhyoussef (2016). Phytochemical screening and metal chelation activity of medicinal plants used to treat mental disorders in Kavango east region of Namibia. Poster presented at the INSPIRE 2016 Sustainable Futures 3rd Green Medicine Symposium, 8-10 September 2016, University of the Western Cape (UWC), Cape Town, South Africa.

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LIST OF ABBREVIATIONS AND/OR ACRONYMS

^{13}C-NMR	Carbon-13 Nuclear Magnetic Resonance
^{1}H-NMR	Proton Nuclear Magnetic Resonance
2D-HETCOR	Two-Dimensional Heteronuclear Correlation Spectroscopy
2D-HPLC	Two-Dimensional High Performance Liquid Chromatography
ACh	Acetylcholine
AChE	Acetylcholinesterase
AD	Alzheimer's disease
AR	Analytical reagent
BBB	Blood brain barrier
BCSFB	Blood cerebrospinal fluid barriers
BuChE	Butylcholinesterase
CE	Capillary electrophoresis
CE-DAD	Capillary electrophoresis-diode array detection
ChAT	Choline acetyltransferase
CNS	Central nervous system
COSY	Homonuclear correlation spectroscopy
DCM/MeOH	Dichloromethane/methanol
DPPH	2,2-diphenyl-1-picrylhydrazyl

DTNB	5,5’-Dithio-Bis-(2-nitrobenzoic acid)
EPSP	Excitatory postsynaptic potential
EtOH	Ethanol
FTMS	Fourier Transform mass spectroscopy
GABA	Gamma-aminobutyric acid
GC-MS	Gas chromatography–mass spectrometry
HBA	Hydroxybenzoic acid
HCA	Hydroxycinnamic acid
HCl	Hydrochloric acid
HDL	High density lipoproteins
HIV-1	Human immunodeficiency virus-1
HPLC	High Performance Liquid Chromatography
HPLC-DAD	High performance liquid chromatography–diode array detection
HPLC-UV	High performance liquid chromatography–ultraviolet detection
LAT	Large neutral amino acid transporter
LC-MS	Liquid chromatography–Mass Spectrometry
LDL	Low density lipoproteins
MIC	Minimum inhibitory concentration

MOA	Monoamine oxidase
MoHSS	Ministry of Health and Social Services
MS	Mass Spectrometry
NaOH	Sodium hydroxide
NMR	Nuclear Magnetic Resonance Spectroscopy
PD	Parkinson's disease
SERT	Serotonin transporter
Tf-R	Transferrin receptor
TLC	Thin Layer Chromatography
UV	Ultraviolet spectroscopy
WHO	World Health Organisation
WIND	National Herbarium of Namibia
NCRST	National Commission on Research, Science and Technology

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This thesis is dedicated to my entire family, **Mr. Theodor Nghifikepunye Nghilalulwa**, **Mrs. Rebekka Nghilalulwa**, **Tarah Haulinenu Mandume Nghilalulwa**, **Tulimevava Shidute Nghilalulwa** and **Rebekka Tsenaye Nghilalulwa,** who have always provided me with the highest love shield, constant support and encouragement.

This is for **YOU GUYS**!!

DECLARATION

I, Theodor Nande Nghifikepunye Nghilalulwa, hereby declare that this study is my own work and is a true reflection of my research and that this work or any part thereof has not been submitted for a degree at any other institution.

Theodor NN Nghilalulwa	_____	October 2022
Name of Student	**Signature**	**Date**

CHAPTER 1: INTRODUCTION

1.1 Background of the study

According to the Namibian Ministry of Health and Social Services (MoHSS), over 8 000 people were treated for mental illnesses at different health facilities countrywide in 2015 (1). Although the number of people suffering from mental disorders who seek the services of traditional healers is currently unknown (1), this data points to the severity of these problems in Namibia and the world at large. A large number of plants including *Laggera decurrens, Ficus glumosa, Psendolachnostylis maprounifolia, Ochna pulchra* and *Ozoroa longipes* are currently used in the treatment or alleviation of symptoms associated with mental illnesses in traditional healing systems in Africa (2). For example, the bark decoction of *Ochna pulchra* was found to exhibit psychoactive effects after an infusion was taken, while the roots of *Securidaca longepedunculata* are used to treat mental disorders by the Venda people of South Africa (2). The use of plants as sources of drugs or lead compounds for drug discovery is well documented (3). Medicinal plants are selected for analysis based on their ethnomedicinal use (3).

Acetylcholine (ACh) is a neurotransmitter of the cholinergic system which mediates an array of functions, including cognition (4). Strong evidence suggests that the decline of ACh in the cholinergic system is a key factor in the pathogenesis and progression of neurodegenerative diseases (4). Acetylcholinesterase (AChE) is an acetylcholine hydrolase enzyme that catalyse the hydrolysis of ACh and other choline esters resulting in the termination of synaptic transmission and impaired cognitive functions (4,5). AChE inhibition is considered to be a viable therapeutic strategy in the management of neurodegenerative disease and mental disorders (5,6).

Neurodegenerative diseases and mental disorders are caused by the loss of cholinergic neuronal activity, where acetylcholine is a significant neurotransmitter. Therefore, increasing central cholinergic activity by inhibiting cholinesterase enzymes such as acetylcholinesterase is currently the main mechanism in preventing neurodegenerative disorders (6).

Plants have proven to be important sources of compounds that exert inhibitory activity against acetylcholinesterase (6). Extraction is a crucial first step in the analysis of medicinal plant extracts for detection and identification purposes (7). Plant extracts exist as a combination of various types of bioactive compounds or phytochemicals (7,8). It is a common practice in the isolation of these bioactive compounds that a number of different separation techniques are used to obtain pure compounds (8). High performance liquid chromatography (HPLC) is a versatile, robust and widely used separation technique in the identification, quantification and purification of bioactive compounds or phytochemicals (9).

1.2 Statement of the problem

Although a large number of plants are widely used in the treatment or alleviation of symptoms associated with mental illnesses in Namibian traditional healing systems, only a small number of these plants have been scientifically evaluated for the presence of phytochemicals and antimicrobial activity, and in most cases, the active compounds have not been identified. The chemical constituents of the majority of plants analysed in this study are still not known and therefore, their safety and effectiveness is not guaranteed.

1.3 Objectives of the study

The objectives of this study were:

a) To screen for the presence of four phytochemical classes (alkaloids, flavonoids, diterpenes and saponins) known to have anti-psychotic effects in five medicinal plants used traditionally to treat mental disorders in the Kavango east region of Namibia.
b) To determine the acetylcholinesterase (AChE) inhibition activity of the plant extracts.
c) To isolate and characterise psychoactive compounds using high performance liquid chromatography (HPLC) in combination with mass spectrometry (MS) and nuclear magnetic resonance (NMR).

1.4 Significance of the study

The study shed light on some of the phytochemicals present in the medicinal plants of interest. The plant extracts were found to inhibit the AChE enzyme which has been implicated in the progression of neurodegenerative diseases. Thus, this study contributed new knowledge and serves as a baseline study towards understanding the chemical composition and role of the secondary metabolites present in these medicinal plants.

1.5 Limitations of the study

The study is not comprehensive in nature as it was only limited to a few selected medicinal plants used in the treatment of mental disorders in Kavango east region. The plants under study are seasonal, so it was not possible to collect plants during the dry season. Lack of a high resolution mass spectrometer at UNAM and the withdrawal of financial support initially committed by the National Commission on Research, Science and Technology (NCRST) to this project greatly hampered the characterisation of individual compounds present in the plants of interest.

CHAPTER 2: LITERATURE REVIEW

2.1 Psychoactive compounds: Classification and effects

Psychoactive or psychotropic substances are chemicals which, when ingested, affect the mind or mental process (10). They can take on the meaning of chemical substances that are utilised to modify the emotional, intellectual and behavioural function of human beings and can be grouped based on the type of action they produce or their therapeutic use (10). These substances are used for various reasons, medicinally, recreationally (to purposefully improve performance or alter consciousness) or as entheogens for ritual, spiritual or shamanic purposes. Ingestion of these compounds usually results in a change of consciousness and mood, which is often pleasant or rewarding to the user (e.g. euphoria or a sense of relaxation) or advantageous (e.g. increased alertness) (11). Plants produce a wide range of chemical compounds that exert different psychoactive effects such as sedation, euphoria, stimulation and drowsiness (10,12). Psychoactive chemicals affect the central nervous system (CNS) in several ways by impacting the release of neurotransmitters such as acetylcholine, or by imitating their actions (12). Psychoactive chemical compounds are grouped according to their actions on the central nervous system as stimulants, hallucinogens, depressants or narcotics (12).

Stimulants: These chemical compounds are responsible for inducing excitement, increased mental awareness and physical activity, decreased fatigue and hunger suppression, for example, caffeine.

Hallucinogens: Result in change in perception, thought and mood that enables one to deviate from normal behaviour, usually inducing a dream-like state, e.g. marijuana.

Depressants: Induce a feeling of dull awareness, decrease physical performance and induce sleep or a trance-like state, e.g. temazepam.

Narcotics: Consumption of these chemical compounds result in depression of the central nervous system. Effects usually include numbness, lethargy and sleep. This category includes opiates, alcoholic beverages and kava. Narcotic chemical compounds are also addictive (12).

Administration of psychoactive compounds follows various modes such as oral ingestion (as a tablet, capsule, liquid or beverage), subcutaneous, intramuscular or intravenous injection, rectal deposition, disimpaction and inhalation by smoking, vaporisation and insufflation. Psychoactive compounds temporarily affect the neurochemistry of a person, bringing a change to the mood, cognition, perception and behaviour patterns of the individual (13). A specific action is rendered by the drug on one or more neurotransmitters or receptors. Subjection of neurons to psychoactive compounds causes changes to the structure and functions of the neurons, as the nervous system tries to regain the homeostasis status disrupted by exposure to these compounds. Agonists (drugs that cause an increase in particular neurotransmitter systems) act by increasing the production of one or more neurotransmitters by either reducing its re-uptake from the synapses or by mimicking their action by binding to the postsynaptic receptor itself. Drugs that reduce neurotransmission activity are termed as antagonists (14). Their operation is centred on inhibiting the synthesis of neurotransmitters or blocking the postsynaptic receptor so that neurotransmitters cannot bind to them. Subjecting a particular neurotransmitter to antagonists can increase the number of receptors specific for that neurotransmitter or the receptors themselves may become more responsive to the neurotransmitter (15).

2.2 The role of AChE in mental health

Acetylcholinesterase is a serine hydrolase, with the main function of terminating signal transmission in the cholinergic system (16). AChE contains a complex protein of α/β

hydrolase fold type with an all-inclusive ellipsoid shape containing a deep groove, normally referred to as the gorge, which is approximately 20 Å (17). AChE acts on acetylcholine, found in the cholinergic system, and has a pronounced effect on motor neurons involved in memory formation. ACh functions in the peripheral nervous system and the central nervous system, serving as a fast-acting point-to-point neurotransmitter at the neuromuscular junction and in the autonomic ganglia (18). In the CNS, ACh is believed to be responsible for learning, memory and mood (19).

The synthesis of ACh takes place at the presynaptic terminal from acetyl coenzyme (acetyl-CoA) and choline, catalysed by the enzyme choline acetyltransferase (ChAT), then stored in the synaptic vesicles in the presynaptic terminal (19,20). Depolarisation of the presynaptic terminal membrane induces synaptic transmission where voltage sensitive Ca^{2+} channels at the presynaptic membrane open up, allowing the influx of Ca^{2+} that signals the release of acetylcholine into the synaptic vesicle. Fusion of synaptic vesicles into the synaptic membrane is facilitated by Ca^{2+} (20). The binding of synaptic vesicles to the presynaptic membrane is aided by a number of proteins, such as vamp, syntaxin, synap and synaptotagmin (21). In the binding process, the implicated proteins form complexes between the synaptic vesicle and the presynaptic terminal, permitting the docking of the vesicles to the membrane upon the Ca^{2+} entry and fusion of the vesicles with presynaptic membrane subsequently releasing the ACh at the neuronal junction (21). After the release of ACh, diffusion takes place across the synaptic cleft and binding of the neurotransmitter to a specific receptor on the postsynaptic membrane (21).

There are two types of cholinergic receptors; nicotinic and muscarinic. The former is found at the synapses, situated between two neurons and at the synapses, in the middle of the neurons and skeletal muscle cells (22). Depolarisation of the skeletal muscle is

triggered by the transfer of ions into the ion channels which brings about the activation of the ion channels. On the other hand, the latter is located at the synapses of nerves with either smooth or cardiac muscle (4,22). Transmission action is set off by signal transduction. Protein receptors at the postsynaptic membrane undergo conformational change once ACh binds to them. In the process, $Na^+ – K^+$ channel in the receptor opens up, allowing Na^+ ions to move into the post synaptic vesicles, yielding a positive charge in the membrane referred to as the excitatory postsynaptic potential (EPSP). After the EPSP reaches the threshold, an action potential begins in the neuron (6). Cleavage of ACh by AChE and butylcholinesterase (BuChE) in the post synaptic receptor then takes place, reducing ACh to acetate and choline, bringing an end to synaptic activity. The last stage of neurotransmission aims to reinstate ACh for subsequent chemical transmission (6). The liberated choline is transported in the pre-synaptic nerve again, where acetylcholine is produced from the combination of choline with acetyl-CoA, aided by choline acetyltransferase. ACh is then stored in synaptic vesicles, bringing an end to the transmission process.

Acetylcholinesterase is a serine hydrolase enzyme that is highly specific to acetylcholine. Acetylcholine degradation occurs at a rate of one molecule of AChE per 25 000 molecules of ACh per second (23). The enzyme's active site is situated 4 Å from the bottom of the molecule, composed of two subsites, the anionic subsite and the esteratic subsite (23). The anionic subsite is uncharged and lipophilic, it is responsible for binding quaternary ammonium ligands of ACh by π-cation interactions (24). The anionic subsite contains Trp84, Phe330 and Glu199, of which Trp84 is the most important residue because it binds ACh (25). The esteratic subsite which consists of catalytic amino acid traid; serine 200 (Ser), histidine 440 (His) and glutamate 327 (Glu), is responsible for amplifying the nucleophilicity of the catalytic serine. This is

because the bonding between His and Ser through strong hydrogen bonding boosts the ability of Ser to mount a nucleophilic attack on the substrate, while Glu stabilises histidinium cation of the transition state (26). In the hydrolysis of ACh, cleavage of the carboxyl ester bond leads to the formation of an acyl-enzyme and a free choline. After this, the acyl-enzyme undergoes nucleophilic attack by a water molecule with the aid of the histidine 440 group, which liberates acetic acid and regenerates the free enzyme (27).

The cholinergic system plays a key role in cognitive functions: memory, selective attention, emotional processing, etc. (28). AChE inhibitors or anti-cholinesterases inhibit acetylcholinesterase enzyme from breaking down ACh, which results in an increase of the level and duration of the neurotransmitter's action. The rapid hydrolysis of acetylcholine by acetylcholinesterase in the cholinergic system has for a long time been implicated as a major factor in the advancement of neurodegenerative and mental disorders (28,29). Neurodegenerative disorder refers to various conditions arising from the chronic breakdown and deterioration of the neurons, specifically those that function in the central nervous system (30). These disorders include Alzheimer's disease, Parkinson's disease, dementia, epilepsy, multiple sclerosis, migraine and other headache disorders, neuroinfections, brain tumours and traumatic disorders of the nervous system (31). In addition, neurodegenerative diseases may be caused by a combination of environmental, genetic and age-related factors. Mental disorders on the other hand, are psychiatric illnesses or diseases that are characterised by abnormalities of thought, feeling or behaviour, resulting in distress or impairment of function (32). Examples include schizophrenia, anxiety and depression. The accumulation and imbalance of glutamate can cause schizophrenia, bipolar disorder and depression (33).

2.3 Evidence of actions of phytochemicals at the central nervous system

The neurological bioactivity of plants towards CNS diseases is due to several mechanisms of action (34). This section gives a review of mechanisms that are well understood and examples of mechanisms pertaining to various diseases that affect the CNS.

Alzheimer's disease (AD) is a chronic, progressive neurodegenerative disorder of the brain associated with the deterioration of everyday living activities such as behaviour, memory and cognition (35). The progression of AD is caused by deficits in central cholinergic activity that results from the loss of ACh. Compounds isolated from plants enhance central cholinergic activity by inhibiting AChE activity, thereby increasing the availability of ACh to restore nicotinic and muscarinic receptors within the brain (35,36). Huperzine A, a lycopodium alkaloid isolated from *Huperzia serrate* moss exhibited both *in vitro* and *in vivo* AChE inhibitory activity. Huperzine A significantly improved the memory and behaviour of Alzheimer's patients in a clinical trial (37). Flavonoids are considered as health-promoting agents as indicated from both *in vitro* and *in vivo* biological assay (38). A large pool of flavonoids have been isolated from medicinal plants and tested for their acetylcholinesterase inhibition potential. Results obtained from flavonoids isolated from *Syzgium samarangense* showed that 2',4'-dihydroxy-3',5'-dimethyl-dihydrochalcone had a high degree of inhibition ability (98.5% inhibition) at a concentration of 0.25 mM (39). Research on quercetin and tiliroside showed that they displayed good inhibition activity with IC_{50} values of 19.8 and 23.5 µM, respectively, which were lower than the control compound, dehyroevodiame (IC_{50} 37.8 µM) (40).

Age-related dementia is also closely linked to the progression of AD (36,39). Thus drugs which enhance memory in AD patients can equally be used to improve

symptoms of people suffering from age related dementia by acting on the CNS through inhibition of AChE activity, causing an increase in the transmission of ACh. It is a neurologically based movement disorder (41). The current available pharmaceutical drugs are able to offer only symptomatic relief for patients suffering from Parkinson's disease (PD). These drugs either increase the levels of dopamine in the brain or mimic the effects of dopamine. Drugs function by inhibiting monoamine oxidase B (MAO B) which deaminate dopamine, increasing the basal central dopamine level of PD patients (42). A study conducted on MAO inhibition activity and specific MAO B inhibition activity of 20 southern African traditionally used plants found that non-polar leaf extracts of *Ruta graveolens* exhibited good MAO inhibitory activity. The IC_{50} value of ethyl acetate extract was 5 µg/mL, while that of petroleum ether extract was 3 µg/mL. Specific MAO inhibition; with IC_{50} values of ethyl acetate extract and petroleum ether extract were calculated to be 7 µg/mL and 3 µg/mL, respectively (43). Depression affects an individual's state of mind, behaviour, health and some cases it can lead to suicide (44). The neurochemistry of depression has been closely linked to the depletion of levels of serotonin, norepinephrine and/or dopamine in the brain (45). Plant extracts with inhibitory activity against serotonin transporters (SERT) have attracted interest from researchers focused on finding new anti-depressant drugs. *Sceletium tortuosum* leaves have been used to affect mood changes by pastoralists and hunter-gatherers in southern Africa. Ethanolic extracts from *Agapanthus campanulatus*, *Boophone disticha*, *Mondia whitei* and *Xysmalobium undulatum* were investigated for functional inhibition of SERT, noradrenalin uptake (NAT) and dopamine uptake (DAT) using COS-7 cells expressing hSERT, hNAT and hDAT (46). All four extracts exhibited antidepressant-like effects of varying degrees in animal models (45). Mesembrine is an alkaloid from *S. tortuosum* which acts as a serotonin

reuptake inhibitor and it has been developed into a drug that is able to treat mild depression and elevation of consumer's mood (47). Baicalin is an example of a flavonoid that has the ability to inhibit prolyl oligopeptidase activity (48).

Prolyl oligopeptidase is linked to neuropsychiatric conditions such as schizophrenia, bipolar affective disorder and other related neuropsychiatric disorders due to the fact that it hydrolyses proline-containing peptides at the carboxyl terminus of proline residues (49). Baicalin can therefore serve as a potential treatment of neuropsychiatric disease. This is primarily due to the inhibition of MAO A and MAO B (49). The MAO enzyme has a significant role in the metabolism of monoamine neurotransmitters, noradrenalin, dopamine and 5-hydroxytryptamine (50).

2.4 The use of medicinal plants in the treatment of mental disorders

There is an increased use of medicinal plants in traditional therapy. In some Asian and African countries, almost 80% of the population makes use of traditional medicine for their primary health care needs (51). Traditional medicine practices include European medicine, traditional African medicine, traditional Chinese medicine, traditional Korean medicine, Ayurveda, Siddha medicine, Unani, ancient Iranian medicine, Iranian (Persian) medicine, Islamic medicine, Ifá and Muti (52). The World Health Organisation (WHO) approximates that over one billion of the world population suffer from illnesses linked to the CNS. A notable number of studies have been conducted on traditional medicinal plants over the past years to find alternative treatments for diseases of the nervous system by identifying structures with activity at the CNS (53). In fact, the very first drug formulated to treat pathological systems of the CNS was sourced from medicinal plants (54). Studies conducted recently show that certain classes of compounds exhibit stimulating or depressing activity of the CNS. These classes of compounds are mainly alkaloids, amino acids and phenolic compounds (54).

The connection between classes of compounds and the biological activity at the CNS was not linear. The search was also not limited only to these classes of compounds (55).

Studies have shown that more than 300 000 seed plants have been used in therapeutic treatment of a wide range diseases and infections, including neurological disorders affecting the central nervous system. About 84 plants out of the total 144 were found to obtain phytochemicals that cause neurological bioactivity (56). The dominant molecular group is alkaloids, with an overall percentage of 38%, followed by flavonoids constituting 16% and terpenic compounds weighing 11% (57). However, not all the action at the CNS was attributed to one constituent in some plant samples, but to a combination of phytochemicals, which highlights the possible synergistic actions to neurological activity (56,57). The plants identified represented 56 families, of which the most represented is the Labiatae (Lamiaceae), with a total percentage of 39%, and it comprise about 210 genera and some 3 500 species (58). The Leguminosae (Fabaceae) family represented 19% of the total plants identified and it consists of about 400 genera and 9 000 species (58).

Over 300 Southern African plant species from 96 families are used in the treatment of mental disorders and related ailments in Africa (59). The use of traditional medicine is widespread, with a large population of black people consulting traditional healers to relieve symptoms associated with neurological disorders. Namibia is no exception to this, with a large number of plants being used to treat mental disorders and related illnesses in traditional medicine systems. However, these will be too many to review here, only the medicinal plants shortlisted for this study will be briefly discussed.

2.4.1 *Laggera decurrens*

Laggera decurrens is a bushy annual aromatic half shrub that grows to a length of up to 2 m. Most parts of the plant are covered in silky, silvery-grey hairs (60). The leaves are narrow in shape, with dimensions of 30 mm × 5 mm with the leaf bases running down the stem as wings. The capitula are situated in branched large terminal inflorescences (60). The plant is distributed throughout tropical Africa and southern Africa. In Yemen, the leaf and root extracts are used to treat stomach pains and acne (58,60). Other medicinal uses include treatment of malaria, headaches, exhaustion and leg problems (58). Research conducted on the antioxidant potential and free-radical-scavenging activity of essential oil of *L. decurrens* yielded yellow oils from aerial parts, 0.70 % (w/w) dry weight. Gas chromatography–mass spectrometry (GC-MS) analysis was used to identify 44 constituents which represented 91.9 % of the total oil of *L. decurrens*. The oil contained a high percentage of oxygenated monoterpenes (46.3 %). The most abundant oxygenated monoterpenes were 3-methoxy-2-methyl-5-(1-methylethyl)-2,5-cyclohexadiene-1,4-dione(3-methoxythymoquinone) with a percentage of 28,1% and thymol (5.7 %). The oil also consisted of 22.7 % oxygenated terpenes, of which eudesma-11-en-4a-ol (7.0 %), tau-cadinol (T-cadinol) (5.1 %) and caryophyllene oxide (3.4 %) were the main compounds (58).

The antimicrobial determination assay results revealed that the essential oil exhibits significant activity against various bacterial organisms. The lowest minimum inhibitory concentration (MIC) values of 0.13 mg/mL were recorded for *Staphylococcus aureus* (60). Oxygenated monoterpenes are reported to account for antimicrobial activity of various essential oils, which comprise compounds such as thymol, carvacrol, thymoquinone, camphor, borneol, linalool and α-fenchol (61). In the same study, the antioxidant ability of the oil was assessed using the 2,2-diphenyl-

1-picrylhydrazyl (DPPH) free radical scavenging activity assay. The oil showed antioxidant activity of 91 % at 500 µg/mL, while ascorbic acid had an antioxidant activity of 96 % at the same concentration (60). The high content of phenolic components like 3-methoxythymoquinone, thymol and carvacrol were thought to be responsible for this effect (62). The results obtained in this study showed that the essential oil of *L. decurrens* portrays potent antimicrobial activity, hence validating its use in traditional healing systems. The biological activity was found to be due to oxygenated monoterpenes (63).

Phytotoxicity assay was conducted on *L. decurrens* leaf samples to examine the phytotoxic activity (60). From the bioassay-guided fractionation of hexane and chloroform extracts of *L. decurrens*, two phytotoxic compounds, namely, 3-hydroxythymoquinone and 5-acetoxy-2-hydroxythymol, were isolated and characterised (60). Results obtained from *Lemna minor* biological assay showed that 3-hydroxythymoquinone and 5-acetoxy-2-hydroxythymol completely killed the indicator organisms at concentrations of 2.5 and 5 mg/L, respectively (60). The related compound, thymoquinone, was however inactive at the tested concentrations (0.3-20 mg/L) and the allelopathic quinone compound, juglone, which was tested for comparison purposes, caused complete necrosis at 20 mg/L (60). The results obtained in this study showed substantial phytotoxic activity in the leaves of *L. decurrens* (60).

2.4.2 *Ficus glumosa*

Ficus glumosa is a plant that can grow up to 15 m high. It contains a roundish crown that may open and close (64). The bark is pale grey to yellowish grey in colour with a smooth to slightly rough texture. *F. glumosa* is geographically distributed across tropical Africa spreading to western Saudi Arabia in the east and South Africa in the south (64). Morphological studies conducted on the bark of *F. glumosa* revealed the

presence of calcium oxalate crystals (36.04 µm) prism type, cork cells (10.84 µm), long lignified fibres (126.96 µm), medullary rays and parenchyma cells (70). Physicochemical analysis revealed an ash value of 9.5 %, insoluble acid value of 2.0 %, water soluble content at 6.5 %, ethanol extractive value at 16.6 % and water extractive value at 12.0 % (70). Nine elements were identified in the powdered stem bark, sodium (Na), potassium (K), copper (Cu), calcium (Ca), zinc (Zn), iron (Fe), cobalt (Co), nickel (Ni) and magnesium (Mg).

In addition, *F. glumosa* has diverse medicinal uses in different countries, including being used to alleviate pain from sprains, toothache, and treat diarrhoea and sore eyes (65). In Zimbabwe, the latex is , diluted in water and administered orally to treat diarrhoea (66). In central Africa and Tanzania, a decoction preparation of the bark is used as a mouth wash to relieve toothache. The bark macerate is applied to the eyes to prevent conjunctivitis in new born babies (46). Research carried out in Senegal and the Ivory Coast revealed that the roots and fruits are used to cure female sterility (67). In Nigeria, the bark is used to treat rheumatism. In East Africa, Cameroon and Senegal, the leaves of *F. glumosa* are used to treat edema, hypertension, diabetes, rheumatism, stomatitis and skin diseases in traditional medicine therapy. Studies conducted in Nigeria on ethanol extracts of *F. glumosa* leaves showed that it possesses hypoglycemic effect (68). In a study conducted in Cameroon, many compounds were isolated from *F. glumosa* stem bark, notably lupeol, catechine, luteolin, genisten, dongnoside, polystachyol, dongnoside E and ceramides (69).

The methanol extract displayed activity against inflammation induced by carrageenan 500 mg/kg with 28 % protection at the end of 3 hours, which was statistically significant ($p<0.05$) (70). Anti-inflammatory activity of the methanolic extract supports the use *F. glumosa* in traditional medicine (70,71).

2.4.3 *Psendolachnostylis maprounifolia*

Psendolachnostylis maprounifolia plant is found in the Democratic Republic of Congo and Tanzania, across southern Africa (72). This plant is a round, single stemmed tree that grows to a maximum height of 12 m (72). In addition, the bark is usually greyish but can change to dark brown.

It is used medicinally in the treatment of stomach aches and abdominal problems, tumours, earache, pneumonia, tuberculosis, anaemia, diarrhoea, dysentery, gonorrhoea and female sterility (73). Knowledge of the phytochemical constituents and biological activity of the plant is unknown.

2.4.4 *Ochna pulchra*

O. pulchra is a small deciduous southern African tree that can reach a maximum length of 5 m. The bark is distinctive, peeling in thin flakes to expose creamy-white under bark. It normally grows in sandy soil and rocky slopes (74,75). It belongs to the tropical family of Ochnaceae, which is widely distributed in Africa and Asia. The *Ochna* genus has about 86 species (75). Plants belonging to this family have been used for food and medicinal purposes for centuries (76). One anthrone and two vismiones compounds were isolated and identified from the root bark of *O. pulchra*. Antimicrobial analysis in the same study showed that *O. pulchra* possesses antimicrobial activity. *O. pulchra* acetone leaf extract were assessed for their antimicrobial activity against 4 bacterial strains; *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecalis* and *Pseudomonas aeruginosa*. The MIC value ranged from 0.039 mg/mL to 1.25 mg/mL after a 12 hour incubation period and 0.078 mg/mL to 1.25 mg/mL after a 36 hour incubation period for all bacterial strains (77).

2.4.5 *Ozoroa longipes*

O. longipes is a multi-stemmed, deciduous shrub with dropping branches. Its bark is dark brown in colour and the leaves are simple, they are circular-like, with a bright green colour. The lateral veins are conspicuous (78). According to research carried out by Chinsembu *et al* (79) on plants used in the management of HIV/AIDS opportunistic diseases in Rundu, Kavango east region, Namibia, the leaves, roots and succulent stems are used in the treatment of skin rashes, tuberculosis and oral candidiasis (79). The roots and succulent stems are pounded, mixed with warm water and the resulting decoction is administered orally to treat tuberculosis or used as a mouth wash to treat oral candidiasis. In addition, fresh leaves are crushed and rubbed onto the affected skin part to treat skin rashes (80).

2.5 Analysis of psychoactive compounds

Plant extracts, either as pure compounds or mixtures, provide unlimited opportunities for the discovery of new drugs because of the unmatched availability of chemical diversity (32). Chemical characterisation of plant extracts is critical for the scientific validation of their uses and preparation of standardised herbal extracts (81). Natural products are mostly isolated after an examination of the crude extracts in a biological assay (82). The bioactive compound, often present only as a minor component of the crude extract, is subsequently fully characterised using a number of analytical techniques. Several chromatographic and spectroscopic techniques can be used for this purpose, namely, high performance liquid chromatography (HPLC), gas chromatography (GC), thin layer chromatography (TLC), capillary electrophoresis (CE), mass spectrometry (MS) and nuclear magnetic resonance (NMR) (83). Hyphenated techniques such as high performance liquid chromatography–diode array detection (HPLC-DAD), high performance liquid chromatography–ultraviolet

(HPLC-UV), capillary electrophoresis–diode array detection (CE-DAD), liquid chromatography – mass spectrometry (LC-MS) and liquid chromatography – nuclear magnetic resonance (LC-NMR) are utilised because of their high efficiency in the separation and characterisation of multicomponent mixtures (84). HPLC fingerprinting has gained popularity as a viable technique in the identification of bioactive compounds in medicinal plant extracts due to both its high separation efficiency and sensitivity (85,86).

CHAPTER 3: RESEARCH METHODS

3.1 Chemicals and reagents

All HPLC-grade solvents: ethanol, methanol and acetonitrile, as well as analytical reagents such as formic acid, sodium bicarbonate, mercuric chloride, potassium iodide granules, hydrochloric acid 32%, sodium hydroxide pellets and copper (II) acetate monohydrate crystals were supplied by Merck Chemical Co. Ltd. (Darmstadt, Germany). Phenolic standard compounds: gallic acid, catechin, chlorogenic acid, 4-hydroxybenzoic acid, vanillic acid, caffeic acid, syringic acid, *p*-coumaric acid, trans-ferulic acid, sinapic acid and quercetin were all purchased from Sigma Aldrich. Acetylcholine iodide, s-butyrylthiocholine, bio reagent 5,5'-dithiobis-(2-nitrobenzoic acid) ≥98% (DTNB), phosphate buffered formalin (pH 7.0), and acetylcholinesterase from Electrophorous Electricus type VI-S were supplied by Sigma-Aldrich (St Louis, Missouri, United States of America). The water used for analysis was milli-Q water, purified using a Milli-Q® Integral water purification system.

3.2 Instrumentation

HPLC analyses were performed on a Waters Alliance e2695 separations module with a quaternary, low-pressure mixing pump, inline vacuum degasser, autosampler and a 2996 PDA detector, controlled by Empower software. The column used was an Agilent Zorbax SB-C18, 150 mm × 4.6 mm, 5 µm.

3.3 Collection of plant material

Fresh plant parts (leaves and roots) of *Laggera decurrens* (Asteraceae, IKSTF0563), *Ficus glumosa* (Moraceae, IKSTF0556), *Psendolachnostylis maprounifolia* (Phyllanthaceae. IKSTF0547), *Ochna pulchra* (Ochnaceae, IKSTF0569) and *Ozoroa longipes* (Anacardiaceae, IKSTF0520) were collected from Kavango east region

(**Figure 1**). Plant materials were taxonomically identified at the National Herbarium of Namibia (WIND). The plant samples were air-dried for a week at room temperature and ground into fine powders using a mechanical blender. The fine powders were then transferred to covered beakers with proper labeling and stored at -20 °C until further analysis.

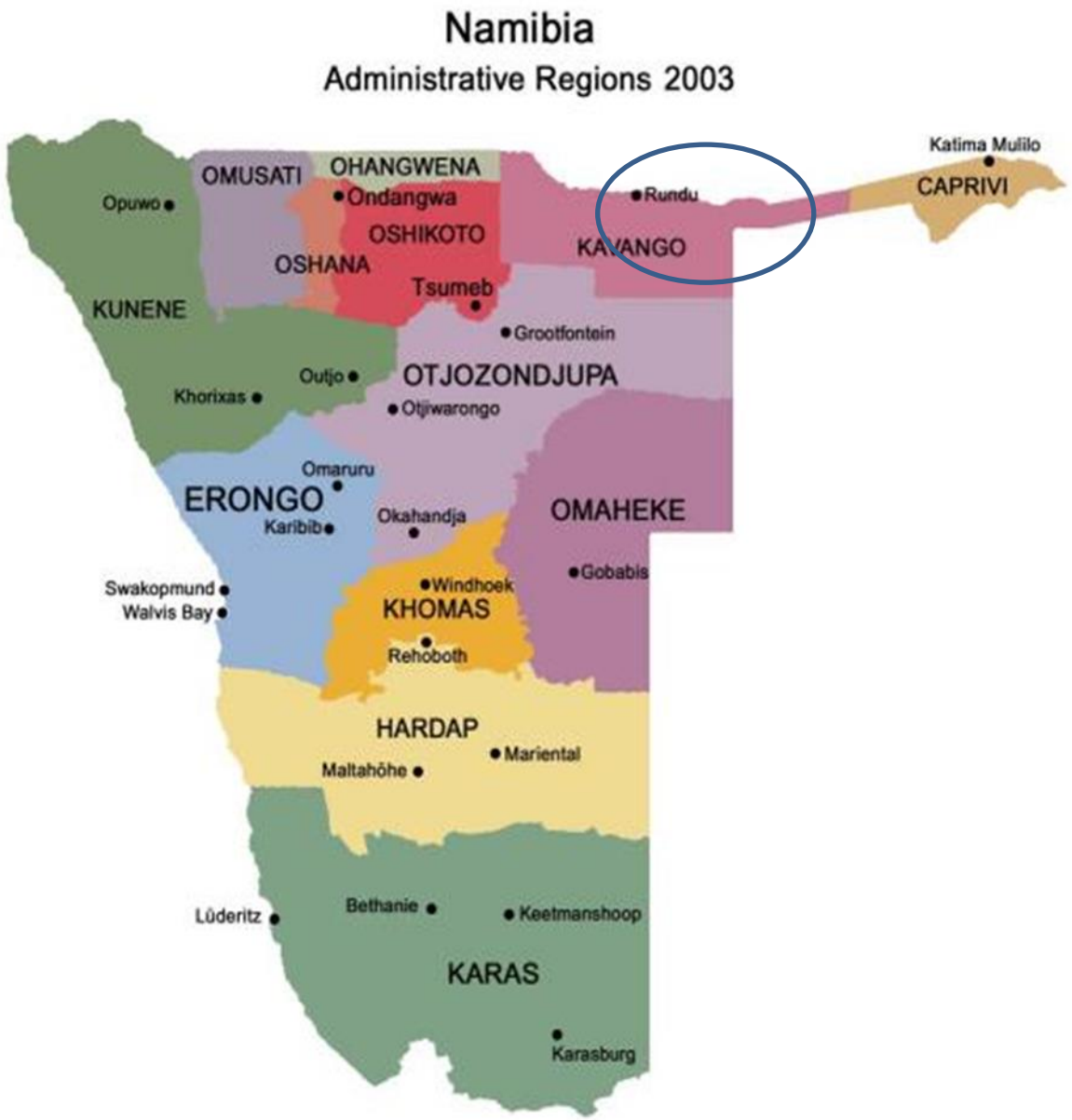

Figure 1: A map showing the area where plant samples were collected.

3.4 Preparation of plant extracts

Ethanolic (EtOH) and dichloromethane/methanol (DCM/MeOH, 50/50, %v/v) extracts were prepared separately according to Harborne (87). About 5.0 g of finely powdered plant material was weighed into a 100 mL glass beaker and an initial volume of 15 mL of the extraction solvent was added. The mixture was placed in a sonication bath for 3 min then centrifuged at 3000 rpm for 3 min and the supernatant was separated from the residue. The residue was extracted again twice with the same solvent using the same procedure. Extracts were combined and filtered through a 0.45 µm filter membrane. The extraction solvent was then evaporated at 40 °C using a rotary evaporator. The extracts were stored in closed vials at -20 °C until analysis.

3.5 Phytochemical screening

Extracts were screened for the presence of four phytochemical classes, alkaloids, diterpenes, saponins and flavonoids using standard procedures described in Tiwari *et al* (88). Procedures used for each class of secondary metabolites are briefly described below.

3.5.1 Detection of alkaloids

Each extract was dissolved in 6.00 mL of 1 % hydrochloric acid (HCl), stirred for 5 min and filtered. Extracts were treated with a few drops of Mayer's reagent. Formation of a yellow coloured precipitate indicated the presence of alkaloids.

3.5.2 Detection of diterpenes

Each extract was dissolved in 1 mL of distilled water and treated with 3 – 4 drops of copper acetate solution. Formation of an emerald green colour indicated the presence of diterpenes (**Figure 2**).

Figure 2: Detection of diterpenes in the medicinal plant extracts.

3.5.3 Detection of saponins

About 0.5 mg of each extract was shaken with 2 mL of Milli-Q water. If the foam produced persisted for at least 10 min, it indicated the presence of saponins (**Figure 3**).

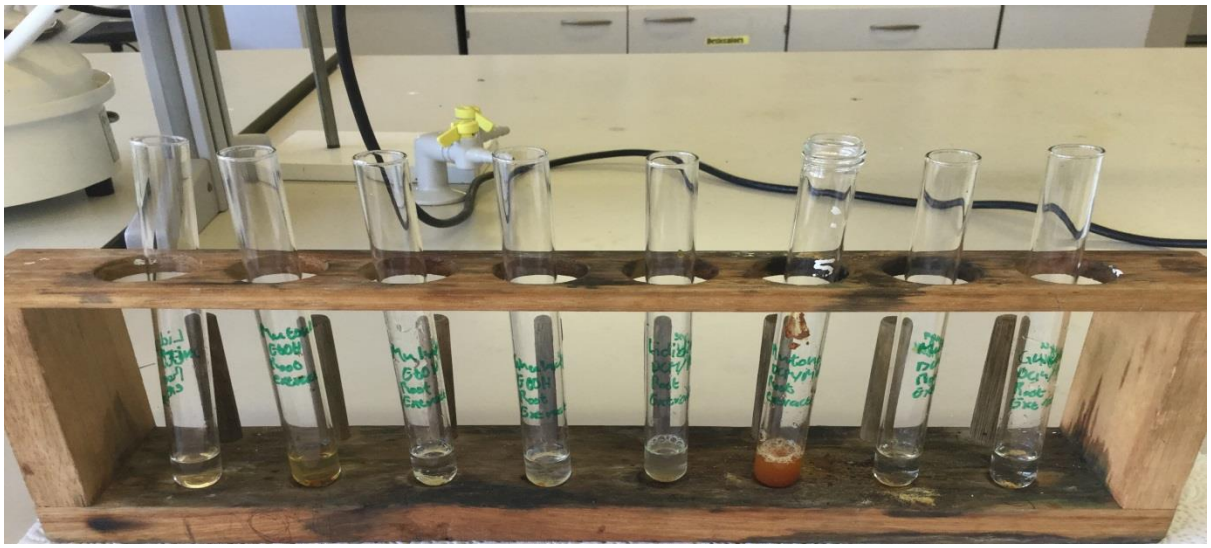

Figure 3: Detection of saponins in the medicinal plant extracts.

3.5.4 Detection of flavonoids

Each extract was treated with a few drops of 10 % sodium hydroxide (NaOH) solution. Formation of an intense yellow colour which becomes colourless on the addition of dilute 8% HCl indicated the presence of flavonoids (**Figure 4**).

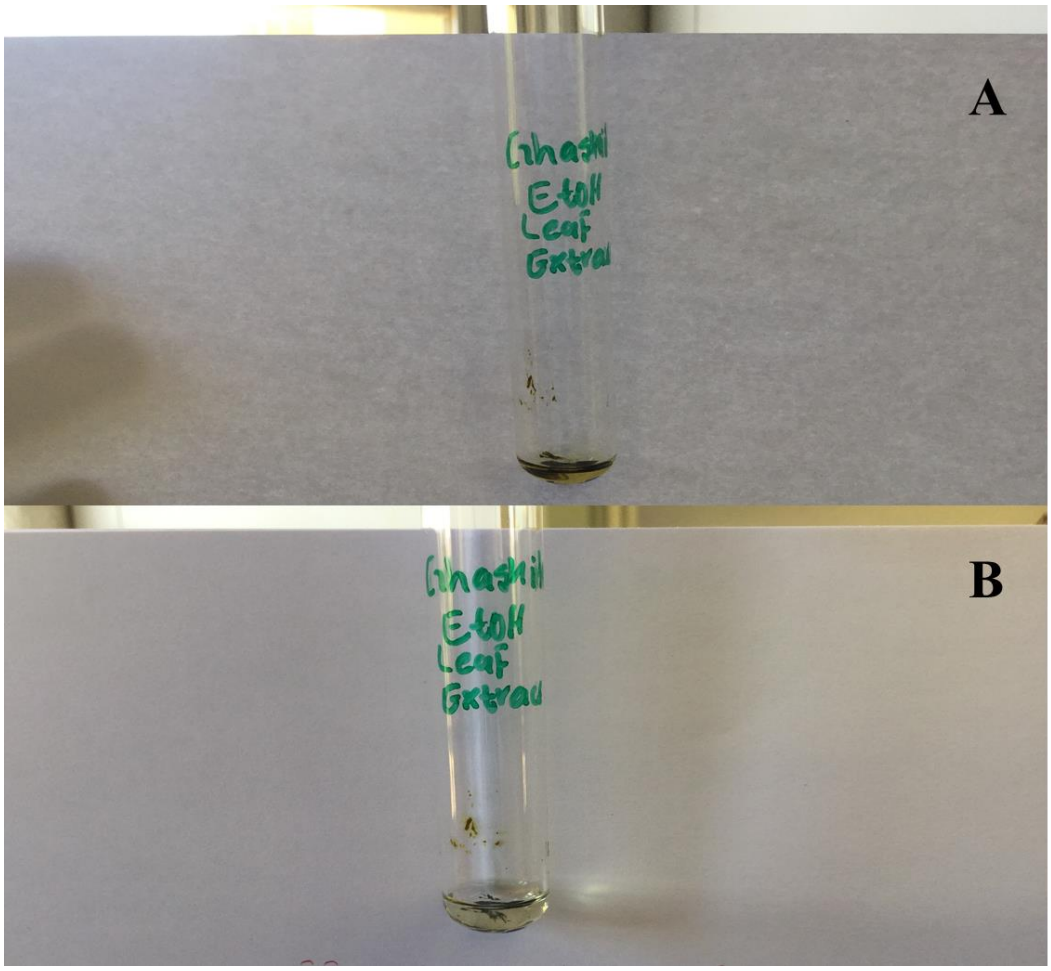

Figure 4: Detection of flavonoids: before (A) and after (B) the addition of 8 % HCl.

3.6 Acetylcholinesterase (AChE) inhibition assay

DCM/MeOH extracts were tested for the inhibitory activity against AChE at concentrations of 50, 100, 150, 200 and 250 µg/mL while ethanolic extracts were tested at concentrations of 5, 10, 50 and 100 µg/mL, using the *in vitro* Ellman's method as described by Nwanna *et al* (88). AChE inhibition activity was determined in a reaction mixture containing 50 µL of the solution of AChE (EC 3.1.1.7) in 1 mL of

0.1 M phosphate buffer (pH 8.0), 50 µL of a solution of 5,5'-dithiobis (2-nitrobenzoic) acid (DTNB) (3.3 Mm in 0.1 M phosphate-buffer solution, pH 7.0) containing $NaHCO_3$ (6 mM), extracts (range of 5-250 µg/mL) and 1125 µL of phosphate buffer (pH 8.0). After incubation for 20 min at 25 °C, 25 µL of 0.05 mM acetylcholine iodide solution was added as the substrate. The inhibition ability of the extracts was determined by monitoring the change in the absorbance reading at 412 nm for 3 min at 25 °C, taking measurements at 15 sec interval using a SpectraMax M2 plate reader. The experiment was performed in duplicate. The positive control was prepared in the same way as the sample, but without the extract. The blank test tube contained all the other reagents except the AChE and the extract. The percentage inhibition of the extracts on AChE was subsequently calculated as follow:

Percentage inhibition = $[(Abs_{ref} - Abs_{ext})/Abs_{ref}] * 100$

Where, Abs_{ref} is the absorbance of the reference or positive control and Abs_{ext} is the absorbance of the extract at intervals of 30 sec for 3 min.

3.7 HPLC analysis of medicinal plant extracts

HPLC separations were performed on a C18 reversed-phase liquid chromatography (RP-LC) column. Extracts were reconstituted in 1 mL of their respective extraction solvents, i.e. dichloromethane/methanol and ethanol (~9 mg/mL) and filtered with a 0.45 µm filter prior to injecting 1 µL into the HPLC system. UV spectral data were recorded in the range 210-700 nm, with selective monitoring at 210, 254, 320, 280 and 370 nm. The step of mobile-phase gradient elution was carried out with different mobile phases of varying ratios on a Zorbax SB-C18 column (4.6 × 150 mm, 5 µm), connected to a guard column. The total run time was 24 min. Gradient elution of two solvents was used, A (0.1% aqueous formic acid, %v/v) and B (acetonitrile, ACN).

The flow was kept constant at 0.80 mL/min. The gradient program began with 95% A and was held at this concentration for 1.30 min. This was followed by reduction to 90% A in 9.70 min. Then the concentration of A was reduced to 82% in 2.70 min. After which, the concentration of eluent B increased to 100% in 4.30 min. Finally, eluent A was changed back to 95% for the last 6 min. The temperature of the column was kept constant at 25 °C.

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Phytochemical screening

The results for the phytochemical screening of the leaves and roots for the five medicinal plants under investigation are presented in **Table 1**. Both ethanol and DCM/MeOH extracts of four medicinal plants tested positive for the presence of flavonoids, diterpenes and saponins, however none of the extracts seem to contain alkaloids. Diterpenes were the most abundant phytochemicals in the plant extracts judging from the deep emerald green colour observed and were also the most occurring phytochemicals in the DCM/MeOH extracts. Saponins were the second most common phytochemicals, but mainly found only in the DCM/MeOH extracts. Saponins are known to possess antimicrobial, antifungal, anti-allergenic, antispasmodic and anti-inflammatory properties (92). Studies carried out on a fenugreek saponin found that it inhibit the progression of AD present in this herb (93). *F. glumosa* tested positive for the presence of flavonoids in both leaf and root parts. Some evidence exist that flavonoids play a role in enzyme and receptor systems of the brain, enabling them to exert neuroprotective effects on the central nervous system and prevent the progression of neurodegenerative diseases such as AD and PD (89).

The medicinal plants under study were found to contain phytochemical classes that purportedly possess neuroprotective properties, which could indicate that they may be helpful in the prevention or progression of neurodegenerative diseases. Future research should focus on identifying the individual chemical constituents to enable detailed study of their properties.

Table 1: Phytochemical analysis of medicinal plants under study.

Plant extracts	Phytochemical classes							
	Alkaloids		Flavonoids		Diterpenes		Saponins	
	EtOH	DCM/MeOH	EtOH	DCM/MeOH	EtOH	DCM/MeOH	EtOH	DCM/MeOH
***O. pulchra* (leaves)**	-	-	++	-	-	-	-	-
***O. longipes* (leaves)**	-	-	-	-	-	-	-	+
O. *longipes* (roots)	-	-	-	-	-	-	-	-
***F. glumosa* (leaves)**	-	-	+	-	++	-	-	+
***F. glumosa* (roots)**	-	-	++	-	-	+	-	-
***L. decurrens* (leaves)**	-	-	-	-	++	++	-	-
***L. decurrens* (roots)**	-	-	-	-	-	+	-	+
***P. maprounifolia* (leaves)**	-	-	-	-	-	++	+	+
***P. maprounifolia* (roots)**	-	-	-	-	-	-	-	++

Key: + Light colour, ++ Dark colour, - Negative

4.2 Acetylcholinesterase inhibition assay

The results for the *in vitro* acetylcholinesterase (AChE) inhibition activity for the DCM/MeOH and ethanol extracts of the leaves of *L. decurrens*, *F. glumosa*, *P. maprounifolia*, *O. pulchra* and *O. longipes* indicate that all the leaf extracts were able to inhibit acetylcholinesterase at all the concentrations studied (**Figures 5 & 6**). However, no trend could be established between the percentage inhibition and the concentration of the extracts. By comparing the results for the DCM/MeOH extracts for different samples, it could be generally concluded that higher inhibition potency was associated with the *L. decurrens* extract, with inhibition percentages ranging from 74–101%. The highest inhibition was recorded at an anomalous value of 101% at a concentration of 200 µg/mL. *O. pulchra* displayed the least inhibition potential with percentage inhibition ranging from 23 to 76%, with the activity being more pronounced at lower concentrations. A commendable inhibition activity was obtained for all the ethanol extracts. The most potent plant was again *L. decurrens*, followed by *P. maprounifolia*. The high inhibition activity displayed by *L. decurrens* extracts is likely to be due to the high concentration of diterpenes detected in the qualitative phytochemical test. Diterpenes and flavonoids have previously been shown to exhibit inhibition activities against key enzymes such as AChE, that are linked to the neurodegeneration (97, 105, 106)

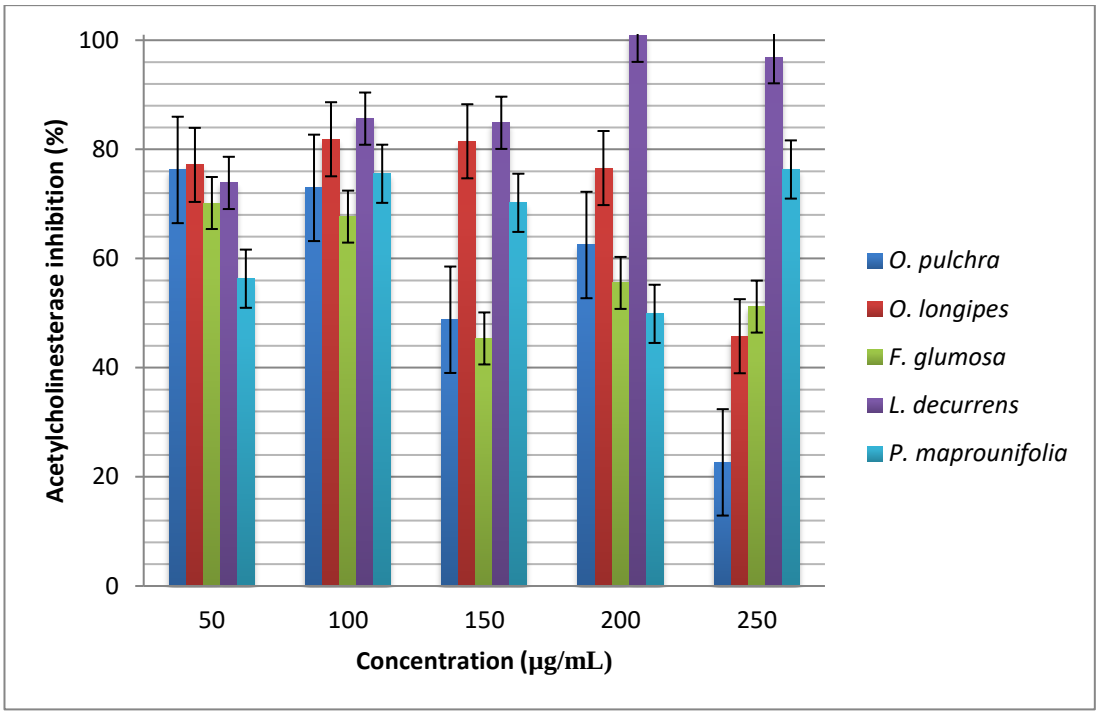

Figure 5: Acetylcholinesterase inhibition by the DCM/MeOH extracts of the leaves of the five medicinal plants.

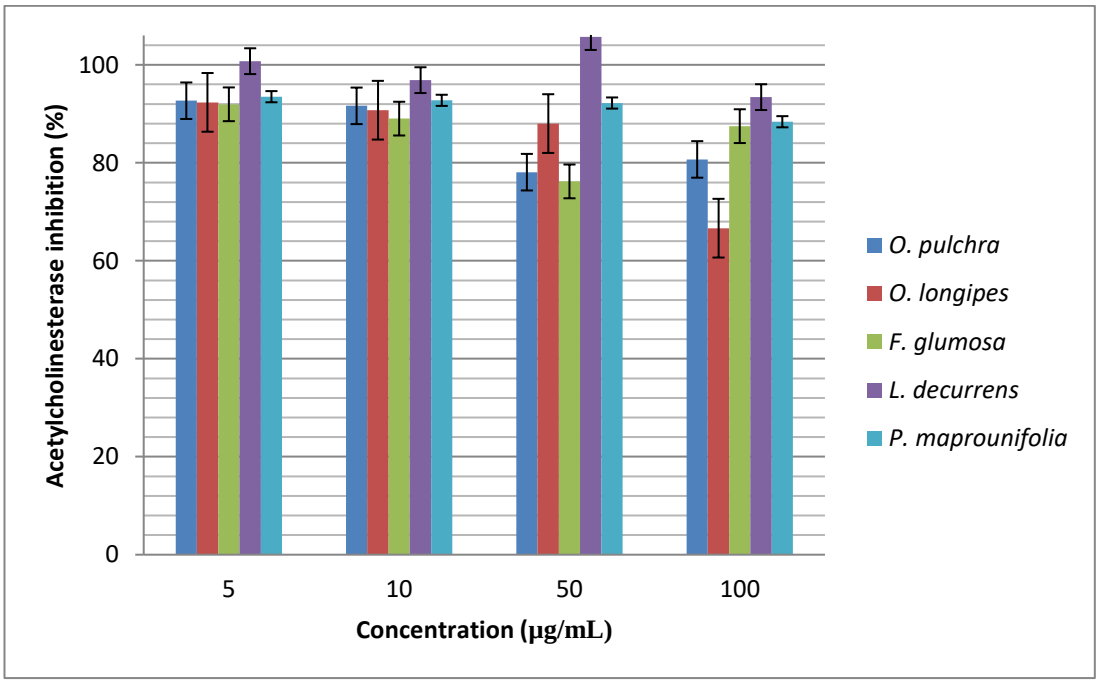

Figure 6: Acetylcholinesterase inhibition by the ethanol extracts of the leaves for the five medicinal plants.

4.3 HPLC-UV analysis of the plant extracts

The HPLC method was initially developed using a mixture of 11 phenolic standards (catechin, quercetin, vanillic acid, 4-hydroxbenzoic acid, sinapic acid, chlorogenic acid, caffeic acid, syringic acid, *t*-ferulic acid, gallic acid and *p*-coumaric acid) belonging to three different phenolic classes (flavanol, flavonol and phenolic acid classes). The UV chromatogram recorded for the optimal separation of the standard mixture at 280 nm is presented in **Figure 7**. Literature reports show that flavonoids and flavones have maximum UV absorbance values at wavelengths of 275-285 nm and 320-350 nm, respectively (97). Phenolic acids derived from hydroxybenzoic acid (HBA) have maximum absorbance values in the region 246-262 nm, as can be seen for chlorogenic acid, caffeic acid, *p*-coumaric acid, trans-ferulic acid and sinapic acid which is similar to the UV spectra data of these compounds. Hydroxycinnamic acid (HCA) derived phenolic acids absorb at two regions: 225-235 and 290-330 nm. Gallic acid, vanillic acid and syringic acid all absorbed in these regions as illustrated in the HPLC chromatogram of phenolic acid standards in **Figure 9** Catechin (flavan-3-ol) has absorption maxima around 220 and 280 nm while quercetin (flavonol) has maxima around 250 and 370 nm. These observations are consistent with literature reports (107). After method optimisation using the standards, the plant extracts were also analysed under the same conditions and chromatograms obtained at 280 nm are illustrated in **Figures 9** and **10**. Better peak shapes were obtained with the DCM/MeOH extracts than the EtOH extracts, which indicates that ethanol was a strong injection solvent under these conditions.

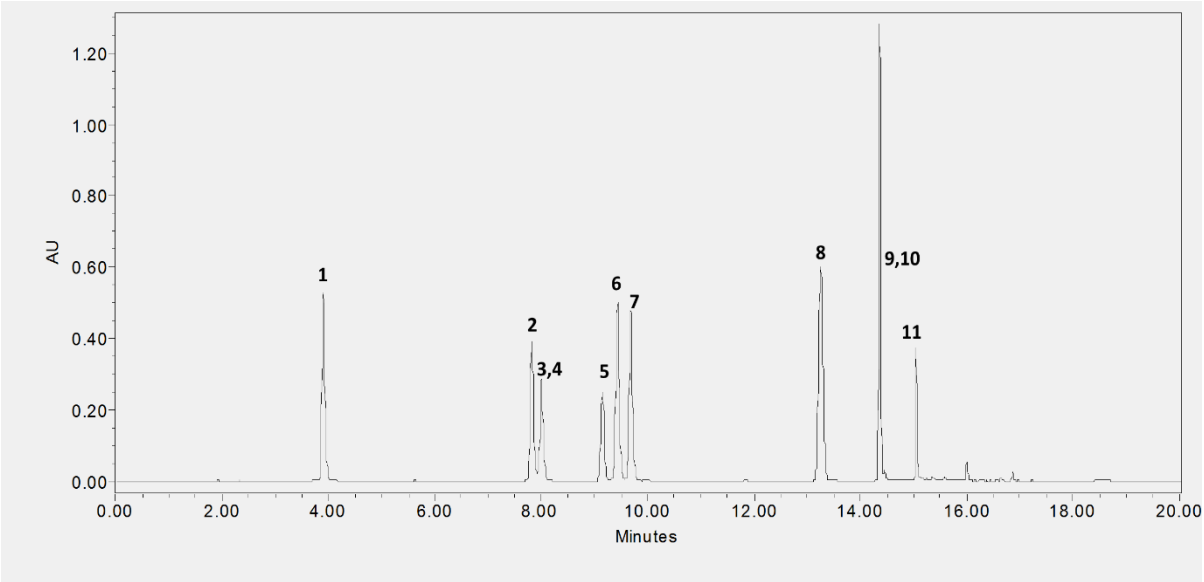

Figure 7: UV chromatograms at 280 nm for the HPLC analysis of phenolic standards.

Peak identity: gallic acid (1), catechin (2), chlorogenic acid (3), 4-hydroxybenzoic acid (4), vanillic acid (5), caffeic acid (6), syringic acid (7), *p*-coumaric acid (8), trans-ferulic acid (9), sinapic acid (10) and quercetin (11).

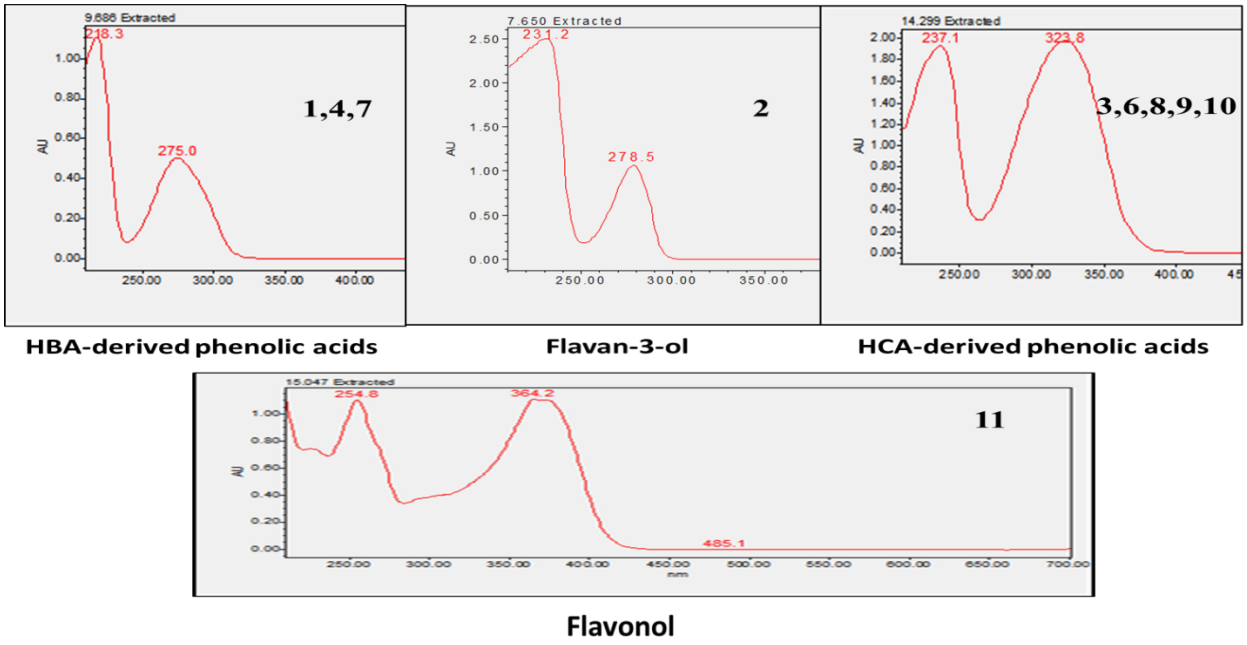

Figure 8: UV absorbance spectra at 210-400 nm for phenolic standards.

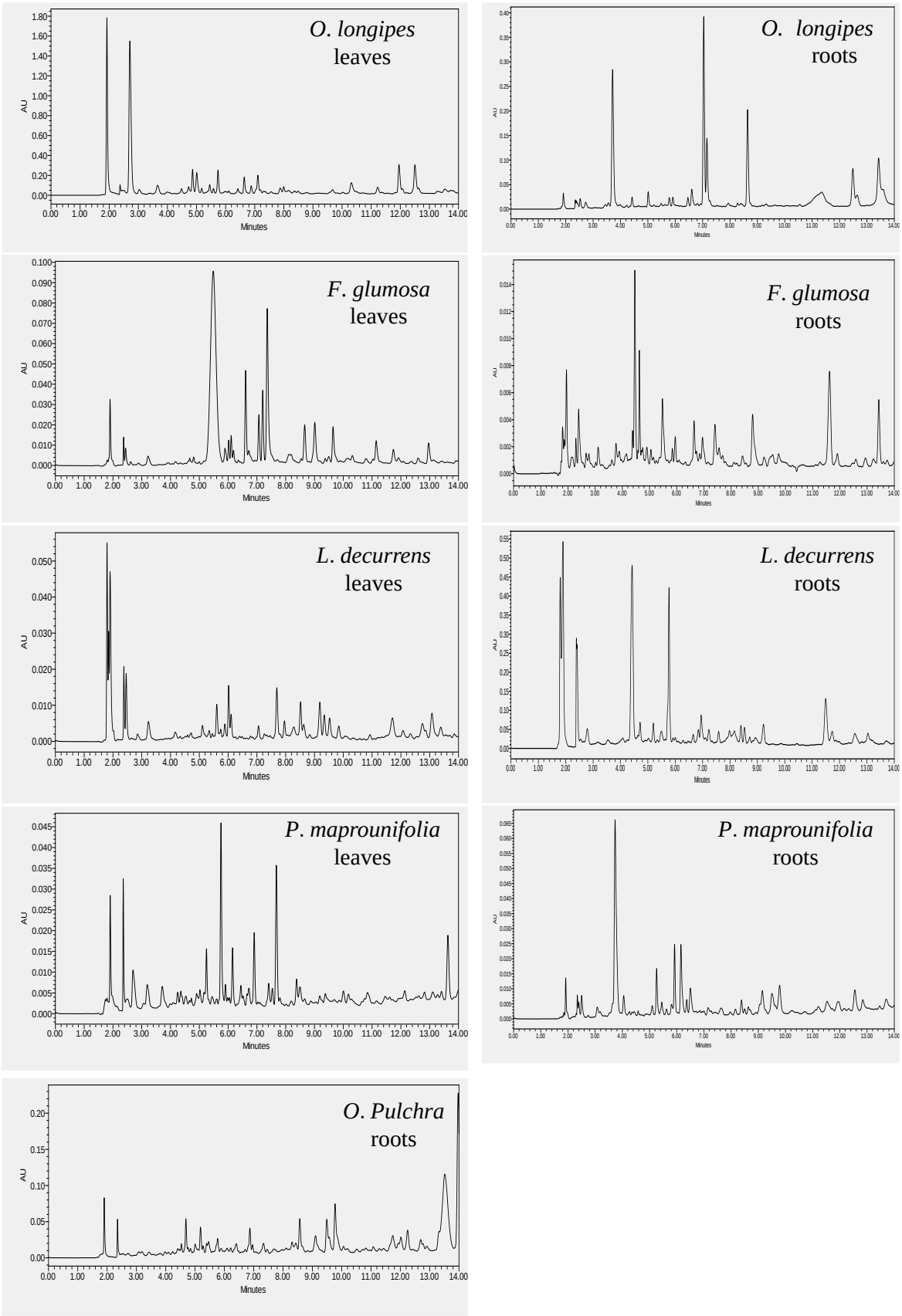

Figure 9: UV chromatograms at 280 nm for the DCM/MeOH extracts of the leaves and roots of medicinal plants under investigation.

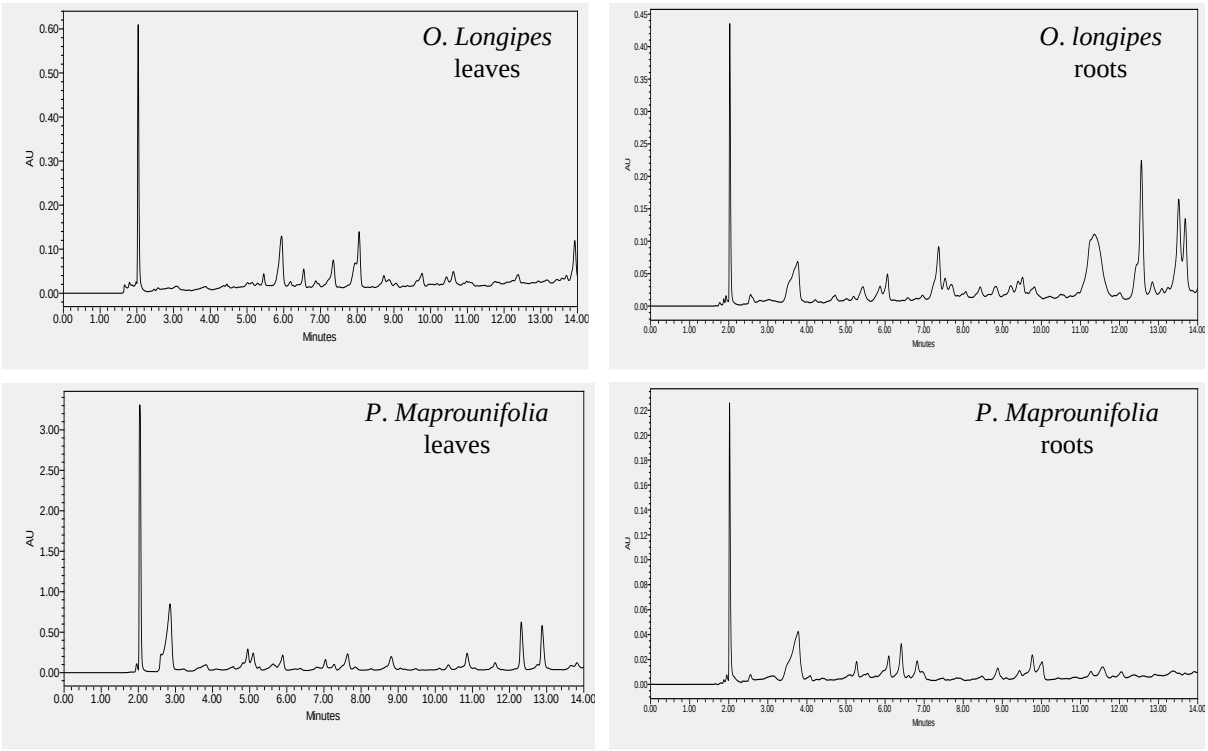

Figure 10: UV chromatograms at 280 nm for the EtOH extracts of the leaves and roots for *P. maprounifolia* and *O. longipes*.

Based on the results obtained, it is clear that a large number of UV-active compounds were present in all the leaf and root extracts. In the absence of additional spectroscopic data, tentative identification of compounds was performed by comparing UV absorption spectra of the standard compounds with those of the individual compounds detected in the plant extracts.

Figures 12, 14, 16 and **18** illustrate the typical UV spectra for the major compounds detected in the DCM/MeOH extracts of two representative medicinal plants, *L. decurrens* and *P. maprounifolia*, which are the two plants found to exhibit high AChE inhibition activities. A comparison of the UV spectra of **Figures 8** (phenolic standards) and **Figures 12, 14, 16** and **18** (plant extracts) shows that the UV absorbance patterns for some of the compounds detected in the plant extracts closely resemble those of the

standard phenolic compounds. It can be seen that all the compounds detected in these medicinal plant extracts absorbed UV in a range of 214-350 nm. For example, the UV absorbance pattern for peaks **1** and **2** in the DCM/MeOH leaf extract of *P. maprounifolia* (**Figure 12**) as well as peaks **5** and **6** in the root extract of the same plant are similar to that of catechin, which could mean that these compounds belong to the flavan-3-ol subclass. Similarly, the spectrum for peak **4** in the leaf extract of the same plant sample resembles those of hydroxycinnamic acid-derived phenolic acids. Notably, the spectra for peaks **1** to **4** in the root extract of *P. maprounifolia* (**Figure 14**) also closely resemble that of gallic acid, which could indicate that these compounds are close relatives of hydroxybenzoic acid-derived phenolic acids. All in all, when closely studying the UV spectra for the compounds detected in the extracts for all the plants, it could be generalised that these plants are rich sources of organic compounds judging from their strong UV absorption capabilities. However, due to lack of powerful spectroscopic instruments such MS and NMR, the individual compounds present in the medicinal plants could not be fully characterised.

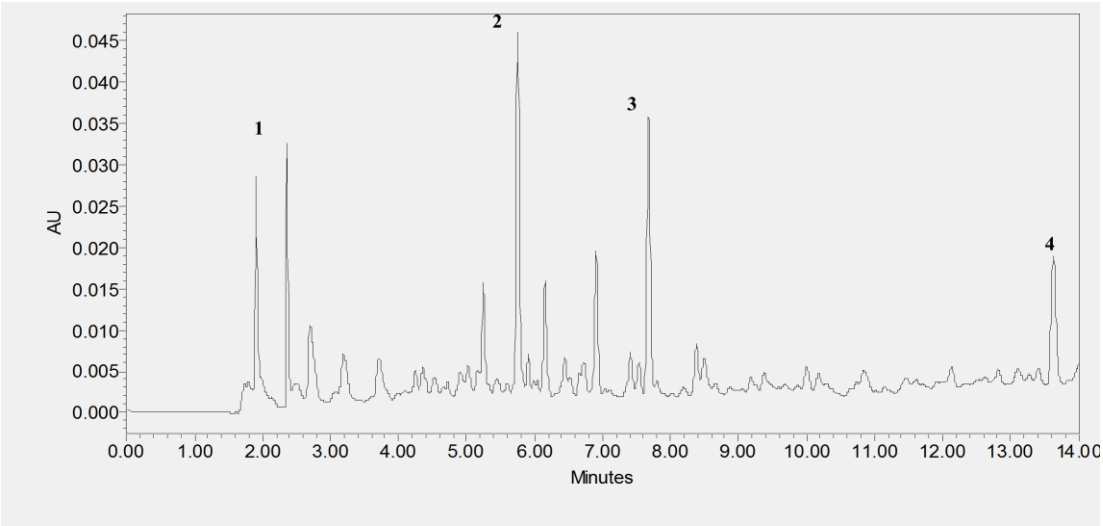

Figure 11: HPLC chromatogram at 280 nm for major peaks detected in the DCM/MeOH extracts of the leaves of *P. maprounifolia*.

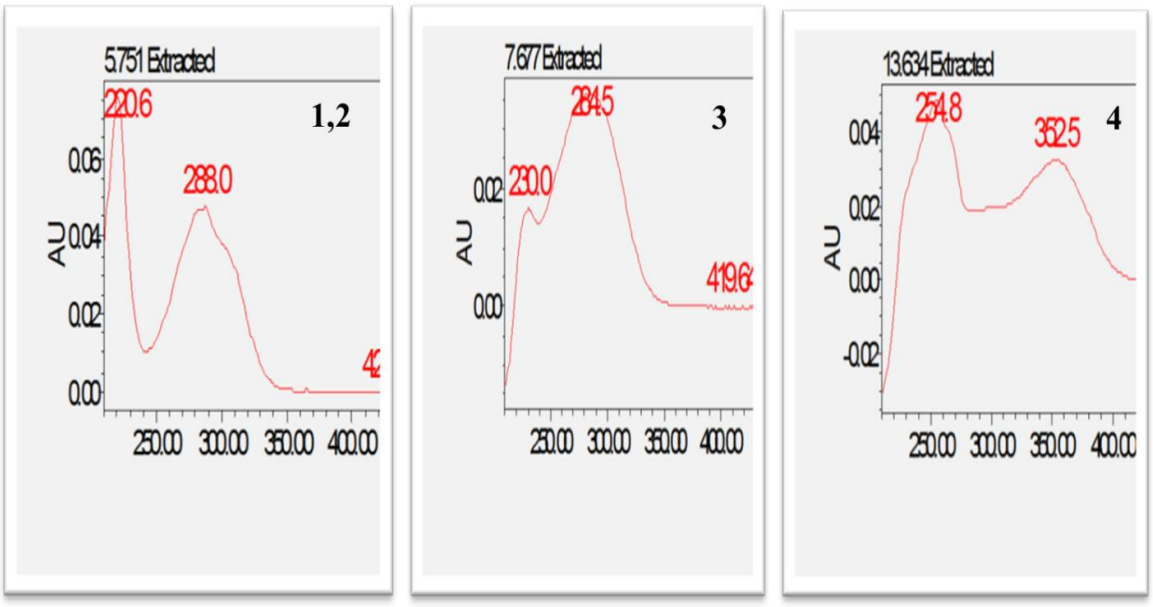

Figure 12: UV absorbance spectra (210-400 nm) for the major peaks detected in the DCM/MeOH extracts of the leaves of *P. maprounifolia*.

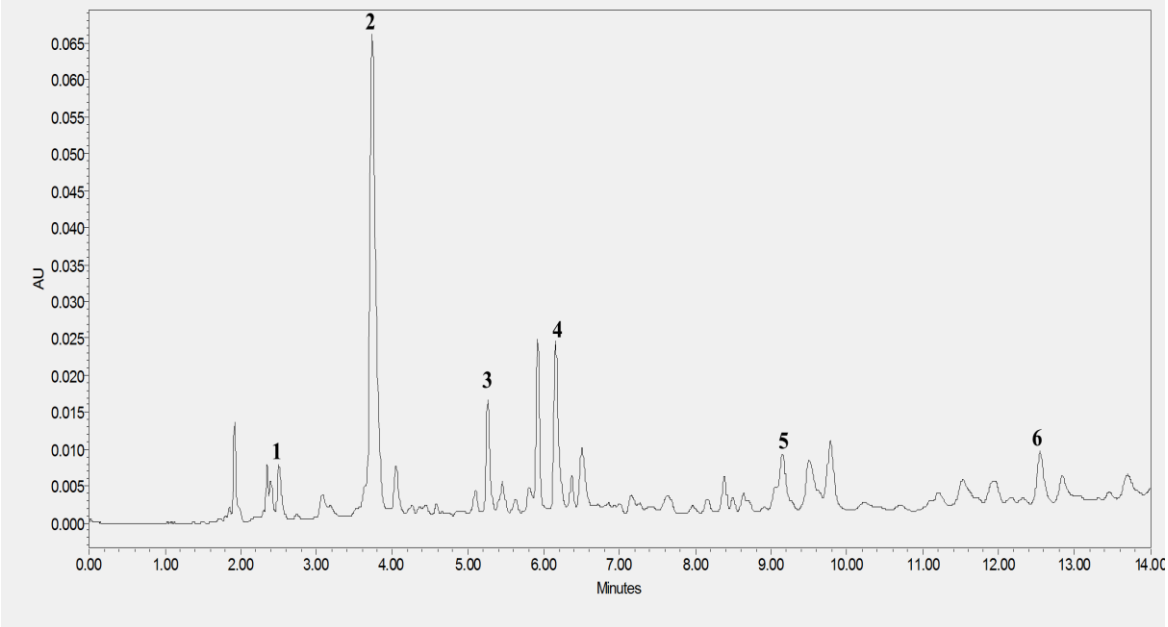

Figure 13: HPLC chromatogram at 280 nm for major peaks detected in the DCM/MeOH extracts of the roots of *P. maprounifolia*.

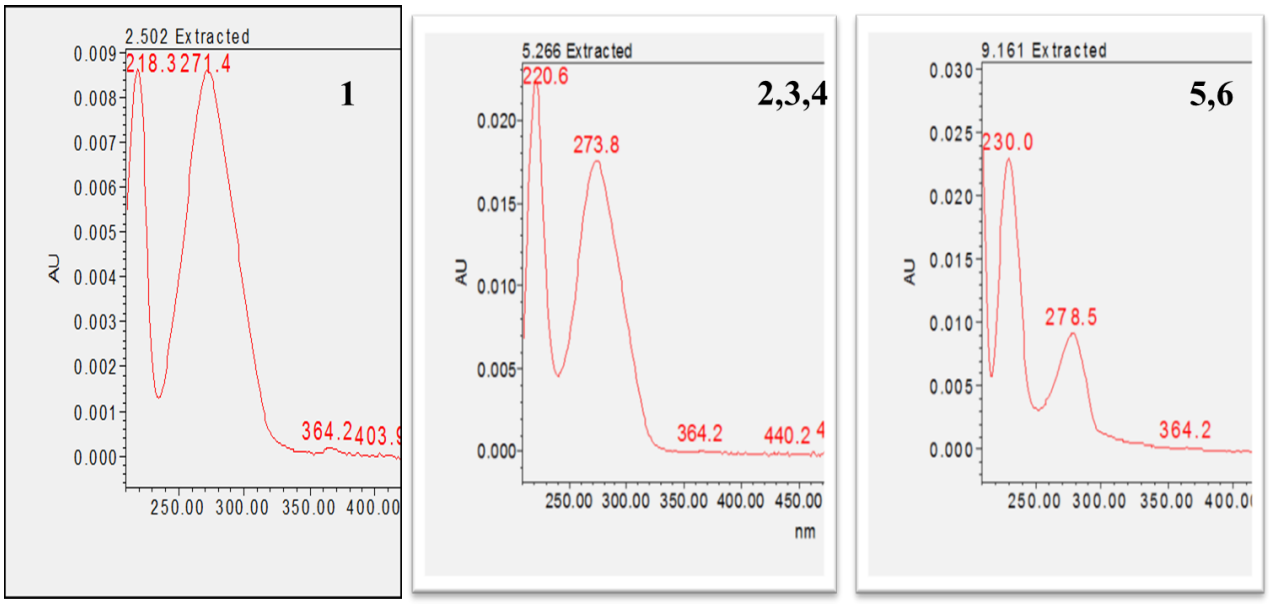

Figure 14: UV absorbance spectra (210-400 nm) for the major peaks detected in the DCM/MeOH extracts of the roots of *P. maprounifolia*.

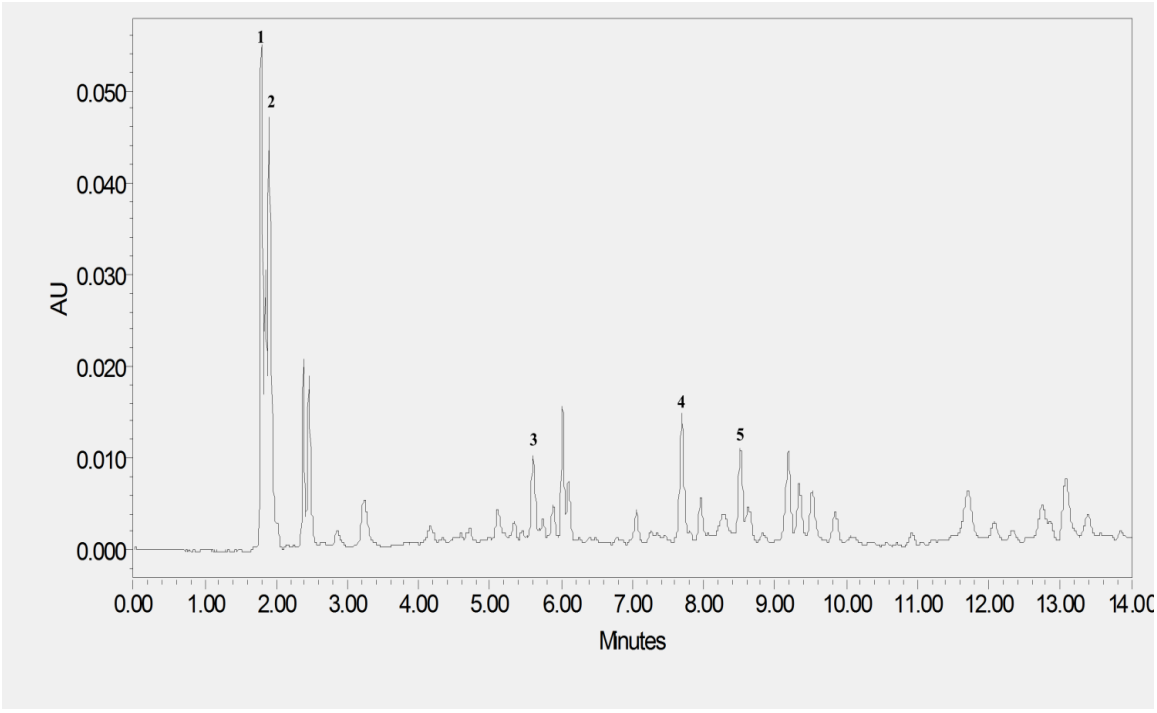

Figure 15: HPLC chromatogram at 280 for the major peaks detected in the DCM/MeOH extracts of leaves of *L. decurrens*.

Figure 16: UV absorbance spectra (210-400 nm) for the major peaks detected in the DCM/MeOH extracts of leaves of *L. decurrens*.

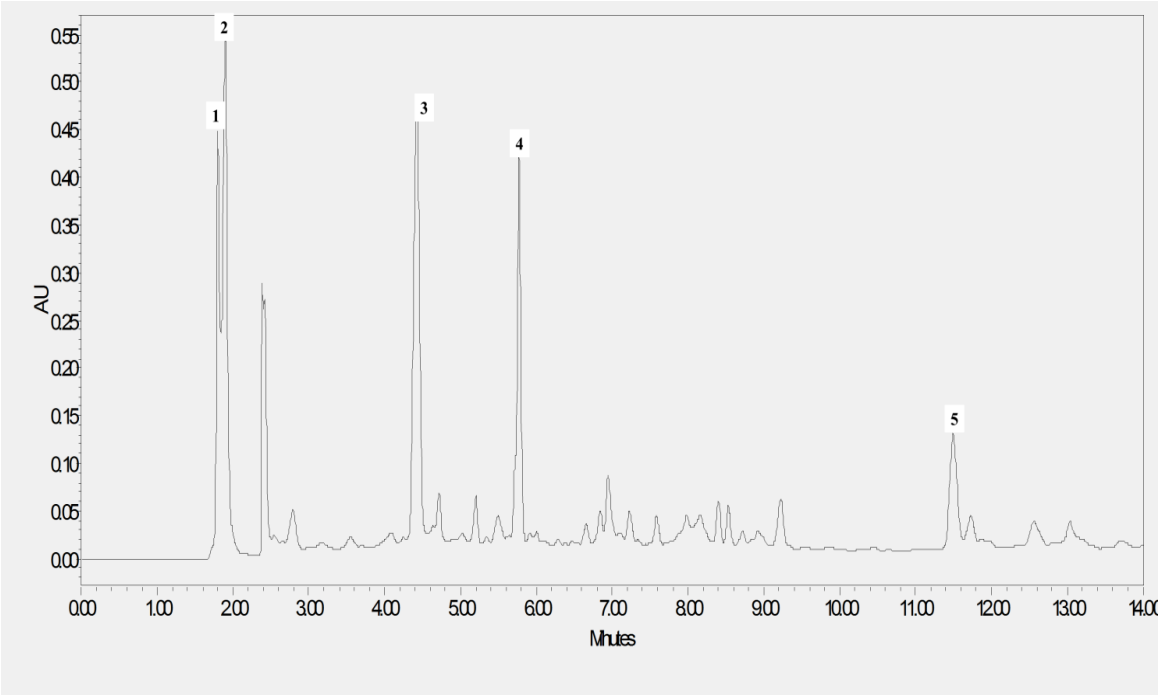

Figure 17: HPLC chromatogram at 280 for the major peaks detected in the DCM/MeOH extracts of roots of *L. decurrens*.

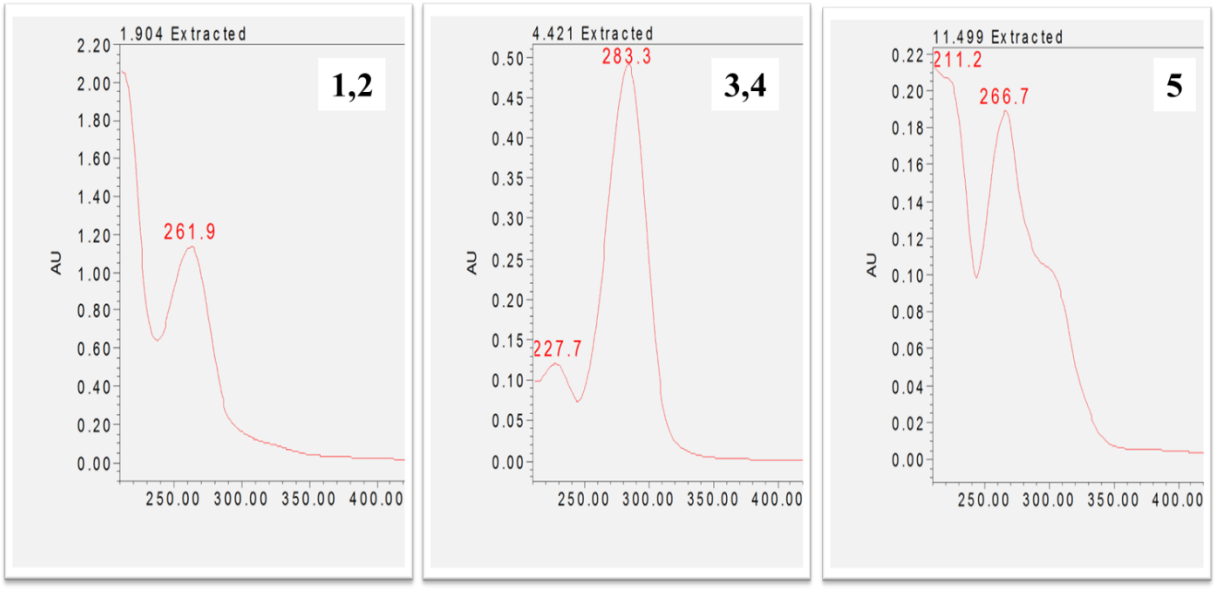

Figure 18: UV absorbance spectra (210-400 nm) for the major peaks detected in the DCM/MeOH extracts of roots of *L. decurrens*.

CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

In the present study, dichloromethane/methanol and ethanol extracts of the leaves and roots of *L. decurrens*, *F. glumosa*, *P. maprounifolia*, *O. pulchra* and *O. longipes* were screened for the presence of alkaloids, saponins, diterpenes and flavonoids and investigated for their inhibition properties against the acetylcholinesterase enzyme. To the best of our knowledge, this is the first research carried out on the evaluation of the AChE inhibition activity of these medicinal plants in Namibia. Some extracts tested positive for the presence of diterpenes, saponins and flavonoids. All the extracts were able to inhibit the AChE enzyme, with *L. decurrens* being the most potent plant species. HPLC coupled with photodiode array detection was employed for the separation and determination of the types of phenolic compounds present in the medicinal plant extracts. The HPLC-UV data revealed that the plant extracts contain numerous UV-active compounds that absorb strongly in the region 210-450 nm and their spectra closely resemble those of common phenolic compounds such as catechin and gallic acid.

5.2 Recommendations

Based on the results obtained from this research, future work should be directed towards the isolation and characterisation of the individual compounds in the extracts of these medicinal plants to identify potent compounds. Isolation and purification of compounds can be carried out by using high resolution LC×LC–MS/MS analysis. The optimised HPLC methods can be coupled to mass spectroscopy (MS) for tentative identification of compounds. In addition, NMR spectroscopy could be employed to elucidate the structures for the chemical compounds whose identities cannot be ascertained from the MS data alone.

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APPENDICES

APPENDIX 1: Ethical Clearance Certificate

UNAM
UNIVERSITY OF NAMIBIA

ETHICAL CLEARANCE CERTIFICATE

Ethical Clearance Reference Number: FOS /227/2017 **Date: 7 June, 2017**

This Ethical Clearance Certificate is issued by the University of Namibia Research Ethics Committee (UREC) in accordance with the University of Namibia's Research Ethics Policy and Guidelines. Ethical approval is given in respect of undertakings contained in the Research Project outlined below. This Certificate is issued on the recommendations of the ethical evaluation done by the Faculty/Centre/Campus Research & Publications Committee sitting with the Postgraduate Studies Committee.

Title of Project: Detection And Identification Of Psychoactive Compounds From Medicinal Plants Used To Treat Mental Disorders In Kavango East Region Of Namibia

Nature/Level of Project: Masters

Researcher: Theodor Nande Nghifikepunye Nghilalulwa

Student Number: 201212181

Faculty: Faculty of Science

Supervisors: Dr K.M. Kalili (Main) Dr A. Cheikhyoussef (Co)

Take note of the following:

(a) Any significant changes in the conditions or undertakings outlined in the approved Proposal must be communicated to the UREC. An application to make amendments may be necessary.

(b) Any breaches of ethical undertakings or practices that have an impact on ethical conduct of the research must be reported to the UREC.

(c) The Principal Researcher must report issues of ethical compliance to the UREC (through the Chairperson of the Faculty/Centre/Campus Research & Publications Committee) at the end of the Project or as may be requested by UREC.

(d) The UREC retains the right to:

(i) Withdraw or amend this Ethical Clearance if any unethical practices (as outlined in the Research Ethics Policy) have been detected or suspected,

(ii) Request for an ethical compliance report at any point during the course of the research.

UREC wishes you the best in your research.

Prof. P. Odonkor: UREC Chairperson

Ms. P. Claassen: UREC Secretary

APPENDIX 2: Research Permission Letter

CENTRE FOR POSTGRADUATE STUDIES

University of Namibia, Private Bag 13301, Windhoek, Namibia
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UNAM
UNIVERSITY OF NAMIBIA

RESEARCH PERMISSION LETTER

Student Name: Theodor N. N. Nghilalulwa

Student number: 201212181

Programme: MSc Chemistry

Approved research title: Detection and identification of psychoactive compounds from medicinal plants used to treat mental disorders in Kavango East Region of Namibia

TO WHOM IT MAY CONCERN

I hereby confirm that the above mentioned student is registered at the University of Namibia for the programme indicated. The proposed study met all the requirements as stipulated in the University guidelines and has been approved by the relevant committees.

The proposal adheres to ethical principles as per attached Ethical Clearance Certificate. Permission is hereby granted to carry out the research as described in the approved proposal.

Best Regards

Dr Marius Hedimbi
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Centre for Postgraduate Studies
Office of the Director
2017 -06- 13
University of Namibia
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