Southern African plants used to treat central nervous system related disorders

GARY IVAN STAFFORD

Submitted in fulfilment of the academic requirements for the degree of

DOCTOR OF PHILOSOPHY

in the

School of Conservation and Biological Sciences

Faculty of Science and Agriculture

University of KwaZulu-Natal

Pietermaritzburg

March 2009

IMPORTANT

POTENTIALLY TOXIC

Caution: This thesis is in no way intended as a guide to the use of medicinal, hallucinogenic or psychotropic plants. Ingestion of some of these plants or plant products may be extremely dangerous. Neither the author nor the University can be held responsible for claims arising from the inappropriate use of plant materials mentioned in this dissertation.

Poisons in small doses are the best medicines;
and useful medicines in too large doses are poisonous
(William Withering, 1789)

DECLARATION

The experimental work described in this thesis was conducted in the Research Centre for Plant Growth and Development, School of Conservation and Biological Sciences at the University of KwaZulu-Natal, Pietermaritzburg from January 2003 to August 2008, under the supervision of Professor J. VAN STADEN (Research Centre for Plant Growth and Development, University of KwaZulu-Natal, Pietermaritzburg) and Professor A.K. JÄGER (Department of Medicinal Chemistry, University of Copenhagen, Denmark).

This thesis, submitted for the degree of Doctor of Philosophy in the Faculty of Science and Agriculture of the University of KwaZulu-Natal, Pietermaritzburg, represents original work by the author, except where the work of others is duly acknowledged in the text. Although the majority of these studies have been published in peer reviewed journals they have not otherwise been submitted in any form for any other degree or diploma.

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GARY I. STAFFORD

August 2008

We certify that the above statement is correct:

……..…….

Professor JOHANNES VAN STADEN

Supervisor

Pietermaritzburg

……

Professor ANNA K. JÄGER

Co-supervisor

Copenhagen

PROLOGUE

A Prologue to a thesis is perhaps a break from tradition but it is felt that due to the involvement of several people in this project it will serve to delineate each participant's contribution. This project was initially conceptualised in 2000 and was to be submitted for a M.Sc. degree. The idea of investigating southern African psychoactive plant use came initially from discussions with my then B.Sc. Honours (4th year) colleague Michael Gardner and Honours Supervisor Dr. Anna K. Jäger. Discussions revolved around the observation that African traditional healing shows similar characteristics to shamanistic practices, in particular the practice of ancestor 'communication'. Unlike other shamanistic cultures, such as those found in South America, very few psychoactive plant species were recognised in southern Africa.

The release of '*People's Plants*' (VAN WYK and GERICKE, 2000) that contained a chapter dedicated to plants that affect the 'mind and mood', stimulated further interest in this topic. After some preliminary research it was clear that this area was in need of further research and that perhaps this topic was too large for an M.Sc. For the next two years while I completed a Masters on the 'Effect of storage on medicinal plants' I continued to gather literature on African psychotropic plants. As I was nearing the completion of my M.Sc. and at last about to tackle 'Southern African plants used to treat central nervous system related disorders' for my Doctorate, J.F. Sobiecki published '*A preliminary inventory of plants used for psychotropic purposes in southern African healing traditions*' (SOBIECKI, 2002). This detailed literature review, accompanied by new data from interviewing traditional healers, superseded the work I had done to this point. Although initially this was a large disappointment, it turned out to be for the best, as it forced me to alter my research emphasis.

Both my initial research and Sobiecki's paper showed that despite the numerous references to potential psychotropic plants in the literature on African traditional medicine, relative little validation and further investigations have occurred. This inspired the continued documentation and extensive screening of these plants in several *in vitro* bioassays.

Professor Anna Jäger, now co-supervisor for my Ph.D. had moved from Research Centre for Plant Growth and Development, Pietermaritzburg to take up a Pharmacognosy Professorship at the Department of Medicinal Chemistry, University of Copenhagen (Denmark). In 2003 I had the pleasure of working with two M.Sc. (Pharmacy) students under her supervision, Nicolaj D. Nielsen and Mikkel Sandager, on the development of the serotonin transporter assay. This radiochemical bioassay was set up at the Research Centre and was used to conduct the first screening of plant extracts for selective serotonin re-uptake inhibition (SSRI) activity. At the same time another two M.Sc. students, Jofrid Risa and Anlaug Risa were working on plant material that I had identified, collected and processed in South Africa. These

were screened for compounds with an affinity for γ-aminobutyric acid (subtype-A)-benzodiazepine ($GABA_A$-benzodiazepine) receptor, and for AChE activity. A year later Ann B. Svenningsen and Katrine Damkjær Madsen came to South Africa to follow through on some of the active plants with an affinity for $GABA_A$-benzodiazepine receptor. Their work lead to the isolation of flavonoid compounds with affinity for the $GABA_A$-benzodiazepine receptor from *Rhus* (*Searsia*) species.

In July 2005 I had the pleasure of travelling to Copenhagen for six weeks to work in Professor Anna Jäger's laboratories. I had brought with me several Amaryllidaceae alkaloids isolated by Dr E. Elgorashi from South African species and successfully tested them in the serotonin transporter assay and for affinity for the $GABA_A$-benzodiazepine receptor. This research resulted in a publication; this research however did not form part of this thesis. I had also brought an additional 46 extracts which were screened in the $GABA_A$-benzodiazepine receptor assay leading to a second publication.

Another two pharmacy M.Sc. students from Professor Anna Jäger's laboratory, J. Peter Almqvist and Stefan A.K. Vangsøe came to South Africa to follow through on some of the active plants that I had discovered while in Copenhagen. This collaboration led to the isolation and identification of active compounds from *Mentha aquatica* in the $GABA_A$-benzodiazepine receptor assay.

That same year I worked closely with Pernille D. Pedersen on the development of a photometric peroxidase linked assay to determine the inhibition of the oxidative deamination of tyramine by monoamine oxidase (MAO) isolated from rat liver. This enabled us to screen twenty plants, used in Zulu traditional medicine to treat several CNS-related ailments, for MAO inhibition and specific MAO-B inhibition activity. Additional researched conducted with Helle T. Olsen on *Mentha aquatica* lead to the isolation and identification of a non-selective MAO inhibitor.

Mikael E. Pedersen, who worked in the Research Centre during his Masters project, is finalizing his Ph.D. at the University of Copenhagen on anti-epileptic and anti-depressive activity of South African plants that were identified through earlier work in this project.

An ambitious project such as this would not have been possible without the co-operation of all those involved. I am extremely grateful to have had the opportunity to have worked with such talented researchers. I am a botanist by training, having had little formal organic chemistry education, I am therefore extremely grateful to the chemists at the University of Copenhagen for providing the NMR data that lead to the identification of the compounds described in this thesis.

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G.I. STAFFORD

March 2009, Pietermaritzburg

PUBLICATIONS FROM THIS RESEARCH

JOURNAL ARTICLES

CHAPTER 2

G.I. Stafford, M.E. Pedersen, J. van Staden, A.K. Jäger. **2008**. Review on plants with CNS-effects used in traditional South African medicine against mental diseases. *Journal of Ethnopharmacology* Accepted, **In Press**.

CHAPTER 3

N.D. Nielsen, M. Sandager, **G.I. Stafford**, J. van Staden and A.K. Jäger. **2004**. Screening of indigenous plants from South Africa for affinity to the serotonin reuptake transport protein. *Journal of Ethnopharmacology* 94, 159-163.

M. Sandager, N.D. Nielsen, **G.I. Stafford**, J. Staden and A.K. Jäger. **2005**. Alkaloids from *Boophane disticha* with affinity to the serotonin transporter in rat brain. *Journal of Ethnopharmacology* 98, 367-370.

CHAPTER 4

J. Risa, A. Risa, A. Adsersen, B. Gauguin, **G.I. Stafford**, J. van Staden and A.K. Jäger. **2004**. Screening of plants used in southern Africa for epilepsy and convulsions in the $GABA_A$-benzodiazepine receptor assay. *Journal of Ethnopharmacology* 93, 177-182.

G.I. Stafford, A.K. Jäger and J. van Staden. **2005**. Activity of traditional South African sedative and potentially CNS-acting plants in the GABA-benzodiazepine receptor assay. *Journal of Ethnopharmacology* 100, 210-215.

A.B. Svenningsen, K. Damkjær Madsen, T. Liljefors, **G.I. Stafford**, J. van Staden and A.K. Jäger. **2006**. Biflavones from *Rhus* species with affinity for the $GABA_A$/benzodiazepine receptor. *Journal of Ethnopharmacology* 103, 276-280.

A.K. Jäger, J.P. Almqvist, S.A.K. Vangsøe, **G.I. Stafford**, A. Adsersen and J. Van Staden. **2007**. Compounds from *Mentha aquatica* with affinity to the GABA-benzodiazepine receptor. *South African Journal of Botany* 73, 518-521.

CHAPTER 5

A. Risa, J. Risa, A. Adsersen, **G.I. Stafford**, J. van Staden and A.K. Jäger. **2004**. Acetylcholinesterase inhibitory activity of plants used as memory-enhancers in traditional. *South African Journal of Botany* 70, 664 – 666.

G.I. Stafford, P.D. Pedersen, A.K. Jäger and J. van Staden. **2007**. Monoamine oxidase inhibition by southern African traditional medicinal plants. *South African Journal of Botany*, 73, 384-390.

H.T. Olsen, **G.I. Stafford**, J. van Staden, S.B. Christensen, A.K. Jäger. **2008**. Isolation of the MAO-inhibitor naringenin from *Mentha aquatica* L. *Journal of Ethnopharmacology* 117, 500-502.

G.I. Stafford, P.D. Pedersen, J.C. Chukwujekwu, A.K. Jäger and J. van Staden. **2009**. *Helichrysums*: antibacterial and monoamine oxidase inhibitory activity of South African summer-rainfall species. **In preparation**.

BOOK CHAPTER

G.I. Stafford, AK Jäger and J van Staden. **2008**. African Psychoactive plants. In: Juliani, R. and Simon, J. (Eds). *African Natural Plant Products: New Discoveries and Challenges in Chemistry and Quality*. American Chemical Society (ACS). **In Press.**

CONFERENCE CONTRIBUTIONS FROM THIS RESEARCH

2008: **4th World Congress on Medicinal and Aromatic Plants (WOCMAP IV)**, Cape Town, South Africa **(Attended)**.

Paper: South African medicinal plants used to treat mental illness (**G.I. Stafford**, M.E. Pedersen, A.K. Jäger and J. van Staden).

7th Joint Meeting of GA, AFERP, ASP, PSE & SIF, Athens, Greece August 3–8, 2008

Poster: Cinnamamides from *Piper capense* with affinity to the benzodiazepine site on the $GABA_A$ receptor (M.E. Pedersen, H.B. Rasmussen, B. Metzler, **G.I. Stafford**, J. van Staden, A.K. Jäger).

Poster: South African traditional medicine inhibits the spontaneous epileptiform discharges in slices of the mouse cerebral cortex (M.E. Pedersen, H.T. Vestergaard, **G.I. Stafford**, J. van Staden, A.K. Jäger).

Poster: Effects of South Africa traditional medicine in animal models of depression (M.E. Pedersen, B. Szewczyk, K. Stachowicz, J. Wieronska, J. Andersen, **G.I. Stafford**, J. van Staden, A. Pilc, A.K. Jäger).

Abstracts published in *Planta Medica* 2008; 74.

2007: **55th Annual Congress on Medicinal Plant Research** (The Society for Medicinal Plant Research), Graz, Austria.

Poster: Psychotropic constituents of *Mentha aquatica* L. (J. Van Staden, **G.I. Stafford**, J.P. Almqvist, S.A.K. Vangsøe, H.T. Olsen, S.B. Christensen, A. Adsersen, A.K. Jäger).

2006: **54th Annual Congress on Medicinal Plant Research** (The Society for Medicinal Plant Research), University of Helsinki, Finland.

Poster: *Helichrysums*: antibacterial and monoamine oxidase inhibitory activity of South African summer-rainfall species (J. van Staden, **G.I. Stafford**, P.D. Pedersen, J.C. Chukwujekwu and A.K. Jäger)

Abstracts published in *Planta Medica* 2006; 72: 961-1089

2004: **30th Annual Congress of SAAB** (South African Association of Botanists), University of KwaZulu-Natal, Durban, SA.

Poster: Screening of South African plants for antidepressive activity as selective serotonin re-uptake inhibitors (SSRI) (**G.I. Stafford**, N.D. Nielsen, M. Sandager, J. van Staden and A.K. Jäger)

2003: **Joint International Conference of SAAB (South African Association of Botanists) and ISE (International Society of Ethnopharmacology)**, University of Pretoria, South Africa **(Attended).**

Paper: CNS-affecting plants traditionally used in southern Africa (**G.I. Stafford**, J. van Staden and A.K. Jäger).

ACKNOWLEGDEMENTS

Professor Johannes Van Staden is thanked for his support, and for the opportunities and facilities afforded to me by the Research Centre for Plant Growth and Development. In addition, Professor Van Staden is thanked for financial support provided in part by way of NRF (National Research Foundation) grant-holder bursaries.

Professor Anna Jäger is thanked for her support, and for the opportunities and facilities afforded to me by the Department of Medicinal Chemistry, University of Copenhagen.

The academic, administrative and technical staff in the Research Centre for Plant Growth and Development and the School of Conservation and Biological Sciences, are thanked for their support and encouragement. In particular I would like to thank:

Dr Marnie Light and **Dr Wendy Stirk**;
Mrs Judy Magnusson and **Mrs Lee Warren**;
Mr Martin Hampton, **Mr Rogan Roth** and **Mr Rennie Noble**.

I an grateful to the advice and guidance offered by the members of my Research Committee, **Dr C. Fennell**, **Dr E. Elgorashi**, **Dr W. Stirk**, **M.E. Light** and **Prof J. Van Staden**.

Mrs Alison Young and her dedicated team are thanked for assisting me with my horticultural needs and in the use of the University Botanical Garden.

Dr David Thompson is thanked for assisting with locating, identifying and collecting of plant material. The Curator and Staff of the John Bews Herbarium, **Christina Potgieter** and **Professor Trevor Edwards**, are also thanked in this regard.

I would like to thank my colleagues, all of whom have become friends, **Nicolaj D. Nielsen, Mikkel Sandager, Jofrid Risa, Anlaug Risa, Ann B. Svenningsen, Katrine Damkjær Madsen, Esam E. Elgorashi, J. Peter Almqvist, Stefan A.K. Vangsøe, Helle T. Olsen, Sara Marie Solbek, Pernille D. Pedersen** and **Mikael E. Pedersen**. I look forward to working with many of you again.

Nikolaj, Rikke and **Mikkel** are thanked for making me feel at home during my stay in Copenhagen and for showing me their beautiful city.

I would also like to acknowledge the kind assistance of the late **Professor Zondi** with regard to the isiZulu discussed in this thesis.

Lastly I would like to thank **Fiona** for her support and encouragement.

ABSTRACT

The majority of the population in South Africa use traditional health care to treat various mental conditions. This thesis has two main objectives; to bring together a comprehensive and detailed record of psychotropic plants used in southern Africa by indigenous peoples for medicinal or cultural purposes. Secondly, this research attempts to investigate the validity and rationale of the use of these plants by screening them in various biological assays for psychotropic activity. Plants were selected, based on their traditional use and availability, and were screened in four assays, which detect biological activity of a useful nature. A number of *in vitro* enzymatic and neuronal signal transduction assays were employed in this thesis, the inhibition of the serotonin reuptake transporter protein (SERT); inhibition of catabolic enzymes (e.g. acetylcholinesterase, monoamine oxidase); $GABA_A$- benzodiazepine receptor binding.

The influence of legislation, past and present, on the state of traditional medicine is highlighted. Aspects of the philosophies and practises of the various practitioners of South African traditional medicine will be discussed. An annotated list compiled from available ethnobotanical literature of plants traditionally used for central nervous system-related purposes is provided. It contains more than 330 species, from 94 families, which are currently used or have been used for cultural, medicinal and recreational purposes related to the central nervous system (CNS). Where available, information pertaining to plant part used, preparation method, dosage, route of administration, known and potentially active constituents are included.

Seventy five extracts from 34 indigenous plant species used in South African traditional medicine or taxonomically related to these were investigated for their affinity to the serotonin reuptake transport protein, making use of an in vitro [^{3}H]-citalopram serotonin reuptake transport protein binding assay. Aqueous and 70% ethanolic extracts of various plant parts were screened and 45 extracts derived from 15 plant species showed affinity. The affinity of 12 extracts from four plants was characterized as high (more than 50% inhibition at 5, 1, and 0.5 mg/ml). Plant species with high affinity to the serotonin reuptake transport protein included *Agapanthus campanulatus*, *Boophone disticha*, *Datura ferox* and *Xysmalobium undulatum*. *Agapanthus campanulatus* yielded high activity in aqueous extracts from leaves and flowers. *B. disticha* showed high activity both in aqueous and ethanolic extracts of leaves and bulbs. *D. ferox* showed high activity in aqueous extracts from the seeds and *X. undulatum* showed high activity in the ethanolic extract of the whole plant.

Two compounds, buphanadrine and buphanamine, were isolated by bioassay-guided fractionation on vacuum-liquid-chromatography (VLC) and preparative thin-layer-chromatography (TLC) from *B. disticha*. The structures of the compounds were determined by ^{1}H and ^{13}C NMR. Fractions were tested for affinity to the serotonin transporter in a binding assay using [^{3}H]-citalopram as a ligand. The IC_{50} values of buphanidrine and buphanamine were 274 μM (K_i = 132 μM) and 1799 μM (K_i = 868 μM), respectively. The two alkaloids were also tested for affinity to the $5HT_{1A}$ receptor, but only showed slight affinity.

Aqueous and ethanol extracts of 43 plants that are traditionally used to treat against epilepsy and convulsions were initially tested in the $GABA_A$-benzodiazepine receptor binding assay, where the binding of ^{3}H-Ro 15-1788 (flumazenil) to the benzodiazepine site is measured. The $GABA_A$-benzodiazepine receptor complex is involved in epilepsy and convulsions. Out of the 118 extracts tested, one aqueous and 18 ethanol extracts showed activity. The most active extracts were the ethanolic leaf extracts of *Searsia tridentata*, *Searsia rehmanniana* and *Hoslundia opposita* and the ethanolic corm extract of *Hypoxis colchicifolia*, which all showed good dose-dependent activity. A further forty-six ethanol extracts from another 35 species, both indigenous and exotic that are traditionally used predominantly as sedatives or to treat various CNS-related ailments were tested in the $GABA_A$-benzodiazepine receptor-binding assay. Out of the 46 extracts tested, seven showed good activity and 10 showed moderate activity. The most active extracts were the ethanolic leaf extracts of *Arctopus echinatus*, *Artemisa afra*, four *Helichrysum* species and *Mentha aquatica* which all showed good dose-dependent activity.

Two biflavonoids with activity in the ^{3}H-Ro 15-1788 (flumazenil) binding assay were isolated by high pressure liquid chromatography (HPLC) fractionation of the ethanol extract of the leaves from *Searsia pyroides*. The structures of the two biflavonoids were elucidated by nuclear magnetic resonance spectroscopy (NMR) to be agathisflavone and amentoflavone. Agathisflavone and amentoflavone competitively inhibited the binding of ^{3}H-Ro 15-1788 with a K_i of 28 and 37 nM, respectively. Extracts of *Searsia dentata* and *Searsia pentheri* were not as active as the extract from *Searsia pyroides*; both were found to contain apigenin and agathisflavone. The monomer apigenin, agathisflavone and amentoflavone were fitted into a pharmacophore model for ligands binding to the $GABA_A$ receptor benzodiazepine site. This reflected the affinities of the compounds in the [^{3}H]-flumazenil binding assay.

Mentha aquatica, a mint that is found in Europe and Africa, is used in Zulu traditional medicine for spiritual purposes. The ethanolic leaf extract showed a strong affinity to the GABA-benzodiazepine receptor. Viridiflorol from the essential oil and (*S*)-naringenin from an ethanolic extract was isolated by

bioassay-guided fractionation using binding to the GABA-benzodiazepine site. Viridiflorol had an IC_{50} of 0.19 M and (*S*)-naringenin of 0.0026 M.

Twenty plants used in Zulu traditional medicine for several CNS-related ailments were screened for MAO inhibition and specific MAO-B inhibition activity. MAO-B inhibitors are currently employed in the treatment of neurodegenerative related illnesses such as Parkinson's and Alzheimer's diseases. A photometric peroxidase linked assay was used to determine the inhibition of the oxidative deamination of tyramine by MAO isolated from rat liver. *Ruta graveolens* exhibited the best MAO inhibitory activity (ethyl acetate leaf extract = IC_{50} 5 ± 1 μg/ml, petroleum ether extract = 3 ± 1 μg/ml) and specific MAO-B inhibition (ethyl acetate leaf extract = IC_{50} 7 ± 6 μg/ml petroleum ether extract = 3 ± 1 μg/ml). *Schotia brachypetala*, *Mentha aquatica* and *Gasteria croucheri* also exhibited good MAO-B inhibition activity.

Six extracts of varying polarity of *Mentha aquatica* were tested in a photometric peroxidase linked MAO bioassay. The 70% ethanol extract had highest inhibitory activity. (*S*)-Naringenin was isolated from the extract by bioassay guided fractionation on VLC and preparative TLC. The structure of the compound was determined by ^{1}H, ^{13}C and ^{13}C-DEPT NMR and optical rotation. The IC_{50} values for MAO inhibition by naringenin were 342 ± 33 μM for the rat liver mitochondrial fraction, 955 ± 129 μM for MAO-A and 288 ± 18 μM for MAO-B respectively.

South African traditional medicine clearly utilizes many botanical species with CNS-related activity. Only a small number of the more than 330 southern African plant species reported to treat or alter the CNS have been scientifically evaluated. To date very few of the active compounds have been isolated and identified.

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ABBREVIATIONS

(n)	noun
(v)	verb
3-MA	3-methoxytyramine
5-HT	5-hydroxytryptamine
A	Arabic
AA	amino acid
AchE	acetylcholinesterase
AcHEI	acetylcholinesterase inhibitor
Ad	adrenaline
AD	Alzheimer's disease
Af	Afrikaans
ATP	adenosine triphosphate
B	Botswana
BBB	blood-brain barrier
BZD	benzodiazepine
CA	catecholamine
CACA	*cis*-4-aminocrotonic acid
cAMP	cyclic adenosine monophosphate
CNS	central nervous system
COMT	catechol-*0*-methyl transferase
D_1	dopamine receptors, type 1
D_2	dopamine receptors, type 2
DA	dopamine
DAG	diacylglycerol
DEPT-NMR	distortionless enhancement through polarisation transfer-nuclear magnetic resonance
DNA	dioxyribose nucleic acid
DOMA	3,4-dihydroxymandelic acid
DOPA	dihydroxyphenylalanine
DOPAC	3,4- dihydroxyphenylacetic acid
DOPEG	3,4-dihydroxyphenylglycol
DOPET	3,4-dihydroxyphenylethanol
DMT	*N,N*-dimethyltryptamine
E	Europeans
EC	Eastern Cape Province
FS	Free State Province
G	Gauteng Province
GABA	γ-aminobutyric acid
$GABA_AR$	$GABA_A$ receptor
Glu	Glutamic acid
Gly	Glycine
GPCR	G-protein coupled receptor
GTP	guanosine triphosphate
HIV	human immunodeficiency virus
HPLC	high performance (pressure) liquid chromatography
HVA	homovanillic acid
IC_{50}	concentration of compounds causing 50% inhibition of radioligand specific binding to the receptors
INA	Inyangas' National Association
IP_3	inositol-1,4,5-triphosphate
K_d	constant of disassociation
KOR	kappa opioid receptor
KZN	KwaZulu-Natal
L	Lesotho
LIM	Limpopo
LSD	lysergic acid diethylamide
M	Mpumalanga Province
MA	monoamine
MAO	monoamine oxidase
MAO-A	monoamine oxidase subtype A
MAO-B	monoamine oxidase subtype B
MAOI	monoamine oxidase inhibitor
MHPG	3-methoxy-4-hydroxy-phenylglycol
MOPET	3-methoxy-4-hydroxyphenylethanol
MPP	1-methyl-4-phenylpyridinium
N	Namibia
NA	noradrenaline
nACh	nicotinic acetylcholine
nACh-R	nicotinic acetylcholine receptor
NC	Northern Cape Province
NCOP	National Council of Provinces
Nd	Ndebele
NE	norepinephrine
NMR	nuclear magnetic resonance
NU	John Bews Herbarium (University of KwaZulu-Natal
NW	North-West Province
PD	Parkinson's disease
PHC	primary health care
PIP_2	phosphatidylinositol-4-5-diphosphate
QSAR	quantitative structure–activity relationship
S	Swaziland
San	San, SA/Namibia/Angola
SERT	serotonin transporter
Sh	Shona, Zimbabwe
SSRI	selective serotonin re-uptake inhibitor
Sth	Sotho (this language group includes Northern Sotho, South Sotho and Tswana)
Sw	Swazi
TA	tricyclic antidepressants
TBPS	*t*-butylbicyclophosphorothionate
TCM	traditional Chinese medicine
THC	tetrahydrocannabinol
THIP	4,5,6,7-tetrahydroisoxazolo-5,4-*c*-pyridin-3-ol
TLC	thin layer chromatography
Ts	Tsonga
UK	United Kingdom
US	United States of America
UV	ultraviolet
V	Venda
VLC	vacuum liquid chromatography
VMAT	vesicular monoamine transporter
VTA	ventral tegmental area
WC	Western Cape Province
WHO	World Health Organisation
X	Xhosa
Z	Zulu

CHAPTER ONE

Introduction:

Plants and the central nervous system

1.1. Introduction

This Chapter will introduce the reader to the intriguing variety of plants that have an effect on the central nervous system (CNS). The basic principles of brain anatomy and function are briefly outlined to provide a framework within which to discuss the effect of plants on the CNS.

Botanically derived preparations, other than for nutritional value, have been used for a variety of spiritual, therapeutic and recreational reasons for thousands of years. Their use is not unique to any one civilization, culture or historical era. Archaeological evidence and pollen analysis from a Neanderthal burial site in modern day Iraq date the use of medicinal plants to 50,000 B.C. (SOLECKI, 1975).

Among the plants used by humans, those able to alter the consciousness and the senses have drawn particular consideration. Often surrounded by mystic superstitions, magical thoughts and religious rituals, they have often been revered (**Figure 1.1**). In the early stages of development, humans needed to explain all natural phenomena, and without an adequate understanding of biological systems, these plants were considered the 'residences of divinities or other spiritual forces', some were even deemed gods (SCHULTES and HOFMANN, 1992).

Figure 1.1. Symbolic figure on the front of the *Journal of Ethnopharmacology*, representing the head of a goddess or a female worshipper, adorned with poppy capsules (DE SMET, 1996).

Plants with an effect on the central nervous system, referred to as psychotropic plants, are still used by modern societies, although most, such as coffee, tea, chocolate and various alcoholic beverages are generally not considered to be psychotropic drugs. The indigenous ethnic groups of sub-Saharan Africa have also used various psychotropic agents, such as alcoholic beverages, psycho-stimulants, and hallucinogens, since earliest pre-historic times. Some of these agents have been used on a global scale (*Datura* sp.), whereas others are more typical or even unique to Africa (SCHULTES, 1981; DE SMET, 1996).

Most research on psychotropic plants has focus on the New World (SCHULTES, 1967; DE SMET, 1996). A large majority of researchers in the field agree that there is insufficient documentation and scientific investigation of African psychotropic plant use (SMITH, CROUCH, GERICKE and HIRST, 1996; VAN WYK and GERICKE, 2000; SOBIECKI, 2002). Psychoactive plants, in particular hallucinogens (psychodysleptics), have been avoided or have been assigned as low priority by researchers due to the stigma attached to substance use and abuse (CARLINI, 2003; WINKELMANN and DOBKIN DE RIOS, 1989). This has excluded the possibility that these plants could also have beneficial properties to treat mental disease and some psychic ailments.

Early literature and documentation of psychotropic plant use is littered with bias and scepticism. In this respect, it is pertinent to quote a sentence from the first description (1651) of a Mexican hallucinogenic plant (*ololiuqui*): "*A thousand visions and satanic hallucinations appeared to them*" (cited in HOFMANN, 1982). CARLINI (2003) also adds that "most psychoactive plants were first used by the so-called primitive cultures; their occasional use by the European occidental culture was relegated to a second plan, being considered as sorcerer's therapeutics and often viewed in a negative light".

However, investigation of psychoactive plants and their mechanisms of action have provided valuable insight into the neurochemistry of many CNS diseases (LEWIN, 1924; NICHOLS, 2004). The observation that serotonin and lysergic acid diethylamide (LSD) share structural and pharmacological properties led to the suggestion that biogenic amines, like serotonin, are involved in mental disorders such as schizophrenia (GADDUM and HAMEED, 1954; WOOLEY and SHAW, 1954). Lysergic acid diethylamide is the analogue of ergot alkaloids produced by *Claviceps purpurea* a fungus associated with incorrectly stored grains. Additionally, the active ingredient in *Rauwolfia serpentina* (resperine) has been shown to deplete biogenic amines and induce depression, therefore suggesting that a lack of serotonin and/or noradrenalin may be the cause of this pathology (VERTULANI and SULSER, 1975). Current basic understanding of mental illness as neurochemical diseases, as well as science's ability to treat these disorders has been greatly enhanced through the study of psychoactive plants.

The ethnobotanical approach to selecting plants for screening programmes has on numerous occasions been shown to be successful (BALICK, 1990; FOURIE, SWART and SNYCKERS, 1992; COX and BALICK, 1994, BALICK and COX, 1996). This thesis hopes to achieve two main objectives: to consolidate literature on the traditional use of psychotropic plants and to screen a large proportion of these plants in relevant assays to validate their traditional use. It is hoped that this will serve to fill the gap in literature with respect to African psychotropic plant use and further validate traditional medicine, thus narrowing the gap between western and traditional therapies. The specific aims and objectives are dealt with in detail at the end of this Chapter (Page 39).

1.2. The central nervous system

This section reviews basic principles of brain anatomy and function to provide a framework within which to discuss the effect of plants on the CNS. The nervous system is the body's major communication system, and is divided into central and peripheral regions. The central nervous system consists of the brain and spinal cord, and the peripheral nervous system consists of all other nerves. Although thought processes and reason are most commonly associated with the CNS, it should also be noted that almost every aspect of physiological function is affected by CNS activity. 'Brain death' is widely accepted as the definition of the end of human life (HOUGHTON, 2005).

The spinal cord controls reflex actions, and relays sensory and motor information between the body and the brain, so that the organism can respond appropriately to its environment. The region of the brain where it meets the spinal cord is called the hindbrain (rhombencephalon), and is composed of the medulla (myelencephalon) and metencephalon (pons and cerebellum) (**Figure 1.2.1**). The medulla is vital to sustaining life, and controls processes such as breathing, heartbeat and blood flow. The medulla also contains receptors for the **opioid drugs**, such as **heroin** and **morphine**, which is why these drugs can cause respiratory depression and death (WORLD HEALTH ORGANIZATION, 2004). The pons is a relay station for signals being carried from the cortex to the cerebellum, which is involved in body movements and coordination.

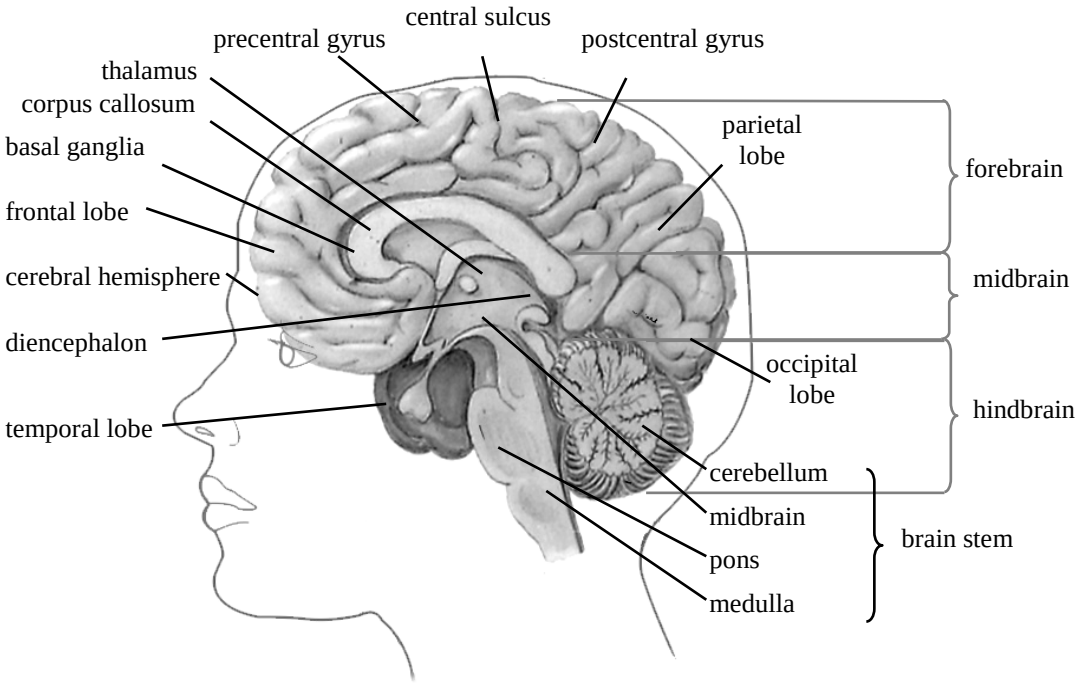


Figure 1.2.1. Anatomy of the human brain.

Above the hindbrain is the midbrain (mesencephalon), which contains two areas that are important with respect to psychotropic plant use, in particular substance dependence. The ventral tegmental area (VTA) is rich in dopamine cell bodies, and projects to the limbic system and forebrain regions. The VTA is involved in signalling the importance of stimuli that are critical to survival such as those associated with feeding and reproduction. However, many psychoactive drugs also have powerful effects on the VTA, which contributes to the development of dependence by signalling to the brain that psychoactive substances are very important from a motivational perspective (WORLD HEALTH ORGANIZATION, 2004). The dopaminergic projection from the VTA to the nucleus accumbens (discussed below, **Figure 1.2.3**) is known as the mesolimbic dopamine system, and is the neurotransmitter system that is most strongly implicated in the **dependence-producing potential** of psychoactive drugs (WISE, 1998).

Another important midbrain structure is the substantia nigra, which also has dopaminergic projections to the forebrain, but these pathways are involved in coordinating and executing movements of the body. Degeneration of neurons in the substantia nigra leads to the characteristic symptoms of **Parkinson disease**.

Lastly, there is the forebrain (prosencephalon), which is composed of the diencephalon and the telencephalon (cerebral hemispheres) (**Figure 1.2.1**). Important areas of the diencephalon (**Figure 1.2.2**) are the thalamus, the hypothalamus, and the posterior lobe of the pituitary gland. The hypothalamus is critical for regulating hormonal signals and basic bodily functions concerning, for example, water balance, body temperature and reproductive hormones as well as responding to changes in these functions. The hypothalamus also secretes hormones that travel to the nearby posterior lobe of the pituitary gland. The thalamus functions as a relay station for sensory and motor information going to and from the cortex to other areas of the brain and body.

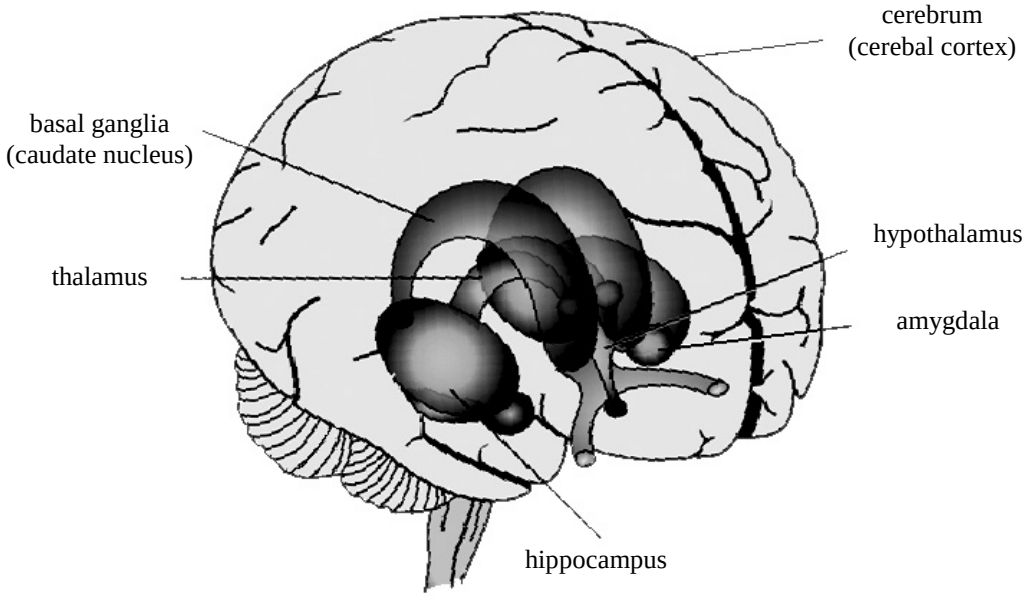


Figure 1.2.2. Structure of the inner human brain, including the limbic system.

The **telencephalon** of the **forebrain** is the most highly developed area of the brain, and is composed of two cerebral hemispheres separated by the longitudinal fissure. The outermost layer of the brain is the **cortex**, which is made up of layers of nerve cells or neurons, and has a highly folded organization that increases its surface area and the number of neurons that it contains. Beneath the cortex run millions of axons that interconnect the neurons and allow the different areas of the brain to communicate and to coordinate behaviour. Each hemisphere of the brain is divided into four lobes: frontal, parietal, temporal, and occipital (**Figure 1.2.1**). Different areas of the cortex are specialized for different functions. The **motor association** cortex, for example, is involved in **coordinating movements of the body**, and the primary motor cortex is involved in executing this function. Similarly, there is a primary sensory cortex that receives information from each of these sense organs (COOPER, BLOOM and ROTH, 1982).

Information from the primary **sensory areas** goes to sensory association areas of the **cortex**, which are involved in perception and memory connected with the sense organs. Here information from several sense organs can be combined to form complex perceptions. The cortex is involved in many aspects of psychotropic plant use, from the primary effects of psychoactive drugs on **sensations and perceptions**, to the complex behaviours and thoughts involved in **drug craving** and uncontrolled use (WORLD HEALTH ORGANIZATION, 2004).

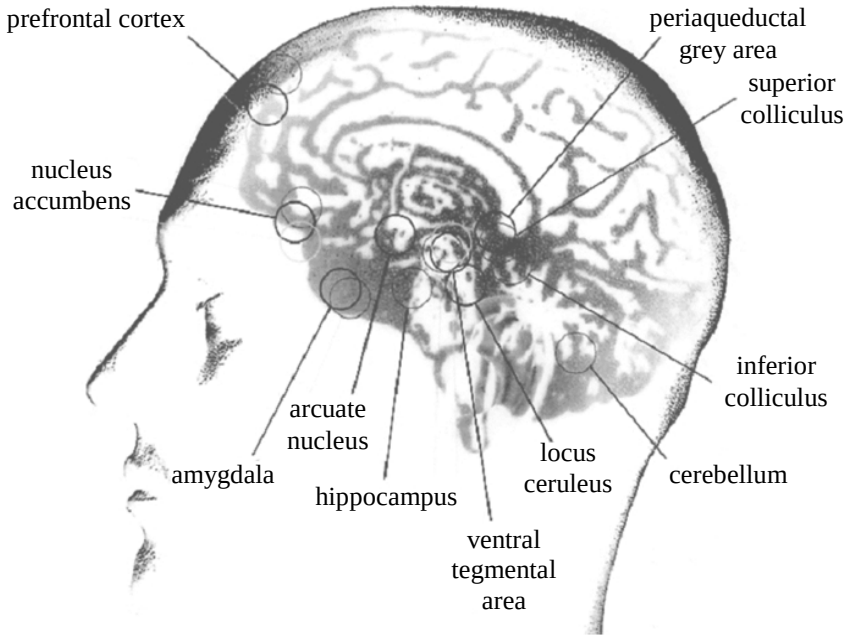


Figure 1.2.3. The 'pleasure circuit', main regions targeted by addictive plant substances. Cocaine and amphetamines target the ventral tegmental area, the neurons of which connect to the 'pleasure circuit' (mesolimbic dopamine system). Opiates also target this region as well as regions activated by the brain's natural opiates, like beta-endorphins. Alcohol also targets the ventral tegmental area and 'pleasure circuit', but goes further. It also affects the cortex (thought), cerebellum (movement), amygdala (emotion) and binding to the $GABA_A$ receptor (sedative) (after GROPPETTI, CERESOLI, MANDELLI and PARENTI, 1990).

Beneath the cortex are several other important structures. The basal ganglia (**Figure 1.2.2**) are structures involved in voluntary motor behaviour and consist of the caudate, putamen, globus pallidus and

amygdala. The caudate and putamen together are known as the striatum. Just below the striatum is a key area for substance dependence and motivation, known as the nucleus accumbens**,** which is made up of core and shell regions (**Figure 1.2.3**). The nucleus accumbens is a very important brain area involved in motivation and learning, and signalling the motivational value of stimuli (DURLACH-MISTELI and VAN REE, 1992; ROBBINS and EVERITT, 1996; CARDINAL**,** PARKINSON, HALL and EVERITT, 2002). Psychoactive substances **increase the production of dopamine** in the nucleus accumbens, which is thought to be an important event in **drug reinforcement** (**Figure 1.2.3**).

The **limbic system** (**Figure 1.2.2**) is an interconnected series of structures that are important in relation to **emotion**, **motivation** and **learning**. The limbic system plays a vital role in the development of **dependence**, and interacts with the cortex and nucleus accumbens. Important structures of the limbic system are the **hippocampus**, which is associated with memory, and the amygdala, which is critical in **emotional regulation**. All of these areas receive sensory information from other brain areas to help coordinate the appropriate **emotional** and **behavioural response** to external stimuli (WORLD HEALTH ORGANIZATION, 2004).

Neurons

Communication in the brain takes place between nerve cells or neurons. Psychoactive substances alter many aspects of communication between neurons, as will be discussed below. The terms ‘neuron’ and ‘synapse’ as components of an integrated nervous system were first described by Sir Charles Sherrington while studying the acetylcholine receptor (nicotinic, nACh-R). Sherrington shared the Nobel Prize for Medicine (1932) with Lord Edgar Adrian for his work on electrical potential differential-based neurotransmission.

Neurons are highly specialized cells that exist in many shapes, sizes and varieties. However, they share the following basic structural regions: cell body or soma, dendrites, axon, and terminal buttons (**Figure 1.2.4**) (CARLSON, 1988). The cell body, or soma, is the metabolic centre of the neuron, and contains the nucleus and other structures that sustain the neuron (**Figure 1.2.4**).

The nucleus plays a role in mature neurons, where it is used to synthesize proteins in response to a wide variety of stimuli. Psychoactive substances can affect the expression of DNA, resulting in short-term or long-term changes in neuronal function, and ultimately, behaviour. Dendrites are highly branched processes extending from the cell body of the neuron, which receive chemical messages from other neurons (**Figure 1.2.5**). This branching, and the presence of dendritic spines (small swellings on the surface of a dendrite with which a terminal button from another neuron forms a synapse), allows many different neurons to converge on a single nerve cell, facilitating the coordination and integration of many complex messages.

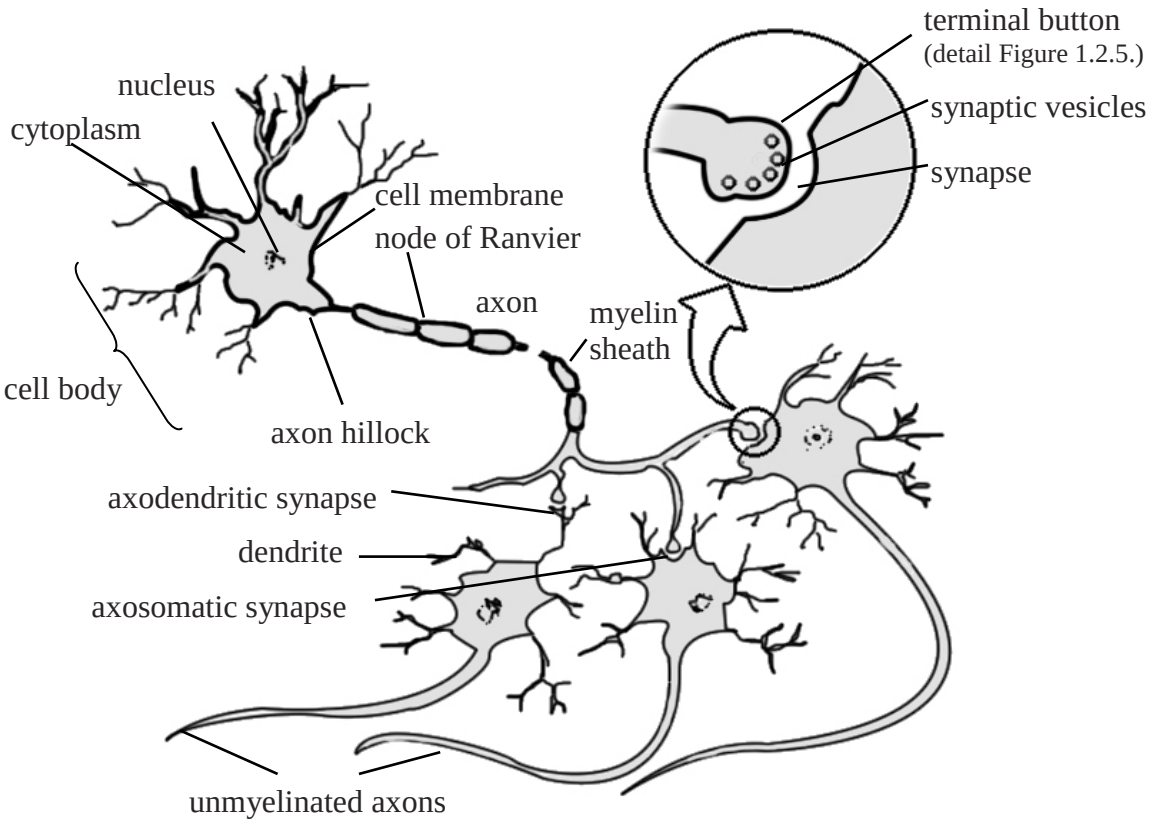


Figure 1.2.4. Structure of a neuron (after PINEL, 1990).

The number of dendritic spines can increase or decrease following exposure to psychoactive substances (SKLAIR-TAVRON, XING SHI, LANE, HARRIS, BUNNEY and NESTLER, 1996; ROBINSON and KOLB, 1999; EISCH, BARROT, SCHAD, SELF and NESTLER, 2000), thus altering communication between neurons, and most likely contributing to the behavioural and neurological effects of the substances. The axon is a long slender process extending from the cell body, which carries information from the cell body to the terminal buttons. Certain chemicals such as neurotransmitters are transported along the axon, and it also propagates nerve impulses. The area where the axon leaves the cell body is known as the axon hillock.

The synapse

The overall architecture of a synapse is illustrated in **Figure 1.2.5.** The presynaptic terminal contains vesicles, which are filled with neurotransmitters. Presynapse and postsynapse are separated by a narrow synaptic cleft into which the neurotransmitters are released from the vesicles via exocytosis. Transmitters diffuse across the synaptic cleft and, after a lag period of about 0.5 ms, bind to a receptor on the postsynaptic cell. The ion permeability of the postsynaptic membrane is changed in the next step causing a sudden change in the corresponding membrane potential. In neurons within the brain, this electric disturbance can induce an action potential, which will result in a change of mental state. Many nerves are excitatory, however, the binding of neurotransmitters to inhibitory receptors on the postsynapse causes

the opening of K^+ and Cl^- ion channels that hyperpolarise the membrane and thus blocks the generation of an action potential. Neuroreceptors are found at the post- and presynaptic membrane. Activation of presynaptic receptors usually leads to an inhibition of neurotransmitter release, whereas their inhibition results in an enhanced release of neurotransmitters. Thus the neurotransmitters and neuroreceptors are the basic elements for signal transduction in the synapses of the central nervous system. These will be discussed in more detail in the following sections.

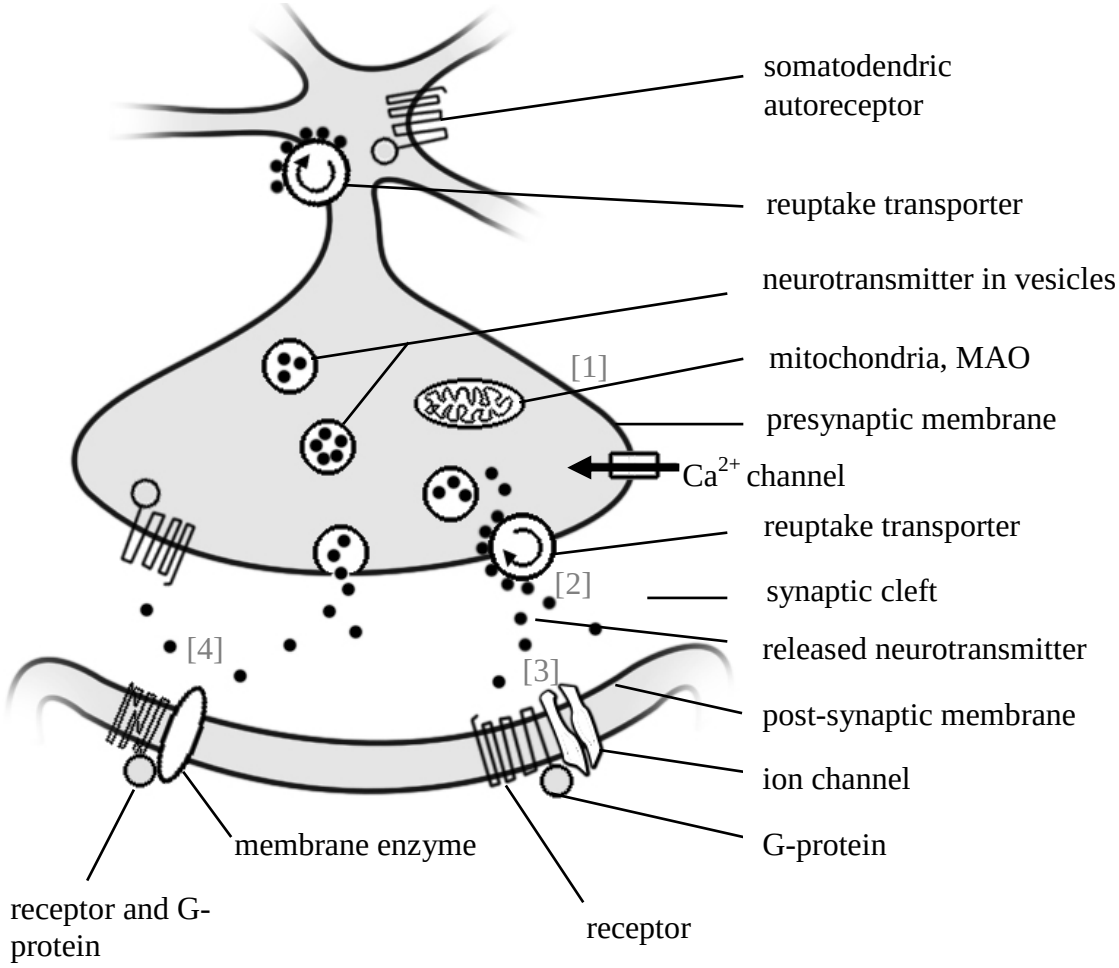


Figure 1.2.5. The monoamine neuron and the site of action of plant compounds screened for in this thesis. Monoamines are synthesized in the presynaptic nerve terminal, stored in the storage vesicles by vesicular monoamine transporter and released by Ca^{2+} -dependent exocytosis. After release, they act on postsynaptic or presynaptic receptors. Most of the monoamine receptors are linked to G proteins, the activation of which will open ion channels or either activate or inactivate membrane enzymes. The inactivation of the monoamine is done by active reuptake into the nerve terminal and/or glial cells using a specific Na^+/Cl^--dependent transporter. After reuptake into the nerve terminal, the monoamine is taken up again by the storage vesicles using the vesicular transporter or exposed to oxidation by MAO. The acute effect of antidepressants on the monoamine system is **[1]** inhibition of neuronal MAO; **[2]** inhibition of the reuptake of the monoamines; **[3]** $GABA_A$- benzodiazepine receptor binding; and **[4]** the inhibition of membrane enzymes, in this case acetylcholinesterase (AchE). Adapted from STAHL (1998) and ELHWUEGI (2004).

Ligand-gated ion channels

Two classes of membrane residing neuroreceptors can be distinguished. The fast ligand-gated channels and the slower G-protein coupled receptor (GPCR). These are structurally remarkably similar across a wide range of animals**.** The ligand-gated channel belongs to the ion-channel complex (**Figure 1.2.6**). When a neurotransmitter binds, a conformational change induces the opening of an ion channel. Depending on the geometry and polarity of the 'gate', a selective permeability of the channel is achieved for Na^+, K^+, Ca^{2+} and Cl^- ions. The driving force is provided via the ion concentration in the cells and the extracellular space. The ligand-gated ion channels include the excitory nicotinic acetylcholine, glutamate/aspartate, ATP_{P2z}, and the 5-HT_3 (serotonin) receptor and the inhibitory glycine and $GABA_A$ receptor (**Table 1.3.1**) (WINK, 2000).

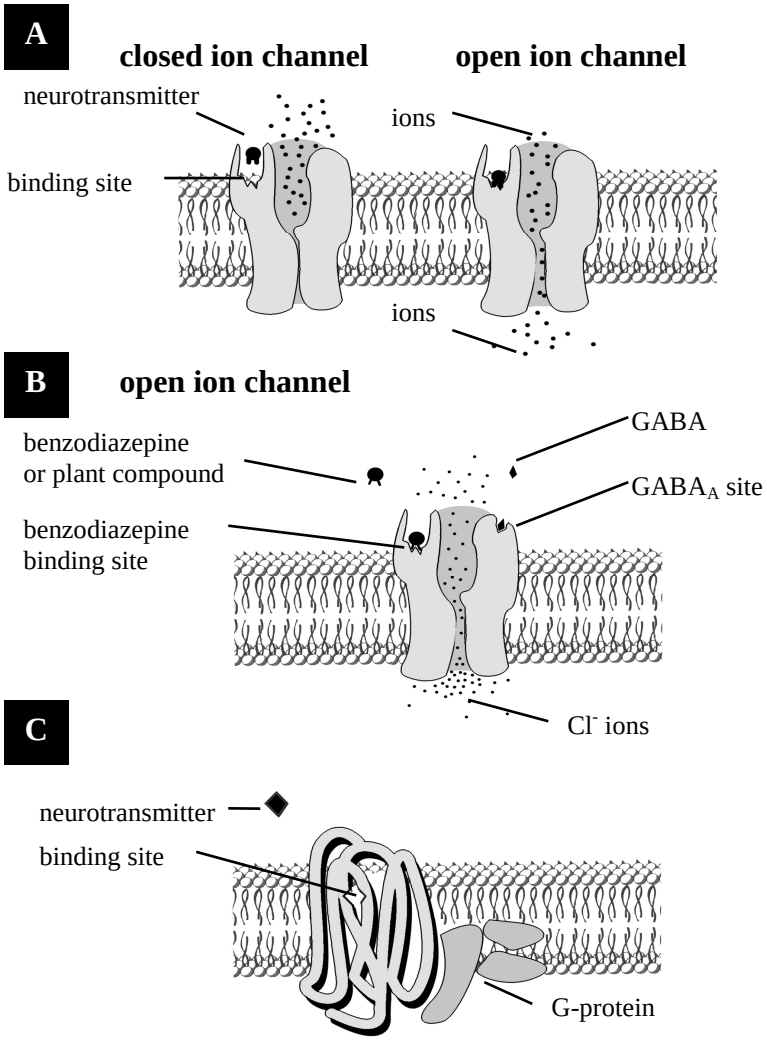


Figure 1.2.6. Schematic illustration of ligand-gated ion channels ([A]: nAchR; [B] $GABA_A$ receptor) and [C] G-protein coupled neuroreceptors (After WINK, 2000).

The G-protein coupled receptors (**Figure 1.2.6 [C]**) are much more numerous and complex than the ligand-gated ion channels (MYSLIVECEK and TROJAN, 2000). They include muscarinic acetylcholine, adenosine, adrenergic, serotonergic (except 5-HT_3), $GABA_B$, glutamate, histamine, and opiate receptors. They share a common architecture, having seven transmembrane domains and three internal and three

external loops each. When the corresponding neurotransmitter binds, the receptor changes its three-dimensional structure, inducing a conformational change in an adjacent G-protein molecule, consisting of three subunits α, β and γ. G-proteins function as an 'on-off switch', which is off when the α-subunit binds GDP. Binding of a ligand to the receptor causes the G-protein to release its bound GDP and bind to GTP, converting the α-subunit to the 'on' state. The α-subunit dissociates and either interacts with an ion channel, or activates/inhibits the enzymes of a second messenger (**Figure 1.2.6**), such as adenyl cyclase (making cAMP, an allosteric regulator of protein kinases and other such proteins), or phospholipase C (splitting phosphatidylinositol-4-5-diphosphate (PIP_2) into inositol-1,4,5-triphosphate (IP_3) which activates Ca^{2+} release channels in the endoplasmic reticulum setting free the second messenger Ca^{2+}) and diacylglycerol (DAG which activates proteinkinase C). Whereas the hydrolysis of GTP (bound to the α-subunit) switches the G-protein back to the inactive state, the second messenger can regulate various ion channels, protein kinases and other proteins (WINK, 2000).

1.3. Neurotransmitters

Sir Hendry Dale (UK) and Otto Loewi (Germany) were awarded the Nobel Prize for Medicine (1936) for their contribution to our understanding of chemical neurotransmission. Their work described the neurotransmitter acetylcholine.

A neurotransmitter can be defined as a chemical substance that is released via the synapse from one neuron and that affects another cell in a specific manner (KANDEL and SCHWARTZ, 1985). A neurotransmitter must also meet the following criteria:

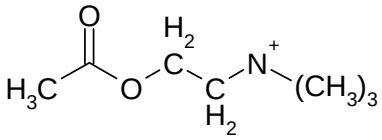
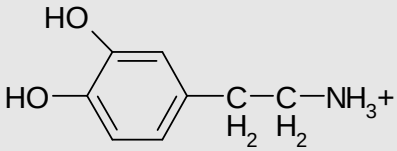
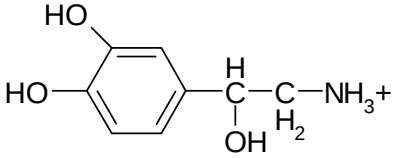
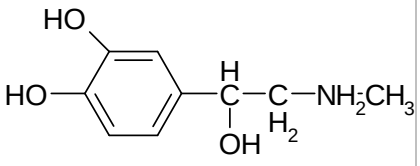
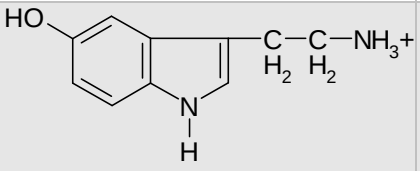
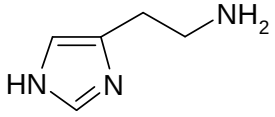
- be synthesized in the neuron;
- be present in the presynaptic neuron;
- be released in sufficient quantity to have a postsynaptic effect;
- have the same effect whether released by natural means (endogenously) or whether applied as a drug (exogenously); and
- it must have a specific mechanism for its removal from the synaptic cleft.

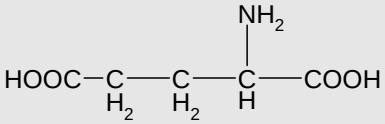
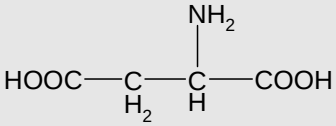
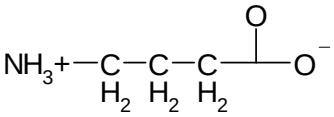
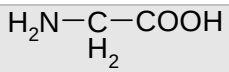
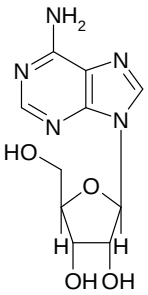
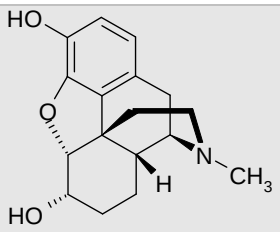
Many types of neurotransmitters have been discovered so far, but in general there are three categories: amino acid neurotransmitters, amino acid-derived neurotransmitters, and peptides (chains of amino acids) (**Table 1.3.1**). The amino acid transmitters include glutamate, GABA, glycine and aspartate. The monoamines, noradrenalin and dopamine (catecholamines) and serotonin (indoleamine) are derived from amino acids. Large molecule peptide neurotransmitters are generally synthesized in the cell body, and transported along the axons to the synapse. Small molecule neurotransmitters can be synthesized in the terminals.

There are distinct regions of the brain where cell bodies for a specific neurotransmitter exist, and other regions or "projection areas" where the axons from those cell bodies project to, and where the

neurotransmitter is ultimately released. Thus, not every neurotransmitter is released in every area of the brain. This allows certain areas of the brain to perform specific functions. Some of the more important neurotransmitters relevant to this work are discussed below, in particular the monoamine neurotransmitters (DAHLSTRÖM and FUXE, 1962), GABA and acetylcholine.

Table 1.3.1. Simplistic characterisation of major neurotransmitters and receptors (After WINK, 2000).

Receptor	Transmitter	Receptor Subtype	Mechanism
Cholinergic	***Monoamines* (*or Biogenic Amines*)**  acetylcholine	nicotinic muscle type nicotinic neuronal type muscarine M_1, M_3 muscarine M_2, M_4	cation channel (Na^+>K^+) cation channel (Na^+>K^+) G-protein (IP_3/DAG) G-protein (cAMP↓)
Dopaminergic	 dopamine	dopamine D_1-D_5	G-protein (cAMP↓↑)
Adrenergic	 noradrenalin  adrenaline	alpha 1_{A-D} alpha 2_{A-D} beta β_1-β_3	G-protein (IP_3/DAG) G-protein (cAMP↓) G-protein (cAMP↑)
Serotonergic	 serotonin	5-$HT_{1A,B,C,D,E,F}$ 5-$HT_{2A,B,C}$ 5-HT_3 5-$HT_{4,5,6,7}$	G-protein (cAMP↓) G-protein (IP_3/DAG) ion channel G-protein (cAMP↑)
Histaminergic	 histamine	H_1 H_2, H_3	G-protein (IP_3/DAG) G-protein (cAMP↑)

Receptor	Transmitter	Receptor Subtype	Mechanism
Glutaminergic	***Amino acids***		
	 glutamate	NMDA	$Na^+/K^+/Ca^{2+}$ channel
		AMPA	$Na^+/K^+/Ca^{2+}$ channel
		kamate	$Na^+/K^+/Ca^{2+}$ channel
		$mGluR_1$, $mGluR_5$	G-protein (IP_3/DAG)
	 aspartate	$mGluR_{2, 3, 4, 6}$	G-protein (cAMP↓)
GABAergic	 GABA	$GABA_A$	Cl^- channel
		$GABA_B$	G-protein (cAMP, Ca^{2+}, K^+)
Glycinergic	 glycine		Cl^- channel
Purinergic	***Other Neurotransmitters***		
	 adenosine	$P_{1\ A1-A3}$	G-protein ($cAMP/IP_3$)
	ATP	P_{2x}, P_{2z}, P_{2T}	cation channel
		P_{2y}, P_{2U}	G-protein (IP_3/DAG)
Opiate	 morphine, endorphins	μ, δ	G-protein (cAMP↓)
		κ	G-protein (Ca^{2+}↓)

The monoamines

The monoamines will be discussed in some detail as they feature prominently in the research presented in this thesis. The monoamines as neurotransmitters share certain properties but differ in their brain distribution, the type of receptors upon which they act and the mechanisms of their actions. In the following sections, the common properties that the monoamines share will be discussed, followed by a brief description about each monoamine in terms of its distribution, the types of receptors they interact with and the mechanisms of the actions.

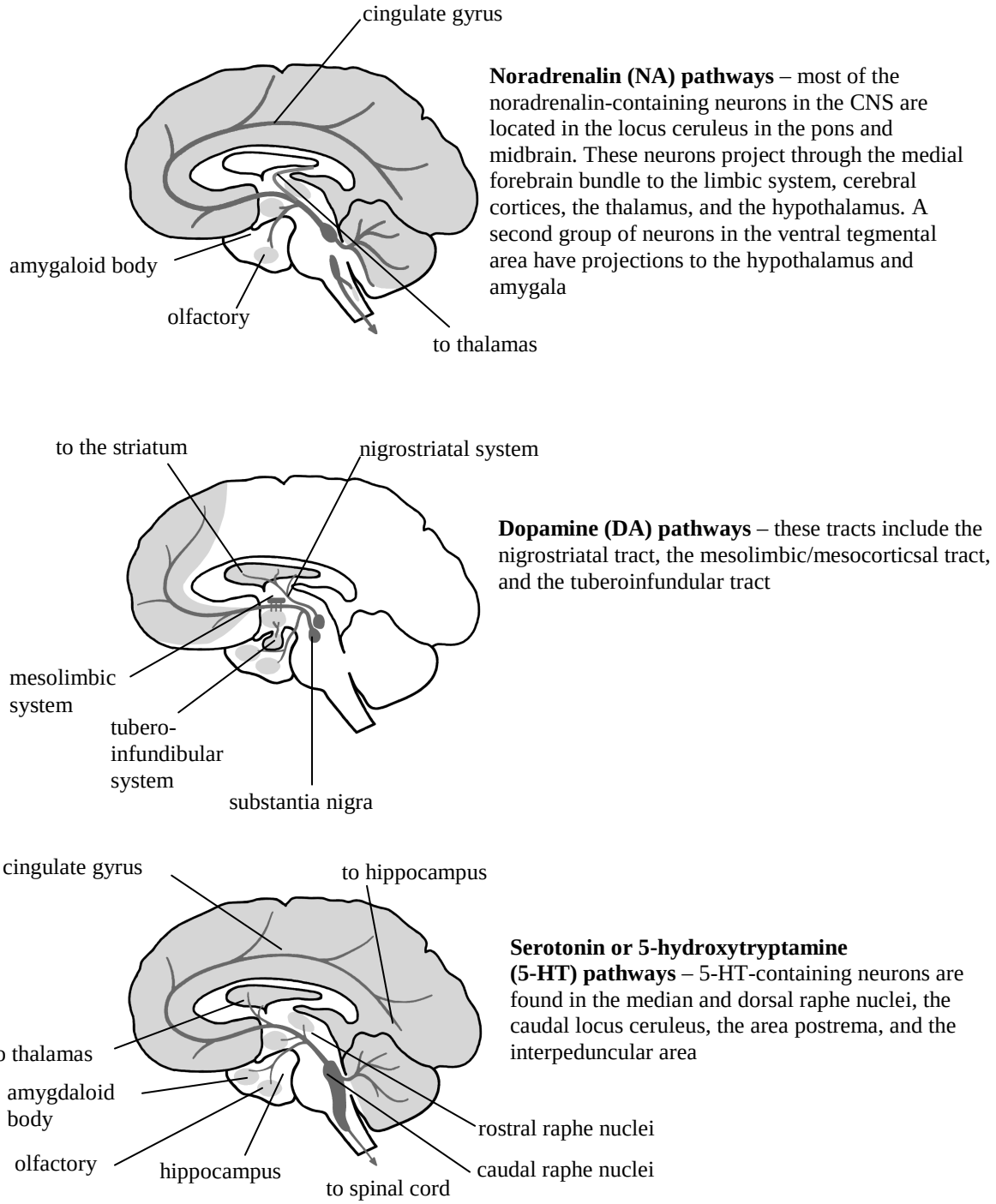


Figure 1.2.8. Neurotransmitter pathways and receptor distribution (adapted from RANG, DALE and RITTER, 1996).

Noradrenalin (NA), adrenaline (Ad) and dopamine (DA) are also known as catecholamines (CA). These monoamines share a common pathway in their synthesis, where they are synthesized from the same precursor (tyrosine) which is converted inside the nerve terminal by tyrosine hydroxylase (the rate-limiting step enzyme) to 3,4-dihydroxyphenylalanine (DOPA). DOPA is then converted to DA, which is converted to NA by dopamine-β-hydroxylase (IVERSON, 1991; BOOIJ, VAN DER DOES, BENKELFAT, BREMNER, JCOWEN, FAVA, GILLIN, LEYTON, MOORE, SMITH and VAN DER

KLOOT, 2002). The other monoamine serotonin is also known as 5-hydroxytryptamine (5-HT). Serotonin is synthesized from tryptophan, which is converted inside the nerve terminal to 5-hydroxytryptophan by tryptophan hydroxylase (the rate-limiting step) then to 5-HT (FULLER, 1980; 1995; RIEDEL, KLAASSEN and SCHMITT, 2002).

All monoamines after their synthesis are concentrated in vesicles at the nerve terminal by a specific vesicular monoamine transporter (VMAT) (NJUS, KELLEY, and HARDABEK, 1986). Using a homology cloning strategy, VMAT-1 and VMAT-2 were successfully isolated in humans. VMAT-1 is primarily present in endocrine and paracrine cells of peripheral organs. On the other hand, VMAT-2 is the predominant monoamine vesicular transporter in the central nervous system (MASSON, SAGNE, HAMON and EL MESTIKAWY, 1999; ELHWUEGI, 2004). The accumulation of intraneuronal monoamines into storage vesicles acts as an amplification step for the overall process of Na^+-dependent uptake of these molecules from the extracellular space. Consequently, it controls their concentration gradient across the plasma membrane. Vesicular accumulation also protects these molecules from leakage and/or intraneuronal metabolism (MASSON, SAGNE, HAMON and EL MESTIKAWY, 1999). Three distinct pools of synaptic vesicles have been identified. The first pool of vesicles, known as 'ready releasable pools', are those that are ready for immediate release upon elevation of intracellular calcium. The second pool is in close proximity to the site of release is known as 'proximal pool' and the third pool, which resides at some distance from the site of release, is known as 'a reserve pool'. It is thought that vesicles are recruited from the reserve pool to the proximal and subsequently to the readily releasable pool (BOEHM and KUBISTA, 2002).

When the action potential reaches the monoamine nerve terminals, it causes the opening of voltage-activated calcium channels. Calcium entry activates calcium sensors, which in turn cause the release machinery to cause vesicle fusion with the presynaptic membrane and the release of the monoamine into the synaptic cleft via exocytosis (**Figure 1.2.5).** The extra cellular calcium concentration-dependent release of the monoamines has a high requirement for energy. This is limited by tetrodotoxin (i.e. action potential-dependent) and subjected to presynaptic modulation through activation of different presynaptic receptors (LLINAS, 1977; VIZI, 2000; ELHWUEGI, 2004).

The released monoamine will act on specific receptors located either on post- synaptic or presynaptic membranes (**Figure 1.2.5**). Stimulation of the postsynaptic receptors results in changes in the properties of the postsynaptic membrane with either a shift in membrane potential when the receptors are coupled to ion channels (known as ionotropic receptors), or biochemical changes in intracellular cyclic nucleotides, protein kinase activity, and related substrate proteins when the receptors are coupled to G-proteins (known as metabotropic receptors, *vide infra*) (STARKE, TAUBE and BOROWSKI, 1977; KALSNER, S., 2000). This stimulation of the presynaptic receptors located on the nerve terminal will regulate the monoamine release triggered by action potential, such as vesicular release, thereby providing a feedback

mechanism that controls the concentration of the transmitter in the synaptic cleft (LANGER, 1980; BOEHM and KUBISTA, 2002). If the regulatory receptors are present on the same neuron releasing the neurotransmitter, then they are called autoreceptors. However, if the regulatory receptors are present on another neuron releasing different neurotransmitters, they are then called heteroreceptors (**Figure 1.2.5**) (ELHWUEGI, 2004). The regulatory receptors may be either ionotropic or metabotropic receptors (BOSKER, KLOMPMAKERS and WESTENBERG, 1997; BOEHM and KUBISTA, 2002).

The non-synaptic transmission is a more recent concept of transmission inside the CNS, where there are functional interactions between neurons without morphological 'synaptic' contacts. This type of neurotransmission is known as 'wireless' interaction between neurons (ELHWUEGI, 2004). In this case, the transmitter released from the axon terminal without a synaptic contact with another neuron will diffuse far away from the release site and activates remote receptors of high affinity on another axon terminal (VIZI, 2000).

The actions of all monoamines are terminated by active reuptake of the monoamines into the presynaptic neuron (known as uptake 1) and/or glial cells. The uptake 1 mechanisms utilize Na^+/Cl^- dependent transporters (LESTER, CAO and MAGER, 1996). These transporters are members of a large family of Na^+/Cl^--containing putative transmembrane domains that control the concentration of the transmitter released into the intrasynaptic and extrasynaptic spaces via rapid reuptake into the nerve terminals, thus maintaining a low concentration of the neurotransmitter at these sites (NELSON, 1998; MASSON, SAGNE, HAMON and EL MESTIKAWY, 1999). Several drugs, including some antidepressants, inhibit these transporters specifically, thus increasing the amount of the monoamines at the synapse. It has been shown that the transporter velocity is increased by hyperpolarization of the membrane and is decreased by depolarization, i.e. the function of the reuptake system is voltage-dependent (SONDERS, ZHU, ZAHNISER, KAVANAUGH and AMARA, 1997). In such a case, it is expected that autoreceptors or heteroreceptors would affect the activity of the reuptake system by changing the membrane potential. This was found to be true where stimulation of dopamine receptors, type 2 (D_2), that causes activation of K^+ currents, i.e. hyperpolarization, increased the activity of dopamine transporter and therefore decreased its availability at the synapse (GINGRICH and CARON, 1993; VIZI, 2000).

The two enzymes that are important in the initial steps of metabolism of the catecholamines, are monoamine oxidase (MAO) and catechol-O-methyltransferase (COMT) (KOPIN, 1985). Both MAO and COMT are widely distributed throughout the body, including the brain; the highest concentrations of each are in the liver and the kidney. However, little or no COMT is found in the monoaminergic neurons (KOPIN, 1985). Two isoforms of MAO (MAO-A and MAO-B) were differentiated on the basis of substrate and inhibitor specificities. MAO-A preferentially metabolizes 5-HT and NA; clorgyline is a specific inhibitor of this enzyme. MAO-B prefers h-phenylethylamine and benzylamine as substrates; selegiline is a selective inhibitor. Dopamine and tryptamine are metabolized equally by both isoforms.

Neurons contain both isoforms of MAO, localized primarily in the outer membrane of mitochondria (SHIH, 1994). Inhibitors of MAO cause an increase in the amount of these amines stored and released from the nerve terminals, thus increasing the monoaminergic activity (See **Chapter 5**).

Noradrenalin - Central noradrenergic pathways

The existence of noradrenalin in the brain was established biochemically in the 1950s. When noradrenalin is applied by microionophoresis to individual cells in the brain, the effect most often is inhibitory, and in most cases it is produced by activation of β-adrenoceptors. Activation of adenylate cyclase with a resultant build-up of cAMP has been unequivocally demonstrated as the mechanism of action in several types of CNS neurons (RANG and DALE, 1987). In some situations, however, noradrenalin has an excitatory effect, which is mediated by either α- or β-adrenoceptors.

There is still much to be learned, as mentioned earlier, about the actual behavioural and physiological responses of these fairly well characterized neuronal mechanisms. Reward studies conducted on animals have shown that drugs that prevent noradrenergic transmission disrupt the 'reward' system. Studies of this kind (ASHTON, 1992) have led to the suggestion that the noradrenergic pathways constitute a 'reward' system, though the relationship of this psychological construct to subjective feelings in man is uncertain. The catecholamine (monoamine) hypothesis of affective disorders, originally formulated by SCHILDKRAUT (1965) suggests that depression results from a functional deficiency of noradrenalin in certain parts of the brain, while mania results from an excess. Various lines of evidence suggest that activation of noradrenergic pathways can produce behavioural arousal. One is that amphetamine-like drugs, which are known to act by releasing catecholamines in the brain, increase wakefulness, alertness and exploratory activity. There is a close relationship between mood and state of arousal. Depressed patients are usually lethargic and unresponsive to external stimuli. This association of symptoms may reflect the dual role of noradrenergic neurons in controlling both mood and arousal (RANG and DALE, 1987).

The understanding that central, as well as peripheral, noradrenergic synapses are involved in blood pressure regulation, derived from investigation of the mechanisms of action of hypotensive drugs such as clonidine and methyldopa, both of which were shown to decrease the discharge of sympathetic nerves emerging from the central nervous system (RANG and DALE, 1987). It was then revealed that they cause obvious hypotension when injected locally into the vasomotor centres. Noradrenalin, injected locally into the region of the vasomotor centres has similar effects. Agonist and antagonists show that these responses are due to activation of α_2-adrenoceptors, which, on the basis of lesion studies, appear to be located post-synaptically (in contrast to most peripheral α_2 –receptors, which are pre-synaptic) (RANG and DALE, 1987). Noradrenergic synapses in the medulla probably form part of the baroreceptor reflex pathway**.**

Dopamine

Recognition of the role of dopamine in the brain, as a transmitter in its own right and not merely as a precursor of noradrenalin, came in the mid-1960s (RANG and DALE, 1987). The synthesis of dopamine follows the same route as that of noradrenalin, namely the conversion of tyrosine to dopa (the rate-limited step, catalysed by tyrosine hydroxylase) followed by decarboxylation (catalysed by dopa decarboxylase).

Dopamine produces inhibitory postsynaptic potentials. It is involved in movement, learning and motivation. Dopamine plays a key role in the neurobiology of dependence. Dopamine receptor genes have also been highly implicated in substance dependence in general, as well as in nicotine and alcohol dependence. There are two major dopamine projections in the brain. One, the mesolimbic pathway, projects from the VTA to the nucleus accumbens. This pathway appears to be directly or indirectly activated by most known psychoactive substances. Closely associated with this is the mesocortical dopamine pathway, which projects from the VTA to regions of the cortex. The second major dopamine pathway projects from the substantia nigra to the striatum, which is known as the nigrostriatal pathway (UNGERSTEDT, 1971). In Parkinson disease, this pathway undergoes degeneration leading to the characteristic movement disorders. Excessive dopamine function in the mesolimbic and mesocortical dopamine systems is thought to underlie the delusions and hallucinations of schizophrenia. It is interesting to note here that certain substances such as cocaine and amphetamine can, in high doses, mimic some of the features of schizophrenia and bipolar disorders through the same basic actions on the dopamine system (RANG and DALE, 1987).

The functions of dopaminergic pathways are better understood than those of pathways involving other transmitters (RANG and DALE, 1987). This is partly due to the availability of selective agonists and antagonist for dopamine receptors, and to the fact that dopaminergic neurons can be selectively destroyed by local injections of 6-hydroxydopamine into small areas of the brain.

Parkinson’s disease is a progressive motor disturbance that occurs mainly in elderly patients, whose main symptoms are rigidity and tremors, together with extreme slowness in initiating voluntary movements (hypokinesia). It is known to be associated with a deficiency of dopamine in the nigro-striatal pathway. Huntington’s chorea, an inherited disease that results in severe involuntary movements, may be the pathological opposite of Parkinsonism, in which symptoms are associated with an excess, rather than a deficit, of dopamine (RANG and DALE, 1987).

The tuberoinfundibular dopaminergic pathway is involved in the control of prolactin secretion. The hypothalamus secretes various mediators, which control the secretion of different hormones from the pituitary gland. One of these, which have an inhibitory effect on prolactin release, is dopamine. This system is of considerable clinical importance. It was observed many years ago that ergot derivatives tend to suppress lactation, whereas anti-psychotic drugs have the opposite effect, even to the point of causing

breast development and lactation in males. Studies on isolated pituitary glands confirmed that dopamine and related agonists strongly inhibit prolactin secretion, an effect that is abolished by many anti-psychotic drugs, which block dopamine receptors. Anti-psychotic drugs are used in South Africa to increase milk-production in breast-feeding mothers. This often results in a dependency on these drugs by the mothers after breast-feeding which is a negative aspect of dopamine antagonists (RANG and DALE, 1987).

The secretion of growth hormone is similarly regulated by dopamine. In normal subjects dopamine receptor activation increases growth hormone secretion, but paradoxically inhibits the excessive secretion responsible for acromegaly, a condition in which bromocriptine (high potency D_2 agonist and partial D_1 agonist) has a useful therapeutic application, provided that it is given before excessive growth has taken place (RANG and DALE, 1987).

Serotonin (5-Hydroxytryptamine)

Interest in serotonin as a possible CNS transmitter dates from 1953 (RANG and DALE, 1987), when lysergic acid diethylamide (LSD), a powerful hallucinogen, acted as a serotonin antagonist in peripheral tissues, and suggested that its central effects might also be related to this action. Its presence in the brain was demonstrated a few years later. It is involved in regulation of mood, arousal, impulsivity, aggression, appetite and anxiety. Serotonin-synthesizing cell bodies are found in the midbrain in a region called the raphe nuclei. These neurons project into many areas of the brain such as the cortex, hypothalamus and limbic system. There are many subtypes of serotonin receptor. In the body, serotonin is found in the gastrointestinal tract, platelets and spinal cord. Most antidepressant drugs work by increasing the action of serotonin in the brain. Serotonin is also involved in the primary actions of some psychoactive compounds such as LSD and ecstasy, and is also implicated in the effects of cocaine, amphetamine, alcohol and nicotine. Serotonin will be discussed in greater detail in **Chapter 3**.

Amino-acid neurotransmitters

γ-aminobutyric acid (GABA)

GABA is particularly abundant in brain tissue and not in other mammalian tissues, where it is found in trace amounts. GABA is thought to act as an inhibitory transmitter in many different CNS pathways. The most detailed studies have been carried out on the cerebellum, cerebral cortex, hippocampus and striatum. GABA mediates inhibitory action throughout the CNS by activating three classes of receptors, $GABA_A$, $GABA_B$ and $GABA_C$ receptors that are classified structurally and pharmacologically (**Table 4.1; Chapter 4**). $GABA_A$ receptors are ligand-gated chloride ion channels and resemble, but not identically, the inhibitory GABA receptor of invertebrates.

It was thought that a GABA-like substance may prove to be effective in controlling epilepsy and other convulsive states, and since GABA itself fails to penetrate the blood-barrier, the search for more lipophilic GABA analogues continues. One such substance is the *p*-chlorophenyl derivative of GABA

(baclofen), which was introduced in 1972 (RANG and DALE, 1987). $GABA_A$ receptors contain modulator binding sites for benzodiazepine (BZD), barbiturates, neurosteroids and ethanol, are activated by GABA, muscimol and inhibited by bicuculline and picrotoxin.

In contrast, $GABA_B$ receptors are members of the 'seven transmembrane' receptor family, which are coupled to either K+ or Ca 2+ channels via G-proteins and are regulated by intracellular secondary messenger systems. $GABA_B$ receptors are selectively activated by GABA and (-)baclofen and are inhibited by phaclofen. Although similar to $GABA_A$ receptors directly associated with a Cl^- ion channel, $GABA_C$ receptors differ markedly from $GABA_A$ and $GABA_B$ receptors in their pharmacological properties. They are insensitive to both bicuculline and baclofen as well as to $GABA_AR$ modulatory drugs like BZDs, barbiturates or neurosteroids, but stimulated by GABA and certain analogues of GABA, such as *cis*-4-aminocrotonic acid (CACA).

A remarkable relationship exists between $GABA_A$ receptors and the actions of the benzodiazepine group of drugs, which have powerful sedative, anxiolytic and antiepileptic effects. These drugs selectively potentiate the effects of GABA on $GABA_A$ receptors in such a way that the binding of GABA is facilitated and its pharmacological activity is enhanced. $GABA_A$ will be discussed in greater detail in **Chapter 4**.

Acetylcholine (Ach)

Acetylcholine is a central and a peripheral neurotransmitter. The symptoms of Parkinson's disease result from a defect in the balance between acetylcholine and dopamine in the basal ganglia. Thus anticholinergic medication is used to treat the parkinsonian adverse effects of antipsychotic medications (e.g. procyclidine and orphenadrine which block the D_2 receptors in the basal ganglia) and idiopathic Parkinson's disease (RANG and DALE, 1987).

An important approach to treat Alzheimer's disease (AD) is directed towards the inhibition of acetylcholinesterase (AChE). Nicotinic (receptor-operated Cl^- ion channels) and muscarinic (which are G-protein-coupled of which there are many sub-types) agonists or drugs that enhance endogenous acetylcholine function appear to be beneficial in the treatment of Alzheimer's disease. Based on the cholinergic hypothesis, a defect in the cholinergic system is involved in AD. The cholinergic system will be discussed in greater detail in **Chapter 5**.

BOX 1.

The classification of psychotropic drugs (RANG, DALE and RITTER, 1996).

Psychotropic drugs are defined as those that affect mood and behaviour. Due to the fact that these are extremely complex functions, arriving at an adequate classification of drug effects is far from straightforward, and no single basis for classification has been found to be satisfactory. Thus

classification is on a chemical basis, which produces categories such as benzodiazepines, butyrophenones etc., but does not give much guide to pharmacological effects. A pharmacological or biochemical classification, on the other hand, is appealing for those drugs whose mechanism of action is reasonably well understood (e.g. monoamine oxidase inhibitors, catecholamine uptake blockers), but there are still many instances (e.g. hallucinogens) where the mechanism of action is too poorly understood to form the basis of a reliable classification. Another possibility is to adopt an empirical classification based on clinical use, and divide drugs into categories such as 'antidepressants', 'antipsychotic agents' and so on, but this has an weakness that some important psychotropic drugs have no clinical use, or their use may have changed or been superseded according to clinical fashion. Amphetamine, for example, a drug with well-characterized effects on mood and behaviour, has had an extremely chequered clinical career and would have been dismissed, revived and reclassified many times if a purely clinical classification had been adopted. Because no single basis for classifying psychotropic drugs is feasible, different authorities tend to offer a variety of hybrid, and often-incompatible schemes. This currents arrangement is not ideal and is often confusing.

The following classification (TYRER, 1982; TREASE and EVANS, 1983) is based on suggestions by the World Health Organization (WHO) in 1976. Although it is not completely indisputable it provides a useful basis for the material discussed in this project.

a. Anxiolytic sedatives

Synonyms: Hypnotics, sedatives, minor tranquillizers

Definition: Drugs that cause sleep and reduce anxiety. Some may be used as anticonvulsants

Examples: Barbiturates, benzodiazepines, ethanol

b. Neuroleptics

Synonyms: Antipsychotic drugs, antischizophrenic drugs, major tranquillisers

Definition: Drugs that are effective in relieving the symptoms of schizophrenic illness

Examples: Phenothiazines, butyrophenones

c. Antidepressant drugs

Synonyms: Thymoleptics

Definition: Drugs that alleviate the symptoms of depressive illness

Examples: Monoamine oxidase A inhibitors (MAOI) e.g. , Serotonin reuptake inhibitors (SSRI), noradrenalin reuptake inhibitors, tricyclic antidepressants (TA)

d. Psychomotor stimulants

Synonyms: Psychostimulants

Definition: Drugs that cause wakefulness and euphoria

Examples: Amphetamine, cocaine, caffeine

e. Psychodysleptics

Synonyms: Hallucinogens, psychotomimetic agents

Definition: Drugs that cause disturbances of perception and behaviour in ways that cannot be simply characterized as sedative or stimulant effects, but resemble the symptoms of schizophrenia

Examples: Lysergic acid diethylamide (LSD), mescaline, phencyclidine.

1.4. Plants and the central nervous system

Allopathic medicines, and the strongly associated disciplines of pharmacology and medicinal chemistry, have their historical roots in an earlier era in which the compounding of herbal remedies for specific illnesses were conducted as largely empirical activities. This was usually with no scientific basis and often, when a therapeutic rationale was provided at all, it was within a theoretical framework that resembled magic or superstition (KRIEG, 1964). Centuries of trial-and-error experimentation by shamans, herbalists, witches, alchemists, and other practitioners, inevitably lead to the discovery of the 'bioactive' properties of certain plants, and of these, it was plants affecting the nervous system and mental functions that often had the most profound and dramatic effects. For instance, the juice of certain poppies (*Papaver somniferum*) had the power to block pain and induce a dream-like sleep; chewing the leaves of other plants (*Catha edulis* or *Erythroxylum cocoa*) could induce a state of wakefulness and mental clarity; consumption of certain mushrooms (*Amanita muscaria*) or seeds (*Ipomoea violaceae*) could plunge the eater into a bizarre alternate reality, complete with visions, voices, and confrontations with gods (or demons). Hemlock (*Conium maculatum*) is known for its potent depression of the nervous system and more so for its most famous victim; Socrates in 399 B.C. The major psychoactive botanicals, active components and their principal molecular targets are given in **Table 1.4.1.**

Due to the unmistakable and occasionally terrifying effects of psychoactive plants, whether consumed accidentally or deliberately, it is little wonder that our predecessors developed a healthy respect early on for the power of plants to heal, as well as to derange and even destroy. Knowledgeable practitioners adapted the use of psychoactive and other biodynamic plants to their own purposes, incorporating them into quasi-medical practices as well as ceremonial and magical activities. Gradually, people became more sophisticated in the phytotherapy, sharing and transferring their knowledge between cultures and generations. New methods of preparing sophisticated, multicomponent 'recipes' were discovered, as were new technologies for extracting the 'essences' or 'spirit' of the plants (McKENNA, 1996).

The discovery of steam distillation in the Middle Ages (STOLL, 1967) was one such turning point, as it marked the appearance of one of the initial pharmaceutical technologies that went beyond the preparation of simple aqueous decoctions. It was not until the nineteenth century, however, that this natural desire to literally isolate the 'spirit' of plants in an ever more purified, concentrated, and potent form, stimulated the discoveries that eventually would form the foundations of modern organic chemistry, pharmacology, and

pharmacotherapeutic medicine. Once again, psychoactive plants assumed center stage as the focus of the new phytochemical technologies. Morphine, from the opium poppy (*Papaver somniferum*), isolated by the German pharmacist Sërtuner in 1803 (JAFFE and MARTIN, 1975), was the first alkaloid to be purified from a plant. This event marked the first scientific application of phytochemical technology that would lead to the isolation of the presumed 'active principles' from many of the important medicinal plants of the era, including cocaine from coca leaves by Squibb in 1885 (KENNEDY, 1985), and mescaline from the dried tops of the peyote cactus by Arthur Heffter in 1896 (HEFFTER, 1896). It was a fortunate accident of nature that these and other highly bioactive plant constituents were alkaloids, and thus amenable to purification using relatively crude isolation methods.

Figure 1.4.1. Louis Lewin M.D. (1850-1929), author of more than 200 publications on the subject of pharmacology and the first researcher to study peyote and kava. (Photo: Clendening Library Portrait Collection)

Several other early scientific investigators can be credited with beginning interdisciplinary research on psychoactive plants and plant substances. As early as 1855, Ernst Freiherr von Bibra published *Die narkotischen Genussmittel und der Mensch*, a study of 17 plants. The non-technical article, *The Seven Sisters of Sleep* (1860) by the British mycologist Mordecai Cooke, was a noteworthy work on narcotic plants. Inspired by von Bibra's work, Karl Hartwich over half a century later published *Die Menschlichen Genussmittel* (1911), a thorough work on about thirty psychoactive plants.

However, the publication of Louis Lewin's *Phantastica* in 1924 began a new era of ethnobotany. Until Lewin, texts on the use of psychotropic plants were purely anthropological, largely concerned with how these plants were used rather than their mode of action. As a world renowned pharmacologist and toxicologist, he was fascinated by both. Lewin travelled extensively and acquired an astounding variety of knowledge on psychotropic plant use. He provided detailed information on all major psychotropic plants of the time, including opium, cocaine, heroin, cannabis, peyote, kava, coffee, tea, cocoa and tobacco. This knowledge forms the foundations of most work in this field.

The isolation of active compounds (e.g. plant alkaloids) contributed to the development and eventual understanding that physiological processes occurring in living organisms were fundamentally not different from physicochemical processes which could be studied and manipulated in a variety of systems, both living and non-living. Chemists found that they could modify the structural characteristics of an isolated natural product using relatively simple methods, and that the pharmacological properties of the modified compound would often be altered as a result, sometimes in unexpected ways. These discoveries further eroded the notion that bioactive plants were the dwelling place of some kind of

mystical 'healing spirit or god' as mentioned earlier. These ideas gained general acceptance and became firmly entrenched in Western views of nature, and have lead to modern medicine, pharmaceutical technology, and medicinal chemistry. Natural products, which provided the foundation of the field in the first place, continue to make their contributions felt in significant ways. Specifically, natural products, whether isolated from plant, animal, or microbial sources, have provided and still provide medicinal chemists and pharmacologists with: (1) pharmacological research tools; (2) novel structural templates; and (3) leads to new pharmacologic mechanisms (McKENNA, 1996).

Natural products provide pharmacologists with useful research tools for elucidating basic pharmacological mechanisms. Many examples could be cited here, but a CNS-related one that should be quite familiar to most neuroscientists is reserpine, the major alkaloid of *Rauwolfia serpentine* or Indian Snake Root. It has been used clinically as an antipsychotic tranquilizer and hypotensive medication (NICKERSON and COLLIER, 1975). While its clinical use has been all but superseded by newer and more sophisticated medications, its ability to deplete neuronal vesicles of their stores of serotonin and catecholamines rendered it a useful tool for pharmacologists studying processes related to neurotransmitter storage, synthesis, and release.

Seventy-five percent of prescription drugs sold in the United States are synthetic, while many of the remaining 25% are semisynthetic derivatives of natural products (FARNSWORTH and MORRIS, 1976; FARNSWORTH, AKERELE, BINGEL, SOEJARTO and GUO, 1985). Natural products also provide medicinal chemists with ideas for novel structural templates; these are new molecular entities, whether synthetic or semi-synthetic, which bear a clear correlation to the natural product which provided the inspiration for their manufacture, but which may have unique pharmacological properties that are not shared by the parent compound.

The last important area where natural products have significant impact on pharmacology lies in their usefulness in identifying new mechanisms of action, and/or novel or previously uncharacterized receptor populations mediating those mechanisms (McKENNA, 1996). Often, a structurally novel class of neuropharmacological agents can lead investigators to the discovery of a new class of receptors with which the compounds interact.

The discovery of cardiovascular activity from the roots of the Indian plant *Rauwolfia serpentine* led to the isolation of reserpine over five decades ago. Reserpine, was brought to the attention of the Western world in 1949 by Vakil who described its use in hypertension; in rapid succession between 1952 and 1958, reserpine was isolated from *Rauwolfia*, its structure determined and its total synthesis achieved (DOHADWALLA, 1985). The indiscriminate use of reserpine as an antihypertensive agent and tranquilizer led to reports of depression and Parkinsonism effects. These findings stimulated further investigation and evidence was found that reserpine depleted not only brain serotonin but also nor-

epinephrine and dopamine (CURZON, 1990). This was a major stimulus for continued research on transmitter amine defects in depression and Parkinson's disease. This in part laid the foundation for the development of many of the modern psychoactive drugs and stimulated a significant interaction between researchers and drug industry.

As the adverse effects of reserpine continued to be revealed through clinical research, interest in the product gradually diminished, particularly when safer antihypertensive drugs were made available, though reserpine is still used in clinical medicine, particularly in low-income population. In deed, there is a revival of interest in its use based on some recent clinical trials, which showed that lower doses of reserpine (0.05–0.1 mg) combined with low doses of thiazide diuretic and hydralazine provides highly effective blood pressure lowering regimen along with renal protective effect; relatively free from conventional side-effects and is perhaps the most cost-effective antihypertensive treatment available today (MILNE and PINKNEY-ATKINSON, 2004).

This development of reserpine clearly illustrates the scientific principle that drugs, in addition to being therapeutic agents, become tools for further understanding of disease and hence design of new drugs. Other compounds, which have been considered invaluable pharmacological "tools" for evaluating the mode of action of other drugs or investigation of basic physiological function, include muscarine and nicotine (pioneer selective agonists for muscarinic and nicotinic receptors respectively), cocaine (catecholamine uptake inhibitor), yohimbine (selective α2 blocker) and himbacine, a prototype of cardio-selective antimuscarinic agents (GILANI, 1998).

Table 1.4.1. Main psychoactive botanicals and their principal molecular targets (adapted from ROTH, LOPEZ, BEISCHEL, WESTKAEMPER and EVANS, 2004)

Plant name **FAMILY** **Country**	**Main active ingredient(s)**	**Principal molecular target(s)**	**Class of target (GPCR, ion channel, transporters, other)**	**Common name**
Amanita muscaria AMANITACEAE Siberia, Asia and northern Europe	Muscimol, ibotenic acid	Muscarinic and metabotropic glutamate receptors (NICOLETTI et al., 1986)	GPCR	Fly agaric, Ibo Tengatake
Areca catechu ARECACEAE Asia	Arecholine, other constituents in the nut include arecaidin, arecaine and choline	Muscarinic cholinergic receptors	GPCR	Betel nut
Artemisia absinthium COMPOSITAE Europe	Thujone	$GABA_A$ receptors and likely other targets	Ion channels	Absinthe
Atropa belladonna SOLANACEAE Europe	Atropine	Muscarinic receptors	GPCR	Belladonna, night shade
Cannabis sativa CANNABACEAE China and Middle East	Tetrahydro-cannabinol	CB-1 cannabinoid receptors (HOWLETT et al., 1990, MAKRIYANNIS and RAPAKA, 1990)	GPCR	Marijuana, dagga.
Catha edulis CELASTRACEAE East Africa and the Middle East	Cathinone	Noradrenalin transporter (ROTHMAN et al., 2003)	Transporter	Kat
Claviceps purpurae[*] HYPOCREACEAE Europe	Ergot alkaloids	5-HT receptors (many)	GPCR	Ergot, St Anthony's Fire
Coffee arabica RUBIACEAE Arabia	Caffeine	Adenosine receptors (SNYDER et al., 1981)	GPCR	Coffee
Conium maculatum APIACEAE Europe	(+)-Coniine, γ-Coniceine	Nicotinic cholinergic (nACh) receptors agonist (POLYA, 2003)	Ion channels	Hemlock
Corynanthe (=*Pausinystalia*) *yohimbe* RUBIACEAE West African	Yohimbine	a2-Adrenergic antagonist	GPCR	Yohimbine

[*] Not a plant, but a fungus that can grow on incorrectly stored grains

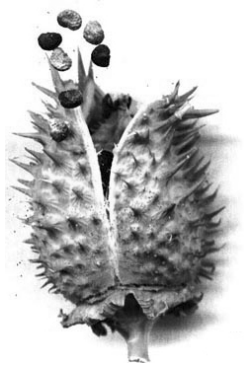
Plant name **FAMILY** **Country**	**Main active ingredient(s)**	**Principal molecular target(s)**	**Class of target (GPCR, ion channel, transporters, other)**	**Common name**
Datura sp. SOLANACEAE South America and Europe	Scopolamine	Muscarinic receptors	GPCR	Jimson weed
Ephedra sinica EPHEDRACEAE China	Ephedrine-related stereoisomers	Noradrenalin transporters (ROTHMAN et al., 2003)	Transporters	Ephedra
Erythroxylum cocoa ERYTHROXYLACEAE South America	Cocaine	Multiple biogenic amine transporters	Transporters	Cocaine, Huanuco
Heimia salicifolia LYTHRACEAE Mexico	Cryogenine	Unknown (? prostaglandin synthetase inhibition) (LEMA et al., 1986)	Enzyme/GPCR indirectly	Sinicuichi
Hyoscyamus niger SOLANACEAE Europe, Middle East	Hyoscamine and other tropanes	Muscarinic receptors	GPCR	Henbane
Hypericum perforatum HYPERICACEAE Europe	Hypericin, hyperforin, amentoflavone and many others	Many GPCR, transporters, kinases, and ion channels (SIMMEN et al., 1999, 2001; BUTTERWECK et al., 2002)	GPCR, ion channels, transporters	St. John's wort
Ipomoea violaceae CONVOLVULACEAE Mexico	Lysergic acid amide	5-HT_{2A} serotonin receptors (GLENNON et al., 1984; NICHOLS, 2004)	GPCR	Morning glory seeds
Lobelia sp. LOBELIACEAE South America	Lobeline	Nicotinic cholinergic (nACh) receptors agonist (POLYA, 2003)	Ion channels	Indian tobacco
Lophophora williamsii CACTACEAE Mexico	Mescaline	5-HT_{2A} serotonin receptors (GLENNON et al., 1984; NICHOLS, 2004)	GPCR	Peyote
Myristica fragrens MYRISTICACEAE New Guinea, East Indies	Myristicin	Unknown	Unknown	Nutmeg
Nicotiana tabacum SOLANACEAE Americas	Nicotine	Nicotinic cholinergic (nACh) receptors	Ion channel	Tobacco

Plant name **FAMILY** **Country**	**Main active ingredient(s)**	**Principal molecular target(s)**	**Class of target (GPCR, ion channel, transporters, other)**	**Common name**
Papaver somniferum PAPAVERACEAE Persia, Asia	Morphine and many related alkaloids	α-opioid receptor (PERT and SNYDER, 1973)	GPCR	Opium
Paulinia cupana SAPINDACEAE Used interchangeably with *Ilex paraguariensis*, and others Brazil, South America	Caffeine	Adenosine receptors (SNYDER et al., 1981)	GPCR	Guarana, Yerbe mate
Piper methysticum PIPERACEAE Pacific Islands	Kava lactones	Multiple ion channels (SINGH and SINGH, 2002)	Ion channels	Kava kava
Psilocybe mexicana STROPHARIACEAE Mexico	Psilocybin	5-HT_{2A} serotonin receptors (GLENNON et al., 1984; NICHOLS, 2004)	GPCR	Psilocybin mushrooms
Psychotria viridis RUBIACEAE Used with *Mimosa tenuiflora*, *Peganum harmala* and *Virola sp*. Amazon Basin	N,N-DMT (and related tryptamines)	5-HT_{2A} serotonin receptor (GLENNON et al., 1984; NICHOLS, 2004)	GPCR	Chacruna, ayahuasca
Salvia divinorum LAMIACEAE Mexico	Salvinorin-A	Kappa opioid receptor (KOR) (ROTH et al., 2002; YAN and ROTH, 2004) (see **Table 1.4.1.**)	GPCR	Salvia, Ska Pastora
Tabernanthe iboga APOCYNACAE Congo, Gabon	Ibogaine	Unknown (see **Table 1.4.2.**)	Unknown (see **Table 1.4.2.**)	Eboga, Ibogaine
Vocanga africana APOCYNACEAE Congo, Gabon	Voacangine	Unknown—related to ibogaine	Unknown	None

The following section will highlight in more detail a few of the highly researched and better documented psychotropic plants already mentioned in **Table 1.4.1**.

Cannabis sativa (Cannabaceae)

Cannabis has had a long history of use (over 5000 years) starting in Central and Northeast Asia with current use spreading worldwide as a recreational drug or as a medicine; albeit unauthorized. A number of countries, notably Canada, have eased restrictions on the use of cannabis for medicinal purposes. Several historic reviews have been written on Cannabis use as a therapeutic drug, the most recent of which are those by RUSSO (2001, 2002) and ELSOHLY (2002). Cannabis is extremely complex in its chemistry due to the enormous number of its constituents and their possible interaction with one another. These compounds represent almost all of the chemical classes, e.g., mono- and sesquiterpenes, steroids, flavonoids, nitrogenous compounds and amino acids, sugars, hydrocarbons, among others. The best-known and the most specific class of Cannabis constituents is the C_{21} terpenophenolic cannabinoids, with (-)-Δ^9-trans-(6a*R*, 10a*R*)-tetrahydrocannabinol (Δ^9-THC) being the most psychologically active constituent (MECHOULAM and GAONI, 1967). The total number of natural compounds identified in *C.sativa* L. in the 1980s was approximately 420 (TURNER, ELSOHLY and BOEREN, 1980; ELSOHLY, BOEREN, TURNER and ELSOHLY, 1984) and 495 in a more recent paper (ELSOHLY and SLADE, 2005).



Datura stramonium Seed capsule

Atropa* and *Datura species (Solanaceae)

Atropine and scopolamine are found in jimson weed (*Datura stramonium*), nightshade (*Atropa belladonna*) and mandrake (*Mandragora officionarum*), and scopolamine alone is found in henbane (*Hyoscyamus niger*) (BROWN and TAYLOR, 2001), all of which are popularly grown as ornamental flowers. *D. stramonium*, a weed that is probably indigenous to tropical America is now widely distributed throughout the world including southern Africa. The seeds are the most potent part of the plants, followed by the roots, stems, leaves, and flowers, and as few as ten seeds are sufficient for psychoactivity. Dissociative rather than entirely hallucinogenic, both chemicals act as CNS depressants and competitively antagonize muscarinic cholinergic receptors. They have considerable application in ophthalmology to dilate pupils (atropine), anaesthesia to decrease secretions and treat bradycardia, toxicology to treat organophosphate and nerve gas poisoning, and in emergency medicine for cardiac arrest. Scopolamine is also used as a treatment for motion sickness. In excess, these plants can cause a toxic delirium that may lasts hours to days, marked by amnesia, confusion, dissociation, hallucinations, delusions, euphoria, and sometimes episodes of bizarre self-injury (GRINSPOON and BAKALAR, 1997). This is evident by the Afrikaans name, *malpitte* which translates as ‘mad seeds’ (VAN WYK and GERICKE, 2000) and its Zulu name *iloyi* which is derived from *–loya*

which means to bewitch, or cast a spell on. In tropical West Africa, *Datura* spp. are used in native beer or in palm wine to add a stupefying or narcotic effect. A drink made from the seeds of *D. metel* (*D. fastuosa* var. *alba*) is given as an intoxicant to Fulani youth to incite them in the 'Sharo contest' or ordeal of manhood (OLIVER-BEVER, 1986). In Tanzania, the leaves of *D. fastuosa* are added to native beer to further its intoxicating effect (BALLY, 1938).

Hypericum perforatum Flower

Hypericum perforatum (Hypericaceae)

St. John's Wort, *Hypericum perforatum*, is a perennial herbaceous plant traditionally used in the treatment of ulcers, burns, abdominal pains and bacterial diseases (MILLS and BONE, 2000). Currently it is a popular botanical used for mild to moderate depression. A total sales figure of about US$ 6 billion for 1998 in Europe alone attests to the popularity of this herb for treating depression (HARRISON, 1998).

The antidepressant actions are believed to be mediated through multiple modes. These include inhibition of monoamine oxidase, catechol-o-methyltransferase and dopamine-β-hydroxylase (THIEDE and WALPER, 1994; KLEBER, OBRY, HIPPELI, SCHNEIDER and ELSTNER, 1999; RON, WILLIS, BONE and MORGAN, 2000), by blocking synaptic reuptake of 5-HT, noradrenalin, dopamine, GABA and L-glutamate (MULLER, 2003), inhibiting nitric oxide synthase (LUO, SUN, MAO, LU and TAN, 2004) and through calcium channel and PDE blockade (GILANI, SHAH, GHAYUR, and MAJEED, 2005). Reports show that *H. perforatum* extracts have a significant antidepressant effect when administered to humans. Numerous clinical trials and meta-analyses assessing the efficacy of *H. perforatum* for the alleviation of mild to moderate depression report that extracts are significantly superior to placebo, similarly effective as standard antidepressant drugs (such as imiprimine and diazepam), and apparently produce significantly fewer side-effects than the synthetic preparations (ERNST, 1995; LINDE, RAMIREZ, MULROW, PAULS, WEIDENHAMMER and MELCHART, 1996; VOLZ, 1997; SCHRADER, MEIER and BRATTSTROM, 1998; PHILLIP, KOHNEN and HILLER, 1999; KIM, STRELTZER and GOEBERT, 1999; SCHULZ, 2002; LINDE and MULROW, 2003).

Of the known constituents of *H. perforatum*, amentoflavone had highest affinity for any tested molecular target, with high affinity for the GABA-benzodiazepine receptor complex (K_i = 6 nM) and moderate affinity for δ-opioid receptors (K_i = 37 nM). Several other compounds have shown affinities in the low nanomolar to micromolar range for several cloned receptors, including various serotonin receptors for amentoflavone (5- HT_{1B}, 5-HT_{1D}, 5-HT_{2C}) and dopamine receptors for hypericin (D_3 and D_4). Another study using fewer receptors (SIMMEN, BURKARD, BERGER, SCHAFFNER and LUNDSTROM, 1999) disclosed low micromolar affinities for various opioid and 5-HT receptor subtypes. Other studies by the same group found that hypericin was a low affinity CRF-1 antagonist (SIMMEN, HIGELIN,

BERGER-BUTER, SCHAFFNER and LUNDSTROM, 2001, SIMMEN, BOBIRNAC, ULLMER, LUBBERT, BERGER BUTER, SCHAFFNER and SCHOEFFTER, 2003). Finally, GOBBI, MOIA, PIRONA, MORIZZONI and MENNINI, (2001) found that hypericin and other constitutents had low micromolar affinities for various peptide (*NPY*-1, *NPY*-2), serotonin, and δ-opioid receptors. Taken together, these results indicate that certain purified substances obtained from *H. perforatum* can interact with a variety of biogenic amine and peptide receptors with low affinities, generally in the micromolar range. With the exception of amentoflavone, which has high affinity for the GABA/benzodiazepine receptor complex and δ-opioid receptors (BUTTERWECK, NAHRSTEDT, EVANS, HUFEISEN, RAUSER, SAVAGE, POPADAK, ERNSBERGER and ROTH, 2002), and hypericin, which has moderate affinity for CRF-1 receptors, the evidence is not yet persuasive that the main molecular targets responsible for the antidepressant actions of these compounds have been discovered. It is most likely that the putative antidepressant actions are mediated by a mixture of compounds, each of which has a complex pharmacological profile (BUTTERWECK, WALL, LIEFLANDER-WULF, WINTERHOFF and NAHRSTEDT, 1997; BUTTERWECK, PETEREIT, WINTERHOFF and NAHRSTEDT, 1998; BUTTERWECK, NAHRSTEDT, EVANS, HUFEISEN, RAUSER, SAVAGE, POPADAK, ERNSBERGER and ROTH, 2002; BUTTERWECK, 2003).

Salvia divinorum (Lamiaceae)

Salvia divinorum is a hallucinogenic plant that has been used by curanderos in Mexico and other Central American countries for centuries for divination and shamanism. The plant was named *S. divinorum* after its use in divination by the Mazatec Indians and was first described by a western observer in 1962 (WASSON, 1962; 1963; OTT, 1996). Other native uses for the plant include the treatment of diarrhoea, headache, and rheumatism. In addition, the plant is used to treat a semi-magical disease known as *panzo´n de barrego*, or swollen belly, which is caused by a curse from an evil sorcerer (VALDES, DIAZ and PAUL, 1983). For many years after its discovery there was considerable controversy regarding the psychoactive potential of *S. divinorum* largely because the active ingredient is less active when taken orally. Additionally, *S. divinorum*'s actions are relatively short-lived and subtle (SIEBERT, 1994; VALDES, 1994). Traditionally it is consumed by chewing fresh leaves or by drinking the juice of the leaves for absorption of the active principle through the oral mucosa (VALDES, DIAZ and PAUL, 1983). These plants are now grown and sold throughout the world for ingestion and for smoking by people who obviously do not have a historical ceremonial connection to this plant. Although the Drug Enforcement Administration (DEA) has not scheduled *S. divinorum*, they are closely monitoring it as a possibly emerging drug of abuse (NATIONAL DRUG INTELLIGENCE CENTER, 2003).

The presumed active ingredient, salvinorin-A (**Figure 1.4.2.**) was independently isolated by two groups in the early 1980s (ORTEGA, BLOUNT and MANCHAND, 1982; VALDES, BUTLER, HATFIELD, PAUL and KOREEDA, 1984) and shown by SIEBERT (1994) to be the main active ingredient more than a decade later. Salvinorin-A defines a novel structural family of hallucinogens in that it is a non-

nitrogenous neoclerodane diterpene of known absolute stereochemistry. Its structure gives no clue regarding its site of action. Subsequently, a large number of other constituents including salvinorin-B, -C, -D, -E, and -F (MUNRO and RIZZACASA, 2003), along with divinaturin-A, -B, and -C (BIGHAM, MUNRO, RIZZACASA and ROBINS-BROWNE, 2003), have been described. All of these are neoclerodane diterpenoids in structure. None of the known diterpenoids, with the exception of salvinorin-A, has any known psychoactive actions and, thus, attention has focused on discovering the mechanism of action of salvinorin-A.

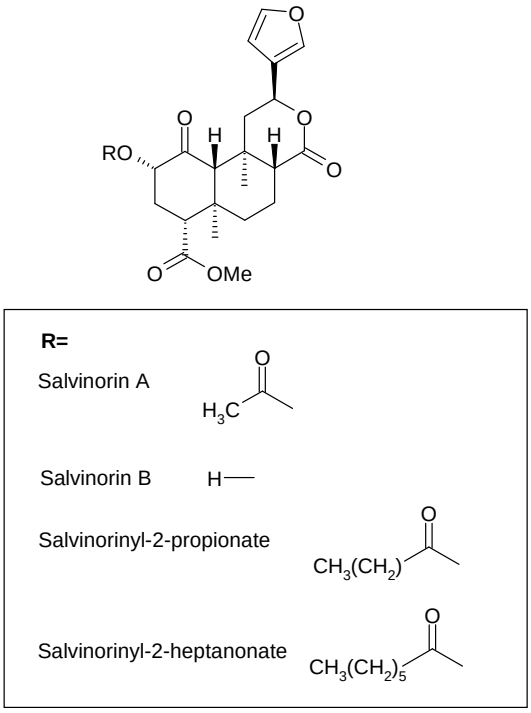


Figure 1.4.2. Non-nitrogenous neoclerodane diterpenes from *S. divinorum.*

There are 59 *Salvia* species in Africa, *S. chamelaeagnea* Berg. and *S. runcinata* L. f. are used traditionally in South Africa by both the Xhosa and Zulu people. An infusion of *S. chamelaeagnea* leaves are used as a remedy for convulsions (WATT and BREYER-BRANDWIJK, 1962). **Table 1.4.2.** shows the psychotropic activity of compounds from *Salvia* species.

Table 1.4.2. Biological activity of compounds from *Salvia* species (excluding neoclerodane diterpenoids salvinorin derivatives) (adapted from POLYA, 2003).

Compound (class)	Species	Biological activity
Cryptotanshinone (diterpine quinine)	*S. miltiorrhiza*	Central Benzodiazepine/$GABA_A$ receptor partial agonist (Flunitrazepam competition, IC_{50} = 2 μM), Tranquillizer
1,2-Didehydromiltirone (diterpine quinine)	*S. miltiorrhiza*	Central Benzodiazepine/$GABA_A$ receptor partial agonist (Flunitrazepam competition, IC_{50} = 1 μM), Tranquillizer

Compound (class)	Species	Biological activity
(-)-1,2-Dihydrotanshinone I (diterpine quinine)	*S. miltiorrhiza*	Central Benzodiazepine/$GABA_A$ receptor partial agonist (Flunitrazepam competition, IC_{50} = 9 μM), Tranquillizer
Methylenecrypto-tanshinquinone (diterpine quinine)	*S. miltiorrhiza*	Central Benzodiazepine/$GABA_A$ receptor partial agonist (Flunitrazepam competition, IC_{50} = 11 μM), Tranquillizer
Methylenetanshinquinone (diterpine quinine)	*S. miltiorrhiza*	Central Benzodiazepine/$GABA_A$ receptor partial agonist (Flunitrazepam competition, IC_{50} = 11 μM), Tranquillizer
4-Methylenemiltirone (diterpine quinine)	*S. miltiorrhiza*	Central Benzodiazepine/$GABA_A$ receptor partial agonist (Flunitrazepam competition, IC_{50} = 2 μM), Tranquillizer
Miltirone (diterpine quinine)	*S. miltiorrhiza*	Central Benzodiazepine/$GABA_A$ receptor partial agonist (Flunitrazepam competition, IC_{50} = 0.3 μM), Tranquillizer
Ro 09-0680 (diterpine quinine)	*S. miltiorrhiza*	Central Benzodiazepine/$GABA_A$ receptor partial agonist (Flunitrazepam competition, IC_{50} = 11 μM), Tranquillizer
Tanshinone I (diterpine quinine)	*S. miltiorrhiza*	Central Benzodiazepine/$GABA_A$ receptor partial agonist (Flunitrazepam competition, IC_{50} = 36 μM), Tranquillizer
Tanshinone IIA (diterpine quinine)	*S. miltiorrhiza*	Central Benzodiazepine/$GABA_A$ receptor partial agonist (Flunitrazepam competition, IC_{50} = 3 μM), Tranquillizer
Picrotoxinin (=Dehydropicrotin) (tutinolide sesquiterpene lactone)	*S. deserta*	$GABA_A$ receptor non-competitive antagonist (Glycine receptor), CNS stimulant, barbiturate antidote, insecticide
Salvanolic acid A (stilbene, phenylpropanoid)	*S. miltiorrhiza*	H^+, K^+-ATPase Anti-peptic ulcer, inhibits gastric H^+ secretion
8-Epiblechnic acid (= des(α-Carboxy-3,4-dihydroxyphenethyl) lithospermic acid (benzofuran)	*S. miltiorrhiza*	Voltage-gated Ca^{2+} ion channel Hypotensive, vasodilator
5-(3-Hydroxypropyl)-7-methoxy-2-(3'-methoxy-4'-hydroxyphenyl)-3-benzo[b]furan-carbaldehyde (benzofuran)	*S. miltiorrhiza*	A_1 Adenosine receptor ligand ((K_i = 17 nM)
Oleanolic acid (triterpene)	*Salvia* spp.	Muscarinic acetylcholine receptor agonist, Uterine smooth muscle contraction
α- and β-Thujone (=α- and β-Thujan-3-one) (monoterpene)	*S. officinalis* and *S. triloba*	Cannabinoid receptors CB1-receptor ligand (rat, , IC_{50} > 10 μM), CB2-receptor ligand (rat, , IC_{50} > 10 μM), Anthelmintic, convulsant, hallucinogenic, intoxicant
α-Pinene (= 2-Pinene) (monoterpine)	*Salvia* spp.	Acetylcholinesterase (IC_{50} = 630 μM), Ataxic, delirium-inducing, dermatitic, irritant, perfume

1.4.1. African psychotropic plants

> *"There is always something new out of Africa" Gaius Plinius Secundus, (circa AD 23 - 79) better known as Pliny the Elder, Roman Natural Historian*

In the following sections several well documented African psychotropics will be reviewed. The relatively unknown southern African psychoactives, the main topic of this thesis will be reviewed in **Chapter 2**.

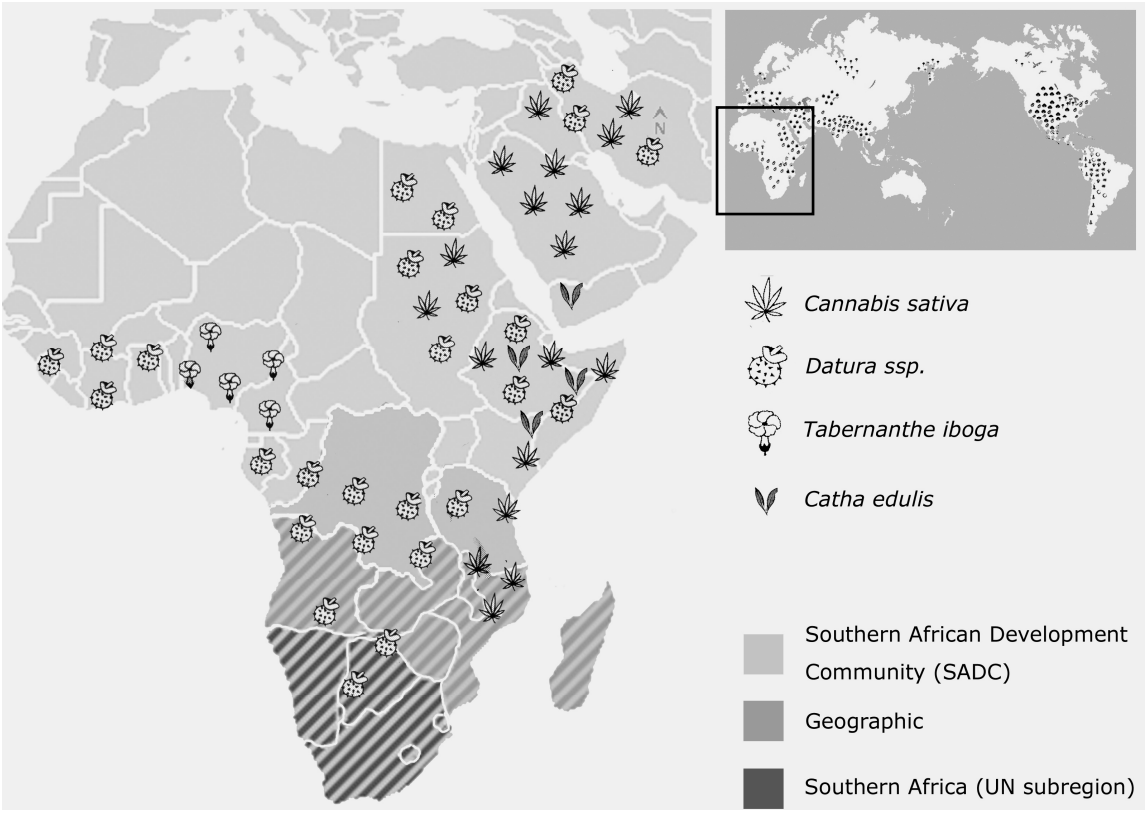


Figure 1.4.3. Distribution of documented African psychotropic plant use, also showing southern African regions (adapted from SCHULTES and HOFMANN, 1992).

Catha edulis (Celastraceae)

The psychotropic properties and use of 'Khat,' has been known for centuries in East Africa, the Middle East, including Ethiopia, Tanzania and North Yemen (CARLINI, 2003). The usual method of ingestion is chewing of the young leaves (NENCINI, AHMED and ELMI, 1986). Recently, this habit has reached other parts of the world (AL-MOTARREB, BAKER and BROADLEY, 2002). Khat induces a clear anorectic effect (ZELGER and CARLINI, 1980), together with euphoria, excitation and cheerful sensation (NENCINI, AHMED and ELMI, 1986). These effects are produced mostly by phenylpropanolamines present in the leaves: cathinone (*S*-α-aminopropiophenone), cathine [(-)-1*S*,2*S*-norpseudoephedrine] and (-)-IR,2*S*-norephedrine (CARLINI, 2003). These substances have pharmacological properties similar to those of D-amphetamine (ZELGER**,** SCHORNO and CARLINI, 1980), as they provoke the release and inhibit the uptake of dopamine in CNS (ZELGER and CARLINI, 1981).

***Cola* species** (Sterculiaceae)

Caffeine is the most widely used psychoactive substance in the world (GILBERT, 1976; BARONE and ROBERTS, 1996). Caffeine is valued because of its stimulant-like behavioural activity on mood and performance. It occurs in several plants which are widely known and employed throughout the world. Wherever these 'caffeine containing plants' were found, indigenous groups have recorded their mildly stimulant effects and have grown habituated to their use. The most well-known sources in contemporary western society are coffee, tea, caffeinated soft drinks, cocoa, chocolate and certain medications.

More tradional sources include *Ilex* and *Paullinia* species valued by South American Indians, whereas *Cola* spp. are an important social drug for West African peoples (**Table 1.4.3.**) (DE SMET, 1990). Kola nuts have also played an important role in Western Africa as a commodity. Whole caravan trains were assembled to purchase them (MADAUS, 1979).The Yoruba farmers of Western Nigeria recognize at least four kinds of kola nuts, which probably belong to three different *Cola* species (*C. acuminata*, *C. nitida* and *C. verticillata*) (DE SMET, 1996).

Table 1.4.3. Botanical sources rich in caffeine other than coffee, tea and cacao (DE SMET, 1990).

FAMILY *Species*	Common name	Utilized plant part	Caffeine content (%)
AQUIFOLIACAEAE			
Ilex guayusa	Guayusa	Leaves	1.8
Ilex paraguariensis	Mate´ [a]	Leaves	0.3 – 2
Ilex vomitoria	Yaupon	Leaves	0.01 – 1.65
SAPINDACEAE			
Paullinia cupana	Guarana	Seeds	2.5 – 5
Paullinia yoco	Yoco	Bark	2.7
STERCULIACEAE			
Cola nitida [b]	Colab	Seeds	1.5 – 3.5

[a] Several other *Ilex* spp. contain caffeine as well and are used as substitutes for Mate´ (e.g. *I. amara*, *I. conocarpa*, *I. theezans*).

[b] Cola nuts may also be obtained from other caffeine-containing *Cola* spp. (e.g. *C. acuminata* and *C. ballayi*).

The notable behavioral effects of caffeine occur after low to moderate doses (50–300 mg) and are increased alertness, energy and ability to concentrate. Moderate caffeine consumption leads very rarely to health risks (BARONE and ROBERTS, 1996). Higher doses of caffeine induce negative effects such as anxiety, restlessness, insomnia and tachychardia. These effects are only evident primarily in a small portion of caffeine-sensitive individuals (BARONE and ROBERTS, 1996).

Left Figure 1.4.4: West African wooden kola nut dish. This specimen probably originates from an Igbo group in Southeast Nigeria. The Igbo call such platters *okwa oji* (dish kola) and use them for serving kola nuts and other foods to their guests (Photograph: DE SMET, 1996).

The effect of caffeine on the release of intracellular calcium and as an inhibitor of cyclic nucleotide phosphodiesterases has been mainly shown *in vitro* at 500 μM - 500 mM concentrations. Doses higher than those usually achieved by human consumption (NEHLIG and DEBRY, 1994) and cannot therefore account for most of the physiological effects of caffeine. One exception is the respiratory stimulant effect of caffeine that appears to be prominently mediated by the inhibition of type-IV phosphodiesterase (HOWELL, 1993). In fact, it is now widely accepted that the main mechanism of action of caffeine occurring at circulating concentrations achieved after the consumption of one or two cups of coffee, is the antagonism at the level of adenosine receptors (NEHLIG and DEBRY, 1994, FREDHOLM, 1995). Indeed, in animals, most pharmacological effects of adenosine in the brain can be suppressed by relatively low concentrations of circulating caffeine, i.e. less than 100 mM, which are attained after the consumption of one to three cups of coffee. Adenosine decreases the firing rate of neurones and exerts an inhibitory effect on synaptic transmission and on the release of most neurotransmitters, while caffeine increases the turnover of many neurotransmitters, including monoamines and acetylcholine (NEHLIG and DEBRY, 1994).

The A_1 and A_{2a} adenosine receptors are the subtypes that are primarily involved in the effects of caffeine, while the A_{2b} and A_3 receptors play only a minor role. The A_1 receptors are negatively linked to adenyl cyclase, while the A_{2a} receptors are positively linked to the enzyme. Adenosine A_1 receptors are widely distributed throughout the brain with high levels in the hippocampus, cerebral and cerebellar cortex, and thalamus (DAVAL, WERCK, NEHLIG and PEREIRA DE VASCONCELOS, 1991). Conversely, A_{2a} receptors are almost exclusively located in the striatum, nucleus accumbens and olfactory tubercle (ONGINI and FREDHOLM, 1996). In the latter regions, A_{2a} receptors are coexpressed with enkephalin and dopamine D_2 receptors in the same kind of striatal neuronal cells (ONGINI and FREDHOLM, 1996). There is direct evidence for a central functional interaction between adenosine A_{2a} and dopamine D_2 receptors. Indeed, the administration of adenosine A_{2a} receptor agonists decreases the affinity of dopamine binding to D_2 receptors in striatal membranes (FERRÉ, VON EULER, JOHANSSON, FREDHOLM and FUXE, 1991). The interaction between adenosine A_{2a} receptors and dopamine D_2 receptors in the striatum might underlie some of the behavioral effects of methylxanthines. By antagonizing the negative modulatory effects of adenosine receptors on dopamine receptors, caffeine

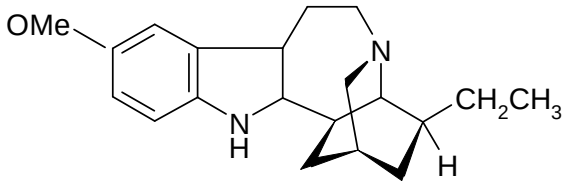
leads to the inhibition and blockage of adenosine A_2 receptors, leading to a potentiation of dopaminergic neurotransmission (GARRETT and GRIFFITHS, 1997). The latter interaction is very interesting since it could explain the adenosine receptor antagonists-induced increase in behaviors related to dopamine (DALY, 1993), such as, e.g. caffeine-induced rotational behavior (GARRETT and HOLTZMAN, 1995).

Piper betle (Piperaceae)

Betel is a preparation of several natural substances chewed for its mild psycho-stimulating effects. Betel is composed of the fruit or nut of the areca palm (*Areca catechu,* Arecaceae), the leaf of the betel pepper (*Piper betle*), and lime (calcium hydroxide). Approximately 200 million persons chew betel regularly mainly throughout the western Pacific basin and south Asia. However, it is reportedly used by the Swahili of Zanzibar (NORTON, 1998) and plants can be found through-out Africa. According to EICHHORN (1911), the Kenyan Shambala (Waschambaa) considered the chewing of betel as a means to preserve the teeth and as a way to soften toothache. In South Africa, the habit is primarily confined to people of Indian or South East Asian origin (SEEDAT and VAN WYK, 1988). Betel chewing is also associated with oral leukoplakia, submucous fibrosis, and squamous cell carcinoma. The active ingredient of the areca nut is arecoline, an alkaloid with properties that mimic acetylcholine. The hydrolyzing action of lime on arecoline produces arecaidine a central nervous system stimulant, which in combination with the betel pepper produces mild euphoria. Along with the mild euphoria, chewers experience cholinergic effects such as diaphoresis, lacrimation, pupillary constriction, and occasionally diarrhoea (MUJUMDAR, KAPADI and PENDSE, 1979). Arecoline can cause bronchoconstriction and may trigger asthma attacks (TAYLOR, AL-JARAD, CONROY and BARNES, 1992). Betel appears to be psychologically and physiologically addictive (PICKWELL, SCHIMELPFENIG and PALINKAS, 1994).

Tabernanthe iboga (Apocynaceae)

The Iboga people living in Gabon and other nearby West African countries chew the roots of this plant at the religious cult of Bwiti (Bouiti) in order to communicate with their ancestors. In large doses the roots of *T. iboga* produce altered states of consciousness and even hallucinations (EMBODEN, 1972). Apart from this religious use, according to European explorers in the 19th century, eating the roots had also stimulant and aphrodisiac effects and greatly increased endurance (POPIK, LAYER and SKOLNICK, 1995). Not unexpectedly, Ibogaine, the main active constituent has been used as a performance enhancing agent by athletes (DE SIO, 1970). Ibogaine was isolated and identified in the beginning of the 20th century (POPIK, LAYER and SKOLNICK, 1995); at least 12 more indole alkaloids have been isolated from the plant (SCHULTES and HOFMANN, 1992; POLYA, 2003). Ibogaine mimics most of the effects of crude *T. iboga* extracts; however, there seem to be some pharmacological differences between both (for a review, see POPIK, LAYER and SKOLNICK, 1995 and **Table 1.4.4.**).



Ibogaine

In the mid-1980s, a new era of interest arose for ibogaine, with the filing of a patent for ibogaine treatment of opiate dependence (SANCHEZ-RAMOS and MASH, 1994). Three other patent filings followed in rapid succession for treatment of cocaine, amphetamine, alcohol and nicotine/ tobacco dependence syndromes.

Ibogaine is currently the only representative of a class of complex indole alkaloids that are particularly abundant in the Apocynaceae family, and it is likely that other naturally occurring alkaloids with related structures will also exhibit interesting CNS activity. Potential candidates worthy of investigation are *Acokanthera oppositifolia* which is administered to treat fits, *Tabernaemontana* species and *Strophanthus* species which are used to treat hysteria (HUTCHINGS, SCOTT, LEWIS and CUNNINGHAM, 1996).

Table 1.4.4. The diverse biological activity of *Tabernanthe* alkaloids (adapted from POLYA, 2003).

Compound	**Biological activity**
Tabernanthine (= 13-Methoxyibogamine)	Benzodiazepine receptor agonist (flunitrazepam displacement (IC_{50} = 150 μM), NMDA-Glutamate-receptor antagonist (K_i = 11 μM), σ1-receptor ligand (IC_{50} = ~1 μM) and σ2-receptor ligand (IC_{50} = 0.2 μM) Opiate (κO)-receptor ligand (K_i = 0.2 μM), μO-receptor (IC_{50} >100 μM ;), δO-receptor (K_i = 3 μM),
(±)-Coronaridine (= Carbomethoxyibogamine)	NMDA-Glutamate-receptor antagonist (K_i = 6 μM), Opiate (μO)-receptor (K_i = 3 μM), δO-receptor ligand (K_i = 8 μM), κO-receptor ligand (K_i = 4 μM), Voltage-gated Na^+ channel antagonist (K_i = 16 μM) cytotoxic, diuretic and oestrogenic

Compound	Biological activity
Ibogaine (=12-Methoxyibogamine)	$5HT_3$-receptor ligand (IC_{50} = 4 μM), Dopamine and 5HT re-uptake transporter inhibitor (IC_{50} = 4 and 0.6 μM respectively) Dopamine receptor (D1) ligand (IC_{50} > 10 μM), Dopamine receptor (D2) ligand (IC_{50} > 10 μM), NMDA-Glutamate-receptor antagonist (K_i = 1 μM), σ1-receptor ligand (IC_{50} = 9 μM) and σ2 receptor ligand (IC_{50} = 0.2 μM), Opiate (κO)-receptor ligand (IC_{50} = 25 μM ; K_i = 2 μM), μO-receptor (K_i = 4 μM), δO-receptor (K_i > 100 μM), Adenosine-receptor (subtype A_1) ligand, Muscarinic acetylcholine-receptor ligand, α1-Adrenergic-receptor (IC_{50} = 7 μM) Voltage-gated Na^+ channel antagonist (K_i = 9 μM) Anticonvulsant, hallucinogen, inhibits morphine dependence, anti-addictive, increases synaptic 5HT)
Noribogaine (=12-Hydroxyibogamine) Metabolite of Ibogaine	Dopamine receptor (D1) ligand (IC_{50} > 10 μM), Dopamine receptor (D2) ligand (IC_{50} > 10 μM), Opiate (κO)-receptor ligand (K_i = 4 μM), μO-receptor (K_i = 0.2 μM),
Ibogamine	NMDA-Glutamate-receptor antagonist (K_i = 6 μM), σ1-receptor ligand (IC_{50} = ~1 μM) and σ2 receptor ligand (IC_{50} = 0.1 μM), Opiate (κO)-receptor ligand (K_i = 3 μM), μO-receptor (K_i > 100 μM), δO-receptor (K_i > 100 μM), Voltage-gated Na^+ channel antagonist (K_i = 8 μM) Brachycardiac activity, cytotoxic and hypotensive
Tubotaiwine	Adenosine-receptor (subtype A_1) ligand. Opiate-receptor ligand (K_i = 2 μM) Analgesic (mouse abdominal relaxant)

Plants and alcoholic beverages

One of the most common traditional psychotropic agents throughout Africa is ethyl alcohol (ethanol). It is known that the imbibing of this reversible general CNS depressant can lead to inebriation. All that is needed to prepare an alcoholic beverage is a 'sugar-providing' plant and the right yeast to transform the sugar in the presence of water into ethyl alcohol and carbon dioxide. Thus the psychotropic effects that these alcoholic preparations produce are not a direct effect of a plant secondary metabolite. This fermentation process is so simple that it is believed that man already knew it before he learnt how to record his own history (LEWIS and ELVIN-LEWIS, 1977). What are of more pertinence to this project are the plants which are added to these beverages to 'fortify' them.

Various additives to alcoholic beverages have been described (DE SMET, 1996, 1998; VAN WYK and GERICKE, 2000) but little is known about their chemical constituents. Examples are; *Anacampseros rhodesica* (Portulacaceae) which according to GELFAND, MAVI, DRUMMOND and NDEMERA (1985), is used in Zimbabwe to initiate hallucinations and to serve as a narcotic additive to beer. WATT and BREYER-BRANDWIJK (1962) also mention that it is thought to be narcotic. They state that it is used as an adulterant in African beer making. In Tanzania, the fruit of *Kigelia aethiopica* (Bignoniaceae) is added to beer to increase its strength. However, this potentiation may be due to the fermentation process rather than to specific *Kigelia* constituents. The drinking of the beer may result in a severe headache, perhaps due to the formation of amyl alcohol (BALLY, 1938; WATT and BREYER-BRANDWIJK, 1962). The Turkana in Kenya use the fruit of the related *Kigelia africana* together with sorghum or sugar to make beer (MORGAN, 1981). *Lachnopylis platyphylla* (Loganiaceae) leaves are used in Tanzania to ferment sugar-cane beer or to increase its intoxicating effects (WATT and BREYER-BRANDWIJK, 1962). In Tanzania, the Chaga of the Kilimanjaro region frequently add the bark of *Rauwolfia caffra* (Apocynaceae) (*msesewe*) to a traditional beer called *mbege*, to give the beer a 'kick' and to conveniently intoxicate a drinker after imbibing only a moderate amount. Different parts of *R. inebrians* and *R. obliquinervis* have also been used for this purpose (BRAUN, 1912; 1925; BALLY, 1938; MADATI, KAYANI, PAZI and NYAMGENDA, 1977). Of the people who habitually drink *mbege* which has been adulterated like this, annual averages of 45 die (MADATI, KAYANI, PAZI and NYAMGENDA, 1977). In Kenya, the stem of *R. caffra* is used for making beer (OMINO and KOKWARO, 1993). African *Rauwolfia* species are known to be rich in indole alkaloids (COURT, 1983). The stem bark of *R. caffra* yields 0.25 mg/g of total alkaloids, which largely consist of ajmaline, norajmaline, ajmalicine and ajmalicinine (NASSER and COURT, 1984).

1.5. Aims of this study

This project has two main objectives. Firstly, to bring together a **comprehensive and detailed record of psychotropic plants** used in southern Africa by indigenous peoples for medicinal or cultural purposes. Secondly, this research attempts to investigate the **validity and rationale of the use of these plants** by screening them in various biological assays for psychotropic activity. Obviously, not all the plants used can be screened in all available bioassays and thus plants were selected, based on their traditional use and availability, and were screened in four assays, which detect biological activity of a useful nature. A number of *in vitro* neuronal signal transduction assays were employed in this thesis, the inhibition of the serotonin reuptake transporter protein (SERT); inhibition of catabolic enzymes (e.g. acetylcholinesterase, monoamine oxidase); $GABA_A$- benzodiazepine receptor binding (**Figure 1.5.1.)**. Activity in these assays indicates that the plants may have potentially useful psychotropic activity.

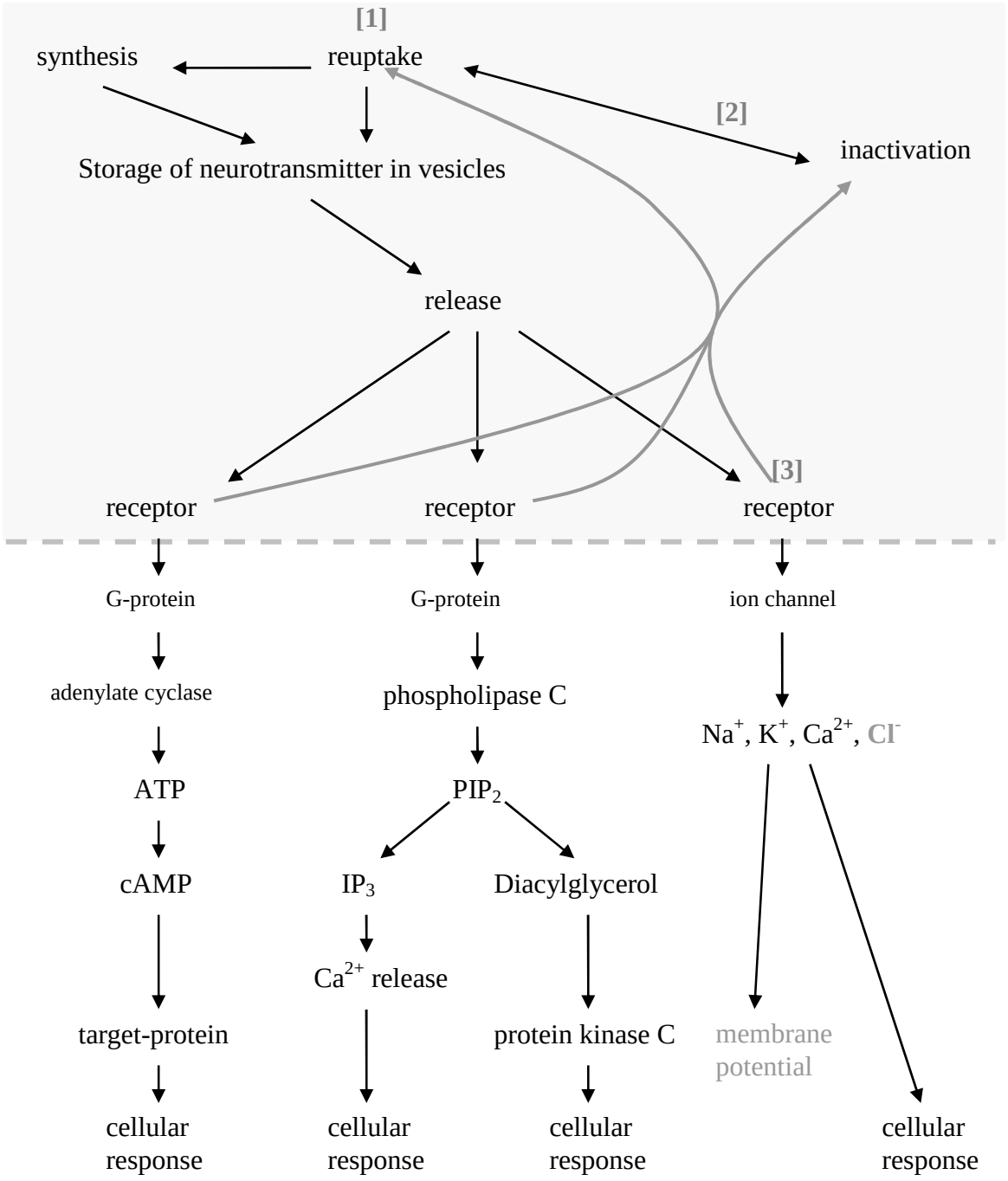


Figure 1.5.1. Schematic illustration of important elements of neuronal signal transduction (Adapted from WINK, 2000). Numbers indicate targets of assays employed in this thesis: **[1]** the inhibition of the serotonin reuptake transporter protein (SERT; see **Chapter 3**); **[2]** inhibition of catabolic enzyme (e.g. acetylcholinesterase and monoamine oxidase; see **Chapter 5**); **[3]** $GABA_A$- benzodiazepine receptor binding (see **Chapter 4**).

CHAPTER TWO

Ethnobotanical literature survey:

South African medicinal plants with central nervous system related activity and use

2.1. Introduction

This Chapter examines the history and current status of African traditional medicine is South Africa. Aspects of the philosophies and methodologies of the various practioners of South African traditional medicine will be discussed. The *materia medica* of southern African traditional medicine, known locally as *muthi*, will be outlined as well as the importance of the indigenous botanical knowledge, including Zulu botanical names. These topics, where appropriate, will be discussed in relation to the use of plants for central nervous system-related purposes. Lastly, an annotated list compiled from available ethnobotanical literature of plants traditionally used for central nervous system-related purposes is provided (**Table 2.2a**). An alphabetical reference list is also supplied (**Table 2.2b**). The advancement of South African traditional medicine is about to get a much needed boost with the recent development of National Policy on African Traditional Medicine and expected establishment of a National Institute of African Traditional Medicine (DRAFT NATIONAL POLICY on ATMSA, 2008). The influence of legislation, past and present, on the state of traditional medicine will be highlighted.

2.1.1. Southern African traditional healing: history and current status

The WHO acknowledges that it is difficult to assign one definition to the broad range of characteristics and elements of traditional medicine, but that a working definition is essential. It thus concluded that a traditional medicine includes:

> "*diverse health practices, approaches, knowledge and beliefs incorporating plant, animal and/or mineral based medicines, spiritual therapies, manual techniques and exercises applied singularly or in combination to maintain well-being, as well as to treat, diagnose or prevent illness*" (WORLD HEALTH ORGANIZATION, 2002).

The terms, 'indigenous', 'traditional' and 'western' medicines and practitioners are frequently used. With the use of these terms, it is acknowledged that they are somewhat inappropriate, politically-loaded and rather unhelpful in describing the principles, philosophies and practices they represent. It is problematic to assign sensitive and precise definitions to these terms and no suitable synonyms are readily available. The WHO draws a distinction between 'traditional medicines' and 'complementary and alternative medicines'.

Complementary and alternative medicines relate to practices such as acupuncture, homeopathy and chiropractic systems. These are considered a '*broad set of health care practices that are not part of a country's own tradition, or not integrated into its dominant health care systems*' and thus fall outside the scope of this Thesis.

Traditional medicine is part of African culture and is intricately linked with the African world view. One of the definitions given for 'African Traditional Medicine' by the WHO Centre for Health Development is the following:

> "*the sum total of all knowledge and practices, whether explicable or not, used in diagnosis, prevention and elimination of physical, mental, or societal imbalance, and relying exclusively on practical experience and observation handed down from generation to generation, whether verbally or in writing*" (WORLD HEALTH ORGANIZATION, 1976).

The Draft National Policy on African Traditional Medicine in South Africa (DRAFT NATIONAL POLICY on ATMSA, 2008) defines African traditional medicine as follows:

> *"African Traditional Medicine is a body of knowledge that has been developed and accumulated by Africans over tens of thousands of years, which is associated with the examination, diagnosis, therapy, treatment, prevention of, or promotion and rehabilitation of the physical, mental, spiritual or social wellbeing of humans and animals".*

The Third World, in particular Africa, is faced with a lack of medical personnel in general but especially in rural areas. In 1982 it was estimated that there was in the region of one western medical practitioner for every 17 500 people (SAVAGE, 1985). More recently, for Sub-Saharan Africa, the estimated ratio of traditional healers to the population is approximately 1:500, while medical doctors have a 1:40 000 ratio to the rest of the population (ABDOOL KARIM, ZIQUBU-PAGE and ARENDSE, 1994). It is unlikely that this situation has improved since 1994 when one considers that population growth far exceeds the current training of medical practitioners. This relative lack of medical personnel is likely to remain in the foreseeable future, and one question that often arises is how the health services for the entire population can be improved? Whatever is done in terms of the allocation of personnel and money is likely to fall far short of what is necessary, and drastic measures are called for. There are a number of these, but one possibility is the utilisation of the services of traditional healers who are indeed numerous and situated where they are required in under developed rural areas.

In 1998 the National Council of Provinces (NCOP) Select Committee on Social Services estimated that there are about 350 000 traditional practitioners in South Africa, providing their services to 60-80% of the

population, yet there was only about 250 000 allopathic (modern) medical personnel in South Africa. This raises another much asked question; can modern medicine and traditional indigenous medicine find common ground in the form of much needed primary health care?

Traditional healing in virtually all its forms has been illegal for more than a century in South Africa. Under the provisions of the SUPPRESSION OF WITCHCRAFT ACT (1957) (first introduced in 1895, last amended in 1970), all forms of divination are outlawed. Divination is the heart of healing in Africa; therefore, all healing is outlawed. One of the main hindrances for the improvement of traditional medicine, especially the medicinal plant aspect, in South Africa has been Clause 36 of the MEDICAL, DENTAL AND SUPPLEMENTARY HEALTH SERVICES PROFESSION ACT (1974). This clause forbade any registered practitioner to practise in collaboration with a non-registered person, and for non-registered persons to perform acts pertaining to the medical or dental professions. The outcome of this legislation was the division of the traditional healers as a group in hundreds of secretive sub-groups. The trust between healers and allopathic practitioners and westerners in general is tenuous, making collaboration near impossible. Compounded by the over-sensationalism created by the media surrounding '*muthi*' murders which has created the perception of traditional healers as being evil, we are left with lack of trust and unity among traditional healers which has proved to be one of the major stumbling blocks in the formation of a unified traditional healers' body. Until this happens traditional healers will not gain recognition from the Department of Health. Some progress has been made, in 1989, nine years after attempts were made by traditional healers to gain recognition; the KwaZulu-Natal legislature changed their laws to allow 'black medicine-men', herbalists and midwives to practise, subject to certain provisions. This change in legislation led to the establishment of a register of traditional healers aided by the longest standing non-governmental organisation in Zululand, the Inyangas' National Association (INA).

Despite these advancements no form of quality control exists amongst those who are registered and it had no recognisable code of ethics. In 1995 the KWAZULU-NATAL HEALTH ACT (1995) proposed further investigations and found that the public called for more legal control. Again all efforts put in place to resolve the situation were hindered by the lack of unity between various healer groups. The University of Zululand, under the guidance of Mrs. A. Hutchings undertook the task of explaining to a large number of traditional healers in KwaZulu-Natal the benefits of a unified traditional healers' council. Their success resulted in the formation of the KZN Traditional Healers' Council. More recently, the South African Medicines and Medical Devices Regulatory Authority Act of 1998 requires all medicines, including traditional medicines, to be registered with the Medicines Control Council. Despite these laws, traditional healers have only rarely been prosecuted simply for plying their trade (ASHFORTH, 2005).

Following recommendations of the WHO (1978), and, like its neighbours Ghana, Tanzania, Zambia and Zimbabwe, South Africa has attempted the 'professionalization' route to traditional healers (LAST and CHAVUNDUKA, 1986; PILLSBURY, 1982). The TRADITIONAL HEALTH PRACTITIONERS ACT (THPA) was gazetted in parliament in May 2005, and is designed to formalize the structure and organization of traditional health practice through the Traditional Health Practitioners Council of South Africa (the THP Council) (THPA, 2004). The THP Council is expected to comprise a maximum of 25 members consisting of registered traditional health practitioners, a representative from the Department of Health, someone from a legal background, a medical practitioner of the Health Professions Council of South Africa (HPCSA), and others. The Council will have to meet at least twice a year (THPA, 2004).

The THPA, which is intended

> "*to establish the Interim Traditional Health Practitioners Council of South Africa; to provide for a regulatory framework to ensure the efficacy, safety and quality of traditional health care services; to provide for the management and control over the registration, training and conduct of practitioners, students and specified categories in the traditional health practitioners profession; and to provide formatters connected therewith*",

has raised concerns amongst western medicinal practitioners and researchers.

To mention two concerns expressed by BERGER (2006); firstly, the regulations governing membership of the THP Council of South Africa calls for 10 traditional health practitioners, one to serve as chair, in addition to a representative from each of the traditional healer categories. In contrast to this overwhelming representation from the traditional sector, the Act provides for the appointment of only one orthodox medical practitioner. Secondly, while the document is almost entirely made up of rules and procedures regarding its constitution it lacks substantive statements that set out standards of skill, knowledge and training, or that address the safety and efficacy specifications required of materials and methods used in the practice of traditional medicine. It leaves it to the Minister of Health to prescribe the necessary qualifications on recommendation of the Council. BURGER (2006) suggests that, given the composition of the council and the absence of mandated academic, scientific expertise, it will be unlikely if the regulations that ultimately emerge will meet the scientific and ethical concerns of medical science and modern standards of patient care. It should be noted that section 19(1) provides that "*No person shall be entitled to practise as a traditional health practitioner within the Republic unless he or she is registered in terms of this Act*" (THPA, 2004). Thus traditional health practitioners not registered with the Council will not be able to recover remuneration "*in respect of any act specially pertaining to the occupation of a traditional health practitioner*" (THPA, 2004). It also states that a person who "*diagnoses, treats or offers to treat, or prescribes treatment or any*

cure for cancer, HIV/AIDS or such other terminal diseases as may be described" shall be guilty of an offence (THPA, 2004).

WREFORD (2005) points out that despite calls, from outside and within government, for collaborative relationships between health systems in South Africa, it is useful to note that the Act includes only passing references to liaison between biomedical and traditional practitioners, and offers no practical suggestions for implementation. The effects the legislation is intended to have on the medical status quo are therefore unclear. WREFORD (2005) predicts that, as in Zimbabwe, despite longstanding experience of similar legislative frameworks, the two systems (traditional and western medicine) continue to operate in parallel rather than in partnership (CHAVUNDUKA, 2004).

Perhaps in response to the concerns mentioned above, and many others, a Draft National Policy on African Traditional Medicine in South Africa (the Policy) was recently gazetted (NATIONAL POLICY on ATMSA, 2008) for public comment. The Policy "*is designed to provide a framework for the institutionalisation of African Traditional Medicine in the South African healthcare system.*" This document is the result of The Presidential Task Team on African Traditional Medicine which was appointed in 2006 to make recommendations with regard to a national policy and an appropriate regulatory and legal framework for the institutionalisation of African traditional medicine in South Africa.

To achieve the institutionalization African traditional medicine the Policy recommended that legislation on African traditional medicine be enacted to provide "*an enabling environment for African Traditional Medicine in its entirety and scope, covering but not limited to:*

- *the regulation of African traditional medicine in South Africa;*
- *registration and regulation of African traditional medicines and medicinal products in South Africa;*
- *protection of African traditional medicine knowledge and intellectual property rights; and*
- *the protection of the rights of persons involved in the discipline of African traditional medicine in South Africa*" (NATIONAL POLICY on ATMSA, 2008).

It is recommended that a National Institute of African Traditional Medicine should be established, which would devise strategies, coordinate, undertake and provide leadership in the research of African traditional medicine and collaborate with other institutions on a needs basis. Research priority areas should be identified and developed and addressed through well developed, scientifically rigorous and ethical research protocols. It is suggested that a national pharmacopoeia of African traditional medicine in South Africa be developed. Conservation issues are also addressed and it is stated that the development of commercial medicinal plants should be limited to cultivated raw materials with wild harvesting only allowed under exceptional

circumstances. A National Ethics Committee for African Traditional Medicine research should be formed, consisting of both orthodox phytochemists and clinical trial specialists with experience in African traditional medicine research, as well as traditional health practitioners (NATIONAL POLICY on ATMSA, 2008). The recommendations contained within the Policy are largely in line with those made by FENNELL, LINDSEY, McGAW, SPARG, STAFFORD, ELGORASHI, GRACE and VAN STADEN (2004) and FENNELL, LIGHT, SPARG, STAFFORD, VAN STADEN, (2004) amongst others.

2.2. African traditional views of illness and treatment

Culture is the lens and guide we use in constructing, defining, and interpreting the world around us. Thus people from different cultural contexts and traditions define and experience events (i.e. illness) in different ways. This is particularly true of views about mental disorders and subsequently their diagnosis and treatment since these cannot be separated from cultural experiences. In order to determine which plants used in African traditional medicine may act on the CNS, an understanding of indigenous or traditional African views of illness and treatments are required. A brief interpretation follows, however, more thorough treatments on African culture as a whole and of specific regions are available (GLUCKMAN, 1956; BERGLUND, 1976; NGUBANE, 1977; LAMBO, 1982; GUMEDE, 1990; INGSTAD, 1989; 1990; GREEN, 1994; PATEL, MUSARA, MARAMBA and BUTAU, 1995; TSEY, 1997; YAMBA, 1997; JACOBSSON, 2002; LIDDELL, BARRETT and BYDAWELL, 2005).

In many traditional cultures, illness is believed to be caused by psychological conflicts or disturbed social relationships that upset an equilibrium which is expressed in the form of physical or mental problems (KLEINMAN, EISENBERG, GOOD, 1978). Although Africa has a diverse culture, there is a common thread, particularly with cultures south of the Sahara, that follows that the disruption of this equilibrium, what the west calls illness, may be caused by external psychological or spiritual factors, or both, that relate to African cosmology and "threaten the intactness of the person" (HEWSON, 1998). In these traditional cultures healing involves restoring this equilibrium. The DRAFT NATIONAL POLICY on ATMSA (2008) states that the philosophy underpinning African traditional medicine is *ubuntu*.

> "***Umuntu ngumuntu ngabantu/motho ke motho ka batho****/a human being is a human being through other human beings*"**.**

Thus an important causal factor considered in African traditional medicine is the status of relations existing between the 'unwell' individual and other human beings, both the living, and the deceased. Thus, philosophy, religion, psychiatry, physiology and biology, are all essential elements within the practice of African traditional medicine.

OKPAKO (1999) proposes that 'ancestor spirit anger' can be understood as a metaphorical reference to emotional stress, similar views are expressed by HORTON (1976). OKPAKO (1999) argues that when divination reveals that an illness is underpinned by ancestor spirit anger, it also invariably points to the probable existence of a hidden guilt. Therefore, treatment usually includes exposing the cause of the guilt and offering of propitiatory sacrifices to the offended ancestral spirits. This is often done through a ritual which is attended by members of the afflicted person's family and community. The immoral conduct by the afflicted person is recognized as a condition for the occurrence of grave illness and ancestor spirits are not considered to be unpredictable agents inflicting illness on their victims at random.

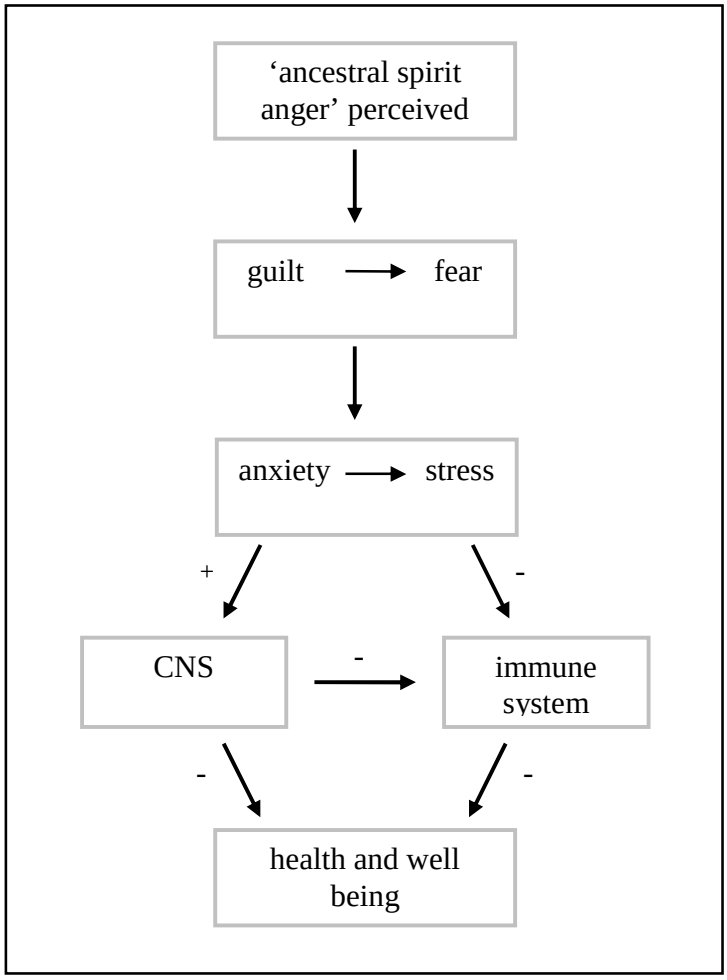


Figure 2.1. African traditional medicine holds that ancestor spirit anger is the cause of serious illness in individuals who have sinned. (+), positive impact; (-), negative impact (From OKPAKO, 1999).

OKPAKO (1999) suggests that in a group that is conditioned to believe that some types of immorality can enrage the ancestors or gods, a hidden sin of this type can give rise to emotional stress that, by "*gnawing at the sinner*", might lead to illness (SAWYERR, 1978) (**Figure 2.1**).

In general, many African cultures group illnesses into three categories (LIDDELL, BARRETT and BYDAWELL, 2005). Illnesses which have no discernible moral or social cause; these tend to be minor

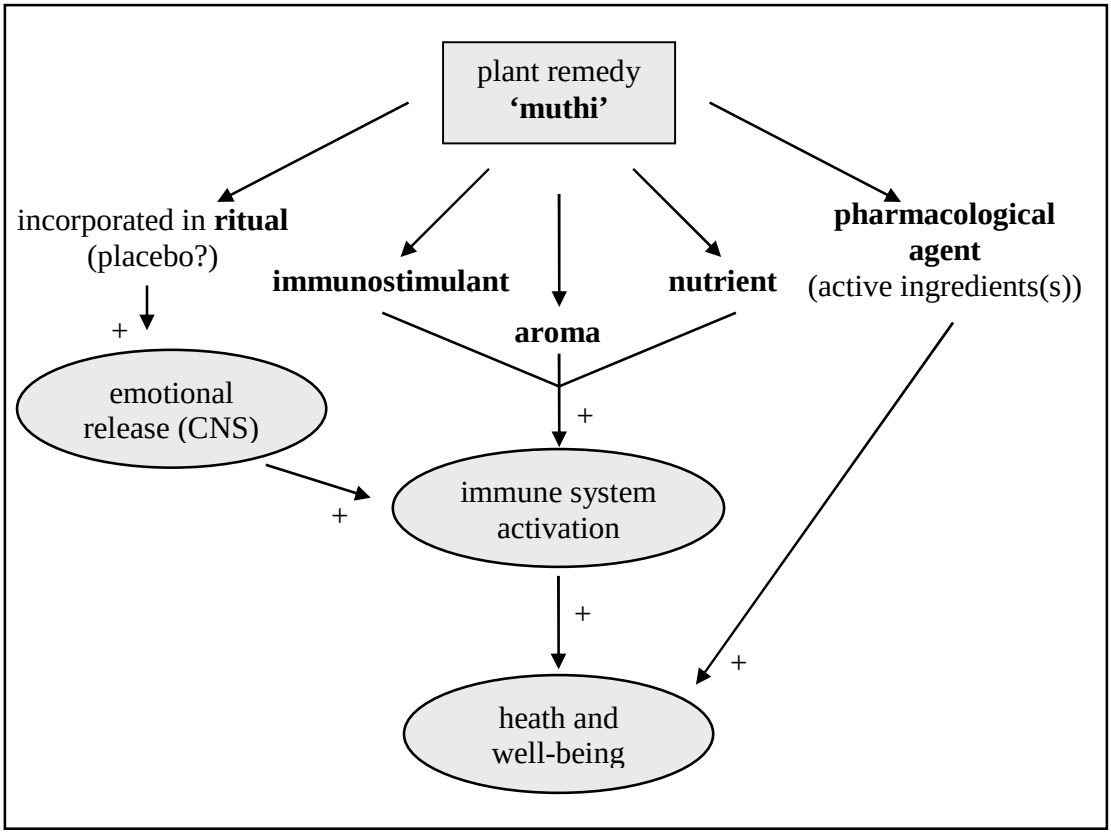


Figure 2.2. Plant remedies in traditional African medicine (TAM). (+), positive impact (From OKPAKO, 1999).

ailments such as rashes and colds. This is the only class of illness that occurs by chance, and for which causes are not sought (YAMBA, 1997). In this situation people aquire herbal treatments without recourse to spiritual evocations (OKPAKO, 1999), similarly as one might treat such ailments in Western medicine without a doctor's prescription.

Some illnesses are considered 'modern diseases' which can be contracted by people anywhere in the world, and which are believed to have been first introduced into Africa by European settlers (GREEN, 1994). Lastly, there is the belief that there are diseases which only African people can contract, and to which all African people are vulnerable (NGUBANE, 1977). A large majority of mental disorders fall under this last category. The 'epidemic' of ***indiki*** spirit possession in Zululand, South Africa from the 1890s to 1914 is an interesting example (PARLE, 2003). ***Indiki*** was first reported among the Zulu of South Africa around 1910 (JUNOD, 1921), and a similar phenomenon ***ufufunyane*** about 20 years later (NGUBANE, 1977).

OKPAKO (1999) suggests that chronic debilitating illnesses in which the cause is not apparent to the healer and the patient is not responding to common therapies, then 'ritual treatment of hidden guilt' becomes a major therapeutic consideration. When a plant remedy is used as part of the treatment regime, its physical characteristics (e.g. aroma, texture, shape, taste, colour and nutrient value) and the rituals attending its preparation and administration (e.g. incantation or song) are more important than its pharmacological constituents (**Figure 2.2**).

Perhaps a better explaination follows that, African cultures define two types of causes for illnesses (GLUCKMAN, 1956; LIDDELL, BARRETT and BYDAWELL, 2005). Firstly, a proximate cause, which accounts for how a disease is contracted (YAMBA, 1997). Infection and contagion from pollutants are examples of proximate causes (INGSTAD, 1990). Secondly, an ultimate cause, which accounts for why a disease is contracted by a particular person. A simple explanation by GREEN (1994) clarifies, 'a mother may recognize that her infant has diarrhoea because flies settled on and contaminated its food (**proximate cause**), but she will also want to establish who sent the flies to harm her child (**ultimate cause**)'. The material types of treatment (i.e. herbal remedies) are often considered of secondary importance and only complementary. The primary concern of African traditional healing is to discover who, or what has caused the imbalance or illness (i.e. the ultimate cause).

As mentioned, three main types of ultimate cause are often raised in explaining illness, contact with pollutants (literal and/or spiritual), sorcery and ancestral punishment. Pollutants are considered to often originate in other people's bodies, and include semen, menstrual discharge, vaginal secretions, and blood (INGSTAD, 1990). Death is also believed to be a pollutant (NGUBANE, 1977). Since contact with pollutants cannot always be avoided, people fortify themselves from contamination by maintaining strict moral codes and observing protective rituals (GREEN, 1994). Illness is often suspected to be inflicted by people who have been offended by a victim's behaviour. Failure to honour filial obligations, violence, or other forms of uncooperative behaviour risk creating a level of offence which elicits kin or neighbours to seek redress through 'witchcraft' (DOUGLAS, 1987). The 'survival' of ancestors in the spirit world depends on them being accorded regular attention from living offspring (e.g. respect paid to ***mizimu*** in Uganda and ***amadlozi*** in South Africa). This attention is manifest in rituals, sacrifices, avoidance of taboos, and high standards of social behavior. Where these requirements are not met, illnesses can be sent as a warning or punishment (NGUBANE, 1977).

To return to ***indiki*** and ***ufufunyane*** mentioned earlier, these are considered examples of illness representation theories that incorporate new diseases. LIDDELL, BARRETT and BYDAWELL (2005) point out that both these 'epidemics' coincided with periods in Zulu history when migrant labour was becoming extremely

disruptive to family life. Migrant labourers were engaged in high-risk occupations such as construction work and underground mining. Men who were killed whilst working away from home were buried near their place of work. It was believed that their spirits were too far away to benefit from sacrifices, and they were unable to monitor the daily activities of their successors. Consequently, these spirits were unable to attain ancestral status. As a last resort, the spirits possessed Zulu men in an attempt to return home with them and assume an ancestral role with a new family. This provided an 'ultimate' explanation for ***indiki*** and ***ufufunyane***. Simultaneously, 'proximate' causes could be located in the sexual infidelities, violations of taboos, lack of ritual observances, and general breakdown in traditional family life that accompanied the prolonged absence of a head of household. The means to avoid spirit possession lay in observing stricter social and moral codes. Branches of *Zizphus mucronata*, known as ***umlahlankosi*** (from ***-lahla*** (v) meaning to bury; ***-nkosi*** (*i-*, *ama-*) (n) meaning king or chief) are used in Zulu culture to return a departed relative's spirit to his or her home.

Both proximate and ultimate causes require treatment if a disease is to be cured. Diseases manifest themselves not only in physical symptoms, such as fever or pain, but in mystical disturbances of the blood commonly described in terms of 'impurities' or 'heat' (INGSTAD, 1990). To treat proximate causes and physical symptoms, people may consult medical personnel and/or traditional healers for appropriate remedies. However, treatment for ultimate causes must also be sought, and since these lie within the mystical or spiritual domain, it is believed that only traditional healers or diviners are capable of useful insights and therapies (BUHRMANN, 1984).

These cultural beliefs make it difficult for outsiders to understand and determine the use, in western terms, of many African medicinal plants. It is difficult for ethnobotanists to separate plants with a so-called 'magical' or 'spiritual' use (i.e. to treat ultimate cause of disease) from those that are medicinal (i.e. to treat proximate cause of disease). Literature on African medicinal plants is replete with references to plants used to counteract curses and appease the ancestors. For example, the plant *Asparagus virgatus* (Syn. = *Protasparagus virgatus*) is known by the Zulu people of South Africa as '***iphinganhloya*****'** which means 'what suppresses the ill-omen or curse' (POOLEY, 2005). Many of these plants are not ingested but are usually carried about on the person in the form of a protective amulet or charm (referred to as '***imfingo***' in Zulu culture**)** or administered by sprinkling onto the person or around the homestead (referred to collectively as '***intelezi***' in Zulu). These are assumed to have no physiological effect on the patient, however, some remedies such as those referred to as '***amakhubalo***' which are plant materials which are ingested, for protection from 'evil' may have a physiological effect on the user. It is therefore not only important to know the symptoms, including the proximate and ultimate causes, but also the methods of administration of the plant material, many of which are often neglected in ethnobotanical literature.

2.2.1. South African traditional health practitioners

The misunderstanding and negative stigma that is associated with traditional medicine in South Africa is largely due to the grouping of all traditional practitioners under the now taboo name of 'witch-doctors'. Traditionally the press has portrayed 'witch-doctors' as a group of evil, money-hungry, con-artists who are responsible for the numerous 'muthi-killings' (the use of human remains). This is unfortunate as these exploits are most likely the work of ***umthakathi*** whose intention is not to heal. The more socially acceptable term of 'sintu healer' has also been used as an umbrella name. This refers to a person who treats patients with the intention to heal, based on his or her tradition and customs. Terms such as traditional healer, traditional medical practitioners and traditional herbalist are anglicised translations that are not entirely correct. The situation is a lot more complicated in Sintu culture with many specialized practitioners each with their own name. The terms '***izinyanga***' and '***izangoma***' (plural) are often used to refer exclusively to the herbalist and diviner respectively. However today the distinction has become blurred in some cases, more often the diviners (***izangoma***) practice both arts (VAN WYK, VAN OUDSTHOORN and GERICKE, 1997).

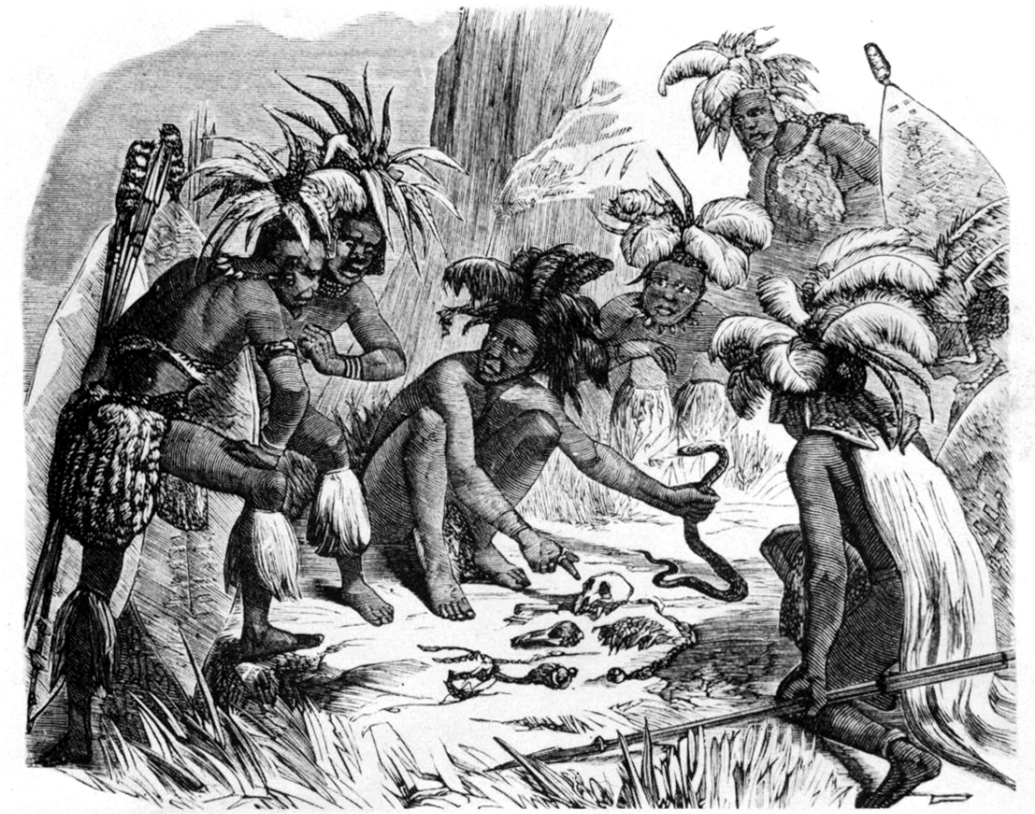
Izinyanga

In western terms the inyanga would be the medicine man or herbalist, of which in South Africa the majority are male. The ***izinyanga's*** (plural) craft is passed from father to son as opposed to that of ***isangoma*** who are chosen by ancestral spirits (***amadlozi***). One may also merely choose to be an inyanga as a profession then be trained by a qualified inyanga or several different izinyanga's.

An ***inyanga*** trains for several years as an apprentice and focuses mainly on diagnosis of illnesses and the identification and use of plants in healing physical illness. Generally the training of an ***inyanga*** is more academic and much longer than that of an ***isangoma*** (KOOPMAN pers. comm.). An ***inyanga*** is often a very skilled botanist with a vast knowledge of the local flora. At the end of their training ***izinyanga's*** can obtain a certificate from the Inyangas' National Association (INA). The ***izinyanga*** specializes in different illness and it is not uncommon for this speciality for curing one particular illness or a group of illnesses to have run in the family for many generations.

Their mode of treatment generally starts with an interview where the patient is asked where they think they acquired the illness or symptoms in question. By combining this information with the observable symptoms, the inyanga will prescribe ***umuthi***. The ***umuthi*** usually consists of plant material, but may also contain animal parts, soils, and water from rivers, dams and seas. Coloured powders, which consist of soils, burnt animal hair and plant ash is sometimes added to the water (***isiwasho***). ***Umuthi*** will be discussed in greater detail in the sections to follow. ***Izinyanga*** also supply protective and good-luck charms that are used mostly

for good intentions. An ***inyanga ebhulayo*** is similar to an ***isangoma*** and practice divination and is considered be similar to a soothsayer**.** The ***inyanga*** is highly respected in the community for the skills that they posses.



A Zulu witchdoctor's school

Plate 2.1. Taken from BRYANT (1966) Zulu Medicine and Medicine Men, an example of Western perceptions of African Traditional medicine.

Isangoma

Amagqirha (isiXhosa singular ***ugqirha***) and ***izangoma*** (isiZulu singular ***isangoma***) were previously referred to as 'witch-doctors' but this term is no longer used due to its derogatory nature. ***Izangoma*** are traditionally considered to be diviners who consult with ancestral spirits (***amadlozi***).

BOX 2.

Important spiritual components to traditional Zulu belief systems (DENT and NYEMBEZI, 1999, BURGLUND, 1976):

amadlozi – (n) spirit of departed person; guardian spirit; snake supposed to be the spirit of departed.

umhlabathi – (n) spirit(s) of departed, used when related to pains in the chest and shoulders (***uhlabo***) which are caused by ancestral sprits when they require a person to become a diviner.
amathongo – (n) spirit of departed person seen in dreams (***isithongo*** – (n) deep sleep)
uNkulunkulu – (n) Deity, God; the ancestral spirit of all mankind (DENT and NYEMBEZI, 1999), also referred to as ***umhlabathi*** (***-hlabathi*** – ground, soil or earth). 'Earth' (***umhlabathi***) is believed to be the mother of ancestral spirit of all mankind and the 'reed' (***uhlanga***) the father.

The role of the ***isangoma*** has changed in recent years as many have similar 'herbalist' skills to those of the ***inyanga***. The majority of the izangoma in South Africa are female although the gender inequality is not as definite as it is in the izinyanga. Like ***izinyanga***, ***izangoma*** show specialization in various areas, for example an ***isangoma*** that specializes in 'smelling-out' bad elements is referred to as an ***isanusi***. According to the way in which ***izangoma*** divine, can be divided into several types. For example, that of ***isangoma sekhanda*** who practices a form of divination that involves communication with the ***amadlozi*** or ***ithongo*** (spirit) through a 'telepathic trance'. These trances are often induced through the inhalation of smoke from burning plant material, in particular ***impepho*** (*Helichrysum* species). The second type is the ***isangoma samathambo***. These izangoma throw bones (***ukubhula***) and various other objects (e.g. shells, dice and seed pods) and according to the position and arrangement of the objects various predictions can be made with the guidance of the ***amadlozi***. Some plants are used in these rituals for example the divining dice of the Lobedu, a Northern Sotho group, were reportedly left overnight in an infusion made from *Lonchocarpus capassa* (raintree) so that divination could occur (KRIGE, 1940). The ***isangoma sabalozi*** apparently asks questions and the ***amadlozi*** will answer in audible whistling which both patient and isangoma can hear, these are then interpreted by the isangoma who then informs the patient.

SWIFT and ASUNI (1975) suggest that healers, because of their knowledge of the ways of their people and the power conferred upon them by their people, can often provide peace of mind to the distressed and thus provide a feeling of protection to the threatened. LAING (1965) and others have initiated a school of thought, much unliked by medical spheres, that indigenous medicine is in many respects better, more holistic, than its modern western counterpart, that it is conducive with 'indigenous world-views' and therefore more effective for 'indigenous patients' than the biomedical approach (HAMMOND-TOOKE, 1989). They argue that traditional healers do not only know their patients as people, but also understand the social matrix in which they move and live and therefore are bound to be more effective.

Umthakathi

These persons, male or female, could be referred to as sorcerers. ***Umthakathi*** (***abathakathi*** plural; ***ubuthakathi*** – (n) witchcraft) tend to go against social norms and the community does often not know their

identity. If they are discovered they will be hunted and killed by the community, similar to that of 'witch-hunts' in medieval Europe. ***Umthakathi*** are believed to know which ***umuthi*** ingredients to mix in order to make ***umuthi wakuthakatha*** (witchcraft medicine) or ***umuthi omnyama*** ('black' *muthi*). This is used for negative purposes such as to kill, cause disorder and stop childbirth. Another example is an *umbhulelo,* which is any poisonous or injurious concoction, which may or may not contain plant material, placed onto a path by an umthakathi in order to harm passers-by.

Several herbal remedies, which may or may not be ingested, are employed by healers and the general public as protection or to counteract the work of umthakathi. These include ***imfingo,*** any medicine used to protect against evil, usually carried about on the person in the form of a protective amulet or charm. ***Amakhubalo***, however, are any 'medicines' such as roots or barks, but not leaves, bulbs or animal powders, which are used for self-fortification and protection from evil. These are nibbled or chewed and are often carried around the neck or on ones person for this purpose. A very common preparation, an ***intelezi*** is any protective medicine or charm against evil, usually administered by "sprinkling" (***ukuchela***) onto the person or around the homestead. This consists of a mixture of plant parts like bulbs (commonly *Clivia* and *Agapanthus* species), leaves and bark that is placed in a container with cold water for use later when it is sprinkled around the homestead or on the person. It can also be boiled and used when bathing (NGWENYA, KOOPMAN and WILLIAMS, 2003). An ***imbozisa*** (***-bozisa*** (v) cause to rot) is any strong-smelling herbal counter-remedy. A number of strongly scented plants (e.g. *Plectranthus* species) are known generally by this term.

Umthakathi are reported to use specific human body parts to use in ***umuthi wakuthakatha***, these are generally taken from young children (SCHOLTZ, PHILLIPS and KNOBEL, 1997). It should be stressed that these ***umthakathi*** are not traditional healers and 'true traditional healers' (***izangoma*** and ***izinyanga***) should not be painted with the same brush.

When a healer sets out to cure a person afflicted by the work of ***umthakathi***, he or she will typically promise that their ***muthi*** will return the evil forces deployed by the ***umthakathi*** to their source, thereby killing the umthakathi. Such violence, however, is considered by the community to be legitimate, for it is executed in the name of defense. ***Umthakathi***, by definition, are engaged in illegitimate uses of the powers of ***muthi*** (ASHFORTH, 2005).

Umuthi *– preparation and administration of African traditional medicine*

When traditional healers, both inyanga and izangoma, administer aid to patients in distress, they almost always dispense substances known generically as ***muthi***. The term ***muthi*** (spelled ***muti*** in Xhosa transliterations) derives from the Nguni root ***-thi***, signifying 'tree'. Usually translated into English as either

'medicine' or 'poison,' with the anodyne 'herbs' used occasionally (ASHFORTH, 2005), ***muthi*** refers to substances fabricated by an experienced or trained hand, substances designed by persons possessing secret knowledge to achieve either positive ends of healing, involving cleansing, strengthening, and protecting persons from evil forces, or negative ends of 'witchcraft' (***ubuthakathi***), bringing illness, misfortune, and death to others or illicit wealth and power to the witch (ASHFORTH, 2005).

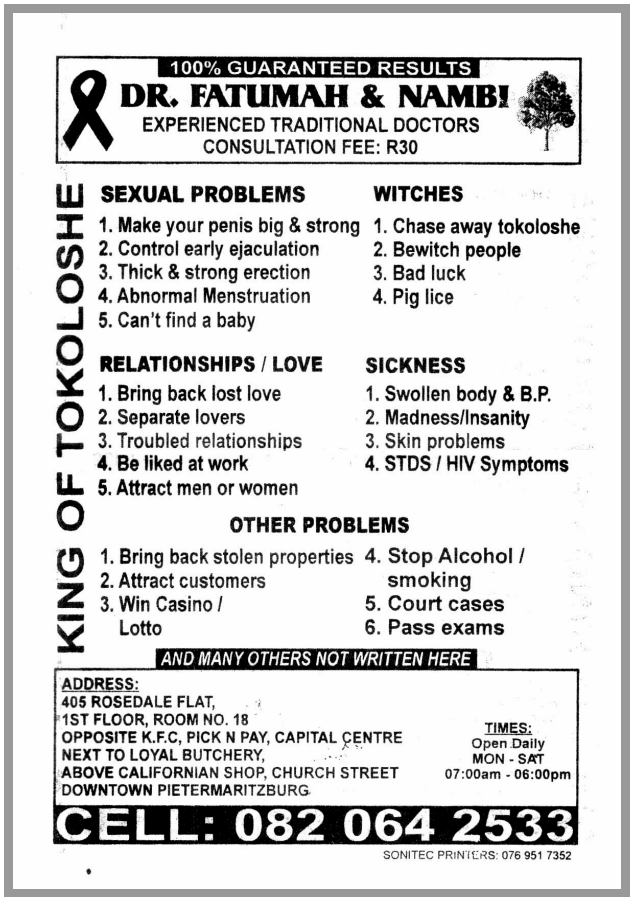


Figure 2.3. An example of flyer advertising a 'traditional healers' abilities and claims.

To clarify whether the ***muthi*** is medicine or poison, the valence of the term can be specified, as in several cultures, by reference to the colours black and white. For example in Zulu, ***umuthi omnyama*** (black ***muthi***) is the harmful poison, and ***umuthi omhlope*** (white ***muthi***) is the healing medicine. According to NGUBANE (1977), colour plays an important role in the symbolism associated with the treatment in Zulu people. The important colours are black, red (***-bomvu***), and white. Black and red are equivocal; and they stand for good and bad, while white represents what is good. Whenever red or black objects or plant materials are used, they generally are always followed by white; however, white is often used alone. Red and black are used to expel bad things from the body, and to strengthen the body against future attacks. White is used to regain good health.

Plant material and mixtures are administered in several forms and by several routes. Several traditional Zulu preparations, ***umbhulelo***, ***imfingo***, ***amakhubalo***, ***intelezi*** and ***imbozisa***, have been discussed. Another important preparation are ***amakhambi*** (plural) which are any green, vegetable medicines, leaves or roots which are used as common house-hold remedies. These are well-known to most Zulu mothers, as are *imbiza* which include a large number of herbs which are boiled into decoctions for scrofula, chest complaints and blood purifying.

Oral ingestion, of preparations made by cold infusions (*isichonco, -chonco* (*isi- izi-*) (n) mixture taken in small doses), hot infusions (***imfudumezelo***, from –***fudumeza*** (v) make warm) or boiled and simmered decoctions (***impheko***, from ***-pheka*** (v) cook) are common. These would normally be swallowed, but sometimes would be held in the mouth by the patient and blown out in a fine spray over the affected body part. Emetics (***umuthi wokuphalaza*** or ***umhlanziso*** from –***phalaza*** (v) vomit) are an extremely common particularly when administering charms of all kinds (i.e. protective charms, lucky charms and love charms). VAN WYK, VAN OUDTSHOORN and GERICKE (1997) mention that among Zulus and other cultural groups, a common health practise is to drink a large volume (up to two litres) of weak, luke-warm herbal infusions (***ukuphalaza*** or ***ukugabha***) and then induce vomiting to cleanse and tone the system. Sea water is also commonly used for this purpose.

The Zulu administer hot stem infusions of *Stapelia gigantea* (***ililo elikhulu*** or ***uzililo***) or cold water infusions of *Psoralea pinnata* (***umhlongani***) (BRYANT, 1966) and infusions or decoctions of *Crassula alba* (***isidwe***) (GERSTNER, 1939) as emetics to treat hysteria (***umhayizo***). ***Umhayizo*** is an illness associated with Zulu teenage girls and is commonly explained as an affliction that is the direct result of witchcraft (***ubuthakathi***). It is believed to be caused by the actions of a jealous or bitter man who performs, usually at a distance from the girl in question, a particular ritual act involving ***umuthi***. This results in screaming and wailing with a possession-like experience of having relinquished control over one's body. These symptoms are a visible manifestation of 'bewitchment', and it is this that calls for treatment. Zulu men and adolescent boys use decoctions of unspecified parts of *Bulbine latifolia* (***ibhucu***), as emetics, in purification rites aimed at the prevention of antisocial behaviour (HUTCHINGS, SCOTT, LEWIS and CUNNINGHAM, 1996). Zulu ***izangoma*** (diviners) use root infusions of *Adenopodia spicata* (***ibobo***) as emetics to increase their divining power (HUTCHINGS, SCOTT, LEWIS and CUNNINGHAM, 1996).

Another common oral method, which appears to be particularly prevalent in Zimbabwe, is the administration of plant material in traditional porridge, for example *Crabbea hirsuta, Tulbaghia leucantha, Steganotaenia araliacea,* the exotic *Bauhinia candicans, Bauhinia thonningii, Vigna unguiculata, Pycnostachys urticifolia,*

Securidaca longepedunculata, Fadogia ancylantha and *Gnidia kraussiana* (**Table 2.2**.). Most of these are used in the treatment of epilepsy or madness (SHONE and DRUMMOND, 1965; GELFAND, MAVI, DRUMMOND and NDEMERA, 1985).

Decoctions, and in some cases infusions, are commonly administered as enemas (***uchatho, isitho, upotsho***). Today this is most commonly done using enema syringes or tubes. However in some rural areas, as in the past, a lubricated, truncated cow's horn is used (VAN WYK, VAN OUDTSHOORN and GERICKE, 1997). Ground bulbs of *Scilla nervosa* (***ingcino*** or ***ingcolo***) are administered in the form of milk enemas to relieve nervous conditions in children in the northern Transvaal (WATT and BREYER-BRANDWIJK, 1962). Some other plants used in this manner to treat mental illness are *Tulbaghia alliacea, Blumea alata, Cissampelos torulosa, Oxygonum* species and *Aptosimum decumbens* (**Table 2.2**.).

Plant materials may be applied directly to the skin, where it is assumed that the active principles are absorbed into the underlying tissues. A common administration method in African traditional medicine is where plant or other material which has been dried, roasted or burnt, may be ground to a fine powder, which is then rubbed into incisions in the skin (***umgcaba*** from ***gabela*** (v) to cut slits). It is claimed that the San in Botswana induce visual hallucinations by rubbing the bulb of *Pancratium tenuifolium* into incisions on the head (DOBKIN DE RIOS, 1986). *Asparagus* roots of an unidentified species are burned, powdered and placed in incisions for febrile convulsions in Zimbabwe (CHINEMANA, DRUMMOND, MAVI and DE ZOYSA, 1985). *Launaea nana, Canthium inerme* and *Lopholaena coriifolia* roots are used as a body wash and are applied into incisions made on the forehead or body to treat convulsions in Zimbabwe (GELFAND, MAVI, DRUMMOND and NDEMERA, 1985). *Swartzia madagascariensis* (***mukosho*** in Shona) roots and pods are taken orally as an infusion or applied to incisions made on the forehead for convulsions in Zimbabwe (GELFAND, MAVI, DRUMMOND and NDEMERA, 1985).

A large variety of plants are taken as fine dried powders, referred to as snuff (***ugwayi*** or ***umakhalis****a*). Some are taken to induce sneezing ((n) ***ukuthimula***), which is believed to aid the expulsion of disease or evil spirits. Traditional healers induce sneezing through the use of snuff frequently. Snuffs are also used for the treatment of respiratory ailments and headaches. The nasal route is an extremely effective route for introducing soluble phytochemicals directly into the cerebral circulation and is often a method used in taking narcotics and stimulants (refer to **Table 2.1**. and **Table 2.3.**).

WATT and BREYER-BRANDWIJK (1962) mention other botanical snuff sources utilized in southern Africa, these include: *Agave americana, Aloe aristata, Aloe marlothii Aloe saponaria* var. *ficksburgensis,*

Aloe sp., *Amaranthus caudatus, Amaranthus spinosus, Artemisia afra, Cussiona spicata, Rhus erosa, Salvia* sp., *Senecio coronatus, Senecio longiflorus, Tagetes minuta* and *Zea mays.*

Table 2.1. Snuffs used in southern African traditional medicine for sedative, narcotic and divination proposes or in the treatment of mental illness (authorities are given in **Table 2.2** page 68).

Species	**Traditional use**
Alepidea amatymbica	The dry rhizome and roots are smoked, or powdered and taken as a snuff by unspecified diviners and healers in South Africa to assist in divination and communication with the ancestors (VAN WYK and GERICKE, 2000).
**Centella asiatica*	Unspecified groups use the dry powdered leaf as a snuff, which produces a calming, sedative effect (VAN WYK and GERICKE, 2000).
Chenopodium ambrosiodes	South African traditional healers use the plant as an intoxicating snuff to communicate with the ancestors (SOBIECKI, 2002)
**Datura stramonium*	Powdered roots and leaves are inhaled as snuff to aid healers in divining in South Africa (HUTCHINGS, SCOTT, LEWIS and CUNNINGHAM, 1996).
Entandrophragma spicatum	The pods are burned and the ashes are mixed with tobacco to make a narcotic snuff (RODIN, 1985).
Erythrophleum lasianthum	The Zulu administer a snuff for hysteria (HUTCHINGS, SCOTT, LEWIS and CUNNINGHAM, 1996).
Gomphocarpus species	Unspecified groups use a snuff of the powdered leaves as a sedative (VAN WYK and GERICKE, 2000).
Lannea schweinfurthii	Unspecified groups in South Africa use the root as a sedative snuff (VAN WYK and GERICKE, 2000)
**Nicotiana tabacum*	Taken as a snuff by southern African diviners at the start of divination, and is also made as a traditional offering to the ancestors (VAN WYK and GERICKE, 2000).
Ocimum canum	The Ndebele use the whole plant mixed with the seed of *Ricinus communis* L.(exotic) and *Chenopodium ambrosidoides* L. as a snuff for madness (GELFAND, MAVI, DRUMMOND and NDEMERA, 1985).
Pachycarpus asperifolius	A powdered snuff is used for hysteria in South Africa (HUTCHINGS, SCOTT, LEWIS and CUNNINGHAM, 1996).
Phytolacca octandra	The shoots are used as a stimulant snuff in Venda (MABOGO, 1990).
Pleiospilos bolusii	Unspecified groups dry and powder it to be used as a snuff (VAN WYK and GERICKE, 2000).
Ptaeroxylon obliquum	The Xhosa use powdered bark traditionally as a snuff for recreational purposes (WATT and BREYER-BRANDWIJK, 1962).
Rabiea albinota	The pulverised plant is reported to be a hallucinogenic additive to tobacco to be smoked or taken as snuff (VAN WYK and GERICKE, 2000).
Rhamnus prinoides	Unspecified groups use ground bark that is administered as snuff for mental disorders in the Transkei (HUTCHINGS, SCOTT, LEWIS and CUNNINGHAM, 1996).
Sceletium tortuosum	The traditionally prepared dried plant material is chewed, smoked, or powdered and inhaled as a snuff. It is used as a sedative, and elevates mood and decreases anxiety, stress and tension (VAN WYK and GERICKE, 2000).
Tephrosia capensis	Dried powdered roots are also used as a snuff for headaches (HUTCHINGS, SCOTT, LEWIS and CUNNINGHAM, 1996).
Turbina oblongata	The Sotho use the leaves as a snuff mixed with tobacco in Lesotho (JACOT GUILLARMOD, 1971).
Xysmalobium undulatum	Powdered tubers are administered as snuff to treat hysteria in Transkei (HUTCHINGS, 1989).

* exotic species

The inhalation of smoke and vapours from burning or heated plant material is a common practise in many cultures. In a recent review on 'medicinal smokes' used throughout the world, 19 species were reported to be used in treating mood disorders, 70 species were reported to have neurological action (MOHAGHEGHZADEH, FARIDI, SHAMS-ARDAKANI and GHASEMI, 2006). These were divided into 8 categories: analgesic (38 spp.), anticonvulsive (9 spp.), stimulant (9 spp.), narcotic (7 spp.), sedative (7 spp.), hallucinogen (3 spp.), strengthener (2 spp.) and as a remedy for vertigo (2 spp.). In seven cases of its use as an analgesic, the smoke is directed at specific organs; in all other cases, it is inhaled. Important families within this group are Asteraceae (11 spp.), Solanaceae (10 spp.), Apiaceae (9 spp.), Fabaceae (7 spp.) and Ranunculaceae (4 spp.) (MOHAGHEGHZADEH, FARIDI, SHAMS-ARDAKANI and GHASEMI, 2006).

A few treatments are administered by steaming them in hot water or by heating over a fire or hot plate and the vapours inhaled. The tubers of *Trichodesma physaloides* are boiled and the steam inhaled for madness in Zimbabwe (GELFAND, MAVI, DRUMMOND and NDEMERA, 1985). In South Africa, leaves and bark of *Maerua angolensis* are heated over a fire without water and the steam is inhaled to treat children with convulsions (MABOGO, 1990; VENTER, 1996). Unspecified parts of *Buxus macowanii* (***umgalagala*** in isiXhosa) are used in a vapour bath to treat mental illness in southern Africa (SIMON and LAMLA, 1991), members of this genus are known to contain alkaloids that inhibit acetylcholinesterase (**Chapter 5**).

Southern African plants that are **burnt and the smoke inhaled** to treat mental illness, for narcotic or divination purposes are listed below:

Alepidea amatymbica
Aspilia pluriseta
Astripomoea malvacea
Blumea alata
Brackenridgea zanguebarica
Bridelia cathartica
Cassia didymobotrya
Cineraria aspera
Clematopsis scabiosifolia
Cullen obtusifolia
Cymbopogon validus
Entada rheedii
Euclea natalensis
Helichrysum species
Hemizygia bracteosa
Leonotis leonurus
Mentha aquatica
Monanthotaxis caffra
Ocimum canum
Pachystigma pygmaeum
Pellaea calomelanos
Rabiea albinota
Sceletium tortuosum
Sesamothamnus lugardii
Sutera species
Sutherlandia frutescens
Sutherlandia microphylla
Tagetes minuta
Tarchonanthus camphorates
Turraea nilotica

Herbal mixtures may also be added to bath water to relieve certain conditions. The Zulu use the leaves of *Uvaria lucida* (***umavumba***) for bathing patients with mental disease in South Africa (HUTCHINGS, SCOTT, LEWIS and CUNNINGHAM, 1996). A bath taken in water in which the bark of *Balanites maughamii* has been infused is reported to be both stimulating and exhilarating (COATES PALGRAVE, 2002). Cold

infusions of *Commelina africana* are used to bathe restless sleepers (WATT and BREYER-BRANDWIJK, 1962). *Desmodium gangeticum* is used in Liberia to bathe children with convulsions (OLIVER-BEVER, 1986). Leaf decoctions of *Clerodendrum myricoides* are used for bathing patients with convulsions in Zimbabwe (GELFAND, MAVI, DRUMMOND and NDEMERA, 1985).

Zulu botanical names

Zulu plant names are extremely descriptive and can often provide insight into the plants cultural and medicinal use. Zulu plants names often provide more than a means of identifying the plant; they also often show a link between Zulu culture and their environment (NGWENYA, KOOPMAN and WILLIAMS, 2003). Much can be learnt about the medicinal use, administration, location and morphology from these names. This is only true where Zulu plant name exhibits a relationship between the plant itself (the 'denotatum' of the name) and the underlying meaning of the name (the 'designatum') (**Figure 2.4**). The Zulu Botanical Knowledge Project, involving the Natal Herbarium, National Botanical Institute and the University of KwaZulu-Natal, was established to capture this botanical and cultural knowledge. A pilot project, focusing on tree regions within KwaZulu-Natal, Bulwer/eLotheni, oNgoye/eNkandla and eNtimbankulu resulted in a small publication which has inspired and assisted in the inclusion of the meanings of some of the Zulu names in **Table 2.2**. Additional assistance was given by Prof Adrian Koopman, a lexicography specialist, in the School of Language, Culture and Communication at the University of KwaZulu-Natal.

A single plant name is often used for a variety of different species in different genuses because of some perceived similarity. When further qualified, however, the new phrasal name will normally only refer to one species, similar to the Latin binomial system. For example the name ***ishongwe*** is used for the following taxonomically related (members of Asclepiadaceae) plants; *Asclepias cucullata, Xysmalobium undulatum, Pachycarpus coronarius, Pachycarpus dealbatus* and *Pachycarpus concolor*. When further qualified, only one species is referred to, for example ***ishongwe elibomvu*** (meaning the red *ishongwe*) for *Pachycarpus asperifolius*; ***ishongwe elibomvu elikhulu*** (meaning the big red ***ishongwe***) for *Pachycarpus natalensis*; and ***ishongwe elincane elibomvu*** (meaning the small red ***ishongwe***) for *Schizoglossum atropurpureum.* However another Asclepiad *Gomphocarpus physocarpus*, has five names, ***umangwazane, umbababa, umqumbuqumbu, uphuphuma*** and ***usingalwesalukazi***, and does not follow the system above.

Another example of a single name that is used for variety of different species because of some perceived similarity is ***impepho***. This time however, the similarity lies in the plants use. ***Impepho*** refers to any species of small everlasting plants with a sweet smell, used for burning as an offering to the ***amadlozi*** (ancestral spirits). This term is used especially for various species of *Helichrysum* again suggesting a taxonomic relationship however taxonomically unrelated species (e.g. *Psammotropha myriantha*) are also used as

impepho. When further qualified, again only one species is referred to, for example; ***impepho-emhlophe*** (white ***impepho***) *Helichrysum aureonitens*, ***impepho-tshani*** (grass ***impepho***) *Psammotropha myriantha*, ***impepho- yamakhosi*** (royal or chief's ***impepho***) *Helichrysum herbaceum).*

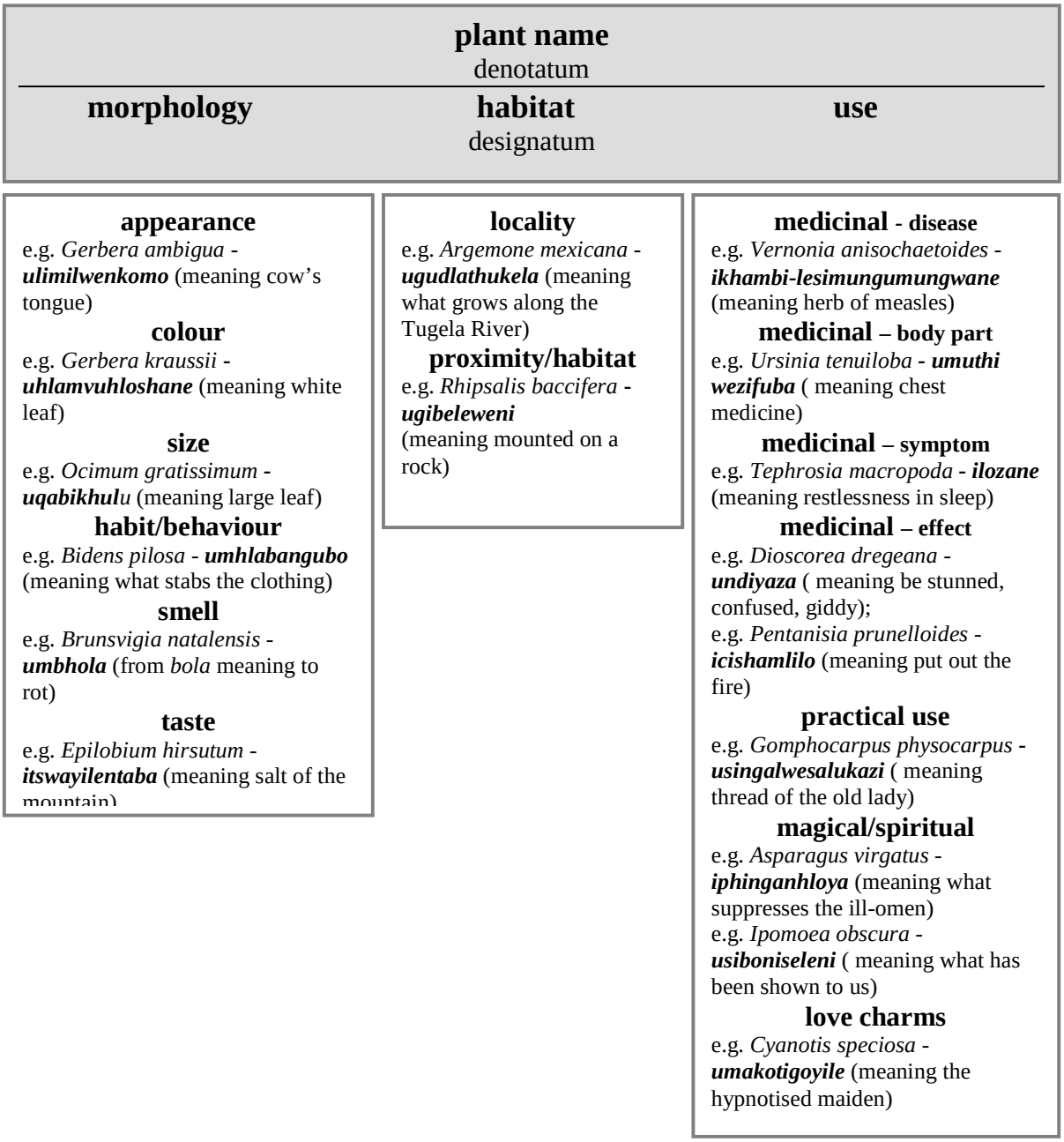


Figure 2.4. Diagram shown examples of the relationship between Zulu botanical names (denotatum) and features of the plant such as habitat, appearance and use (designatum). After KOOPMAN pers. comm.

An example of a plant which shows a relationship between its Zulu name and its medicinal use is *Dioscorea dregeana.* The tuber, used in traditional medicine to treat insanity and fits (HUTCHINGS, SCOTT, LEWIS and CUNNINGHAM, 1996), has three Zulu names which refer to the conditions treated and the effect (**see**

Figure 2.4). ***Ilabatheka*** refers to any medicine for causing/treating madness or excitement, ***undiyaza*** means to be stunned or confused and ***udakwa*** is to be drunk or inebriated. Where possible the meanings of the Zulu names of plants have been incorporated in **Table 2.2**.

Not all Zulu plant names have a clear relationship between underlying meaning and the plant itself. There are a vast number of plant names where designatum and denotatum overlap (e.g. ***ihlukwe*** refers to any plant that 'western taxonomy' refers to as *Zantedeschia aethiopica* and has no other meaning). These are mainly single-stemmed words where the 'underlying meaning' and the 'surface meaning' are the same. In some instances several underlying meanings are perfectly clear but there are no apparent reasons to link the meaning to any attribute of the plant (eg. *Callilepsis laureola* is known in isiZulu as ***amafuthomhlaba***, meaning fat of the earth which has no obvious relation to the plant). Occasionally there are ambiguous meanings where plant names where the underlying meaning could be taken in two ways, either of which relates sensibly to aspects of the plant.

2.3. Southern African psychoactive plants

A closer look at plants used in African traditional medicine reveals a large number of plants with potential psychoactive properties. One study in South Africa list 306 plants from 94 families with reported psychoactive uses in southern Africa (SOBIECKI, 2002). Thirty plants belonging to 21 families and traditionally used in southern Nigeria by herbalists for the management of mental disorders (including amnesia, insomnia and senile dementia) have been reported (NWOSU, 1999). NEUWINGER (2000) cites more than 1 750 potential psychoactive traditional African medicinal applications (**Figure 2.5**); the majority (more than 600) of treatments were aphrodisiacs and sexual stimulants. What is also of interest is the large number of treatments with potential CNS suppressant effects, for example 300 plants for treating epilepsy and convulsions alone, possibly indicating the high prevalence of this disease in Africa. In addition to these there are plants to calm the insane, to treat insomnia, hysteria and tranquilizers (NEUWINGER, 2000), which could work on a similar physiological system (i.e. CNS suppressant).

Mental health problems constitute a serious problem in southern Africa. Not surprisingly, a large number of plants, over 320 species from 94 familiess, are used by traditional healers in their treatment of these ailments (**Table 2.2** at the end of this Chapter). Nearly 150 plant species from 63 families are used for the indications epilepsy and convulsions, possibly reflecting the severity of this problem in the population. The families most often represented are Fabaceae (15 species), Asteraceae (13 species) and Lamiaceae (9 species). Over 40 plant species are used for ailments that could loosely be described as depression. These plants come from 26 families. The number of plants used for dementia and age-related mental problems is lower with only 15

species from 7 families recorded, which could be due to a previous demographic situation, where traditional healers less frequently encountered very old patients.

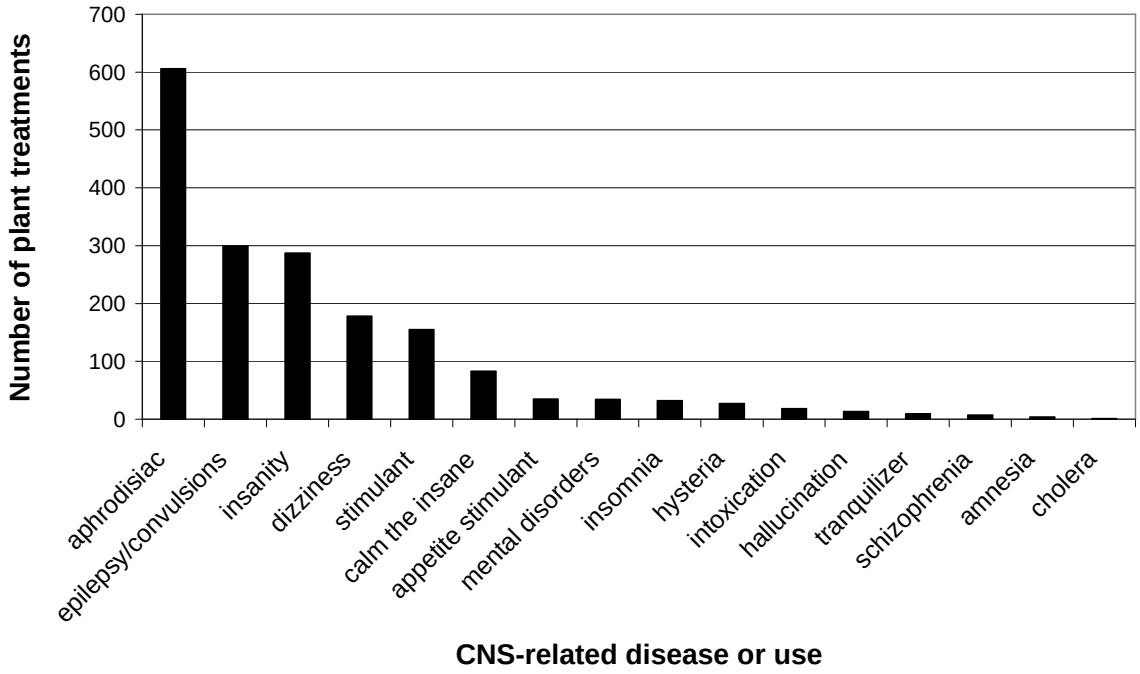


Figure 2.5. Number of African traditional plant treatments with potential psychoactive properties based on their traditional uses reported in NEUWINGER (2000).

Plants used to induce dreams, visions, trances and hallucinations

Psychoactive plants have often featured in the religious and medicinal practices of numerous cultures around the world. In shamanic societies, hallucinogenic plants, and the altered mental state they induce, are often employed by the community spiritual practitioner, or shaman, in various religious and healing rituals. Shamanism simplistically involves practitioners who voluntarily or with the aid of psychoactive substances, dance and/or music; enter an altered mental state. In this state they experience themselves or their spirits travelling to other realms where they reportedly interact with other entities in order to acquire knowledge and power to heal people in their communities (ELIADE, 1987; WALSH, 1990). African traditional medicinal and cultural practices are considered to fall under shamanistic practices (LAMBRECHT, 1998).

Possibly the most culturally important hallucinogenic plant in southern Africa is *Boophone disticha* (***incotho***) (DE SMET, 1996). LAYDEVANT (1932) described its use in initiation ceremonies of the South African Basotho. The boys were given food mixed with the bulb and are instructed it would imbue them with the qualities of their ancestors and that it would tend to make men of them. The signs of intoxication were regarded as a token that the spirit of manhood had entered their bodies. Traditional healers and patients in

South Africa drink *Boophone disticha* bulb infusions to induce hallucinations for divinatory purposes, and also as a medicine to treat mental diseases. However, many injuries result from the toxic use of this plant (SOBIECKI, 2002). Other Amaryllidaceae species, *Crinum* species and *Pancratium tenuofolium* are used in a similar way to *B. disticha* (SOBIECKI, 2002). A plant resembling *Tulbaghia capensis* is reported to be used in South Africa with *B. disticha* to induce visions (*isibonakalo* mental vision) (SOBIECKI, 2002). *Dioscorea dregeana* is often mixed with *Boophane disticha* reportedly to synergize the visionary experience and is a popular psychoactive plant sold on the ***muthi*** markets in Johannesburg (SOBIECKI, 2002). These and other species that may induce an altered mental state and are used in African traditional medicine will be discussed in detail in the rest of this section.

Several species have been identified as being used to induce or enhance **dreams**. The Zulu use unidentified species of *Agapanthus* for inducing visions and dreams (***iphupho***) in South Africa (SOBIECKI, 2002). Smoking the roots of *Alepidea amatymbica* is reported to result in mild sedation and vivid dreams (VAN WYK and GERICKE, 2000). An unidentified plant corresponding to the name ***umhlambamasi***, possibly *Rauvolfia caffra*, is used by the Zulu to enable one to hear one's ancestors in one's dreams (MANANA, 1968). The root of *Silene capensis* are used by Xhosa diviners for spiritual purposes and inducing dreams (HIRST, 1997b). *Pleurostylia capensis* is used to encourage sleep to bring good dreams in the cape of South Africa (DE JAGER, 1963).

Unspecified groups in South Africa crush two to four seeds of *Ipomoea alba* in water and the resulting liquid is taken orally at night to induce vivid dreams, while the seeds of an unknown Convolvulaceae are used to induce dreams and communication with the ancestors (VAN WYK and GERICKE, 2000). The roots of *Albizia adianthifolia* are used for improving memory, and inducing dreams about medicinal plants in Venda (MABOGO, 1990). Tobacco smoked in a pipe made from the large *Entada rheedii* (***umbhone*** or ***intindili*** in isiZulu) seeds have been reported to cause vivid dreaming in South Africa (VAN WYK and GERICKE, 2000), and are also reported by MANANA (1968) to be used to remember dreams. A plant called ***umnono*** identified as *Strychnos henningsii* by SOBIECKI (2002) is used to induce visionary dreams associated with the ancestors. An unidentified plant with the name *ubuvimbha*, possibly *Withania somnifera*, is taken to induce clear dreams (MANANA, 1968).

The bark of *Brackenridgea zanguebarica* is reported to be smoked to induce **visions** and is also used as a protection against evil and bad omens in South Africa (SOBIECKI, 2002). *Eleutherine bulbosa* (***ababomvu***) is believed to have magical and hallucinatory properties (HUTCHINGS, SCOTT, LEWIS and CUNNINGHAM, 1996), and is reported to be an ingredient of a psychoactive mixture used to induce visions in South Africa (SOBIECKI, 2002). Diviners swallow roots of *Monadenium lugardiae* in the Piet Retief area

of South Africa to obtain clear vision before important meetings, while roots are reported to induce hallucinations and delirium if taken in sufficient quantities (WATT and BREYER-BRANDWIJK, 1962). *Synaptolepis kirkii* (***uvuma-omhlophe***) root infusions have been used as purifying ritual emetics and face and body washes to assist South African diviners to 'see' in a metaphysical sense (VAN WYK and GERICKE, 2000).

Four plants, *Indigofera flaviscans*, *Ferraria glutinosa*, *Loranthus oleifolius* and *Plumbago zeylancia*, are reportedly used by the !Kung (San) in the Kalahari to induce a **trance-like state** referred to as '***kia***'. This is described as an altered state of consciousness considered to be a prerequisite for healing practices (KATZ, 1982, WINKELMAN and DOBKIN DE RIOS, 1989). Smoke from burning ***impepho*** (*Helichrysum* species) is inhaled by the Zulu diviners to induce trances in South Africa (HUTCHINGS, SCOTT, LEWIS and CUNNINGHAM, 1996). *Turraea floribunda* (***umadlozana***) roots are used by Zulu diviners to enter the 'neurotic' state needed for divining dances (HUTCHINGS, SCOTT, LEWIS and CUNNINGHAM, 1996).

Some southern African plants have been reported to possess **hallucinogenic** properties but these have yet to be investigated. Pulverized *Rabiea albinota* is reported to be a hallucinogenic additive to tobacco to be smoked or taken as snuff (VAN WYK and GERICKE, 2000). *Anacampseros rhodesica* is a beer additive, and is reported to have hallucinogenic and narcotic activity (GELFAND, MAVI, DRUMMOND and NDEMERA, 1985; VAN WYK and GERICKE, 2000). Leaf decoctions of *Cissampelos torulosa* are reported to be administered as enemas for hallucinations in the Transkei (HUTCHINGS, SCOTT, LEWIS and CUNNINGHAM, 1996). It is not clear whether this plant is used to treat or induce hallucinations.

The Zulu use unspecified parts of *Datura metel* that are mixed with an unidentified *Dioscorea* species as hypnotic drugs against hysterical fits in girls (GERSTNER, 1941). *Datura metel* is also ingested and used as a hallucinogen or entheogen in the Tsonga girls' initiation schools in Mozambique and the Northern Province of South Africa (JOHNSTON, 1972).

Several plants are used by healers themselves to aid in the **divination** process. For example root infusions of *Pittosporum viridiflorum* are used for accuracy in divining by the Sotho (HUTCHINGS, SCOTT, LEWIS and CUNNINGHAM, 1996); diviners are also reported to use the plant to induce altered states of consciousness for purposes of divining (SOBIECKI, 2002). *Myosotis afropalustris* is used in the initiation of Sotho diviners and to treat 'people with the spirit' that suggests a type of mental disturbance or spiritual calling (LAYDEVANT, 1932). Zulu izangoma (diviners) use root infusions of *Adenopodia spicata* (***ibobo***) as emetics to increase their divining power in South Africa (HUTCHINGS, SCOTT, LEWIS and CUNNINGHAM, 1996). The Kgatla, a Tswana group, use unspecified parts of *Mundulea sericea* as a

'divining medicine' (WATT and BREYER-BRANDWIJK, 1962), it is also considered to be a very powerful magical plant, known as ***mukundandou*** by the Vhavenda (MABOGO, 1990). MONNIG (1967) describes how the Pedi use *Helinus integrifolius* in the initiation of diviners to 'strengthen his memory and give the initiate keen powers of observation'. This plant has important spiritual uses among the Zulu and Xhosa in Johannesburg (SOBIECKI, 2002).

Stephania species are used by Sotho diviners 'to discover' things and are used with divining bones (Phillips, 1917). It is not clear whether the plant is ingested. Flowers of *Nymphaea nouchali* are used by South African diviners, and tinctures of flowers are stimulant, aphrodisiac and euphoriant in low doses (VAN WYK and GERICKE, 2000). Flowers are sold on the ***muthi*** markets in Johannesburg for divinatory purposes (SOBIECKI, 2002). *Adenia gummifera* (***impinda***) is reported to have psychoactive properties that are used by the Zulu in divination on the east coast of South Africa (SOBIECKI, 2002). An unidentified *Kaempferia* species (now refered to as *Siphonochilus* perhaps *S. kirkii* (Hook.) B.L.Burtt) is chewed by Lobedu (a Northern Sotho group) traditional healers before divining (KRIGE, 1940). *Euclea divinorum* (magic guarri) is said to be used for divination in Africa, hence the name *E. divinorum* (SOBIECKI, 2002). Sotho diviners use unspecified parts *Crabbea hirsuta* in conjunction with divining dice in South Africa (WATT and BREYER-BRANDWIJK, 1962).

As mentioned already there are surprisingly few traditional African treatments for **memory** loss (amnesia), to improve cognition or treat age-related neurodegenerative diseases like Alzheimer's and Parkinson's disease. Four species are reported to be used in southern Nigeria by herbalists for amnesia (NWOSU, 1999). These include *Boerhaavia diffusa* (Nyctaginaceae), the roots of which are reported to contain liriodendrin (lignan) a Ca^{2+} channel blocker (POLYA, 2003). Decoctions of male inflorescences of *Carica papaya* (Caricaceae) with *Zingiber officinale* (Zingiberaceae) and *Pauridiantha viridiflora* (Rubiaceae) are also used to treat amnesia. *C. papaya* contains (*S*)-(-)-cotinine (pyridine pyrrolidinone), a major brain metabolite of nicotine, which is also an acetylcholine receptor agonist (POLYA, 2003). In southern Africa there appears to be only three references to plants that improve memory. *Albizia adianthifolia* roots are used for improving memory by the Venda (MABOGO, 1990). An infusion of *Aptosimum decumbens* or chewing the leaves is reported to improve the memory (RODIN, 1985). MONNIG (1967) describes how the Sotho (Pedi) use *Helinus integrifolius* in the initiation of diviners to "strengthen his memory and give the initiate keen powers of observation".

Several plants are associated with dealing with **death** of a family or community member. The Zulu regard *Cunonia capensis* (***umaphethu, umhlahlane, umuthi wokuzila***), *Gerrardina foliosa*, *Nuxia floribunda* and *Turraea floribunda* (***umadlozana***) as a strengthening medicines that is taken after the death of a kraal (family

or community) member (GERSTNER, 1939). A mixture containing *Schotia brachypetala* (***ihlusi, ihluze, umgxamu*** and ***uvovovo***) is often taken and also washed with during purification rites after the death of a relative (PUJOL, 1990). As mentined death is believed to be a 'pollutant' (NGUBANE, 1977). The Vhavenda traditionally used decoctions made from root bark of *Lannea schweinfurthii* mixed with a fungus found growing on the roots to help family members to forget a recently deceased relative (MABOGO, 1990).

The remainder of this Chapter consists of a **Table (2.2a**) listing plants that are and have been used for central nervous system-related purposes. This has been compiled from numerous literature sources including books, theses, journal articles and magazine articles. All botanical names, authorities and annotations (morphology and distribution) of the species are taken from GERMISHUIZEN and MEYER (2003). **Table 2.2b** contains an alphabetical list of plant species which can be used to locate specific species in **Table 2.2a**. Part of this table has been published, in particular the plants used to treat epilepsy, convulsions, depression and age-related mental illnesses (STAFFORD, PEDERSEN, VAN STADEN and JÄGER 2008).

The following publications relate to this chapter:

- **G.I. Stafford**, M.E. Pedersen, J. van Staden, A.K. Jäger. **2008**. Review on plants with CNS-effects used in traditional South African medicine against mental diseases. *Journal of Ethnopharmacology* 119, 513–537.

Table 2.2a. Southern African plants traditionally used for central nervous system-related purposes. Plants are arranged in taxonomic families alphabetically. An alphabetical species reference list follows in **Table 2.2b**.

FAMILY ***Species*** Colloquial name[1] – meaning[2] Annotations and distribution for South Africa[3]		**Traditional use, ethnobotanical information and known active constituents**
ACANTHACEAE ***Crabbea hirsuta*** Harv. Syn: *C. cirsioides* (Nees) Nees; *C. robusta* N.E.Br. letsuijana; mereko (S) Perennial. Herb. Ht 0.05–1.25 m. Alt 5–2100 m. LIM, NW, G, M, S, FS, KZN, L, EC	**001**	In Zimbabwe, the roots are administered in porridge to treat **madness** (GELFAND et al., 1985). ***C. velutina*** S. Moore is smoked for its reported narcotic effect in Uganda by the Mbatyaimeku (TABUTI et al., 2003)
Thunbergia dregeana Ness isiphondo (Z) - (n) entrance at side of kraal Perennial. Herb, creeper. Ht 0.4–1 m. Alt 5–765 m. S, KZN, WC, EC	**002**	Strong infusions of root are taken by men in South Africa for diseases (unspecified) reputed to have been acquired from wives who have been the victims of **sorcery** (WATT and BREYER-BRANDWIJK, 1962). Other ***Thunbergia*** spp. are used as **love charms** (BATTEN and BOKELMANN, 1966; HUTCHINGS et al., 1996).
ADIANTACEAE (Pteridaceae) ***Pellaea calomelanos*** (Swartz) Link mumvuriwedombo (Sh) Perennial. Herb, geophyte, lithophyte. Ht 150–250 mm. Alt 100–2100 m. N, B, LIM, NW, G, M, S, FS, KZN, L, NC, WC, EC	**003**	Taken as an infusion or smoked to treat **convulsions** in Zimbabwe (GELFAND et al., 1985). The Kwena and Kgatla administer milk decoctions of rhizome to frightened children at night (HUTCHINGS et al., 1996) presumably to **calm** them.
AGAVACEAE ***Agave*** spieces	**004**	Zulu use grated root decoctions of an unidentified species to treat high blood pressure and **stress** (HUTCHINGS et al., 1996). An exotic genus of which three species are found in SA; ***A. americana*** **L.**, ***A. decipiens*** **Baker,** ***A. vivipara*** **L.**
ALLIACEAE ***Agapanthus africanus*** (L.) Hoffmanns. (sometime placed in Agapanthaceae) Syn: *A. minor* Lodd.,*A. umbellatus* L'Hér., *Crinum africanum* L., *Mauhlia africana* (L.) Dahl, *Mauhlia linearis* Thunb., *Tulbaghia heisteri* Fabric leta-la-phofu (S); ubane(-oluncane), uhlakahla (Z)	**005**	A decoction of *A. africanus* and other unspecified herbs are used by Southern Sotho diviners to **develop memory** and make initiates mentally fit for their work (WALKER, 1996). Perennial. Herb, geophyte. Ht 0.45–0.6 m. Alt 55–1130 m. WC
Agapanthus campanulatus F.M.Leight. (sometime placed in Agapanthaceae) Syn: *A. patens* F.M.Leight. leta-la-phofu (S); ubani (Z) Perennial. Herb, geophyte. Ht 0.4–1.52 m. Alt 425–2125 m. FS, KZN, EC	**006**	Used in the **initiation** of traditional healers (HUTCHINGS et al., 1996, CALLAWAY, 1991). Various parts are used by the Sotho to treat people with a type of **mental illness** known as 'the spirit' (LAYDEVANT, 1932). The Zulu are reported to use unidentified species of *Agapanthus* for **inducing visions** (*imibono*) and **dreams** (SOBIECKI, 2002). Extracts exhibited SSRI activity (NIELSEN et al., 2003; Chapter 3). *Agapanthus campanulatus* exhibited high affinity to the serotonin re-uptake transporter protein in aqueous extracts from leaves and flowers.

FAMILY ***Species*** Colloquial name[1] – meaning[2] Annotations and distribution for South Africa[3]		**Traditional use, ethnobotanical information and known active constituents**
Agapanthus praecox Willd. (sometime placed in Agapanthaceae) ubani (Z)	**007**	Used in the **initiation** of traditional healers (HUTCHINGS et al., 1996). Three subspecies: ***A. praecox*** Willd. subsp**. *minimus*** (Lindl.) F.M.Leight. (Syn: *A. longispathus* F.M.Leight., *A. umbellatus* L'Hér. var. *minimus* Lindl.), Perennial. Herb. Ht 0.4–0.85 m. Alt 5–600 m. WC, EC ***A. praecox*** Willd. subsp. ***orientalis*** (F.M.Leight.) F.M.Leight. (Syn: *A. orientalis* F.M.Leight., *A. umbellatus* L'Hér. var. *maximus* Edwards) Perennial. Herb. Ht up to 1.21 m. Alt 15–1435 m. KZN, EC ***A. praecox*** Willd. subsp. ***praecox*** Perennial. Herb. Ht 0.8–1 m. Alt? KZN, EC
Tulbaghia alliaceae L.f. ishaladi lezinyoka (Z) Perennial. Herb, geophyte. Ht 0.26–0.45 m. Alt 50–2250 m. WC	**008**	Rhizome infusion administer as enemas for **fits** in Transkei (HUTCHINGS et al., 1996). The Zulu name, *ishaladi lezinyoka* means 'garlic/shallots of the snakes', the plant parts smell of garlic and is used by Zulu as a snake repellent (HUTCHINGS et al., 1996).
Tulbaghia leucantha Baker Syn: *T. dieterlenii* E.Phillips false garlic, mhondya (Sh) Perennial. Herb. Ht 0.1–0.25 m. Alt 30–2325 m. N, LIM, NW, G, M, S, FS, KZN, L, NC, WC, EC	**009**	Unspecified parts are eaten in porridge to **treat madness** in Zimbabwe (GELFAND et al., 1985)
Tulbaghia violaceae Harv. Syn: *Omentaria cepacea* Salisb., *T. cepacea* L.f. isihaqa (Z) -*haqa* (v) surround, encircle, enclose	**010**	In Transkei the bulb is rubbed on the body as **protection** from evil spirits before ritual **dancing** by diviners (HUTCHINGS et al., 1996). Leaves are rubbed on the head of restless young children (BATTEN and BOKELMANN, 1966) presumably to **calm** them. Perennial. Herb. Ht 0.2–0.45 m. Alt 3–1220 m. KZN, WC, EC
AMARYLLIDACEAE ***Ammocharis coranica*** (Ker-Gawl.) Herb. boka (S) icukudo, incukudwane, isidiya, umbhodiya (Z) -diya (isi-, izi-) (n) goat skin worn by women over shoulders to cover breasts Perennial. Geophyte. Ht ± 350 mm. Alt? N, B, LIM, NW, M, S, FS, KZN, L, NC, WC, EC	**012**	Used to treat serious **afflictions** (unspecified) caused by **witchcraft** (HULME, 1954). Known to contain buphanidrine (GIBBS, 1974) which exhibited affinity to the serotonin transporter (SERT) protein (SANDAGER et al., 2005). The bulbs of *Ammocharis coranica* have yielded many alkaloids: lycorine, acetylcaranine, crinamine, 1-*O*-acetyllycorine (a potent AChE inhibitor **Chapter 5**; ELGORASHI et al., 2004), hippadine, 6a-hydroxypowelline, hamayne, 1-*O*-acetyl-9-*O*-demethylpluviine, 24-methylenecycloartan-3b-ol, cycloeucalenol, cycloeucalenone and 24-methylenepollinastanone (KOORBANALLY et al., 2000). A healer from the Nongoma District of Zululand reported that A. coranica was used as a substitute for *Boophone disticha* (Amaryllidaceae) when the latter was unavailable, for the treatment of mentally ill patients (KOORBANALLY et al., 2000).

FAMILY ***Species*** Colloquial name[1] – meaning[2] Annotations and distribution for South Africa[3]		**Traditional use, ethnobotanical information and known active constituents**
Boophone disticha (L.f.) Herb. Syn: *B. longepedicellata* Pax bushman poison bulb; leshoma (S); incwadi (X); incotho; incwadi (Z) -ncwadi (i-, izi-) (n) book, refers to the leaf arrangement as the come out of the bulb Perennial. Geophyte. Ht 200–300 mm. Alt 50–1800 m. N, B, LIM, G, M, S, FS, KZN, L, WC, EC Similar species: ***B. haemanthoides*** F.M.Leight. Syn: *B. disticha* (L.f.) Herb. var. *ernesti-ruschii* Dinter & G.M.Schulze Perennial. Geophyte. Ht 200–300 mm. Alt 15–800 m. N, NC, WC	**013**	Weak decoctions of bulb scales given to **sedate** violent, psychotic patents (GORDON, 1947; VAN WYK and GERICKE, 2000). WATT and BREYER-BRANDWIJK (1962) report that concoctions of the bulb, when taken orally, cause sedation, analgesia, visual hallucinations, unconsciousness, irrational behaviour, talkativeness or coma. Traditional healers and patients in South Africa drink bulb infusions to induce **hallucinations** for divinatory purposes, and also as a medicine to treat **mental illness** (SOBIECKI, 2002). The bulb is traditionally used in Zimbabwe to arouse ancestral spirits (NYAZEMA, 1984; GELFAND et al., 1985). Among the Sotho in the Bethlehem District of Orange Free State (South Africa), a decoction of the bulb of *Boophone disticha* was sometimes taken as an enema for suicidal purposes (WATT and BREYER-BRANDWIJK, 1962). Alkaloids isolated from the bulb include buphanamine, buphanidrine, buphanine buphanisine, haemanthamine, nerbowdine, undulatine, lycorine, crinamidine, crinine, 3-O-acetylnerbowdine, ambelline, buphacetine and distchamine (RAFFAUF, 1970; VILADOMAT et al., 1997). Other compounds that have also been isolated are a volatile oil, containing furfuraldehyde, acetovanillone, chelidonic acid, copper, laevulose, pentatriacontane, ipuranol and a mixture of free and combined fatty acids (Watt and Beyer-Brandwijk, 1962). Alkaloids, buphanidrine and buphanamine exhibited an affinity to the **serotonin transporter** (SERT) protein (SANDAGER et al., 2005).
Crinum species umduze, umnduze (Z) ***C. bulbispermum*** (Burm.f.) Milne-Redh. & Schweick. lelutla, mototsw (S) umnduze (Z) ***C. macowanii*** Baker Syn: *C. gouwsii* Traub, *C. macowanii* Baker subsp. *confusum* I.Verd. ***C. moorei*** Hook.f. *C. imbricatum* Baker, *C. macowanii* Baker, *C. schmidtii* Regel	**014**	Cherylline was isolated from ***Crinum moorei*** (ELGORASHI et al., 2001a) and showed good affinity to the **serotonin transporter** (SERT) protein (ELGORASHI et al., 2004). An unidentified species is reported to be used in a similar manner as ***Boophone disticha*** for inducing hallucinations (SOBIECKI, 2002). Alkaloids have also been isolated from ***Crinum bulbispermum*** (ELGORASHI and VAN STADEN, 2003), ***Crinum macowanii*** (ELGORASHI et al., 2001b). These together with alkaloids from ***Cyrtanthus falcatus*** (ELGORASHI et al., 1999) have some weak to moderate acetylcholinesterase inhibitory activity (ELGORASHI et al., 2004). This prompted further quantitative-structure-activity relationship studies on these alkaloids (ELGORASHI et al., 2006).
Pancratium tenuifolium Hochst. ex A.Rich Syn: *Chapmanolirion juttae* Dinter, *P. chapmannii* Harv.	**015**	Reportedly used by San in Botswana to induce **hallucinations** by rubbing the bulb into incisions on the head (DOBKIN DE RIOS, 1986).
Scadoxus multiflorus (Martyn) Raf. Syn: *Haemanthus katharinae* Baker, *Haemanthus multiflorus* Martyn, *Haemanthus otaviensis* Dinter, *Haemanthus sacculus* E.Phillips	**016**	Reported to contain galanthamine (0.002%) an **AChE inhibitor** (JASPERSEN-SCHIB, 1970). According to POOLEY (2005) there are two subspecies which have different Zulu names: ***S. multiflorus*** (Martyn) Raf. subsp**. *katharinae*** (Baker) Friis & Nordal Syn: *Haemanthus katharinae* Baker indunjana, ubukhoswane (Z) Perennial. Geophyte. Ht 0.75–1.2 m. Alt? M, S, KZN, EC ***S. multiflorus*** (Martyn) Raf. subsp. ***multiflorus*** Syn: *Haemanthus multiflorus* Martyn, *Haemanthus otaviensis* Dinter, *Haemanthus sacculus* E.Phillips isiphompo, isiphungo (Z) -phungo (isi-, izi-) (n) medicine for drinking Perennial. Geophyte. Ht 0.5–1 m. Alt? N, B, LIM, M, S, KZN

FAMILY ***Species*** Colloquial name[1] – meaning[2] Annotations and distribution for South Africa[3]		**Traditional use, ethnobotanical information and known active constituents**
Scadoxus puniceus (L.) Friis & Nordal Syn: *Haemanthus magnificus* Herb., *Haemanthus natalensis* Pappe ex Hook., *Haemanthus puniceus* L. var. *puniceus* idumbe-lika-nhloyile, idumbelentaba, isiphompo, umgola (Z)	**017**	Known to cause **CNS excitation** or **depression** and **visual** disturbances (VEALE et al., 1992). Perennial. Geophyte. Ht 0.5–0.75 m. Alt 15–2100 m. B, LIM, NW, G, M, S, FS, KZN, EC
ANACARDACEAE		
Harpephyllum caffrum Bernh. Ex Krauss Perennial. Tree. Ht 2–15 m. Alt 15–1400 m. LIM, M, S, KZN, EC	**018**	In Transkei, root decoctions are taken for paralysis thought to have been contracted from walking over an area that has been 'polluted' through **sorcery** (HUTCHINGS et al., 1996).
Lannea discolor (Sond.) Engl. Syn: *Odina discolor* Sond. live-long; luvale (Lu); isiganganyane (Z)	**019**	The Luvale of Zambia use the leaves to prevent **fits** (WATT and BREYER-BRANDWIJK, 1962). Perennial. Tree. Ht 0.75–12 m. Alt 305–1480 m. N, B, LIM, NW, G, M, S
Lannea schweinfurthii (Engl.) Engl. mulivhadza (V) Subspecies: ***L. schweinfurthii*** (Engl.) Engl. var. ***stuhlmannii*** (Engl.) Kokwaro Syn: *L. kirkii* Burtt Davy, *L. stuhlmannii* (Engl.) Engl. Perennial. Tree. Ht 2–18 m. Alt 30–1370 m. LIM, M, S ***L. schweinfurthii*** (Engl.) Engl. var. ***tomentosa*** (Dunkley) Kokwaro Syn: *L. stuhlmannii* (Engl.) Engl. var. *tomentosa* Dunkley Perennial. Tree. Ht 3–15 m. Alt 1050–1200 m. N, B	**020**	The Vhavenda traditionally used decoctions made from root bark mixed with a fungus found growing on the roots to help family members to **forget** a recently passed away relative (MABOGO, 1990). They are also used as a protection against a sleeping sickness known as *vhulangwane* and to help people **forget** all unpleasant events. The roots are covered with a dense layer of very fine root hairs, that are reportedly used as a **sedative** snuff, and the smoke of the burned roots is inhaled as a **sedative** (VAN WYK and GERICKE, 2000)
Searsia chirindensis (Baker f.) Moffett Syn: *Rhus chirindensis* Bak. f.	**021**	Bark is used to strengthen the body, stimulate circulation and for rheumatism (PUJOL, 1990), bark decoctions are traditionally administered for **mental disturbances** in Transkei (HUTCHINGS et al., 1996).
Searsia natalensis (Bernh. ex Krauss) F.A.Barkley Syn: *Rhus natalensis* Bernh. ex krauss	**022**	Roots are used for **fits** in children (WATT and BREYER-BRANDWIJK, 1962).
Searsia pyroides (Burch.) Moffett Syn: *Rhus pyroides* Burch. mufokosiana (Sh)	**023**	In Zimbabwe the roots are use in infusions to treat **delirium** (GELFAND et al., 1985).
ANNONACEAE		
Annona senegalensis Pers. Syn: *A. arenaria* Thonn. ex Schumach., *A. chrysophylla* Bojer Perennial. Shrub or tree. Ht 1.5–8 m. Alt 15–1538 m. B, LIM, M, S, KZN	**024**	The roots are mixed with *Trema orientalis* to treat madness; roots are used to treat **madness**, to ward off dizziness and to induce **forgetfulness** in small children, thus facilitating weaning processes by the Zulu (PALMER and PITMAN, 1972). The Vhavenda use roots for snakebites, venereal diseases, diarrhoea, dysentery, blood in the faeces, headaches and as **protective** charms against witchcraft (MABOGO, 1990). An *Annona* sp. is used in Ghana as an epilepsy treatment (IRVINE, 1961).

FAMILY ***Species*** Colloquial name[1] – meaning[2] Annotations and distribution for South Africa[3]		**Traditional use, ethnobotanical information and known active constituents**
Artabotrys brachypetalus Benth. mukosvo (Sh) Perennial. Tree, shrub or climber. Ht 0.9–4 m. Alt 305–1400 m. B, LIM	**025**	Root infusions are drunk to treat **convulsions** in Malawi (GELFAND et al., 1985; SOBIECKI, 2002). *Artabotrys* spp. Is used in Madagascar as a **stimulant** (GITHENS, 1949; SOBIECKI, 2002).
Monanthotaxis caffra (Sond.) Verdc. Syn: *Guatteria caffra* Sond., *Popowia caffra* (Sond.) Benth. dwaba berry; umgogi wezinhlanya (Z)	**026**	Root material is smoked for **hysteria** and administered as emetics for bad **dreams** (GERSTNER, 1941; DOKE and VILAKAZI, 1972). Perennial. Tree, shrub or climber. Ht 1–10 m. Alt 10–1190 m. M, S, KZN, EC
Uvaria lucida Benth. Syn: *U. gazensis* Baker f., *U. virens* N.E.Br. umavumba (Z) –*vumba* (n) odour , umazwenda –omnyama (Z) – darkness or ill omen of the land	**027**	Stems are used in Zulu traditional medicine (CUNNINGHAM, 1988); the leaves are used for bathing patients with **mental illnesses** (SOBIECKI, 2002). In Tanzania, roots are used in decoctions to treat **mental disease** (HEDBERG et al., 1982). Perennial. Shrub or climber. Ht 1.5–10 m. Alt 0–100 m. LIM, S, KZN
APIACEAE ***Alepidea amatymbica*** Eckl. & Zeyh. ikathazo (Z) -*khathazo* (n) a kind of herb used as a remedy (Dent and Nyembezi, 1999)	**028**	The dry rhizome and roots are smoked, or powdered and taken as a snuff by Zulu diviners and healers to assist in **divination** and communication with ancestors (HUTCHINGS et al., 1996; VAN WYK and GERICKE, 2000). Smoking the roots reportedly results in mild **sedation** and vivid **dreams** (VAN WYK and GERICKE, 2000). Antihypertensive, antimicrobial and diuretic effects have been indicated in tests on animals (VAN WYK and GERICKE, 2000). Three subspecies are described: ***A. amatymbica*** Eckl. & Zeyh. var. ***amatymbica*** Perennial. Herb. Ht ± 0.1 m, when in flower up to 2.1 m. Alt 243–2620 m. LIM, G, M, S, FS, KZN, L, EC ***A. amatymbica*** Eckl. & Zeyh. var. ***aquatica*** (Kuntze) Weim. Perennial. Herb. Ht up to 0.1 m, when in flower up to 2.1 m. Alt 795–1220 m. EC ***A. amatymbica*** Eckl. & Zeyh. var. ***microbracteata***Weim. Perennial. Herb. Ht up to 0.1 m, when in flower up to 2.1 m. Alt ± 800 m. KZN Similar species include: **A. natalensis** J.M.Wood & M.S.Evans Syn: *A. baurii* Kuntze Perennial. Herb. Ht up to 0.2 m, when in flower up to 0.6 m. Alt up to 2650 m. FS, KZN, L, EC
Arctopus echinatus L. Perennial. Herb. Ht up to 0.1 m. Alt 50–1700 m. WC, EC	**029**	WATT (1967) mentions *A. echinatus* as being administered, together with potassium nitrate, for **epilepsy**. He states that in this form it may cause **drowsiness**. This plant was held in great esteem as a "comfort to the sick", hence the Afrikaans vernacular name *sieketroos* (PAPPE, 1847, 1857). It is thought to have been adopted from the Khoi-San by the Early Cape Settlers (PAPPE, 1847, 1857). Their resinous roots are chemically similar to those of ***Alepidea amatymbica*** **Eckl. and Zeyh.** (*ikhathazo* in Zulu), a well-known Zulu and Sotho medicinal plant, with an equally rich ethnobotanical history (VAN WYK et al., 1997; VAN WYK and GERICKE, 2000; MAGEE et al., 2007).

FAMILY ***Species*** Colloquial name[1] – meaning[2] Annotations and distribution for South Africa[3]		**Traditional use, ethnobotanical information and known active constituents**
Centella asiatica (L.) Urb. Perennial. Herb, climber. Ht up to 0.2 m. Alt 5–2000 m. N, B, LIM, NW, G, M, S, FS, KZN, L, NC, WC, EC	**030**	Dried powdered leaves are used by unspecified groups as a snuff, which reportedly produces a calming, **sedative** effect (VAN WYK and GERICKE, 2000). Triterpenes have been demonstrated to exhibit **tranquillising**, **anxiolytic** activity and stress relief (VAN WYK and GERICKE, 2000). In parts of India it is given with milk to improve **memory** against dementia and aging (AHUJA, 1965). Constituents in *Centella asiatica* include essential oil, triterpenoid saponins, such as asiaticocide, brahmoside and thankuniside, alkaloids (hydrocotyline) and some bitter principles (CHEVALLIER, 1996). Sedative action has been shown by triterpenes, such as those present in *Centella asiatica* (BRINKHAUS et al., 2000; WIJEWEERA et al., 2006). These triterpenes exhibit anxiolytic activity that is thought to be due to cholinergic mechanisms. Furthermore, an extract from *C. asiatica* was shown to exert a dose-dependent increase in GABA levels, in rat brain (CHATTERJEE et al., 1992).
Heteromorpha trifoliata (Wendl.) Eckl. & Zeyh. Now ***H. arborescens*** (Spreng.) Cham. & Schltdl. var. ***abyssinica*** (A.Rich.) H.Wolff Syn: *Bupleurum trifoliatum* H.L.Wendl., *H. abyssinica* Hochst. ex A.Rich. var. *abyssinica*, *H. abyssinica* Hochst. ex A.Rich. var. *simplicifolia* A.Rich., *H. arborescens* (Spreng.) Cham. & Schltdl. var. *trifoliata* (H.L.Wendl.) Sond. umbangandlala (Z) Perennial. Tree, shrub. Ht up to 15 m. Alt up to 2800 m. B, LIM, NW, G, M, S, FS, KZN, L, NC, WC, EC	**031**	Sotho use leaf decoctions that are administered for **mental and nervous diseases** (HUTCHINGS et al., 1996; WATT and BREYER-BRANDWIJK, 1962). It is also used by Sotho to increase the 'power' of the chief (JACOT GUILLARMOOD, 1971); and in the Transkei warm leaf infusions, sometimes with the addition of salt, are administered tree times a day to patients suffering **mental disturbances** (HUTCHINGS et al., 1996). In Botswana, root infusions are taken for weakness by men (TEICHLER, 1971; HEDBERG and STAUGARD, 1989). A plant known as *umbangandlala* (Z) is used to treat **nervous debilities** and **impotency** (PUJOL, 1990), the root (1 teaspoon powdered in 500 ml milk and boiled) is drunk twice a day.
Lichtensteinia interrupta (Thunb.) Sond. Syn: *L. kolbeana* Bolus, *L. pyrethrifolia* Cham. & Schltdl. Perennial. Herb. Ht 0.5–1.2 m. Alt 500–1700 m. KZN, WC, EC	**032**	Roots are used for narcotic drinks and powdered herbs used as **snuff** in the Cape (HUTCHINGS et al., 1996), plants are reputed to be used in **sorcery** to cause people or crops to decay or die and are used as counter remedies (GERSTNER, 1941)
Steganotaenia araliacea Hochst. Perennial. Tree. Ht up to 10 m. Alt 200–1200 m. N, B, LIM, M	**033**	The Shona of Zimbabwe use root infusions taken in porridge to treat **epilepsy** (GELFAND et al., 1985).
APOCYNACEAE ***Acokanthera oppositifolia*** (Lam.) Codd Syn: *A. venenata* in sense of Stapf, not of G.Don, misapplied name ubuhlungu-benyoka (Z) *–hlungu* (n) pain, *-nyoka* (n) snake – pain of the snake	**034**	Leaf infusions are administered against **fits** in the Transkei but are reputed to be very poisonous if 'too strong' (quantities not specified) (HUTCHINGS et al., 1996). Twig decoctions are taken as battle '**charms**' by Thembu and large twigs are burned in huts for protection against evil spirits known as *impundulu* (HUTCHINGS et al., 1996).

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Carissa edulis Vahl Syn: *Azima pubescens* Suess.	**035**	In Malawi the roots are mixed with the roots of ***Securidaca longipedunculata*** (Polygalaceae) and used in a body wash to treat **epilepsy** (GELFAND et al., 1985).
Oncinotis tenuiloba Stapf Syn: *O. inandensis* J.M.Wood & M.S.Evans	**036**	Used to counteract **witchcraft** as medicines made from the plant are believed to be powerful enough to confuse sorcerers (PALMER and PITMAN, 1972).
Pleiocarpa pycnantha (K. Schum.) Stapf. Syn: *P. swynnertonii* S.Moore	**037**	The roots are chewed as a **stimulant** by an unspecified group in South Africa (COATES PALGRAVE, 2002).
Rauvolfia caffra Sond. Syn: *R. natalensis* Sond. quinine-tree (E), kinaboom (A) Perennial. Tree. Ht 2–21 m. Alt 0–1400 m. LIM, NW, G, M, S, KZN, WC, EC	**038**	Decoctions of the bark are used in South Africa as a **tranquilliser** for **hysteria**, and for **insomnia**, and the dried leaves are used as a snuff for headaches (VAN WYK and GERICKE, 2000). In the Transkei, bark is used by traditional healers as a **tranquilliser** for patients believed to be 'possessed by spirites' (BROSTER, 1981). Valley Trust healers in Natal reportedly use bark of *umhlambamanzi* (Z) to ward off 'evil spirits' (HUTCHINGS et al., 1996). Reserpine is used as a hypotensive agent in arterial hypertension, as a **tranquillizer** in anxiety states and in psychoses with **hallucinations** and delirium (FATTORUSSO and RITTER, 1967). On account of their sedative, tranquilizing and hypotensive properties of reserpine, other *Rauvilfia* species have been investigated and numerus alkaloids isolated (KERHARO and ADAM, 1974). The pharmacologically important alkaloids reserpine and rescinnamine occur in the root but not in the leaves (GOODWIN and BUNNEY, 1971; HABIB and COURT, 1974, KHAN, 1986). Reserpine has sedative and tranquillising effects but is not hypnotic. It acts through the CNS and is active only in the presence of the hypothalamus and dienceohalon. Reserpine appears to act as an antimetabolite of serotonin and catecholamine and decreases the serotonin content of the nerve centers.
Strophanthus gerrardii Stapf Ubuhlungubendlovu (Z) - consists of *ubuhlungu* ('pain') and *bendlovu* ('of the elephant') Perennial. Climber. Ht 2–12 m. Alt 20–550 m. M, S, KZN	**039**	Species known as *ubuhlungu(b)endlovu* are reported to be used for **hysteria** (DOKE and VILAKAZI, 1972), Fruit is also used as traditional medicine (CUNNINGHAM, 1988).
Strophanthus petersianus Klotzch Syn: *S. grandiflorus* (N.E.Br.) Gilg Perennial. Shrub, climber. Ht 2–15 m. Alt 20–975 m. LIM, KZN	**040**	Unspecified parts are used by herbalists as a **charm** against evil (method of administration unknown) (WATT and BREYER-BRANDWIJK, 1962).
Strophanthus speciosus (Ward & Harv.) Reber Perennial. Shrub, climber. Ht 0.9–12 m. Alt 50–1750 m. LIM, M, KZN, EC	**041**	Reported to be used by Xhoza to 'render someone awe-inspiring (*isitunzi*)' (SIMON and LAMLA, 1991)
Wrightia natalensis Stapf Perennial. Tree, shrub. Ht 1.6–18 m. Alt 20–1250 m. LIM, S, KZN	**042**	Roots and bark are used as an **aphrodisiac** by the Vhavenda (MABOGO, 1990).

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AQUIFOLIACEAE		
Ilex mitis (L.) Radlk. var. *mitis* Syn: *I. capensis* Sond. African/Cape holy Perennial. Tree or shrub. Ht 3–30 m. Alt 10–2130 m. LIM, NW, G, M, S, FS, KZN, L, WC, EC	**043 0**	In Lesotho diviners use plants (parts and administration not specified) in protective rituals to protect patients from sorcery (WATT and BREYER-BRANDWIJK, 1962).
ARACEAE		
Acorus calamus L. (sometime under Acoraceae) ikalamuzi (Z)	**044**	Ground rhizomes are used for **nervous disorders** (PUJOL, 1990), they are also reported to be ground and mixed with dagga (*Cannabis sativa* L.) by dagga smokers to mask the distinctive smell (HUTCHINGS et al., 1996), they are widely used as tonics and **stimulants** (HUTCHINGS et al., 1996), rhizomes are reported to be bitter and have eupeptic, antithermic, emmenagogic and **tranquillizing** effects (CHIEJ, 1984), rhizomes and roots have **sedative** and analgesic properties (WATT, 1967, LEWIS and ELVIN-LEWIS, 1977), **hallucinogenic** effects and narcotic effects on cobras are also reported (HUTCHINGS et al., 1996). Asarone (chemically similar to mescaline) and β-asarone (chemically similar to myristicin and kava alkaloids) are presumed to be active **hallucinogenic** principles (LEWIS and ELVIN-LEWIS, 1977).
ARALIACEAE		
Cussonia arborea A. Rich.	**045**	Unspecified groups in Malawi ingest leaf infusions, together with leaves of *Ipomoea batatas* (Convolvulaceae) and *Musa* species to **treat madness** (GELFAND et al., 1985). ***Cussonia arborea*** is not found in South Africa.
Cussonia longissima Hutch. & Dalz..	**046**	In Ghana, unspecified parts are used to treat **convulsions** in children (IRVINE, 1961). The Sotho people use leaf decoctions together with other unspecified pants to treat **mental disease** (WATT and BREYER-BRANDWIJK, 1962).
Cussonia paniculata Eckl & Zeyh..	**047**	In southern Africa, theSotho use a decoction of the leaves together with other unspecified plants to treat early **mental illness** (WATT and BREYER-BRANDWIJK, 1962; VAN WYK and GERICKE, 2000). There are two sub species, ***C. paniculata*** Eckl. & Zeyh. subsp. ***paniculata*** (Perennial. Tree. Ht 1–4 m. Alt 300–2000 m. KZN, NC, WC, EC), ***C. paniculata*** Eckl. & Zeyh. subsp. ***sinuata*** (Reyneke & Kok) De Winter (Perennial. Tree. Ht 2–5 m. Alt 900–1980 m. B, LIM, NW, G, M, S, FS, KZN, L).
Cussonia spicata Thunb. Perennial. Tree. Ht 3–17 m. Alt 5–1800 m. LIM, NW, G, M, S, KZN, L, WC, EC	**048**	Root bark decoctions are administered for **mental illness** in Tanzania (CHHABRA et al., 1984). The bark is used for 'magical' purposes in South Africa (HUTCHINGS et al., 1996) and in Tzaneen, South Africa healers use it to treat a "mind that goes bad" (SOBIECKI, 2002).
Schefflera umbillifera (Sond.) Baill. Syn: *Cussonia chartacea* Schinz, *Cussonia umbellifera* Sond. Perennial. Tree. Ht 3–15 m. Alt 60–1980 m. LIM, M, S, KZN, EC	**049**	Leaves have been used for rheumatism, colic and to treat **insanity** in unspecified parts of southern Africa (WATT and BREYER-BRANDWIJK, 1962).

FAMILY ***Species*** Colloquial name[1] – meaning[2] Annotations and distribution for South Africa[3]		**Traditional use, ethnobotanical information and known active constituents**
ASCLEPIADACEAE		
Cynanchum obtusifolium L.f. Syn: *C. capense* R.Br., *C. dregeanum* Decne., *Cynoctonum brownii* Meisn. Perennial. Climber. Ht 2–10 m. Alt 0–250 m. KZN, WC, EC	**050**	Root and leaf infusions are taken by Zulu as emetics for illnesses (details not given) believed to be caused by **witchcraft** (HULME, 1954).
Gomphocarpus physocarpus E.Mey. Syn: *Asclepias brasiliensis* (E.Fourn.) Schltr., *Asclepias physocarpa* (E.Mey.) Schltr., *G. brasiliensis* E.Fourn., *G. fruticosus* (L.) Aiton f. forma *brasiliensis* (E.Fourn.) Briq. umangwazane, umbababa, umqumbuqumbu, uphuphuma, usingalwesalukazi (Z)	**051**	Leaves used to '**strengthen body'** (PUJOL, 1990), powdered leaf is used as **sedative** (VAN WYK and GERICKE, 2000). The root of *Asclepias lineolata* Schltr is used in Zambia as a narcotic to catch wild birds (GILGES, 1953). Annual or perennial. Herb. Ht 1–2.5 m. Alt 30–700 (–900) m. LIM, NW, M, S, KZN, WC, EC
Pachycarpus asperifolius Meisn. Syn: *Asclepias valida* (Schltr.) Schltr., *Gomphocarpus asperifolius* (Meisn.) Walp., *Gomphocarpus validus* Schltr., *P. inconstans* N.E.Br., *P. validus* (Schltr.) N.E.Br.	**052**	Underground parts (tubers) are dried and powdered and taken as snuff for headaches and to treat **hysteria** by the Xhosa (HUTCHINGS et al., 1996). Perennial. Geophytic herb. Ht 0.3–1.1 m. Alt 60–1700 m. LIM, G, M, S, KZN, EC
Secamone gerrardii Harv. ex Benth.	**053**	The Zulu are report to treat 'spinal disease' with powdered roots of ***S. gerrardii*** that are mixed in a complex formula reported to contain ***Pterocelastrus rostratus* Walp.**, ***Ocotea bullata* (Burch.) Baill.**, ***Sarcophyte sanguinea* Sparrm**. or ***Hydnora* sp.** together with the dried body of a fruit bat. This mixture is rubbed into incisions made along the affected part (BRYANT, 1966; HUTCHINGS et al., 1996).
Sisyranthus huttoniae (S. Moore) S. Moore	**054**	Medicinal usage related to '**ancestral' spirits** (HUTCHINGS et al., 1996).
Sisyranthus trichostomus K. Schum.	**055**	Root and leaf infusions taken by Zulu as emetics to prevent bad **dreams** (HULME, 1954).
Stapelia gigantea N.E. Br. Syn: *S. cyclista* C.A.Lückh., *S. gigantea* N.E.Br. var. *pallida* E.Phillips, S. marlothii N.E.Br., *S. nobilis* N.E.Br., *S. youngii* N.E.Br. ililo elikhulu (Z) -*lilo* (n) lamentation, *khulu* (adj) large	**056**	Hot stem infusions are administered by Zulu healers as emetics to treat **hysteria** (BRYANT, 1966). An unidentified plant called *uzililo* (Z), which is most likely *Stapelia* spp. or *Huernia hystrix*, is used by Zulu to treat **mental disorders**. Plants are reputed by Zulu to be used to prepare a medicine capable of causing death (HULME, 1954). Anthraquinones have been reported (WATT and BREYER-BRANDWIJK, 1962).

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Xysmalobium undulatum (L.) Aiton.f. ishongwane (fruit, Z), ishongwe (X, Z), uzara, milkweed (E)	**057**	Roots administered in the Transkei by Xhosa to treat **hysteria** (HUTCHINGS et al., 1996). Roots contain several glycosides, and extracts have exhibited weak CNS **depressant** and **antidepressant** activity (HUTCHINGS et al., 1996). Leaf extracts exhibited SSRI (antidepressant) activity (NIELSEN et al., 2003). Two Subspecies: ***X. undulatum*** (L.) Aiton f. var. ***ensifolium*** Burch. Ex Scott-Elliot Syn: *X. ensifolium* (Burch. ex Scott-Elliot) N.E.Br. Perennial. Geophytic herb. Ht ± 1 m. Alt 1000–1300 m. N, B, NW, FS, NC, EC ***X. undulatum*** (L.) Aiton f. var. ***undulatum*** Syn: *Asclepias undulata* L., *Gomphocarpus undulatus (*L.) Schltr. *X. ambiguum* N.E.Br., *X. amplifolium* Weim. *X. dilatatum* Weim., *X. lapathifolium* K.Schum., not of Decne., *X. leucotrichum* (Schltr.) N.E.Br., *X. prismatostigma* K.Schum. Perennial. Geophytic herb. Ht 0.5–1.8 m. Alt 80–2000 m. N, B, LIM, NW, G, M, S, FS, KZN, L, NC, WC, EC
ASPARAGACEAE ***Asparagus*** species ***Asparagus virgatus*** Baker Syn: *Protasparagus virgatus* (Baker) Oberm. iphinganhloya (Z) - means what suppresses the ill-omen, it is used as a protective charm to ward off the effects of evil (Pooley, 2005).	**058**	Roots of an unidentified species are burned, the ash powdered and placed in incisions for febrile **convulsions** in Zimbabwe (CHINEMANA et al., 1985). ***A. africanus* Lam.** (Syn: *Protasparagus africanus* (Lam.) Oberm.) is used to treat **mental illness** and disturbances in east Africa (KOKWARO, 1976). The Zulu name *iphinganhloya* means what suppresses the ill-omen, it is used as a protective charm to ward off the effects of evil (POOLEY, 2005).
ASPHODELACEAE ***Aloe ferox*** Mill.	**059**	The nectar is said to be **narcotic** and to produce weakness of the joints if injested in large quantities (WATT and BREYER-BRANDWIJK, 1962).
Anthericum species Now ***Chlorophytum*** Ker Gawl. (Anthericaceae) see ***Chlorophytum blepharophyllum*** Bak.below inkomfe yentaba (Z)	**060**	A decoction of an *Anthericum* species is a remedy for **hysteria** among the Southern Sotho (WALKER, 1996).
Bulbine frutescens (L.) Willd. Syn: *Anthericum frutescens* L., *Anthericum fruticosum* Salisb., *Anthericum incurvum* Thunb., *Anthericum rostratum* Jacq. *B. caulescens* L., *B. rostrata* Willd. (*B. triebneri* Dinter Perennial. Dwarf shrub, succulent. Ht 300–800 mm. Alt 5–2285 m. N, G, S, FS, KZN, L, NC, WC, EC ibhucu (Z) *bhucu* (ideo) of rotting, *-bhucu* (isi- izi-) (n) rotten matter	**061**	Root and leaf infusions are administered by Zulu healers as emetics to patients thought to be going **mad** as a result of being **bewitched** (HULME, 1954).

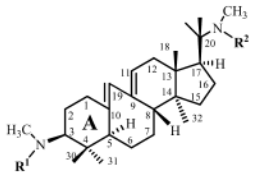
FAMILY ***Species*** Colloquial name[1] – meaning[2] Annotations and distribution for South Africa[3]		**Traditional use, ethnobotanical information and known active constituents**
Bulbine latifolia (L.f.) Roem. & Schult. Syn: *Anthericum latifolium* L.f. (*B. brunsvigiaefolia* Baker, *B. ensifolia* Baker, *B. natalensis* Baker, *B. transvaalensis* Baker Perennial. Herb, geophyte, succulent. Ht 250–910 mm. Alt 35–1405 m. G, KZN, WC, EC ibhucu (Z)	**062**	Decoctions are taken by groups of Zulu men and adolescents as emetics in purification rites aimed at **preventing 'anti-social behaviour'** (HUTCHINGS et al., 1996). Xhosa use underground organs (tubers) to make decoctions used to treat **convulsions** in children (BROSTER, 1982; PUJOL, 1990). The root is boiled in water for a few minutes and the infusion taken in small doses twice daily, two teaspoons for **convulsions** (PUJOL, 1990).
Chlorophytum blepharophyllum Bak.	**063**	The Shona prepare an ointment made from the root that is applied to the face for the treatment of **madness** (GELFAND et al., 1985).
Gasteria croucheri (Hook.f.) Baker Syn: *Aloe croucheri* Hook.f., *G. disticha* (L.) Haw. var. *natalensis* Baker, *G. ensifolia* Haw. impundu (Z) Perennial. Herb, succulent. Ht 0.25–0.6 m. Alt 0–1000 m. KZN, EC	**064**	Used to treat girls with **hysteria** in South Africa (HULME, 1954). ***Impundu* (Z) –** (n) gatepost, bulbous roots are placed between the gateposts at the kraal entrance to cause forgetfulness in evil-doers.
ASTERACEAE		
Achyrocline stenoptera (DC.) Hilliard & Burtt (now *Helichysum stenopterum* DC) imphepho (Z)	**065**	Inhaled by the Zulu diviners (*izangoma*) to **induce trances** (HUTCHINGS et al., 1996). Used in a similar way as many *Helichrysum* species as *imphepho*, where the leaves and stems are burned to invoke the goodwill of the **ancestral spirits** (HUTCHINGS et al., 1996).
Arctotheca calendula (L.) Levyns	**066**	Unspecified groups in Southern Africa use the juice from this plant as an antidote to strychnine (WATT, 1967). **Narcotic** effects have been observed in rabbits (VAN DER WALT and STEYN, 1940).
Arctotis arctotoides (L.f.) O. Hoffm. ubushwa (X)	**067**	Leaf juice is a Xhosa treatment for **epilepsy** in South Africa (WATT and BREYER-BRANDWIJK, 1962).
Aspilia pluriseta Schweinf. mukushamvura (Sh)	**068**	In Zimbabwe the root is burned and the **smoke** inhaled to treat **delirium** (GELFAND et al., 1985).
Aster bakeranus Burtt Davy ex C.A. Sm. Syn: *A. asper* J.M.Wood & M.S.Evans, *A. bakerianus* Burtt Davy ex C.A.Sm. subsp. *albiflorus* Lippert, *A. bakerianus* Burtt Davy ex C.A.Sm. subsp. *angustifolius* Lippert, *A. bakerianus* Burtt Davy ex C.A.Sm. subsp. *intermedius* Lippert, *A. bakerianus* Burtt Davy ex C.A.Sm. subsp. *ovalis* Lippert, *A. bakerianus* Burtt Davy ex C.A.Sm. subsp. *septentrionalis* Lippert, *A. grauii* Lippert *A. hispidus* (Thunb.) Baker, *Calendula hispida* Thunb., *Diplopappus asper* Less., *Diplopappus natalensis* Sch.Bip., *Felicia asper* Burtt Davy phoa (S); noxgxekana (X); udlatshana, umaqhunsula, umhlungwana (Z)	**069**	Roots of the plant known as *udlutshane* and reported to be *A. bakeranus* are used by Zulu people to treat **psychiatric disturbances** (HUTCHINGS et al., 1996). Perennial. Herb. Ht 0.1–0.75 m. Alt 10–2285 m. G, M, S, FS, KZN, L, EC Similar species: ***A. harveyanus*** Kuntze Syn: *A. harveyanus* Kuntze subsp. *corymbosus* Lippert, *A. harveyanus* Kuntze subsp. *gracilis* Lippert, *A. harveyanus* Kuntze subsp. *robustus* Lippert, *A. harveyanus* Kuntze subsp. *xylophyllus* (Klatt) Lippert, *A. serrulatus* Harv. var. *xylophyllus* Klatt, *A. xylophyllus* Klatt, *Diplopappus serrulatus* Harv., *Felicia serrulata* (Harv.) Burtt Davy Perennial. Herb. Ht 0.1–1 m. Alt 60–2075 m. LIM, NW, G, M, S, KZN, EC

FAMILY ***Species*** Colloquial name[1] – meaning[2] Annotations and distribution for South Africa[3]		**Traditional use, ethnobotanical information and known active constituents**
Athrixia heterophylla (Thunb.) Less.	**070**	Early record of root decoctions together with ***Anemone caffra*** (Ranunculaceae) being used in South Africa to **treat mental** disease (SMITH, 1888).
Berkheya discolor (DC.) O. Hoffm. & Muschl. Syn: *B. kuntzei* O.Hoffm., *B. microcephala* (DC.) R.A.Dyer, *B. polyacantha* S.Moore, *B. spinulosa* N.E.Br., *Stobaea acarnoides* DC., *Stobaea discolor* DC., *Stobaea microcephala* DC.	**071**	In Lesotho a decoction of unspecified parts, most likely leaves, is used to **pacify nervous patients** (JACOT GUILLARMOD, 1971). Perennial. Herb. Ht 0.3–0.9 m. Alt 460–2045 m. NW, FS, KZN, L, EC
Blumea alata (D. Don) DC. rutapatsikidzi (Sh)	**072**	Leaves are used in Zimbabwe for enemas to treat **convulsions** (GELFAND et al., 1985).
Brachylaena elliptica (Thunb.) DC. igqeba elimnyama/isiduli (selathi) (Z)	**073**	Roots are used by Zulu healers as a substitute for the roots of ***Vernonia neocorymbosa*** (Asteraceae), which are used to treat **hysterics** (GERSTNER, 1939; HUTCHINGS et al., 1996).
Callilepis laureola DC. amafuthomhlaba, ihlamvu, impila (Z) Syn: *C. glabra* DC., *C. hispida* DC., *C. laureola* DC. var. *glabra* (DC.) Harv., *C. laureola* DC. var. *hispida* (DC.) Harv. Perennial. Herb. Ht 0.3–1 m. Alt 0–2150 m. M, S, FS, KZN, EC	**074**	The Zulu use the toxic root as a protective '**charm**' which is placed under the pillow to stop bad **dreams** (HUTCHINGS et al., 1996). Root-stock is toxic and can be fatal if ingested even in small quantities; some symptoms include confusion and convulsions (VEALE, 1987).
Cenia sericea DC. Syn:***Cotula sericea*** L.f. Perennial. Suffrutex. Ht 0.05–0.3 m. Alt 3–200 m. WC, EC	**075**	Used in the eastern Cape region of South Africa to assist with restful **sleep** and to break high fever (BATTEN and BOKELMANN, 1966).
Chrysanthemoides monilifera (L.) T. Norl. motlempe (S)	**076**	In Lesotho, leafy branches are burned as a cure in the huts of **mad** men (JACOT GUILLARMOD, 1971). Several subspecies: ***C. monilifera*** (L.) Norl. subsp. ***canescens*** (DC.) Norl. Syn: *Osteospermum pisiferum* L. var. *canescens* DC. Perennial. Shrub, succulent. Ht 0.3–3.65 m. Alt 765–2210 m. M, FS, KZN, L, EC ***C. monilifera*** (L.) Norl. subsp. ***monilifera*** Syn: *Lepisiphon dentatus* Turcz., *Osteospermum moniliferum* L. Perennial. Shrub, succulent. Ht 0.1–3 m. Alt 5–1760 m. WC, EC ***C. monilifera*** (L.) Norl. subsp. ***pisifera*** (L.) Norl. Syn: *C. pisiformis* Medik., *Osteospermum moniliferum* L. var. *pisiferum* (L.) Harv., *Osteospermum pisiferum* L. Perennial. Shrub, succulent. Ht 0.3–3 m. Alt 5–1370 m. NC, WC, EC ***C. monilifera*** (L.) Norl. subsp. ***rotundata*** (DC.) Norl. Syn: *Osteospermum moniliferum* L. var. *rotundatum* (DC.) Harv., *Osteospermum rotundatum* DC. Perennial. Shrub, succulent. Ht 0.25–3 m. Alt 5–500 m. KZN, EC ***C. monilifera*** (L.) Norl. subsp. ***septentrionalis*** Norl. Syn: *Osteospermum moniliferum* L. forma *foliis-subintegris* Engl. Perennial. Shrub, succulent. Ht 0.5–3 m. Alt 1200–2400 m. LIM ***C. monilifera*** (L.) Norl. subsp. ***subcanescens*** (DC.) Norl. Syn: *Osteospermum moniliferum* L. var. *angustifolium* DC., *Osteospermum subcanescens* DC. Perennial. Shrub, succulent. Ht 1–3 m. Alt 15–1219 m. WC, EC

FAMILY ***Species*** Colloquial name[1] – meaning[2] Annotations and distribution for South Africa[3]		**Traditional use, ethnobotanical information and known active constituents**
Cineraria aspera Thunb. moholu-oa-pela (S) Annual, occ. perennial. Herb or suffrutex. Ht 0.15–1.5 m. Alt 1200–2500 m. NW, G, M, FS, KZN, L, NC, WC, EC	**077**	In Lesotho the leaves are smoked to treat respiratory problems, and are said to be as **intoxicating** as *Cannabis* (PHILIPS, 1917; JACOT GUILLARMOD, 1971).
Conyza scabrida DC. Syn: *Baccharis ivaefolia* L., *C. ivifolia* (L.) Less., *Pluchea scabrida* DC. uhlabo (Z) Perennial. Shrub. Ht 0.38–1.2 m. Alt 5–1920 m. N, B, LIM, NW, G, M, S, FS, KZN, L, NC, WC, EC	**078**	Early records in South Africa suggest that leaf decoctions are administered to children suffering from **convulsions** (SMITH, 1895).
Dicoma anomala Sond. Two subspecies: ***D. anomala*** Sond. subsp. ***anomala*** Perennial. Herb, prostrate. Length up to 0.3 m. Alt 152–2200 m. LIM, NW, G, M, S, FS, KZN, L, EC ***D. anomala*** Sond. subsp. ***gerrardii*** (Harv. ex F.C.Wilson) S.Ortíz & Rodr.Oubiña Perennial. Herb. Ht 0.1□0.4 m. Alt up to 2335 m. N, B, LIM, NW, G, M, S, FS, KZN, NC	**079**	Unspecified parts are used for treating **hysteria** in South Africa (LAYDEVANT, 1932). In Zimabawe and South Africa, the root has a great reputation as a panacea (VAN WYK and GERICKE, 2000).
Dicoma shinzii O. Hoffm. Syn: *D. arenicola* Muschl. ex Dinter, *D. lanata* Muschl. ex Dinter Perennial. Herb, prostrate. Length up to 0.3 m. Alt 550–1900 m. N, B, LIM, NW, FS, NC	**080**	Used to treat febrile **convulsions** (referred to as 'arm movements' possible the result of a fever) in children by the San in the Kalahari (VAN WYK and GERICKE, 2000).
Ethulia conyzoides L. f.	**081**	Unspecified parts are used by Zulu to treat **madness** (BRYANT, 1966; DOKE and VILAKAZI, 1972)
Helichrysum aureonitens Sch. Bip. Syn: *H. helodes* Hiern impepho-emhlophe (Z) - white *Helichrysum* inkondlwane (Z) a plant used by the Zulu to make fires	**082**	Leaves and stems are widely used as incense to invoke the goodwill of the **ancestors** and in other forms of traditional medicine (HUTCHINGS et al., 1996). 'They are probably used by the *izangoma* (Zulu diviners) **to induce trances**' (HUTCHINGS et al., 1996). The 'white' (*emhlophe*) in the Zulu name does not refer to the colour of any part of the plant itself, but rather to the clarity of thought brought about by using this plant in **divination**. Several species have shown other biological activities, MEYER, et al. (1996) has shown th.inhibition of herpes simplex virus type 1 by aqueous extracts from shoots of *Helichrysum aureonites*.
Helichrysum species imphepho (Z) NOTE: this is a large genus (± 600 species) mainly found in Africa. Approx. 244 species are found in South Africa.	**083**	*Imphepho* (Z) can refer to ***H. decorum* DC., *H. epapposum* Bolus** (Syn: *H. flavum* Burtt Davy, *H. inerme* Moeser excl. *var. brachycladum* Moeser)**, *H. gymnocomum* DC., *H. natalitum* DC., *H. nudifolium* (L.) Less., *H. odoratissimum* (L.) Sweet** (Syn: *Gnaphalium odoratissimum* L.) or ***H. stenopterum* DC**. (*Achyrocline stenoptera* (DC.) Hilliard & B.L.Burtt). All of these are used as incense to invoke the goodwill of the **ancestors** (HUTCHINGS et al., 1996). ***H. herbaceum* (Andrews) Sweet** (Syn: *H. squamosum* of authors, not of (Jacq.) Thunb., *Xeranthemum herbaceum* Andrews) is refered to as *imphepho-yamakhosi* 'the *Helichrysum* of **ancestral spirits**', and is used as other *imphepho* (HUTCHINGS et al., 1996).

FAMILY ***Species*** Colloquial name[1] – meaning[2] Annotations and distribution for South Africa[3]		**Traditional use, ethnobotanical information and known active constituents**
Helichrysum odoratissimum (L.) Sweet	**084**	Leaves and stems are widely used as incense to invoke the goodwill of the **ancestors** and in other forms of traditional medicine (HUTCHINGS et al., 1996); The smoke is reportedly **sedative**, and helpful for **insomnia**. The smoke from ***H. foetidum* (L.) Moench** and ***H. stenopterum* DC.** are inhaled by healers in KwaZulu- Natal to induce a **trance** (VAN WYK and GERICKE, 2000).
Launaea nana (Bak.) Chiov. Syn: *Lactuca nana* Baker Perennial. Herb. Ht up to 0.05 m. Alt 500–800 m. LIM, M, KZN	**085**	The Shona of Zimbabwe use the roots in a body wash as well as applying them to incisions made on the forehead to treat **convulsions** (GELFAND et al., 1985). This species is not found in South Africa.
Lopholaena coriifolia (Sond.) E.Phillips & C.A.Sm. Syn: *L. bainesii* (Oliv. & Hiern) S.Moore, *L. randii* S.Moore, *Othonna coriifolia* Sond.	**086**	The Shona of Zimbabwe apply the roots to incisions made on the forehead to treat **convulsions** (GELFAND et al., 1985). Perennial. Shrub, succulent. Ht 0.3–2 m. Alt 765–1830 m. LIM, NW, G, M, S
Oncosiphon piluliferum (L.f.) Källersjö Syn: *Cotula globifera* Thunb., *Cotula pilulifera* L.f., *Matricaria globifera* (Thunb.) Fenzl ex Harv., *Matricaria pilulifera* (L.f.) Druce *Pentzia globifera* (Thunb.) Hutch., *Pentzia pilulifera* (L.f.) Fourc., *Tanacetum obtusum* Thunb.	**087**	Reported used as part of a European remedy for treating **convulsions** in the cape of South Africa (Watt, 1967). Annual. Herb. Ht 0.1–0.6 m. Alt 8–1645 m. NW, G, M, FS, KZN, L, NC, WC, EC
Oncosiphon suffruticosum (L.) Källersjö Syn: *Cotula tanacetifolia* L., *Matricaria multiflora* Fenzl ex Harv., *Pentzia suffruticosa* (L.) Hutch. ex Merxm., *Pentzia tanacetifolia* (L.) Hutch., *Tanacetum suffruticosum* L.	**088**	Used by unspecified groups in South Africa to treat infantile **convulsions** (VAN WYK and GERICKE, 2000). Fresh plant material that is crushed with ***Exomis microphylla*** (Chenopodiaceae) or ***Ruta graveolens*** (Rutaceae, *wynruit* (A)) to treat infantile **convulsions** (VAN WYK and GERICKE, 2000). Annual. Herb. Ht 0.1–0.6 m. Alt 15–1500 m. N, NC, WC
Printzia pyrifolia Less. Perennial. Shrub. Ht 0.9–1.6 m. Alt 600–1700 m. FS, KZN, L, EC	**090**	Roots are used by Zulu healers as a substitute for the roots of *Vernonia neocorymbosa*, which are used to **treat hysterics** (GERSTNER, 1939; HUTCHINGS et al., 1996)
Senecio discodregeanus Hilliard & B.L.Burtt. Syn: *S. dregeanus* DC. var. *discoideus* DC.	**091**	Unspecified parts are used to treat **madmen** in Lesotho (PHILLIPS, 1917; JACOT GUILLARMOD, 1971). Perennial. Herb. Ht 0.4–1 m. Alt 50–2375 m. G, M, S, FS, KZN, L, EC
Solanecio angulatus (Vahl) C. Jeffrey Syn: *Crassocephalum bojeri* (DC.) Robyns, *Crassocephalum subscandens* (A.Rich.) S.Moore	**092**	The whole plant is boiled in water and the resultant infusion is drunk to treat **madness** in Zimbabwe (GELFAND et al., 1985). Perennial. Shrub, climber, succulent. Ht up to 30 m. Alt 10–945 m. LIM, KZN
****Tagetes minuta*** L. Annual. Herb. Ht up to 3 m. Alt 14–2125 m. N, B, LIM, NW, G, M, S, FS, KZN, L, WC, EC	**094**	The leaves together with ***Kalanchoe brachyloba*** (Crassulaceae) and ***Mentha aquatica*** (Lamiaceae) are burnt and the smoke inhaled for **mental illness** in Venda, South Africa (ARNOLD and GULUMIAN, 1984).

FAMILY ***Species*** Colloquial name[1] – meaning[2] Annotations and distribution for South Africa[3]		**Traditional use, ethnobotanical information and known active constituents**
Tarchonanthus camphoratus L. Syn: *T. abyssinicus* Sch.Bip. ex Schweinf. & Asch., *T. camphoratus* L. var. *litakunensis* (DC.) Harv., *T. litakunensis* DC., *T. procerus* Salisb Perennial. Tree or shrub. Ht 1–8 m. Alt 750–2135 m. N, B, LIM, NW, G, M, FS, NC	**095**	Dried leaves are reported to have slightly **narcotic** effects when smoked (WATT and BREYER-BRANDWIJK, 1962). Smoking the dried leaves in a pipe is **sedative** (VAN WYK and GERICKE, 2000).
Vernonia adoensis Sch. Bip. ex Walp.	**096**	The root is chewed to treat **madness** in Ndebele of Zimbabwe (GELFAND et al., 1985)
Vernonia amygdalina Del.	**097**	Malawian women who want their beer to be 'strong' are said to fortify it by rubbing the inside of the pots used to brew the **beer** with leaves of *V. amygdalina.* This is reported to make the beer more **intoxicating** (WILLIAMSON, 1974).
Vernonia neocorymbosa Hilliard	**098**	Roots are used to treat **hysteria** by Zulu (GERSTNER, 1939). Macerated leaves are used for treating **epilepsy** by the Swazi (WATT and BREYER-BRANDWIJK, 1962).
BALANITACEAE ***Balanites maughamii*** Sprague	**099**	Roots and bark are ingredients in infusions used in important protective **rituals** against evil spirits by Zulu traditional healers (PALMER and PITMAN, 1972). Froth from the infusions is licked from a bowl, without the use of the hands, three times a day. The remainder is thrown of the roof or spilled at the entrance. Bark infusions are said to make a **stimulating** and **exhilarating** bath (HUTCHINGS et al., 1996; COATES PELGRAVE, 2002)
BEGONIACEAE ***Begonia*** species	**100**	The Zulu are reported to take ***B. homonyma*** **Steud**. root decoctions to counteract *idliso* (poison believed to be administered in food) (HUTCHINGS et al., 1996). Several species including ***B. sutherlandii*** **Hook. f**. are used as **protective** charms and detect threats to the kraal. These may or may not be ingested (HUTCHINGS et al., 1996).
BIGNONIACEAE ***Markhamia obtusifolia*** (Bak.) Sprague Syn: *Dolichandrone obtusifolia* Baker, as '*Dolichandra*' Perennial. Shrub or tree. Ht 0.6−15 m. Alt 910−945 m. N, B, LIM?	**101**	The roots are used for children with **convulsions** in East Africa and Malawi (WILLIAMSON, 1974).
Tecoma capensis (Thunb.) Lindl. Syn: *Bignonia capensis* Thunb., *Gelseminum capense* (Thunb.) Kuntze, *Tecomaria capensis* (Thunb.) Spach, *Tecomaria krebsii* Klotzsch, *Tecomaria petersii* Klotzsch Perennial. Shrub, scrambler or small tree. Ht 0.5−7.5(–10) m. Alt 15−1645 m. LIM, M, S, KZN, WC?, EC	**102**	Dried powdered bark infusions are taken for **sleeplessness** (ROBERTS, 1990); reported to **induce sleep** (HUTCHINGS et al., 1996). Note: the sinking of *Tecomaria* under *Tecoma* is questionable and a close study of the two genera is needed. Recent authors vary in their use of the two names (GERMISHUIZEN and MEYER, 2003).

FAMILY ***Species*** Colloquial name[1] – meaning[2] Annotations and distribution for South Africa[3]		**Traditional use, ethnobotanical information and known active constituents**
BOMBACEAE ***Adansonia digitata*** L. Perennial. Tree. Ht 3–18 m. Alt 100–1060 m. N, B, LIM	**103**	Bark, leaves and roots are infused with the whole plant of ***Myrothamnus flabellifolius* Welw.** and the preparation is ingested to **treat madness** by the Shona of Zimbabwe (GELFAND et al., 1985).
BORAGINACEAE ***Lithospermum cinereum*** A.DC. Perennial. Herb. Ht 0.1–0.3 m. Alt 1020–2300 m. N, NW, FS, KZN, L, EC	**104**	The plant is used as a **sedative** by the Sotho (ASHTON, 1943). Details of the administration were not given.
Myosotis afropalustris C.H. Wright lephukhuphukhu (Z) – *phukuphuku* (isi- izi-) (n) foolish person Perennial. Herb. Ht up to 0.5 m. Alt 1800–3200 m. FS, KZN, L, EC	**105**	Zulu make decoction from the whole plant to **treat hysteria**, a cupful is administered daily or every second day for a month (WATT and BREYER-BRANDWIJK, 1962). Other ingredients may include ***Agapanthus africanus, Cissus, Galium*** and ***Clematis*** species. Similar decoctions are used by the Sotho for treating **hysteria** and also in the training of healers to develop **memory** and make initiates (*ithwasa*) mentally fit for their work (WATT and BREYER-BRANDWIJK, 1962).
Trichodesma physaloides (Fenzl) A.DC. Syn: *Friedrichsthalia physaloides* Fenzl, *T. droogmansianum* De Wild. & T.Durand, *T. glabrescens* Gürke, *T. ringoetii* De Wild.	**106**	The tubers are boiled and the stem inhaled to treat **madness** by the Shona of Zimbabwe (GELFAND et al., 1985). Perennial. Herb. Ht up to 0.5 m. Alt 610–1980 m. LIM, G, M, S, KZN
BUXACEAE ***Buxus macowanii*** Oliv.	**107**	Unspecified parts are used in a vapour bath to treat **mental illness** in southern Africa (SIMON and LAMLA, 1991). Buxamine-B and buxamine-C (below), isolated from Pakistan species *Buxus papillosa* and *Buxus hyrcana* species, have been found to inhibit AChE noncompetitively in a concentration dependent fashion. The IC_{50} values of buxamine-B and buxamine-C were 74 and 7.5 μM, respectively (ATTA-UR-RAHMAN et al., 2001; 2004; KHALID et al., 2004).  Buxamine-B: R^1 = H, R^2 = CH_3 Buxamine-C: R^1 = CH_3, R^2 = H
CAMPANULACEAE ***Wahlenbergia grandiflora*** V. Brehm. ushayindoda omhlope (Z) –*shaya* (v) strike or punish, *-doda* (n) man; *-mhlophe* (v) white or clearly	**108**	Roots are chewed by Zulu boys as a **protective charm** to make them invisible when they have let the cattle stray (HULME, 1954). The powdered roots of *W. undulata* (L. f.) A. DC. which is similar in appearance is traditionally used by Zulu warriors as pre-battle washes (HULME, 1954).

FAMILY ***Species*** Colloquial name[1] – meaning[2] Annotations and distribution for South Africa[3]		**Traditional use, ethnobotanical information and known active constituents**
CANNABACEAE ***Cannabis sativa*** L.	**109**	Widely used in traditional medicine throughout the world. Decoctions are sometimes taken for hypertension, epilepsy (HUTCHINGS et al., 1996); reported to be used as an analgesic and hypnotic in the treatment of depressive mental conditions (OLIVER-BEVER, 1986); and also as a sedative in equine colic (MERCK, 1989); smoking or ingestion of the drug produces a state of euphoria followed by intellectual excitement, illusions, hallucinations, inco-ordination of movement and drowsiness (OLIVER-BEVER, 1986). Has been used in Europe and America to treat epilepsy (FORMUKONG et al. 1989).
CANELLACEAE ***Warburgia salutaris*** (Bertol.f.) Chiov. Syn: *W. breyeri* Pott Perennial. Tree, shrub. Ht 2.5–10 m. Alt 5–1757 m. LIM, M, KZN	**110**	Bark is used in emetics or purgatives for febrile complaints and for rheumatism or the ailments known as *isibhobo* (-bhobo (Z) (n) sharp pain in chest) or *amanxeba* (-nxeba (Z) (n) wound), which are traditionally thought to be caused be **sorcery** (HUTCHINGS et al., 1996)
CAPPARACEAE ***Bosica albitrunca*** (Burch.) Gilg & Ben. umlalampisi (Z) –*lala* (n) sleep, lie down; *mpisi* (n) hyena	**111**	Known as *fructus simulo*, the unripe fruit is used to treat **epilepsy** in southern Africa (WATT, 1967; VAN WYK and GERICKE, 2000). The Zulu are reported to use this plant for **magical purposes** (POOLEY, 1993).
Cadaba natalensis Sond. amangwe (-emnyama) (Z) *ngwe* (n) leopard –*nyama* (n) darkness, ill omen	**112**	Black powder from the burnt plant is used by the Zulu as an inoculation against *ukugcucaba* and *ukucinda* (**evil spirts**) (HUTCHINGS et al., 1996).
Capparis sepiaria L. usondeza (Z) –*sondeza* (v) bring close	**113**	Xhosa herbalists use root decoctions administered as emetics as protective measures to counteract **witchcraft** or render its users, such as soldiers and others wishing to escape detection, invisible (WATT and BREYER-BRANDWIJK, 1962). Traditional Zulu application for protection against lightning and to ward off evil (POOLEY, 1993).
Capparis tomentosa Lam.	**114**	Unspecified parts are a Zulu application used to **treat madness**, as **love charm** emetics and also as charms against lightning or misfortune (GERSTNER, 1941), similar to *C. sepiaria*. Infusions of root are administered for a treatment of **madness** by the Wemba (WATT and BREYER-BRANDWIJK, 1962; HUTCHINGS et al., 1996). The Xhosa also use this plant to treat **madness** (PUJOL, 1990). According to PUJOL (1990) this is also the first plant Zulus turn to 'in order to eradicate germs' after a person has passed away. In these circumstances they will cook the root bark in the house and let it soak for several days and then the whole family will take two cups of this mixture per day.
Maerua angolensis DC.	**115**	In South Africa the Venda use the leaves and bark which are heated over a fire, without water, and the resultant vapour or steam is inhaled to treat children with **convulsions** (MABOGO, 1990; VENTER, 1996).

FAMILY ***Species*** Colloquial name[1] – meaning[2] Annotations and distribution for South Africa[3]		**Traditional use, ethnobotanical information and known active constituents**
CARYOPHYLLACEAE		
Dianthus crenatus Thunb. iningizimu (Z) –*ningi* (n) the greater number, many; –*zimu* (n) cannibal; *zimu* (ideo) of becoming stout isidala (Z) – old way of doing things	**116**	Cold water infusions are taken as emetics by Zulu diviners to clear their **vision** and sharpen their divining faculties (HULME, 1954). The root infusions are also taken as **love charm** emetics and the face is washed in the froth (saponins?) that the infusion produces when whisked vigorously (HULME, 1954; WATT and BREYER-BRANDWIJK, 1962).
Silene burchellii Otth	**117**	Unspecified parts are used in making various important medicines by the Sotho (JACOT GUILLARMOD, 1971). In unspecified parts of southern Africa *Silene burchellii* is used in **tonic** baths taken after severe illness and to combat sleepness (WATT and BREYER-BRANDWIJK, 1962).
Silene capensis Ott. ex DC. undlela ziimhlophe (X) 'white paths'	**118**	In South Africa unspecified parts are used to treat **delirium** (WATT and BREYER-BRANDWIJK, 1962). The Xhosa diviners use the pungent sweet smelling root for spiritual purposes and inducing **dreams** (HIRST, 1997b). The root of *undlela ziimhlophe* is finely ground and mixed vigorously with a forked stick until it produces a white froth, most like caused by saponins. This is consumed by novice diviners on an empty stomach to **enhance dreaming**. The objective is to full one stomach with the foam to a point that causes regurgitation (HIRST, 1990; 2005).
CELASTRACEAE		
Cassine papillosa (Hochst.) Kuntze	**119**	These trees are believed by the Zulu to have powerful **magic** properties and unspecified parts are used to blunt the effects of evil spirits (PALMER and PITMAN, 1972).
Catha edulis (Vahl) Forssk. ex Endl.	**120**	Leaves and twigs that are chewed or taken as a hot infusion are a Xhosa treatment for **stimulating** the CNS (HIRST, 1997a). Bark decoctions are taken in southern Africa as **nerve tonics** (PUJOL, 1990); chewed leaves and twigs, and also tea from the leaves are sometimes used as **stimulants** in the Cape but are more extensively used in North and East Africa and Arabia, where young leaves are chewed to inhibit the sensations of hunger and fatigue and for their stimulating effects on the CNS (WATT and BREYER-BRANDWIJK, 1962; IWA, 1993); Leaves are reported to have short lasting stimulant effects, producing a marked sustaining effect with release from fatigue and hunger as well as euphoric effects (WATT and BREYER-BRANDWIJK, 1962). Chewing a twig of about ten leaves produces wakefulness, mental alertness and a feeling of well-being. Sedative effects are produced by 50 mg but 300 - 400 mg produces hyperexcitability, mydriasis, spinal convulsions and death from respiratory paralysis. Physical dependence does not apparently occur and deaths are rare (DUKE, 1985).
Gymnosporia species Refer to *Maytenus*		

FAMILY ***Species*** Colloquial name[1] – meaning[2] Annotations and distribution for South Africa[3]		**Traditional use, ethnobotanical information and known active constituents**
Hartogiella schinoides (Spreng.) Codd ***Cassine schinoides*** (Spreng.) R.H.Archer *Elaeodendron schinoides* Spreng., *Hartogia angustifolia* Turcz., *Hartogia capensis* L., *Hartogia capensis* L. var. *lanceolata* Sond., *Hartogia capensis* L. var. *latifolia* Sond., *Hartogia capensis* L. var. *multiflora* (Eckl. & Zeyh.) Sond., *Hartogia capensis* L. var. *riparia* (Eckl. & Zeyh.) Sond., *Hartogia schinoides* C.A.Sm., *Hartogiella schinoides* (Spreng.) Codd, *Schrebera schinoides* Thunb. Perennial. Tree, shrub. Ht 2–5 m. Alt 100–1800 m. WC, EC	**121**	The leaves appear to have similar **stimulant** activity to Catha edulis when chewed. Reported activities include chewing the leaf to **quench thirst** (astringent rather than quenching, therefore a possible suppressant and **appetite suppressent**. Also used to suppress fatigue (VAN WYK and GERICKE, 2000)
Maytenus senegalensis (Lam.) Excell Syn: *Gymnosporia senegalensis* (Lam.) Loes.	**122**	The Shona reportedly use the root which is chewed, and the leaves which are rubbed on the face, to treat **epilepsy** (GELFAND et al., 1985). COATES PALGRAVE (2002) mentions the inclusion of this plant in **beer** as an **aphrodisiac**.
Maytenus heterphylla (Eckl. & Zeyh.) N.K.B. Robson Syn: *Gymnosporia heterophylla* (Eckl. & Zeyh.) Loes.	**123**	In various parts of East Africa, root decoctions are taken for **epilepsy** (KOKWARO, 1976).
Pleurostylia capensis (Turcz.) Loes Syn: *Cathastrum capense* Turcz., *P. africana* Loes. Perennial. Tree or shrub. Ht 2–18 m. Alt 30–1640 m. LIM, M, S, KZN, EC	**124**	In the cape of South Africa unspecified parts are used to encourage sleep and to bring good dreams (DE JAGER, 1963).
Pterocelastrus rostratus Walp.	**125**	Bark is used as an antidote to sorcery by Zulu (DOKE and VILAKAZI, 1972). Roots also used in a treatment to treat spinal disease (BRYANT, 1966) which comprises powdered roots of *Secamone gerrardii*, *P. rostratus* Walp., *Ocotea bullata*, *Sarcophyte sanguinea* Sparrm. or *Hydnora* sp. together with the dried body of a fruit bat. This mixture is rubbed into incisions made along the affected part (BRYANT, 1966; HUTCHINGS et al., 1996).
CHENOPODIACEAE ***Chenopodium ambrosiodes*** L. insukumbili (Z)	**126**	The leaves are used in Zimbabwe by unspecified groups to treat madness and convulsions (GELFAND et al., 1985). South African traditional healers use the plant as an intoxicating snuff which assists in communication with the ancestors (SOBIECKI, 2002). In Madigascar infusions of leaves and sap are used to **treat nervous complaints** (GURIB-FAKIM et al. 1993).
Exomis microphylla (Thunb.)	**127**	Early accounts recall that Europeans and Africans have used a milk decoction of the leaf in treatments of epilepsy in South Africa (SMITH, 1888; WATT, 1967). Refer to *Oncosiphon suffruticosum* (Asteraceae); used in a treatment for infantile convulsions, together with *Ruta graveolens* (Rutaceae) (VAN WYK and GERICKE, 2000).

FAMILY ***Species*** Colloquial name[1] – meaning[2] Annotations and distribution for South Africa[3]		**Traditional use, ethnobotanical information and known active constituents**
CHRYSOBALANACEAE ***Parinari capensis*** Harv	**128**	Root infusions taken orally and to wash the body are used by the Shona of Zimbabwe to treat delirium (GELFAND et al., 1985).
CLUSIACEAE ***Hypericum perforatum*** L. (sometimes placed in HYPERICACEAE) Saint John's wort	**129**	This plant is indigenous to Europe, where it has a long history of use, particularly to treat mild **depression**, **anxiety** and **sleeping-disorders** (VAN WYK and GERICKE, 2000). There are several indigenous *Hypericum* species, *H. aethiopicum* Thunb., *H. lalandii* Choisy, *H. natalense* J.M.Wood & M.S.Evans, *H. oligandrum* Milne-Redh., *H. revolutum* Vahl (Syn: *H. lanceolatum* Lam., *H. leucoptychodes* Steud. ex A.Rich.), *H. roeperianum* Schimp. ex A.Rich. and *H. wilmsii* R.Keller. Although many of these are used in African medicine, none are reported to be used for CNS-related ailments.
Harungana madagascariensis Lam. ex Poir.	**130**	Leaves are used as a **stimulant** in Madagascar (PERNET and MEYER, 1957).
COMBRETACEAE ***Combretum adenogonium*** Steud. ex A. Rich. Syn: *Combretum ternifolium* Engl. & Diels	**131**	Unspecified parts are used in Zimbabwe for the treatment of **convulsions** in children (GELFAND et al., 1985). Unspecified parts of ***Combretum ternifolium*** are used in Zimbabwe for convulsions in children (GILGES, 1953; 1955).
Combretum microphyllum Klotzsch	**132**	Unspecified groups in Zambia use unspecified parts to treat **lunacy** (WATT and BREYER-BRANDWIJK, 1962).
Combretum molle R. Br. ex G. Don	**133**	Roots are used for **convulsions** and as an **aphrodisiac** in Zimbabwe (GELFAND et al., 1985).
Terminalia species	**134**	An emetic of roots of ***T. phanerophlebia*** **Engl. & Diels** and ***T. sericea*** **Burch. ex DC.** are used to cause and protect against an illness called *amanxebha* (GERSTNER, 1941; WATT and BREYER-BRANDWIJK, 1962; HUTCHINGS et al., 1996). Traditionally believed to be caused by **witchcraft** this ailment takes the form of a pain in the chest and shoulders (WATT and BREYER-BRANDWIJK, 1962). Roots of ***T. stenostachya*** **Engl. & Diels** to treat **epilepsy** in Zimbabwe (GELFAND et al., 1985).
COMMELINACEAE ***Commelina africana*** L.	**135**	Cold infusions are used to **bathe restless sleepers**, especially children (WATT and BREYER-BRANDWIJK, 1962); root decoctions are administered by Xhosa orally for **fits** (BOLOFO and JOHNSON, 1988; HUTCHINGS et al., 1996). Plants are used in decoctions with ***Tephrosia capensis*** (Fabaceae) for heart complaints and **nervous ailments** in Lesotho (WATT and BREYER-BRANDWIJK, 1962).
CONVOLVULACEAE ***Astripomoea malvacea*** (Klotzsch) A. Meeuse	**136**	The roots and leaves are burned and the smoke inhaled to treat **madness** by the Shona in Zimbabwe (GELFAND et al., 1985).

FAMILY ***Species*** Colloquial name[1] – meaning[2] Annotations and distribution for South Africa[3]		**Traditional use, ethnobotanical information and known active constituents**
Ipomoea species ***Ipomoea obscura*** usiboniseleni (Z) – (*ideo*) what has been shown to us; used in traditional medicine as a hallucinogenic (Pooley, 2005) ***Ipomoea ficifolia*** ikhambilesihlungu (Z) - of poisonous herb; used to treat snake-bites. ***Ipomoea crassipes*** Uvimbukhalo (Z) -what blocks up the loins; used in traditional medicine to treat dysentery.	**137**	Unspecified groups in Zimbabwe use ***I. batatas* (L.) Lam**. with ***Cussonia arborea*** in the treatment of **madness** (GELFAND et al., 1985). This tropical American ornamental climber (***I. tricolor* Cav.**) common in many gardens has been reported to be **hallucinogenic** when 200 to 500 seeds are chewed in South Africa (GELFAND et al., 1985). Two to four seeds of ***I. alba* L**. (another tropical American species) crushed in water and taken at night result in vivid **dreams**, and the seeds of an unknown Convolvulaceae are used to induce dreams and communication with the ancestors (VAN WYK and GERICKE, 2000). The root of ***I. ommaneyi* Rendle** is used as a decoction taken orally to treat **convulsions** (GELFAND et al., 1985) and as an infusion taken orally as an **aphrodisiac** (VAN WYK and GERICKE, 2000). The active substances in the seeds of various species of *Ipomoea* and other members of the Convolvulaceae are alkaloids such as ergine, lysergol, and various clavines which are well described **hallucinogens** (VAN WYK and GERICKE, 2000).
CRASSULACEAE ***Cotyledon orbiculata*** L.	**138**	Fresh leaf juice used by unspecified groups in the treatment of **epilepsy** in the Cape (HUTCHINGS et al., 1996), half cup doses are taken two or three times a day. Also used by the Sotho for various diseases (unspecified) and as a protective 'charm'.
Crassula alba Forssk. isidwe (Z)	**139**	Infusions and decoctions are used as emetics to treat **hysteria** by the Zulu (GERSTNER, 1939).
Crassula arborescens (Mill.) Willd.	**140**	There are early reports of unspecified parts being used in South Africa to treat **epilepsy** (PAPPE, 1857).
Kalanchoe brachyloba Welw. ex Britten	**141**	The leaves are used together with leaves of *Tagetes minuta* in the treatment of **mental illness** by the Venda (ARNOLD and GULUMIAN, 1984). *K. thyrsiflora* Harv. is used by the Sotho as a charm to smooth away difficulties and root decoctions are administered to pregnant women who do not feel well (WATT and BREYER-BRANDWIJK, 1962)
CUCURBITACEAE ***Cucumis hirsutus*** Sond. uthangazane (Z) thanga 'pumpkin' + azi 'biggish' + ane 'smallish', i.e. a plant resembling a medium-sized pumpkin.	**142**	Roots used to treat **convulsions** by the Shona in Zimbabwe. Several cases of suspected poisoning from ingestion of the roots have been reported (BRYANT, 1966; GELFAND et al., 1985). Decoctions made from the leaves or fruit of ***C. africanus* L. f.** are used by Xhosa healers as emetics for patients thought to have been bewitched (SMITH, 1895). Fruit if ***C. myriocarpus* Naud.** is used to make dogs ferocious in Zimbabwe (GELFAND et al., 1985).
Momordica balsamina L inkaka, intshungu (Z)	**143**	Decoctions or cold infusions of the runners are used to soothe 'squeamish stomachs' (BRYANT, 1966). ***M. foetida*** Schumach also used for the same purpose. As well as in Paraguay the roots are used to treat **epilepsy** (WATT and BREYER-BRANDWIJK, 1962).
CUNONIACEAE ***Cunonia capensis*** L. umaphethu, umhlahlane, umuthi wokuzila	**144**	The Zulu regard this plant as a **strengthening medicine** that is taken after the death of a kraal member (GERSTNER, 1939).

FAMILY ***Species*** Colloquial name[1] – meaning[2] Annotations and distribution for South Africa[3]		**Traditional use, ethnobotanical information and known active constituents**
CYATHEACEAE		
Cyathea dregei Kunze isikhomakhoma (Z)	**145**	Plants known as *isikhomakhoma* are used by the Zulu in infusions taken to counteract the effects of witchcraft (HUTCHINGS et al., 1996).
DIOSCOREACEAE		
Dioscorea diversiflora Griseb. udakwa (Z) *–dakwa* (v) be drunk, be intoxicated	**146**	Unspecified groups in southern Africa use tubers to treat **hysterical fits** (WATT and BREYER-BRANDWIJK, 1962).
Dioscorea dregeana Baker ilabatheka (Z) – (*n*) for causing madness or excitement; undiyaza (Z) - be stunned, confused, giddy' udakwa (Z)-being drunk.	**147**	Tuber is Zulu remedy for **hysteria**, **convulsions** and **epilepsy** (WATT, 1967). In southern Africa tubers are used for **hysterical fits** and to cure **insanity** (GERSTNER, 1941; WATT and BREYER-BRANDWIJK, 1962). The Zulu administer small pieces of root, boiled in water, for **nervous spasms** and cramps (PUJOL, 1990). Cold infusions are used by Xhosa as soporifics. Maize cobs boiled in strong tuber decoctions are used to inebriate monkeys so that they can be easily caught (HUTCHINGS et al., 1996); Two teaspoons of fresh macerate from the tuber are reputed to make a person drunk and **intoxicated** (GERSTNER, 1939). Tubers are eaten, after being soaked in running water for several days, as a famine food by the Mpondo (WATT and BREYER-BRANDWIJK, 1962). Insufficiently soaked tubers are reported to produce paralysis of the legs and raw tubers can produce narcosis.
EBENACEAE		
Euclea crispa (Thunb.) Guerke idungamuzi (Z) male plant; umsekisane (Z) female plant	**148**	In Zimbabwe roots of ***E. crispa*** and ***E. divinorum*** **Hiern** are used to treat **epilepsy** and **convulsions** (GELFAND et al., 1985). ***E. natalensis*** **A. DC.** root is burned and the smoke inhaled as a **hypnotic** (VAN WYK and GERICKE, 2000), and root decoctions are used by venda to treat **epilepsy** (ARNOLD and GULUMIAN, 1984). The wood of ***E. schimperi*** **(A. DC.) Dandy** is never used as firewood in South Africa as it is believed to lead to domestic quarrels (WATT and BREYER-BRANDWIJK, 1962).
Diospyros lyciodes Desf.	**149**	The Venda use root decoctions and other unspecified ingredients for treating **epilepsy** (ARNOLD and GULUMIAN, 1984). The Shona of Zimbabwe drink root infusions to treat **epilepsy** (GELFAND et al., 1985).
EUPHORBIACEAE		
Antidesma venosum E. Mey. ex Tul.	**150**	The toxic roots (WATT and Breyer-Brandwijk, 1962) are used as infusion is used to treat **epilepsy** and are used in magic rituals in Malawi (WILLIAMSON, 1974).
Bridelia cathartica Bertol.f.	**151**	Smoke from burnt roots is inhaled to treat **epilepsy** in Zimbabwe (GELFAND et al., 1985). The Zulu use unspecified parts in **love charm** treatments and for patients thought to have been bewitched (GERSTNER, 1941).
Croton gratissimus Burch. ihubeshane-elikhulu	**152**	The fumes from ground leaves mixed with those of other *Croton* species place on hot coals are inhaled to treat **insomnia** (PALMER and PITMAN, 1972).

FAMILY ***Species*** Colloquial name[1] – meaning[2] Annotations and distribution for South Africa[3]		**Traditional use, ethnobotanical information and known active constituents**
Croton sylvaticus Hoscht. umzilanyoni (Z) *–zila* (um-, imi- (n) track; *-nyoni* (n) bird, lightning (column of birds?)	**153**	Bark known as *umzilanyoni* and reported to be *C. sylvaticus* is boiled with salt and herbs to make a tonic for listlessness (HUTCHINGS et al., 1996).
Euphorbia species ***Euphorbia bupleurifolia*** Jacq. inkamasane (Z) -what squeezes out a little maas ***Euphorbia natalensis*** Bernh. inkamasane and inkalamasane (Z)-what weeps maas	**154**	***E. decussate*** **E. Mey. ex Boiss.** is reported to have been used as a fermenting agent in beer making in the Cape (ENGELBRECHT, 1936). ***E. helioscopia*** **L.**, ***E. pubescens*** **Vahl** and ***E. tiruealli*** **L.** are reported to be used as a **narcotic** by unspecified groups in South Africa (WATT, 1967).
Flueggea virosa (Roxb. ex Willd.) Voigt.	**155**	Leaf sap is reported to be used to treat **epilepsy** and **mental illness** in East Africa (HAERDI, 1964).
Jatropha curcas L. inhlakuva (Z) also the Zulu name for castor-oil bean (*Ricinus communis*)	**156**	Roots and leaf infusions of this exotic species are used for **convulsions** and **fits** by unspecified groupd in Africa (ADESINA, 1982). Roots are administered as tonics to infants in Zimbabwe (GELFAND et al., 1985). The leaves ***Ricinus communis*** **L.** (Euphobiaceae) also and exotic is taken orally to **treat madness** in Zimabawe (GELFAND et al., 1985).
Margaritaria discoidea (Baill.) Webster	**157**	Ash from bark is an ingredient in an ointment applied to scarifications on the body as a **stimulant** and tonic (WATT and BREYER-BRANDWIJK, 1962).
Monadenium lugardiae N.E. Br. umhlebe (Z) *–hleba* (v) whisper, speak of evil umhuwa (Z)	**158**	Roots are swallowed by diviners in the Piet Retief area KwaZulu-Natal to obtain **clear vision** before important meetings (WATT and BREYER-BRANDWIJK, 1962). Latex from young shoots is reported to have anaesthetic effects, while the roots are reported to induce **hallucinations** and **delirium** if taken in sufficient (unspecified amount) quantities (WATT and BREYER-BRANDWIJK, 1962).
Phyllanthus reticulates Poir	**159**	Froth (saponins) from mixtures of root bark and other ingredients, which have been briskly stirred in water, are licked without use of hands to give Zulu healers and initiates clear and penetrating **vision** (HUTCHINGS et al., 1996). Root bark infusions are taken by Zulu as emetics or used as bathing '**charms**' to conceal secrets from diviners (PALMER and PITMAN, 1972).
Tragia meyeriana Müll. Arg. ubangalala (Z)	**160**	Roots used by Zulu to cause **sexual excitement** (DOKE and VILAKAZI, 1972). Roots of an unidentified *Tragia* species used as a **stimulant** and tonic in South Africa (PUJOL, 1990).
FABACEAE ***Abrus precatorius*** L. Syn: *A. squamulosus* E.Mey. umkoka (Z)	**161**	Roots are used as sedatives and **anticonvulsants** in Amerindian and African medicine (HUTCHINGS et al., 1996). Extracts from the roots have moderate **sedative** effect (ADESINA, 1982). The leaves with palm oil are taken to treat **convulsions** in Ghana and Gabon (DALZIEL, 1937). DESAI and RUPAWALA (1966) report on the antifertility of the steroidal oil derived of the seeds of *Abrus precatorius* L. from India.

FAMILY ***Species*** Colloquial name[1] – meaning[2] Annotations and distribution for South Africa[3]		**Traditional use, ethnobotanical information and known active constituents**
Acacia amythethophylla Steud. ex A. Rich.	**162**	Root infusions are taken to treat **convulsions** in Zimbabwe (GELFAND et al., 1985). *A. nigrescens* Oliv. roots are applied to the body in an ointment to treat **convulsions** in Zimbabwe (GELFAND et al., 1985).
Acacia karoo Hayne isikhombe, umunga (Z)	**163**	In Zimbabwe the roots are used for general body pains, **dizziness**, **convulsions** and as an **aphrodiasiac** (GELFAND et al., 1985).
Acacia nilotica (L.) Willd. ex Del.	**164**	Bark and root decoctions are drunk by Masai youths to aquire strength and courage and are reported to have an **intoxicating** effect (WILLIAMSON, 1974). Bark decoctions are also taken by the Masai as nerve **stimulants** (WATT and BREYER-BRANDWIJK, 1962).
Adenopodia spicata (E. Mey.) Presl ibobo (Z), umlungumabele	**165**	Root infusions are taken by Zulu diviners (iZangoma) as emetics to increase their diving powers (WATT and BREYER-BRANDWIJK, 1962; HUTCHINGS et al., 1996).
Albizia adianthifolia (Schumach.) W.F. Wight muvhadangoma (V)	**166**	The Venda healers use the roots for improving **memory**, inducing **dreams** about medicinal plants (MABOGO, 1990)
Argyrolobium tomentosum (Andr.) Druce umadlozana (Z) – derived from *amadlozi* (n) ancestral spirits umlomomnandi (Z) - pleasant voice	**167**	Root infusions taken by Zulu diviners (*sangoma*) to sharpen their **vision** (HULME, 1954) and communicate with ancestral spirits (*amadlozi*). It appears that grassland species with bright yellow flowers that are easily seen, even at a distance, such as *Helichrysum* and *Argyrolobium* species are often burned and or taken for 'sharpening of vision'.
Bauhinia thonningii Schumach	**168**	Powdered root, as well as those from *B. candicans* Benth., are taken in porridge to treat **convulsions** by the Shona of Zimbabwe (GELFAND et al., 1985).
Bolusanthus speciosus (H. Bol.) Harms	**169**	Roots reported to have **sleep-inducing** effect by the Tsonga people (LIENGME, 1981).
Caesalpinia bondac (L.) Roxb.	**170**	Unspecified groups in southern Africa use the plant to treat infantile **convulsions** (WATT, 1967). An unidentified *Caesalpinia* species is used as a Chopi remedy for **convulsions** (WATT and BREYER-BRANDWIJK, 1962).
Chamaecrista mimosoides (L.) Greene imbubu yotaboni (Z), umbonisela (Z)	**171**	Cold water root infusions are taken and rubbed on the body to **remember** 'forgotten **dreams'** by Zulu (HULME, 1954). Used by Xhosa and Mfengu to **induce sleep** by being placed under pillows or sleeping mats (WATT and BREYER-BRANDWIJK, 1962). The Sotho use plant decoctions for **loss of appetite** in children (WATT and BREYER-BRANDWIJK, 1962; HUTCHINGS et al., 1996).
Dalbergia obovata E. May.	**172**	Ash from the burnt plant is used in snuff, although no narcotic effects are reported (WATT and BREYER-BRANDWIJK, 1962; HUTCHINGS et al., 1996).

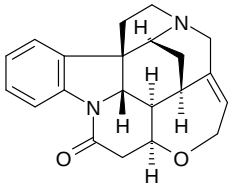
FAMILY ***Species*** Colloquial name[1] – meaning[2] Annotations and distribution for South Africa[3]		**Traditional use, ethnobotanical information and known active constituents**
Desmodium barbatum (L.) Benth. Syn: *D. dimorphum* Welw. ex Baker var. *argyreum* Welw. ex Baker, *Nicolsonia barbata* (L.) DC. var. *argyrae* (Welw. ex Baker) Schindl.	**173**	Infused together with *Faurea saligna* (Proteaceae), it is taken orally once a day for five days to treat **epilepsy** in Zimbabwe (GELFAND et al., 1985).
Elephantorrhiza elephantina (Burch.) Skeels intolwana (Z) ugweje (Z) –(n) reddish object; enormous red underground rhizome.	**174**	Zulus take root decoctions as emetics to mitigate the anger of the ancestors. These are taken every morning for a period of seven to ten days and are reported to have no side effects (HUTCHINGS et al., 1996). Roots are also used by the Zulu as emetics for love charms (GERSTNER, 1939), and in Zimbabwe roots are taken as **aphrodisiacs** (GELFAND et al., 1985).
Entada rheedii Spreng. umbhone (Z), intindili (Z) –*ntindili* (i-, izi-) (n) destitute person	**175**	Tobacco smoked from pipes made from the seeds has been reported to cause vivid **dreaming** (VAN WYK and GERICKE, 2000). Powdered seeds are used by the Zulus for making *umego* medicines. These are taken as a protection against pollutant effects of tracks believed to be left by harmful animals and sorcerers (HUTCHINGS et al., 1996).
Eriosema cordatum E. Mey. umhlabankunzi, uqontsi (Z)	**176**	Hot milk infusions of roots and powdered root infusions have been reported on several occasions to be taken by Zulu for **impotency** (HULME, 1954; BRYANT, 1966; HUTCHINGS et al., 1996). Also used by the Sotho in a mixture with other unspecified plants to stimulate the bulls to mate in the spring (JACOT GUILLARMOD, 1971).
Eriosema distinctum N.E. Br. ubangalala omkhulu (Z)	**177**	Decoctions from pounded boiled roots are used by Zulu for urinary ailments and **impotence** (HULME, 1954).
Eriosema salignum E. Mey. iqonsi, ubangalala (Z)	**178**	Hot milk infusions of roots or cold water infusions of root bark taken in small doses at night and the morning by Zulu for **impotence** (HULME, 1954).
Erythrophleum lasianthum Corbishley	**179**	Powdered bark is administered by Zulus as snuff to treat **hysteria** (HUTCHINGS et al., 1996). Early records report the use of the bark by Zulu to counteract suspected sorcery (GERSTNER, 1939), and to increase the **potency of palm wine** in unspecified parts of Africa (PALMER and PITMAN, 1972). Recently it has been report that the bark is used in South Africa for treating **mental illness** and the snuff of the bark is a mild intoxicant (SOBIECKI, 2002).
Indigofera hilaris Eckl. & Zeyh. isikhubabende (Z)	**180**	Suspected of poisoning cattle at certain seasons and is repoted to produce **delirium** and paralysis (WATT and BREYER-BRANDWIJK, 1962).
Millettia grandis (E.Mey.) Skeels	**181**	The Zulu use mixture of roots ground with those of *Croton* species with one part lion fat, a little ground lion bone and one portion python fat that is burned in homes as a **tranquilliser** and to **dispel worries** (PALMER and PITMAN, 1972). In unspecified parts of southern Africa, residues from evaporated ground roasted roots mixed with water are licked from fingers to **induce sleep** (PALMER and PITMAN, 1972).

FAMILY ***Species*** Colloquial name[1] – meaning[2] Annotations and distribution for South Africa[3]		**Traditional use, ethnobotanical information and known active constituents**
Mimosa pudica L. ***Mimosa pigra*** L. imbune (Z) *–buna* (v) wilt, droop, become emaciated umazifisa (Z)	**182**	Unspecified parts are used to treat children's **convulsions** in Madagascar (JENKINS, 1987). In Mauritius, decoctions of young leaves and stems are given for **insomnia** and **nervousness** (GURIB-FAKEM et al., 1993). Leaves are also used for **insomnia** in Ecuador (SCHULTS and RAFFAUF, 1990). The Zulu name *umazifisa* means 'self-desire' and the plant is used as an ingredient in preparations designed to make an enemy impotent (HUTCHINGS et al., 1996).
Newtonia hildebrandtii (Vatke) Torre udongolokamadilika (Z), umfomothi (Z) man medicine	**183**	Drops of a decoction made from roasted ground bark mixed with water and elephant dung are licked from the hands to drive away 'starts' while sleeping (PALMER and PITMAN, 1972)
Psoralea pinnata L. umhlongani (Z), umhlonishwa (Z)	**184**	Cold water infusions from roots, mixed with the roots of *Helinus integrifolius* (Soap creeper, Rhamnaceae) and pounded and stirred until the liquid froths (saponins), are taken as emetics by healers afflicted with **mental disturbances** associated with their calling (to be a sangoma) (BRYANT, 1966).
Rhynchosia nervosa Benth. & Harv. ubangalala (Z)	**185**	***Rhynchosia*** species are used in Zulu culture as **love charm** emetics (GERSTNER, 1941; BRYANT, 1966; PUJOL, 1990). Roots of species known as *ubangalala* (Z) are cooked in milk and taken in mouthfuls as **aphrodisiacs** (DOKE and VILAKAZI, 1972). Seeds of ***R. pyramadilis* (Lam.) Urb**. and ***R. longeracemosa* Mart. & Gal.** have **narcotic** properties which are utilised by some South Mexican Indian tribes (ALLEN and ALLEN, 1981). ***R. pyramadilis*** is classed as a narcotic **hallucinogen** and is also reported to have **aphrodisiac** properties (DUKE, 1985).
Schotia brachypetala Sond. ihlusi, ihluze, umgxamu, uvovovo	**186**	Largely bark and roots used for **nervous conditions** (VAN WYK and GERICKE, 2000), smoke from leaves also inhaled (Hutchings et al., 1996). The bark is used in a red-bark mixture known as *ikhubalo* to ward off evil or cure unspecified ailments (HUTCHINGS et al., 1996). These mixtures are often taken and also washed with during purification rites after the death of a relative. They are also used to **strengthen** the body and to steam the face (PUJOL, 1990).
Senna didymobotrya (Fresenius) N.W. Irwin & R.C. Barneby Syn. *Cassia didymobotrya* Fresen.	**187**	The roots are burned and the smoke inhaled to treat **madness**, while decoctions of the root are taken orally for **convulsions** in Zimbabwe (GELFAND et al., 1985). Seeds of ***S. occidentalis* (L.) Link** are used in India to treat **convulsions** in children (LAL and GUPTA, 1973).
Senna petersiana (Bolle) J.M. Lock Syn. *Cassia petersiana* Bolle	**188**	A decoction of the roots together with ***Diospyros lycioides*** (Ebenaceae) and ***Euclea natalensis*** (Ebenaceae) are taken to treat **epilepsy** in Venda, South Africa (ARNOLD and GULUMIAN, 1984).
Tephrosia capensis (Jacq.) Pers. isidamvulu, isikhwali (Z)	**189**	The Sotho used cooked roots for palpitations and decoctions of the plant with ***Commelina africana*** (Commelinaceae) for weak hearts and nervousness. Reported to be toxic (WATT and BREYER-BRANDWIJK, 1962).

FAMILY ***Species*** Colloquial name[1] – meaning[2] Annotations and distribution for South Africa[3]		**Traditional use, ethnobotanical information and known active constituents**
Vigna species	**190**	The Zulus use several *Vigna* species (***V. luteola* (Jacq.), *V. unguiculata* (L.) Walp.** and ***V. vexillata* (L.) A. Rich.**) as emetic **love charms** (GERSTNER, 1939). ***V. unguiculata*** root is used in infusions prepared with porridge to treat **epilepsy** in Zimbabwe (GELFAND et al., 1985).
FLACOURTIACEAE ***Casearia gladiiformis*** Mast. umjuluka (Z) –*juluka* (v) perspire, sweat	**191**	Bark is used by Zulu in traditional medicine, and the ashes of bark are used as a snuff (PALMER and PITMAN, 1972). The Tsonga used this plant to treat 'possessed' or **mad people** (JUNOD, 1962; SOBIECKI, 2002). The Zulu use an unidentified plant referred to as *umjuluka*, for treating **mental illness** (MANANA, 1968).
Gerrardina foliosa Oliv.	**192**	**Strengthening** medicine made of unspecified parts is taken after the death of a kraal member (GERSTNER, 1941).
FUMARIACEAE ***Cysticapnos pruinosa*** (Bernh.) Liden	**193**	Unspecified parts are used to comfort and drug **morning, sorrowful people** in Lesotho (JACOT GUILLARMOD, 1971).
GENTIANACEAE ***Chironia krebsii*** Griseb.	**194**	Used for relieving uneasiness during pregnancy by the Sotho (WATT and BREYER-BRANDWIJK, 1962). Preparations from *C. baccifera* L. are reported to produce **sleepiness** and perspiration (WATT and BREYER-BRANDWIJK, 1962).
HYACINTHACEAE ***Albuca fastigiata*** Dryand. ***Albuca nelsonii*** N.E. Br. umaphipha-intelezi	**195**	Infusions made from bulbs of *A. fastigiata* are used to treat *idliso* (Z), an illness believed by the Zulu to be caused by poisons administered in food by jealous rivals (HUTCHINGS et al., 1996), and also used as **protective charms** (CUNNINGHAM, 1988). Infusions made from *A. nelsonii* bulbs and the tubers of a *Kniphofia* species known as *icacane* are taken as emetics against sorcery (HUTCHINGS et al., 1996).
Ledebouria cooperi (Hook. f.) Jessop Syn: *Scilla adlamii* Baker, *Scilla cinerascens* Van der Merwe, *Scilla cooperi* Hook.f., *Scilla galpinii* Baker, *Scilla glaucescens* Van der Merwe, *Scilla inandensis* Baker, *Scilla petiolata* Van der Merwe, *Scilla pusilla* Baker, *Scilla rogersii* Baker, *Scilla rupestris* Van der Merwe, *Scilla saturata* Baker icubudwana (Z)	**196**	Bulbs are used to **inebriate** Sotho boys during circumcision rituals (WATT and BREYER-BRANDWIJK, 1962). This medicine is reportedly made with *Phygelius capensis* (Scrophulariaceae) and causes the boys to appear stunned, stupefied and to fall asleep (HUTCHINGS et al., 1996).
Schizocarphus nervosus (Burch.) Van der Merwe Syn. *Ornithogalum nervosum* Burch., *S. acerosus* Van der Merwe, *S. gerrardii* (Baker) Van der Merwe, *S. rigidifolius* (Kunth) Van der Merwe, *Scilla gerrardii* Baker, *Scilla nervosa* (Burch.) Jessop, *Scilla rigidifolia* Baker, *Scilla rigidifolia* Baker var. *acerosa* Van der Merwe, *Scilla rigidifolia* Baker var. *nervosa* Baker ingcino, ingcolo (Z)	**197**	Ground bulbs in milk are used as an enema to **relieve nervous** conditions in children by unspecified groups in Limpopo (northern Transvaal) (WATT and BREYER-BRANDWIJK, 1962).

FAMILY ***Species*** Colloquial name[1] – meaning[2] Annotations and distribution for South Africa[3]		**Traditional use, ethnobotanical information and known active constituents**
HYPOXIDACEAE		
Hypoxis colchicifolia Bak. ilabatheka-elimnyama (Z)	**198**	The Zulu are reported to use corm infusions for treating **hysteria** (GERSTNER, 1939). Reported to be able to cause **delirium** and to treat it (WATT and BREYER-BRANDWIJK, 1962). Unspecified parts used by the Xhosa to treat **insanity** (SIMON and LAMLA, 1991).
Hypoxis hemerocallidea Fisch. & C.A. Mey	**199**	Corm infusions used to treat **insanity** in South Africa (PUJOL, 1990).
IRIDACEAE		
Belamcanda chinensis (L.) DC. indawoluthi emnyama (Z)	**200**	Roots are reported used by the Zulu to allay **hysterical crying** (BRYANT, 1966). The early missionary MAYR (1907) also reports on the use of a plant known as *indawoluti* as an emetic to treat hysterics.
Eleutherine bulbosa (Miller.) Urban ababomvu (Z) *–bomvu* - red	**201**	Tropical American species commonly grown outside Zulu homesteads as protective charms (HUTCHINGS et al., 1996). Also believed to have magical and **hallucinatory** propertied by unspecified groups (HUTCHINGS et al., 1996).
Ferraria glutinosa (Bak.) Rendle	**202**	Reported as having **psychoactive** properties and to aid novice San healers to enter *kia* or **trance**-like state (LEE, 1979; KATZ, 1982; WINKELMAN and DOBKIN DE RIOS, 1989).
Gladiolus papilio Hook. f. ibutha, igulusha (Z)	**203**	Root infusions take by the Zulu as emetics to **bring good fortune** (HULME, 1954).
Moraea spathulata (L.f) Klatt	**204**	Preventative medicine made by the Zulu with unspecified parts which is believed to 'blunt' **witches** deeds (GERSTNER, 1939). Toxic to live stock with symptoms of convulsions leading to death from respiratory failure (WATT and BREYER-BRANDWIJK, 1962).
Sparaxis grandiflora (Delaroche) Ker-Gawl. indawoluthi emhlophe (Z)	**205**	Used by the Zulu as an antidote against suspected **sorcery** (GERSTNER, 1938). Administration method was not given.
LAMIACEAE		
Ballota africana (L.) Benth. Syn: *Marrubium africanum* L. Perennial. Dwarf shrub, herb. Ht 0.3–1.2 m. Alt 3–1525 m. N, FS, NC, WC, EC	**206**	Infusions or brandy tinctures are used, in the Western Cape for the treatment of **hysteria** and **insomnia** (VAN WYK and GERICKE, 2000)
Becium grandiflorum (Lam.) Pichi-Serm. Syn: *B. obovatum* (E.Mey. ex Benth.)	**207**	Plants burnt as **protective charms** in Zimbabwe (GELFAND et al., 1985). Unspecified parts used as cleansers against evil spirits in Kenya (GITHINJI and KOKWARO, 1993).
Hemizygia bracteosa (Benth.) Briq.	**208**	The leaves are smoked or chewed by the San in Botswana to give energy for dancing and as a **stimulant** (VAN WYK and GERICKE, 2000). The Shona of Zimbabwe are reported to used powdered leaves orally to treat **fits** (GELFAND et al., 1985).
Hoslundia opposite Vahl	**209**	In Zimbabwe root infusions are used to treat **fits** and **epilepsy** (GELFAND et al., 1985). *Hoslundia* species are used in West Africa to treat **epilepsy** and **mental illness** (AYENSU, 1978).

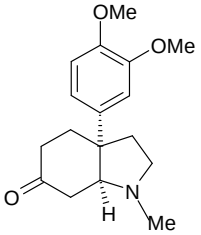
FAMILY ***Species*** Colloquial name[1] – meaning[2] Annotations and distribution for South Africa[3]		**Traditional use, ethnobotanical information and known active constituents**
Leonotis leonurus (L.) R.Br.	**210**	This plant is reported to be mildly **narcotic** (WATT and BREYER-BRANDWIJK, 1962). Leaves are reported to have been smoked for partial paralysis and **epilepsy** (WATT and BREYER-BRANDWIJK, 1962; HUTCHINGS et al., 1996). Aqueous extracts are reported to have **anticonvulsant** activity in animal studies (BIENVENU et al., 2002).
Leucas martinicensis (Jacq.) R. Br. amandetshi (Z)	**211**	In Zimbabwe, leaf infusions are used to **stimulate poor appetite** (GELFAND et al., 1985). Used by the Zulu for feverish conditions in children (GERSTNER, 1941).
Mentha aquatica L.	**212**	Used as a **stimulant** (WILLIAMSON and EVANS, 1988) and known to contain flavones (BURZANSKA-HERMANN et al. 1977). Mixed with leafs of *Tagetes minuta* L. burned and the smoke inhaled for treating **mental illness** in Venda (ARNOLD and GULUMIAN, 1984). Leaf extracts exhibited SSRI activity (NIELSEN et al., 2003). JERKOVIC and MASTELIC (2001) report on the composition of free and glycosidically bound volatiles of *Mentha aquatica* L
Ocimim canum Sims	**213**	The Ndebele of Zimbabwe use the whole plant together with the seeds of *Ricinus communis* and *Chenopodium ambrosidiodes* as snuff to **treat madness** (GELFAND et al., 1985). Unspecified groups in Malawi burn the leaves and inhale the smoke, and also wash the body of the patient with decoctions of the leaves, to treat **convulsions** (GELFAND et al., 1985). In central Africa the leaves together with those of *Cymbopogon densiflorus* Stapf. are macerated and used as a treatment for **epilepsy** (WATT, 1967). *Ocimum* species are used in west Africa to treat **delirium** (AYENSU, 1978).
Pycnostachys urticifolia Hook	**214**	The roots are eaten in porridge, or applied to the face and used in washes to treat **madness** and **convulsions** in Zimbabwe (GELFAND et al., 1985).
Salvia chamelaeagnea Berg.	**215**	Unspecified groups in the cape of South Africa used an infusion of the dried leaves as a treatment for **convulsions** (WATT and BREYER-BRANDWIJK, 1962).
Stachys thunbergii Benth.	**216**	Unspecified groups use the plant in combination with *Valeriana capensis* (Valerianaceae) to treat **hysteria** and **insomnia** in South Africa (VAN WYK and GERICKE, 2000). *S. aethiopica* L. is reportedly burnt in huts to cure feverish delirium in Lesotho (JACOT GUILLARMOD, 1971).
Syncolostemon parviflorus E. Mey. ex Benth. umnandi (Z) (n) pleasant taste, tasty, sweetness	**217**	Milk infusions administered by Zulu as emetics to adults to treat loss of appetite (GERSTNER, 1941; WATT and BREYER-BRANDWIJK, 1962; POOLEY, 2005).
Tinnea zambesiaca Bak.	**218**	The Shona of Zimbabwe use the roots and leaves as a body wash for **convulsions** (GELFAND et al., 1985).
LAURACEAE ***Cinnamomum camphora*** (L.) T. Ness & C.H. Eberm.	**219**	Although not an indigenous plant it has become a popular traditional medicine to treat a variety of complaints, used to treat **hysteria** (WATT and BREYER-BRANDWIJK, 1962)

FAMILY ***Species*** Colloquial name[1] – meaning[2] Annotations and distribution for South Africa[3]		**Traditional use, ethnobotanical information and known active constituents**
Ocotea bullata (Burch.) Baill. unukani (Z) *-nuka* (n) smell, strong odour	**220**	Unspecified parts used as an emetic for emotional and **nervous disorders** in South Africa (PUJOL, 1990).
LOBELIACEAE ***Lobelia decurrentifolia*** (Kuntze) K. Schum. Syn: *Dortmannia decurrentifolia* Kuntze Annual. Herb. Ht? Alt ± 100 m. EC	**221**	The Sotho are reported to believe this plant has powerful **spiritual** uses and it is believed to have the power to stop 'wolves' (which is puzzling as they do not occur in southern Africa) and the spirits of the **ancestors** (LAYDEVANT, 1939; SOBIECKI, 2002).
LOGANIACEAE (Buddlejaceae and Strychnaceae) ***Buddleja*** (L.) species	**222**	Used together with *Heteromorpha trifoliate* (Wendl.) Eckl. & Zeyh. and *Cussonia paniculata* Eckl & Zeyh. by Sotho in South Africa to treat early **nervous** and **mental illnesses** (WATT and BREYER-BRANDWIJK, 1927 cited in SOBIECKI, 2002).
Nuxia floribunda Benth. Syn: *Lachnopylis floribunda* (Benth.) C.A.Sm. Perennial. Tree or shrub. Ht 2–17 m. Alt 30–1980 m. LIM, M, S, KZN, WC, EC	**223**	Bark is used in Zulu traditional medicine as a **strengthening** medicine taken after the death of a kraal member (GERSTNER, 1941). Leaves reported to be used for infantile **convulsions** and in rituals in unspecified parts of Africa (IWU, 1993).
***Strychnos* s**pecies *Strychnos decussata* (Pappe) Gilg *Strychnos henningsii* Gilg umnono (Z)  Srychnine	**224**	These species contains often contain strychnine and other indole alkaloids which are powerful **CNS stimulants**. The Zulu use pinches of scraped and ground root bark of ***S. decussate*** as snuff (PALMER and PITTMEN, 1972**).** ***S. henningsii*** is reported to be used to induce visionary **dreams** associated with ancestral spirits (SOBIECKI, 2002).
LORANTHACEAE ***Loranthus oleifolius*** (Wendl.) Cham. & Schlechtd. Syn: *Tapinanthus oleifolius* (J.C.Wendl.) Danser, *L. meyeri* C.Presl, *L. meyeri* C.Presl var. *inachabensis* Engl., *L. namaquensis* Harv., *L. namaquensis* Harv. var. *ligustrifolius* Engl., *L. speciosus* F.Dietr., *T. namaquensis* (Harv.) Tiegh., Perennial. Shrub, parasite. Ht ± 1.5 m. Alt 70–1950 m. N, B, LIM, NW, FS, NC	**225**	Suspected as being used by the !Kung San to facilitate *kia*, a **trance**-like state (WINKELMAN and DOBKIN DE RIOS, 1989). An unidentified species is used by the Shona of Zimbabwe to treat **epilepsy**, **madness** and **convulsions** (GELFAND et al., 1985).

FAMILY ***Species*** Colloquial name[1] – meaning[2] Annotations and distribution for South Africa[3]		**Traditional use, ethnobotanical information and known active constituents**
MALPIGHIACAE ***Sphedamnocarpus*** species One species in South Africa: ***Sphedamnocarpus pruriens*** (A.Juss.) Szyszyl. ***Sphedamnocarpus pruriens*** subsp. *galphimiifolius* (A.Juss.) P.D.de Villiers & D.J.Botha Perennial. Shrub, climber. Ht 0.3–5 m. Alt 15–2000 m. B, LIM, NW, S, FS, KZN ***Sphedamnocarpus pruriens*** subsp. *pruriens* Perennial. Shrub, climber. Ht 0.1–5 m. Alt 30–2500 m. N, B, LIM, NW, G, M, S, KZN, NC	**227**	Root decoctions of ***S. galphimiifolius*** **(A. Juss.) Szyszyl** are taken orally to treat **mental illness** by the Venda (ARNOLD and GULUMIAN, 1984). Unspecified parts of ***S. pruriens*** **(Juss.) Szyszyl** are used by the Chopi with ***Securidaca longepedunculata*** (Polygalaceae) for people believed to be possessed by evil spirits (WATT and BREYER-BRANDWIJK, 1962). Roots are used by Venda to treat **mental illness** (MABOGO, 1990).
MALVACEAE ***Azanza garckeana*** (F. Hoffm.) Exell & Hillc. Syn: *Shantzia garckeana* (F.Hoffm.) Lewton, *Thespesia garckeana* F.Hoffm. Perennial. Tree, shrub. Ht 4–10 m. Alt up to 2000 m.N, B, LIM	**228**	Root decoctions are taken orally to treat **mental illness** in Zimbabwe (GELFAND et al., 1985).
Hibiscus pusillus Thunb. uguqukile (Z) –*guqu* (ideo) of changing; turning over; 'having turned'	**229**	Emetics made from the roots are taken for bad **dreams** and by Zulu men as **love charms** (WATT and BREYER-BRANDWIJK, 1962).
Malva parviflora L.	**230**	The Sotho reported to give root decoctions to persons who have lost near relatives (WATT and BREYER-BRANDWIJK, 1962). Leaf infusions taken by Europeans as a **nerve tonic** (WATT and BREYER-BRANDWIJK, 1962).
Thespesia acutiloba (Bak. f.) Exell & Mendonca	**231**	The roots are used in baths taken to **refresh** the body and dispel troublesome **spirits** by the Zulu (PALMER and PITTMEN, 1972).
MELIACEAE ***Ekebergia capensis*** Sparrm. umnyamathi (Z) – *nyama* (n) darkness, ill omen – *thi* (n) tree, medicine, stick, poison	**232**	Leaves are crushed in cold water and the resulting extract is introduced to the nostrils to treat **mental problems** including **madness** among the Zulu (PUJOL, 1990). Bark of *umnyamathi* reported to be *E. capensis* is used to treat listlessness, undue **exhaustion** and to ward off evil in by the Zulu in KwaZulu-Natal (HUTCHINGS et al., 1996). Bark is used to protect chiefs against witchcraft and is also taken in **love charm** emetics (GERSTNER, 1941).
Entandrophragma spicatum (C. DC.) Sprague	**233**	The cigar shaped pods are burned and the ash mixed with tobacco to make a **snuff** (RODIN, 1985).

FAMILY ***Species*** Colloquial name[1] – meaning[2] Annotations and distribution for South Africa[3]		**Traditional use, ethnobotanical information and known active constituents**
Melia azedarach L.	**234**	Decoctions made from a large handful of freshly pounded leaves of this exotic in two litres of water are administered as emetics to **epileptic** patients after a **fit** (HUTCHINGS et al., 1996). The fruit is applied externally to treat **convulsions**, spasms and nervous pain (HUTCHINGS et al., 1996). Reported to have **CNS-suppressant** activity (WATT, 1967) and all parts considered to be toxic (WATT and BREYER-BRANDWIJK, 1962). Symptoms include mental confusion, stupor and convulsions. Root bark extracts have been shown to have sedative and depressant properties in animal studies in Nigeria (ADESINA, 1982).
Turraea floribunda Hochst. umadlozana (Z) -*dlozane* (isi-, izi-) (n) nape of the neck, -*dlozi* (i-, ama-) (n) spirit of the departed, guardian spirit, ancestral spirit	**235**	Roots are used by diviners to enter the **neurotic** state needed for divining dances (WATT and BREYER-BRANDWIJK, 1962; HUTCHINGS et al., 1996). Emetics made from bark are taken to prevent the fearful **dreams** thought to be symptomatic of heart weakness (BRYANT, 1966). Strengthening medicine made from unspecified parts are taken after the death of a kraal member (GERSTNER, 1941). Reported to be poisonous if overdoses are taken, amounts not specified (WATT and BREYER-BRANDWIJK, 1962).
Turraea nilotica Kotschy & Peyr	**236**	Root infusions are used to treat **epilepsy**, leaves are burnt and the smoke inhaled for treating **madness** in Zimbabwe (GELFAND et al., 1985).
Nymania capensis (Thunb.) Lindb.	**237**	Used by the Europeans in the Cape of South Africa to treat **convulsions** (WATT, 1967) and early accounts suggest that the Hottentots (KHOI) of Namaqualand also use unspecified parts to treat **convulsions** (LAIDLER, 1928).
MELIANTHACEAE ***Bersama lucens*** (Hochst.) Szyszyl. isindiyandiya, undiyaza (Z)	**238**	The Zulu use a tincture of the bark as an emetic to **calm nervous disorders** (HUTCHINGS and VAN STADEN, 1994). Bark is also used by the Zulu as protective **charms** against evil spirits (HUTCHINGS et al., 1996) and to confuse one's opponents in court (DOKE and VILAKAZI, 1972).
Bersama tysoniana Oliv.	**239**	Bark is used by Xhosa to treat hysteria (WATT and BREYER-BRANDWIJK, 1962). Bark is probably used in a similar way as ***B. lucens*** by the Zulu (HUTCHINGS et al., 1996).
Melianthus comosus Vahl ibonya (Z) –*bhonya* (v) flog, beat on the body; (n) barren beast	**240**	Roots reported to be toxic and causing human death. Root infusions act as violent **depressants**, producing emesis and also exerting marked cardiac action with fatal results (WATT and BREYER-BRANDWIJK, 1962).
MENISPERMACEAE ***Cissampelos*** species umbombo (Z) – (n) bridge of the nose	**241**	***C. torulosa*** **E. Mey. ex Harv.** leaf decoctions are administered as enemas for **hallucinations** in the Transkei (HUTCHINGS et al., 1996). Small portions of the rhizome of ***C. capensis*** **L. f.**, which are rich in bisbenzylisoquinoline alkaloids, are reported to have **sedative** effects when chewed (VAN WYK and GERICKE, 2000). ***C. mucronata*** **A. Rich.** are used as **sexual stimulants** (HUTCHINGS et al., 1996).

FAMILY ***Species*** Colloquial name[1] – meaning[2] Annotations and distribution for South Africa[3]		**Traditional use, ethnobotanical information and known active constituents**
Stephania species	**242**	Early records suggest that an unverified species is used by Sotho **diviners** to discover things and is used in conjunction with the divining bones (PHILIPS, 1917). Epistaphine from the aerial parts of ***Stephania abyssinica*** **(Dill. & Rich.)** Walp. has significant **adrenergic neurone blocking** activity (RAY et al., 1979).
MESEMBRYANTHEMACEAE		
Aptenia cordifolia (L. f.) Schwant. ibohlololo (Z) (ideo) of subsiding umjuluka (Z) –*juluka* (v) perspire	**243**	Used by the Zulu to make **love charm** medicine, and is an ingredient in black powder (*uncolozi omncane* or *ungcolosi*) for vaccination against **sorcery** and to counteract perspiration (GERSTNER, 1941; DOKE and VILAKAZI, 1972). For the treatment of nervous complaints, leaf and stem preparations are rubbed into scarifications (CROUCH et al., 2000).
Conophytum species toontjies (A)	**244**	The genus is reputed to have **narcotic** properties (WATT, 1967), also believed to have **sedative** properties (VAN WYK and GERICKE, 2000).
Khadi acutipetala (N.E. Br.) N.E. Br. khadiwortel (A)	**245**	Although the roots a largely used as a fermentation agent, possible due to associated fungi, it is suggested that mesembrine-type alkaloids in the root may contribute to the beers **intoxicating** properties (VAN WYK and GERICKE, 2000).
Mesembryanthemum species ikhambi-lamabulawo (Z) -*bulawo* (n) body weakness as a result of witchcraft.	**246**	Emetics made from a handful of leaves in boiling water are administered by the Zulu for the fearful **dreams** believed to be symptomatic of heart weakness (BRYANT, 1966), although the name suggests there is **witchcraft** involved.
Pleiospilos bolusii (Hook. f.) N.E. Br. Syn: *Mesembryanthemum bolusii* Hook.f., *P. barbarae* Karrer, *P. beaufortensis* L.Bolus duimpiesnuif (A)	**247**	The plant is dried and powdered and used as a snuff (VAN WYK and GERICKE, 2000).
Rabiea albinota (Haw) N.E. Br. s'keng-keng (Griqua)	**248**	The pulverised plant is reported to be a **hallucinogenic** additive to tobacco to be smoked or taken as **snuff** (VAN WYK and GERICKE, 2000).

FAMILY ***Species*** Colloquial name[1] – meaning[2] Annotations and distribution for South Africa[3]		**Traditional use, ethnobotanical information and known active constituents**
Sceletium species ***Sceletium emarcidum*** (Thunb.) L.Bolus ex H.Jacobsen Syn: *Mesembryanthemum anatomicum* Haw. var. *anatomicum*, *Mesembryanthemum anatomicum* Haw. var. *emarcidum* (Thunb.)DC., *Mesembryanthemum anatomicum* Haw. var. *fragile* Haw., *Mesembryanthemum emarcidum* Thunb., *S. anatomicum* (Haw.) L.Bolus, *S. dejagerae* L.Bolus, *Tetracoilanthus anatomicus* (Haw.) Rappa & Camarrone ***Sceletium tortuosum*** (L.) N.E. Br. Syn: *Mesembryanthemum concavum* Haw., *Mesembryanthemum tortuosum* L., *Pentacoilanthus tortuosus* (L.) Rappa & Camarrone, *Phyllobolus tortuosus* (L.) Bittrich, *S. boreale* L.Bolus, *S. compactum* L.Bolus, *S. concavum* (Haw.) Schwantes, *S. framesii* L.Bolus, *S. gracile* L.Bolus, *S. joubertii* L.Bolus, *S. namaquense* L.Bolus var. *namaquense*, S. namaquense L.Bolus var. *subglobosum* L.Bolus, *S. ovatum* L.Bolus, *S. tugwelliae* L.Bolus kougoed (A) –chewing substance  mesembrine	**249**	It has been suggested that this plant has been used by pastoralists and hunter-gatherers as a **mood-altering substance** since prehistoric-times (VAN WYK and GERICKE, 2000). Kougoed refers to a traditional preparation made from ***S. emarcidum* (Thunb.) L.Bolus ex H.Jacobsen** or ***S. tortuosum* (L.) N.E. Br.**, which is used as a **stimulant** with an effect not unlike that of tobacco. To prepare the plant material, usually a whole plant including the roots, it is crushed between stones, after which it is then placed into a closed container for several days to 'ferment'. On the eighth day the kougoed is spread out to dry in the sun. This is then chewed, smoked or powdered and inhaled as snuff (SMITH et al., 1996; VAN WYK and GERICKE, 2000). Sceletium tortuosum contains mesembrine and the related alkaloids mesembranol and mesembranone (SMITH, et al., 1998). Mesembrine is known for its effects on the central nervous system. The compounds also act as serotonin-uptake inhibitors, and in specified doses act as anti-depressants, minor tranquilizers and anxiolytics used in the treatment of mild to moderate depression, psychological and psychiatric disorders where anxiety is present, major depressive episodes, alcohol and drug dependence, bulimia nervosa, and obsessive-compulsive disorders (U.S.Patent 6 288 104) (GERICKE and VAN WYK, 2001).
Trichodiadema species	**250**	***T. intonsum* (Haw.) Schwant.** and ***T. stellatum* (Mill.) Schwant**. are used as fermentation agents, possible due to associated fungi. It is suggested that mesembrine-type alkaloids present in many Mesembryanthemaceae may contribute to the beers **intoxicating** properties (WATT and BREYER-BRANDWIJK, 1962; DOLD et al., 1999; VAN WYK and GERICKE, 2000).
MYROTHAMNACEAE ***Myrothamnus flabellifolius*** Welw.	**251**	Unspecified parts are administered by healers in Zimbabwe to treat **epilepsy** and **madness** (GELFAND et al., 1985).
MYRSINACEAE ***Maesa lanceolata*** Forssk. umaguqu (Z) uphophopho (Z)	**252**	Bark is used to make a stimulating drink by the Masai of east Africa (WATT and BREYER-BRANDWIJK, 1962; HUTCHINGS et al., 1996). Healers in South Africa are reported to use this plant for spiritual purposes associated with ancestral spirit (*amadlozi*) worship (SOBIECKI, 2002).

FAMILY ***Species*** Colloquial name[1] – meaning[2] Annotations and distribution for South Africa[3]		**Traditional use, ethnobotanical information and known active constituents**
Rapanea melanophloeos (L.) Mez	**253**	Roots and bark are used to treat palpitations [possibly as a result of **anxiety** or stress] in various parts of the Transkei (HUTCHINGS et al., 1996). Ground bark infusions are taken three times a day by persons who '**feel like crying**' (HUTCHINGS et al., 1996).
NYMPHACEAE ***Nymphaea nouchali*** Burm. f. izibu (Z) ikhubalo lechanti (Z) - *ichanti*: a mythical animal of Zulu folktales	**254**	Flowers are thought to have been used in Egypt as a **narcotic** to induce **trances** (EMBODEN, 1989). The fresh and dry flowers have been used by **diviners** in South Africa. Tinctures of the flowers are used as **stimulants**, **aphrodisiacs** and **euphoriants** in low doses, but **tranquillizing** in higher doses (VAN WYK and GERICKE, 2000).
OCHNACEAE ***Brackenridgea zanguebarica*** Oliver.	**255**	The powdered root is rubbed on the body to treat **mental illness** by the Vavenda (ARNOLD and GULUMIAN, 1984).
OLEACEAE ***Olea woodiana*** Knobl. isadlulambazo, umhlwazimamba, umnqumo (Z)	**256**	The bark is used in South Africa as a **nerve tonic** and reputed to have **stimulating** properties that induce a good feeling by the Zulu (PUJOL, 1990). The bark is also reported to **stimulate the appetite** and the leaves are also used for their stimulating properties (PUJOL, 1990). Also in this family is ***Jasminum multipartitum*** Hochst. which is reported to be used for stress, an infusion is said to be relaxing (ASHWELL, 1994).
ORCHIDACEAE ***Ansellia africana*** Lindl. imfe-nkawu (Z)	**257**	Stem infusions are taken as antidotes to bad **dreams** and smoke from burning roots is inhailed for the same purposes by the Zulu (HUTCHINGS et al., 1996). The leaves are stems are used to make an infusion for treating **madness** in the Mpika district of Zambia (GELFAND et al., 1985). Used for various protective **charm** purposes and also as an **aphrodisiac** in Zimbabwe (GELFAND et al., 1985).
Brachycorythis ovata Lindl. imfeyamasele yentaba (Z)	**258**	Root decoctions of ***Brachycorythis ovata*** and ***Ceratandra grandiflora*** are used for the treatment of madness in the Eastern Cape (Transkei) (BATTEN and BOKELMANN, 1966).
Ceratandra grandiflora Eckl. ex Bauer	**259**	
Corycium nigrescens Sond. umabelembuca (Z)	**260**	Root infusions are taken as emetic **charms** to ward off evil (HULME, 1954).
Disa polygonoides Lindl. ihlamvu elibomvu, umklakleshe (Z)	**261**	Tuber infusions are administered to patients who have lost the power of speech due to illness (HULME, 1954).
Eulophia species umahayiza (Z) -*hayiza* (v) be hysterical	**262**	Unidentified species known as *umahayiza* (Z) are reported to be used as emetics to treat **hysterical fits** (GERSTNER, 1941)
PASSIFLORACEAE ***Adenia gummifera*** (Harv.) Harms imfulwa (Z), impinda (Z) (n) a recurrence	**263**	Infusions made from root (approximately 150 mm long and 30 mm thick) in three to four litres of boiling water are administered as emetic **tonics** or s**timulants** for seediness or **depression** caused by a febrile conditions known as *umkhuhlane* (common cold or fever) to the Zulu (BRYANT, 1966). The Shona of Zimbawe reportedly use root infusions to treat **madness** and **epilepsy** (GELFAND et al., 1985).

FAMILY ***Species*** Colloquial name[1] – meaning[2] Annotations and distribution for South Africa[3]		**Traditional use, ethnobotanical information and known active constituents**
PEDALIACEAE ***Sesamothamnus lugardii*** N.E. Br. ex Stapf	**264**	Leaves are rubbed on the face together with smoke from burned leaves which is inhaled to treat **convulsions** in Zimbabwe (GELFAND et al., 1985).
PERIPLOCACEAE ***Mondia whitei*** (Hook. f.) Skeels Syn: *Chlorocodon whitei* Hook.f. umondi (Z)	**265**	The Zulu chew the roots to **stimulate appetite** (BRYANT, 1966; GERSTNER, 1941). Roots are used as an **aphrodisiac** in Zimbabwe (WATT and BREYER-BRANDWIJK, 1962; GELFAND et al., 1985).The Shambala use root infusions to **treat fits** in children (WATT and BREYER-BRANDWIJK, 1962) and by unspecified groups in South Africa to **treat stress** and **tension** in adults (VAN WYK and GERICKE, 2000).
Raphionacme species *R. hirsuta* (E. Mey.) R.A. Dyer ex E. Phillips Syn: *Apoxyanthera pubescens* Hochst., *Brachystelma hirsutum* E.Mey., *Mafekingia parquetiana* Baill., *R. divaricata* Harv., *R. divaricata* Harv. var. *glabra* N.E.Br., *R. hirsuta* (E.Mey.) R.A.Dyer ex E.Phillips var. *glabra* (N.E.Br.), R.A.Dyer, *R. obovata* Turcz., *R. pubescens* (Hochst.) Hochst., *R. purpurea* Harv.	**266**	***R. hirsuta*** is reportedly used in Lesotho to make an energizing and highly **intoxicating beer** (JACOT GUILLARMOD, 1971). Underground parts of various *Raphionacme* species are used in treatments for **madness** in Zimbabwe (GELFAND et al., 1985).
PHYTOLACCACEAE ***Phytolacca*** species ***Phytolacca heptandra*** Retz. Syn: P. stricta O.Hoffm., Pircunia stricta Hoffm. ***Phytolacca octandra*** L. Syn: *P. americana* L. var. *mexicana* L.	**267**	Unspecified groups use *P. heptandra* in emetics for **delirium** (HUTCHINGS et al., 1996). The shoots of *P. octandra* are used as a **stimulant snuff** in Venda (MABOGO, 1990). The roots of the exotic *Phytolacca americana* L.* are reported to have a slightly narcotic effect (MARTINDALE, 1967). SOBIECKI (2002) describes its psychoactive use in southern Africa as uncertain.
PIPERACEAE ***Piper capense*** L. f.	**268**	Root is used by unspecified groups in southern Africa as a **sexual stimulant**, and is reported to cause **sleepiness** (VAN WYK and GERICKE, 2000). ***P. betel* L.** the betel vine is used in wrapping betel nut (*Areca catechu*) which is chewed for its mild psycho-stimulating effects. Approximately 200 million persons chew betel regularly mainly throughout the western Pacific basin and south Asia but this practice has spread throughout the world.
PITTOSPORACEAE ***Pittosporum viridiflorum*** Sims umkhwenkhwe (X, Z), umfusamvu (Z)	**269**	Root infusions are used for accuracy in **divining** and for protecting patients from **witchcraft** by the Sotho (WATT and BREYER-BRANDWIJK, 1962). Root infusions are also used in Zimbabwe as enemas to treat **dizziness** (GELFAND et al., 1985). Bark decoctions are reported to ease pain and to produce **restfulness** (WATT and BREYER-BRANDWIJK, 1962).
PLUMBAGINACEAE ***Plumbago auriculata*** Lam. umasheleshele (Z)	**270**	Pounded root infusions are administered by the Zulu as emetics to dispel bad **dreams** (HULME, 1954). The powdered roots are used by the Xhosa as a **snuff** (WATT and BREYER-BRANDWIJK, 1962). Extracts of the roots of *P. zeylancia* L. had **sedative** activity (ADESINA, 1982). *P. zeylancia* is also suspected to have been used by San to induce **trance** state (WINKELMAN and DOBKIN DE RIOS, 1989).

FAMILY ***Species*** Colloquial name[1] – meaning[2] Annotations and distribution for South Africa[3]		**Traditional use, ethnobotanical information and known active constituents**
POACEAE ***Cymbopogon nardus*** (L.) Rendle Syn: *C. afronardus* Stapf, *C. validus* (Stapf) Stapf ex Burtt Davy *validus* (Stapf.) Stapf. ex Burtt. Davy isicunge (Z)	**271**	The Zulu use the whole plant, boiled with milk and drunk twice daily (two small cups) to **strengthen the nervous system** and to stimulate the body (PUJOL, 1990). This grass is reported to be used to **reduce the appetite** and **revitalise the nerves of moody people** (HUTCHINGS et al., 1996).
POLYGALACEAE ***Nylandtia spinosa*** (L.) Dumort. Syn: *Mundia spinosa* (L.) DC.	**272**	Unspecified parts are used to treat **hysteria** and **sleeplessness** in the Cape of South Africa (BATTEN and BOKELMANN, 1966) and early records of this plant suggest that it is a **narcotic** (KLING, 1923).
Securidaca longipedunculata Fresen.	**273**	Unspecified parts of ***Sphedamnocarpus pruriens*** (Malpighiaceae) are used by the Chopi with ***S. longepedunculata*** for treating people believed to be possessed by **evil spirits** (WATT and BREYER-BRANDWIJK, 1962). Unspecified groups in Zimbabwe used powdered root mixed in porridge and eaten to treat **epilepsy** and **convulsions** (GELFAND et al., 1985). The plant is reported to contain the toxic indole alkaloid securinine and some ergot alkaloids (VAN WYK and GERICKE, 2000). A diterpene, a flavonol glycoside, and a phytosterol glycoside have been isolated from *Securidaca longipedunculata* and *Entada abyssinica* (DEBELLA et al., 2000).
POLYGONACEAE ***Emex australis*** Steinh inkunzama (Z)	**274**	Boiled leaves of this exotic weed are used to **stimulate the appetite** by the Xhosa (WATT and BREYER-BRANDWIJK, 1962).
Oxygonum species	**275**	Roots of unspecified *Oxygonum* species, possibly *O. dregeanum* Meisn. are used in medicines taken to treat **convulsions** in Zimbabwe (GELFAND et al., 1985). The Kwanyama Ovambos use an infusion of an unidentified species as an enema to treat epileptic children (LOEB et al., 1956).
PORTULACACEAE ***Avonia rhodesica*** (N.E.Br.) G.D.Rowley Syn: *Anacampseros rhodesica* N.E. Br.	**276**	The plant is used by unspecified groups as a **beer additive** 'to improve the kick', and is reported to have **hallucinogenic** and **narcotic** properties (GELFAND et al., 1985; VAN WYK and GERICKE, 2000).
Talinum species	**277**	Several species are edible when fresh and eaten as a green vegetable in may parts of southern Africa (VAN WYK and GERICKE, 2000). Root infusions of *T. caffrum* (Thunb.) Eckl. & Zeyh., or *impunyu* (Z) are taken for **nervousness** (GERSTNER, 1941). Tuber decoctions of *T. crispatulum* Dinter ex V. Poelln. are used by unspecified groups in Botswana for heart palpitations, possible caused by **anxiety** (HEDBERG and STAUGARD, 1989).
PROTEACEAE ***Faurea saligna*** Harv.	**278**	Infused together with ***Desmodium barbatum*** (Fabaceae), it is taken orally once a day for five days to treat **epilepsy** in Zimbabwe (GELFAND et al., 1985).
PTAEROXYLACEAE (Rutaceae) ***Ptaeroxylon obliquum*** (Thunb.) Radlk. Syn: *P. utile* Eckl. & Zeyh. sneezewood (E), umthati (X)	**279**	Alcoholic extracts of the wood are used by unspecified groups to treat patients suffering from **fits** (HUTCHINGS et al., 1996). The Xhosa are reported to use the bark as a **snuff** for recreational purposes (WATT and BREYER-BRANDWIJK, 1962).

FAMILY ***Species*** Colloquial name[1] – meaning[2] Annotations and distribution for South Africa[3]		**Traditional use, ethnobotanical information and known active constituents**
RANUNCULACEAE		
Anemone caffra Eckl. & Zeyh. Syn: *Pulsatilla caffra* Eckl. & Zeyh. ***Anemone fanninii*** Harv. ex Mast. umanzamnyama (Z) – black or dark waters	**280**	Zulu believe the roots are used to produce hatred (DOKE and VILAKAZI, 1972). The burnt black roots are used 'to make other people black' possibly indicating **sorcery** (GERSTNER, 1941; Hutchings et al., 1996). In the Transkei the ground inner root is taken as **snuff** to treat **dizziness** (HUTCHINGS et al., 1996). *A. caffra* mixed with *Athrixia heterophylla* (Asteraceae) are used to treat unspecified **mental disease** by the Xhosa (WATT and BREYER-BRANDWIJK, 1962). Roots are used to make love potions and also in preparations to stimulate breast development in girls (BATTEN and BOKELMANN, 1966). ***A. fanninii*** is reportedly used by the Zulu in the same way as ***A. caffra*** (HUTCHINGS et al., 1996). FELIX (1931) reported the use of the roots of an *Anemone* species in South Africa to treat insanity.
Clematis villosa DC. subsp. *villosa* Syn: *Clematopsis scabiosaefolia* DC., *Clematopsis scabiosifolia* (DC.) Hutch. subsp. *stanleyi* Brummitt, *Clematopsis stanleyi* (Hook.) Hutch., *Clematopsis villosa* Hutch. subsp. *stanleyi* (Hook.) Kuntze	**281**	The roots are burned and the smoke inhaled to treat **madness** in Zimbabwe (GELFAND et al., 1985).
Ranunculus species isishoshokazana (Z)	**282**	The Pondo traditionally use root infusions to wash patients suffering from sicknesses thought to be caused by **ancestral spirits** (WATT and BREYER-BRANDWIJK, 1962; HUTCHINGS et al., 1996).
RHAMNACEAE		
Helinus integrifolius (Lam.) Kuntze. Syn: *H. ovatus* E.Mey. ex Sond., *H. scandens* (Eckl. & Zeyh.) A.Rich. soap creeper (E), uphuphuphu (Z) –(ideo) frothing	**283**	Roots used in Zulu traditional medicine to treat **hysteria** (BRYANT, 1966 ; POOLEY, 2005). An emetic for **hysteria** is made from the roots, pounded with the roots of ***Psoralea pinnata* L.** (Fabaceae) and stirred with cold water until it froths (saponins) (BRYANT, 1966).
Rhamnus prinoides L'Herit. Syn: *Celtis rhamnifolia* C.Presl dogwood (Z)	**284**	Unspecified groups use powdered bark that is administered as snuff to treat **mental disorders** in the Transkei (HUTCHINGS et al., 1996). The Chagga are reported to use the roots to inhance the **narcotic** effects of traditional **beer** (WATT and BREYER-BRANDWIJK, 1962).
ROSACEAE		
Rubus ludwigii Eckl. & Zeyh. Syn: *R. rhodacantha* E.Mey. imencemence, itshalo, unomhloshane (Z)	**285**	Roots are used to treat **fits** in the Transkei (HUTCHINGS et al., 1996). European settlers in the Cape of South Africa used roots of ***R. pinnatus* Willd.** to treat **convulsions** (WATT and BREYER-BRANDWIJK, 1962).
RUBIACEAE		
Burchellia bubalina (L.f.) Sims Syn: *B. capensis* R.Br., *Lonicera bubalina* L.f.	**286**	Cold water infusions of pounded roots are taken as emetics against bad **dreams** (HULME, 1954). Roots are sometimes ingredients in Zulu **love charm** emetics (GERSTNER, 1941).
Canthium inerme (L.f.) Kuntze Syn: *C. ventosum* (L.) Kuntze, *Lycium inerme* L.f.	**287**	The roots are applied into incisions on the body to treat **madness** caused by chronic illness by the Shona of Zimbabwe (GELFAND et al., 1985). *Canthium* species are used in west Africa to treat **madness** (AYENSU, 1978).

FAMILY ***Species*** Colloquial name[1] – meaning[2] Annotations and distribution for South Africa[3]		**Traditional use, ethnobotanical information and known active constituents**
Catunaregam species Syn: *C. spinosa* (Thunb.) Tirveng. thorny bone-apple NOTE: *C. spinosa* (Thunb) Tirveng. [*Xeromphis obovata* (Hochst.) Keay] occurs in China and is not found in Africa.	**288**	Unspecified parts used as an **aphrodisiac** in East Africa (VERDCOURT and TRUMP, 1969). Roots are used in drinks taken at riruals to induce emesis, faintness, intoxification and even death in Tete region of Mozambique (WATT and BREYER-BRANDWIJK, 1962). In Zimbabwe the roots are used to treat nausea, **epilepsy** and **dizziness** (GELFAND et al., 1985).
Fadogia species *F. ancylantha* Hiern	**289**	The powdered roots are taken in porridge to treat **madness** in Zimbabwe (GELFAND et al., 1985).
Galium capense Thunb.	**290**	Used to treat people considered to 'with the spirit', a type of **mental disturbance** or spiritual calling (LAYDEVANT, 1932; SOBIECKI, 2002)
Gardenia species	**291**	Roots of ***G. ternifolia*** **Schumach. & Thonn.** are used to treat **madness** in Malawi, while the bark is used as an ointment for treating **convulsions** in Zimbabwe (GELFAND et al., 1985). Unspecified parts of ***G. volkensii*** **K. Schum.** are taken orally in South Africa to treat epilepsy (VENTER, 1996).
Kohautia amatymbica Eckl. & Zeyh. Syn: *Hedyotis amatymbica* (Eckl. & Zeyh.) Steud; *Oldenlandia amatymbica* (Eckl. & Zeyh.) Kuntze umfana-ozacile (Z) - thin boy	**292**	Used by unspecified groups to **improve appetite** of infants (POOLEY, 2005). The Xhosa administer root infusions to babies as protective **charms** against evil (WATT and BREYER-BRANDWIJK, 1962). The Zulu use this plant for **love charm** emetics (GERSTNER, 1941).
Nenax microphylla (Sond.) Salter Syn: *Ambraria microphylla* Sond. daggapit (A)	**293**	Seeds were used by the people of the Karoo as a substitute for *Cannabis* (VAN WYK and GERICKE, 2000); presumably they have a **narcotic** effect.
Pachystigma pygmaeum (Schltr) Robyns Syn: *Vangueria pygmaea* Schltr.; *Vangueria setosa* Conrath	**294**	The roots are burned and the smoke inhaled to treat **convulsions** in Zimbabwe (GELFAND et al., 1985).
Pygmaeothamnus zeyheri (Sond.) Robyns Syn: *Pachystigma zeyheri* Sond.; *P. zeyheri* (Sond.) Robyns var. *oatesii* Robyns; *Vangueria zeyheri* (Sond.) Sond.	**295**	Root infusions are taken orally and used to wash the patients with **delirium** in Zimbabwe (GELFAND et al., 1985).
Vangueriopsis lanciflora (Hiern) Robyns Syn: *Canthium lanciflorum* Hiern; *Vangueria lateritia* Dinter	**296**	Root infusions are dropped into the nose to treat **madness** by unspecified groups in Malawi (GELFAND et al., 1985).
RUTACEAE ***Clausena anisata*** (Willd.) Hook. f. ex Benth.	**297**	Used by Xhosa to treat **mental disease** and **schizophrenia** (PUJOL, 1990). Pounded roots of a plant known as *umnukambhiba* (Z) reported to be *C. anisata* are used by the Zulu in an emetic for illness believed to be inflicted by evil spirits or by the ancestors (HUTCHINGS et al., 1996). Aqueous extracts of the roots have shown behavioural and anticonvulsant activity in animal studies (MAKANJU, 1983).

FAMILY ***Species*** Colloquial name[1] – meaning[2] Annotations and distribution for South Africa[3]		**Traditional use, ethnobotanical information and known active constituents**
Ruta graveolens L.	**298**	Herb and oil of this plant used to treat **hysteria** in South Africa (WATT and BREYER-BRANDWIJK, 1962). The plant is traditionally use in Europe for **hysteria** (VAN WYK et al., 1997)
Zanthoxylum capense (Thunb.) Harv. Syn *Fagara capensis* Thunb.	**299**	Used as an **epilepsy** remedy among Europeans (WATT and BREYER-BRANDWIJK, 1962). The Zulu administer root decoctions of ***Z. davyi* (Verdoorn) Waterm.** as **tonics** to humans and animals (WATT and BREYER-BRANDWIJK, 1962). A species of *Zanthoxylum* is used to treat **mental illness** in Gabon (WALKER, 1953).
SANTALACEAE		
Osyridicarpos schimperianus (Hochst. ex A. Rich.) A. DC. Syn: *O. natalensis* A.DC. umalala (Z) – (n) sleeping Perennial. Shrub. Ht 1–4 m. Alt up to 1980 m. LIM, M, S, KZN, EC	**300**	Leaves and stems are used to make babies **sleep** (MANANA, 1968; HUTCHINGS et al., 1996).
Osyris lanceolata Hochst. & Steud. Syn: *O. abyssinica* Hochst. ex A.Rich. Perennial. Shrub. Ht 1.5–4 m. Alt up to 3000 m. N, B, LIM, NW, G, M, S, FS, KZN, L, NC, EC	**301**	Bark infusions are used in Zimbabwe to **treat madness** (GELFAND et al., 1985).
SAPOTACEAE		
Englerophytum magalismontanum (Sond.) T.D.Penn. Syn: *Bequaertiodendron magalismontanum* (Sond.) Heine & J.H.Hemsl., *Chrysophyllum magalismontanum* Sond., *Pouteria magalismontana* (Sond.) A.Meeuse umnugumabele (Z) stamvrug (A)	**302**	Fruit and roots used are used to treat **epilepsy** by the Kgatla (WATT and BREYER-BRANDWIJK, 1962). A strong alcoholic drink known in Afrikaans as *mampoer* is made from the fruit (WATT and BREYER-BRANDWIJK, 1962). Unspecified groups use an infusion from roots and fruit to treat **epilepsy** in South Africa (COATES PELGRAVE, 2002). Perennial. Tree or shrub. Ht 1–17 m. Alt 550–1830 m. B, LIM, NW, G, M, S, KZN
Sideroxylon inerme L. amaSethole (-amhlophe) (Z) Perennial. Tree or shrub. Ht 1–15 m. Alt 2–1092 m. LIM, M, S, KZN, WC, EC	**303**	Emetics made from unspecified parts are taken to dispel bad **dreams** (WATT and BREYER-BRANDWIJK, 1962).
Vitellariopsis marginata (N.E. Br.) Aubrév. Syn: *Austromimusops marginata* (N.E.Br.) A.Meeuse amaSethole (Z) Perennial. Tree or shrub. Ht 4–10 m. Alt 25–700 m. M, S, KZN, EC	**304**	The Zulus use the root of this tree which is dark red in making of 'psychoactive medicine that **cure moody people** rendered neurotic by way of witchcraft' (PUJOL, 1990). Root and leaf decoctions are also taken orally or as enemas, by Zulus as blood purifiers, strengtheners and **sexual stimulants** (HUTCHINGS et al., 1996). Root infusions are taken twice daily for *idliso*, poisoning in Zulu culture thought to be caused by sorcery (HUTCHINGS et al., 1996).
SCROPHULARIACEAE		
Aptosimum decumbens Schinz Perennial. Dwarf shrub. Ht 0.02–0.1 m. Length 0.12−0.5 m. Alt 915−1555 m. N, B	**305**	An infusion of the plant or chewing leaves is reported to improve **memory** by the Kwanyama of Namibia (RODIN, 1985).

FAMILY ***Species*** Colloquial name[1] – meaning[2] Annotations and distribution for South Africa[3]		**Traditional use, ethnobotanical information and known active constituents**
Harveya speciosa Bernh. ex Krauss (now in Orobanchaceae) Syn: *Cycnium tubatum* Benth., *H. tubata* (E.Mey. ex Drège) Hook. ex Steud., *H. tubata* (E.Mey. ex Drège) Reuter, *Orobanche tubata* E.Mey. ex Drège, Perennial. Herb, parasite. Ht 0.15−± 1 m. Alt 45−± 2600 m. LIM, G?, S, FS, KZN, L, EC	**306**	Un specified parts used by Sotho for **mental illness** (WATT and BREYER-BRANDWIJK, 1962).
Harveya huttonii Hiern. (now in Orobanchaceae) Annual? Herb, parasite. Ht 0.15−0.4 m. Alt 915−1415 m. EC	**307**	Roots are chewed by unspecified groups as a **sedative** for nervous tension in the eastern Cape region of South Africa (BATTEN and BOKELMANN, 1966).
Phygelius capensis E. Mey. ex Benth. mafifi matso (S) Only two species in South Africa: ***P. aequalis*** Harv. ex Hiern Perennial. Shrub, dwarf shrub, herb. Ht 0.45–2 m. Alt 915–2680 m. LIM, M, S, FS, KZN, L, EC ***P. capensis*** E.Mey. ex Benth. Perennial. Shrub, dwarf shrub, herb. Ht 0.3–1.5 m. Alt 1065–2895 m. M, FS, KZN, L, WC, EC	**308**	Used with the bulbs of *Ledebouria cooperi* (Hyacinthaceae) to **inebriate** Sotho boys during circumcision rituals (WATT and BREYER-BRANDWIJK, 1962). This medicine reportedly causes the boys to appear stunned, stupefied and to fall asleep (HUTCHINGS et al., 1996).
Sutera atropurpurea (Benth.) Hiern Now ***Jamesbrittenia atropurpurea*** (Benth.) Hilliard subsp. ***atropurpurea*** Syn: *Chaenostoma croceum* (Eckl. ex Benth.) Wettst. ex Diels, *Lyperia atropurpurea* Benth. in part, *Lyperia crocea* Eckl. ex Benth., *Manulea atropurpurea* (Benth.) Kuntze, *Sutera atropurpurea* (Benth.) Hiern	**309**	The whole plant is burned and the smoke inhaled to treat **madness** in Zimbabwe (GELFAND et al., 1985). Perennial. Dwarf shrub or shrub. Ht ± 1 m. Alt 300− 2000 m. NW, G, FS, L, NC, WC, EC
SIMAROUBACEAE ****Ailanthus altissima*** (Mill.) Swingle Tree of Heaven (E)	**310**	Root bark of this Chinese exotic reportedly used to treat **epilepsy** in South Africa (POTTER, 1932).
SOLANACEAE ****Datura ferox*** L. iloyi (Z) Annual. Shrub or herb. Ht 0.2–1.5 m. Alt 5–1713 m. N, B, LIM, NW, G, M, FS, KZN, L, NC, WC, EC	**311**	Leaves used to **sedate hysterical** and **psychotic** patients, also to treat **insomnia** (VAN WYK and GERICKE, 2000).

FAMILY ***Species*** Colloquial name[1] – meaning[2] Annotations and distribution for South Africa[3]		**Traditional use, ethnobotanical information and known active constituents**
****Datura stramonium*** L. Syn: *D. tatula* L. iloyi (Z) *-loya* (v) bewitch, hypnotise Annual. Shrub or herb. Ht 0.35–2 m. Alt 45–2380 m. N, B, LIM, NW, G, M, S, FS, KZN, L, NC, WC, EC	**312**	Leaves used to **sedate hysterical** and **psychotic** patients, also to treat **insomnia** (VAN WYK and GERICKE, 2000). Used by Zulu as a hypnotic drug against **hysteria** and as a 'diviner's aid' (POOLEY, 2005).
****Nicotiana tabacum*** L. Annual, occ. perennial. Shrub or herb. Ht up to 1.2 m. Alt 50–1465 m. LIM, KZN, WC, EC	**313**	Often taken as a snuff by southern African diviners at the start of **divination**, and is also made as an offering to ancestors (VAN WYK and GERICKE, 2000).
Withania somnifera (L.) Dun Syn: *Physalis somnifera* L., *W. microphysalis* Suess ubuvimbha (Z) *–vimba* (v) prevent, close up, stop Perennial. Shrub or dwarf shrub, herb. Ht 0.2–2 m. Alt 15–2300 m. N, B, LIM, NW, G, M, S, FS, KZN, L, NC, WC, EC	**314**	In southern Africa infusions, decoctions and tinctures of *Withania* root are taken as an **adaptogenic tonic**, as well as a **sedative** and hypnotic (VAN WYK and GERICKE, 2000). In east Africa the roots are also reported to have **narcotic** and **anti-epileptic** effects (OLIVER-BEVER, 1986). An unidentified plant named *ubuvimba* by the Zulu, reputed to be *W. somnifera*, is taken to induce clear **dreams** (MANANA, 1968). In Ayurvedic medicine (*ashwaghanda*) is used for many conditions, notabily as a brain tonic to help the elderly with learning and **memory** retention (VAN WYK and GERICKE, 2000).
STERCULIACEAE ***Hermannia hyssopifolia*** L. Perennial. Shrub, dwarf shrub. Ht 0.3–3 m. Alt 5–700 m. WC, EC	**315**	Root decoctions are used among Europeans as an old Cape remedy for **fits** (WATT and BREYER-BRANDWIJK, 1962). The Plant refered to as *umakotegoyile* by the Zulu (***Hermannia sandersonii* Harv.**) means 'hypnotised bride'. *-makoti* bride *-egoyile* (v) being hypnotised
THYMELAEACEAE ***Gnidia kraussiana*** Meisn. Syn: *G. hoepfneriana* Gilg, *Lasiosiphon hoepfnerianus* Vatke ex Gilg, *Lasiosiphon kraussii* Meisn. imfuzane (Z), umsilawengwe Perennial. Shrub, dwarf shrub. Ht 0.1–3 m. Alt 30–2200 m. N, B, LIM, NW, G, M, S, FS, KZN, L, NC, EC	**316**	The powdered tuber is taken in porridge to treat **madness** and **poor appetite** by the Shona of Zimbabwe (GELFAND et al., 1985). The Zulu use *imfuzane*, reported to be *G. kraussiana* to treat **mental disorders** (MANANA, 1968). The Sotho inhale smoke from burning ***G. anthylloides* (L.f) Gilg** to stop bad **dreams** (WATT and BREYER-BRANDWIJK, 1962).
Synaptolepis kirkii Oliv. uvuma-omhlophe (Z) *-vuma* (v) clap hands during the process of divining *-mhlophe* (adv) white or clarity Perennial. Shrub. Ht 1.21–3.04 m. Alt 6–155 m. LIM, S, KZN	**317**	Root infusions have been used as purifying ritual emetics and face and body washes to assist South African diviners with **visions** (VAN WYK and GERICKE, 2000). Root infusions also used to treat **epilepsy** by the Karanga (WATT and BREYER-BRANDWIJK, 1962). In eastern Tanzania, dried powdered inner roots are taken in tea as an **aphrodisiac** (HUTCHINGS et al., 1996).

FAMILY ***Species*** Colloquial name[1] – meaning[2] Annotations and distribution for South Africa[3]		**Traditional use, ethnobotanical information and known active constituents**
TILIACEAE ***Corchorus asplenifolius*** Burch. Syn: *C. serrifolius* Burch. ubangalala (Z) Perennial. Herb. Ht 0.05–0.91 m. Alt 303–1675 m. N, B, LIM, NW, G, M, S, FS, KZN, NC	**318**	The roots are taken together with *Eriosema* species (Fabaceae) as **aphrodisiacs** (GERSTNER, 1939).
VALERIANACEAE ***Valeriana capensis*** Thunb.	**319**	Unspecified groups use the plant in combination with ***Stachys thunbergii*** (Lamiaceae) to treat **hysteria** and **insomnia** in South Africa (VAN WYK and GERICKE, 2000). Varieties: ***V. capensis*** Thunb. var. ***capensis*** Perennial. Herb. Ht 0.15–1.5 m. Alt 305–3333 m. LIM, NW, G, M, S, FS, KZN, L, WC, EC ***V. capensis*** Thunb. var. ***lanceolata*** N.E.Br. Perennial. Herb. Ht 0.2–0.6 m. Alt 1200–3000 m. KZN, L, EC ***V. capensis*** Thunb. var. ***nana*** B.L.Burtt Perennial. Herb. Ht 0.05–0.75 m. Alt 2200–3333 m. KZN, L, EC
VERBENACEAE ***Clerodendrum*** species	**320**	Weak teas of ***C. glabrum* E. Mey.** are taken at night by the Sotho (Tswana) to **aid sleep** (ROBERTS, 1990). Pounded leaves are placed in armpits and under neck to **induce sleep** and as a treatment for **convulsion** in children by the Lobedu, a Northern Sotho group (WATT and BREYER-BRANDWIJK, 1962). Leaf decoctions of ***C. myricoides* (Hochst.) Vatke** are used for bathing patients who suffer from **convulsions** in Zimbabwe (GELFAND et al., 1985). *C. ternatum* Schinz roots are taken orally in sweet beer to treat **epilepsy** in Zimbabwe (GELFAND et al., 1985).
Lantana rugosa Thunb. Syn: *L. salviifolia* Jacq. Perennial. Shrub. Ht up to 1 m. Alt 30–2160 m. N, B, LIM, NW, G, M, S, FS, KZN, L, NC, WC, EC	**321**	Fruit is report to be edible but is reputed to have **narcotic** effects in birds (WATT and BREYER-BRANDWIJK, 1962).
Lippia javanica (Burm. f.) Speng. Syn: *Lantana galpiniana* H.Pearson, *L. asperifolia* Rich., *Verbena javanica* Burm.f. Perennial. Shrub. Ht 0.7–5 m. Alt 5–1920 m. B, LIM, NW, G, M, S, FS, KZN, EC	**322**	Unspecified groups in Zimbabwe use the leaves to treat **convulsions** (GELFAND et al., 1985). The plant is used as a treatment for **madness** in Malawi (WILLIAMSON, 1974).
Vitex rehmannii Gürke (now in Lamiaceae) umluthu (Z), unduly (Z) *-luthu* (ideo) of aiming true; *-lutha* (v) mislead, make a fool of.	**323**	Unspecified parts used to treat **hysteric fits** by the Zulu (GERSTNER, 1941). Perennial. Tree. Ht 2–3 m. Alt 60–1646 m. LIM, NW, G, M, S, KZN

FAMILY ***Species*** Colloquial name[1] – meaning[2] Annotations and distribution for South Africa[3]		**Traditional use, ethnobotanical information and known active constituents**
Vitex wilmsii Gürke Now ***V. obovata*** subsp. ***obovata*** (now in Lamiaceae) Syn: *V. reflexa* H.Pearson, *V. wilmsii* Gürke var. *reflexa* (H.Pearson) W.Piep. Perennial. Tree. Ht 3–4 m. Alt 550–1370 m. LIM, M, S, KZN, EC umluthu (Z)	**324**	Bark infusions are taken as purifying emetics by adults when a kraal member is dying. Unspecified parts used to treat **hysteric fits** by the Zulu (GERSTNER, 1941). Subspecies: ***V. obovata*** E.Mey. subsp. ***wilmsii*** (Gürke) C.L.Bredenkamp & D.J.Botha Syn: *V. wilmsii* Gürke var. *wilmsii* Perennial. Tree. Ht 3–4 m. Alt? LIM, G, M, S
VISCACEAE ***Viscum*** species indembu, iphakama (Z) ***V. anceps*** E.Mey. ex Sprague Syn: *Aspidixia anceps* E.Mey. ex Tiegh. Perennial. Shrub, parasite. Ht 0.5–1 m. Alt 5–915 m. KZN, EC	**325**	Unspecified parts, most likely the whole plant, of ***V. capense*** **L. f.** reported to be used by the Xhosa in the Cape to treat **epilepsy** (WATT and BREYER-BRANDWIJK, 1962). Unspecified parts of ***V. anceps*** **E. Mey. ex Sprague** is administered internally to treat **hysteria** in the Transkei. The plants are reportedly not used when in flower and overdosing causes drowsiness (HUTCHINGS et al., 1996).
VITACEAE ***Rhoicissus tridentata*** (L.f.) Wild. Et R.B.Drumm Syn: *Cissus cuneifolia* Eckl. & Zeyh., *R. cuneifolia* (Eckl. & Zeyh.) Planch., *R. erythrodes* (Fresen.) Planch.	**326**	Shona take a root infusion orally for the treatment of **madness** (GELFAND ET AL., 1985). Perennial. Climber. Ht indefinite. Alt 60–1800 m. B, LIM, NW, G, M, S, FS, KZN, L, EC
ZINGIBERACEAE ***Siphonochilus aethiopicus*** (Schweinf.) B.L. Burtt Syn: *Kaempferia aethiopica* (Schweinf.) Benth., *Kaempferia ethelae* J.M.Wood, *Kaempferia natalensis* Schltr. & K.Schum., *S. natalensis* (Schltr. & K.Schum.) J.M.Wood & Franks indungulo (Z) –*dungula* (v) put enemy to flight, isiphephetho (Z) –*phephetha* (v) be blown away; soothe by blowing air; destroy.	**327**	Roots are used by Zulu to treat **hysteria** (GERSTNER, 1938). Over-dosage of medicine made from *S. aethiopicus* reported to **stupefy** horses (WATT AND BREYER-BRANDWIJK, 1962). Unspecified groups in South Africa use rhizome infusions to treat **epilepsy** and **hysteria** (VAN WYK AND GERICKE, 2000). Perennial. Herb, geophyte. Ht 0.15–0.45 m. Alt 610–915 m. LIM, M, S, KZN, EC

[1] **Ethnic groups: (A)** = Arabic, **(Af)** = Afrikaans: SA, **(E)** = Europeans: SA, **((Nd)** = Ndebele: SA, **(Sth)** = Sotho (this language group includes Northern Sotho, South Sotho and Tswana), **(San)** = San: SA/Namibia/Angola, **(Sh)** = Shona: Zimbabwe, **(Sw)** = Swazi: SA, **(Ts)** = Tsonga: SA, **(V)** = Venda: SA, **(X)** = Xhosa: SA, (**Z)** = Zulu: SA.
[2] Translation of isiZulu names was achieved using NGWENYA, KOOPMAN and WILLIAMS (2003) and DENT and NYEMBEZI (1999).
[3] Annotations are taken from GERMISHUIZEN and MEYER (2003).

Table 2.2b. Alphabetical list of Southern African plants traditionally used for psychoactive purposes

Species	Code
Abrus precatorius L.	**161**
Syn: *A. squamulosus* E.Mey.	
Acacia amythethophylla Steud. ex A. Rich.	**162**
Acacia karoo Hayne	**163**
Acacia nilotica (L.) Willd. ex Del.	**164**
Achyrocline stenoptera (DC.) Hilliard & Burtt (Syn: *Helichysum stenopterum* DC)	**065**
Acokanthera oppositifolia (Lam.) Codd	**034**
Syn: *A. venenata* in sense of Stapf, not of G.Don.	
Acorus calamus L.	**044**
Adansonia digitata L.	**103**
Adenia gummifera (Harv.) Harms	**263**
Adenopodia spicata (E. Mey.) Presl	**165**
Agapanthus africanus (L.) Hoffmanns.	**005**
Syn: *A. minor* Lodd.,*A. umbellatus* L'Hér., *Crinum africanum* L., *Mauhlia africana* (L.) Dahl, *Mauhlia linearis* Thunb., *Tulbaghia heisteri* Fabric	
Agapanthus campanulatus F.M.Leight. (Syn: *A. patens* F.M.Leight.	**006**
Agapanthus praecox Willd.	**007**
Agave species	**004**
Ailanthus altissima (Mill.) Swingle	**310**
Albizia adianthifolia (Schumach.) W.F. Wight	**166**
Albuca fastigiata Dryand.	**195**
Albuca nelsonii N.E. Br.	
Alepidea amatymbica Eckl. & Zeyh.	**028**
Aloe ferox Mill.	**059**
Ammocharis coranica (Ker-Gawl.) Herb.	**012**
Anemone species	**280**
Annona senegalensis Pers.	**024**
Syn: *A. arenaria* Thonn. ex Schumach., *A. chrysophylla* Bojer	
Ansellia africana Lindl.	**257**
Anthericum species	**060**
Antidesma venosum E. Mey. ex Tul.	**150**
Aptenia cordifolia (L. f.) Schwant.	**243**
Aptosimum decumbens Schinz	**305**
Arctopus echinatus L.	**029**
Arctotheca calendula (L.) Levyns	**066**
Arctotis arctotoides (L. f.) O. Hoffm.	**067**
Argyrolobium tomentosum (Andr.) Druce	**167**
Artabotrys brachypetalus Benth.	**025**
Asparagus species	**058**
Aspilia pluriseta Schweinf.	**068**
Aster bakeranus Burtt Davy ex C.A. Sm.	**069**
Astripomoea malvacea (Klotzsch) A. Meeuse	**136**
Athrixia heterophylla (Thunb.) Less.	**070**
Avonia rhodesica (N.E.Br.) G.D.Rowley	**276**
Syn: *Anacampseros rhodesica* N.E. Br.	
Azanza garckeana (F. Hoffm.) Exell & Hillc.	**228**
Balanites maughamii Sprague	**099**
Ballota africana (L.) Benth.	**206**
Bauhinia thonningii Schumach	**168**
Becium grandiflorum (Lam.) Pichi-Serm.	**207**
Begonia species	**100**
Belamcanda chinensis (L.) DC.	**200**
Berkheya discolor (DC.) O. Hoffm. & Muschl.	**071**
Bersama lucens (Hochst.) Szyszyl.	**238**

Species	Code
Bersama tysoniana Oliv.	**239**
Blumea alata (D. Don) DC.	**072**
Bolusanthus speciosus (H. Bol.) Harms	**169**
Boophone disticha (L.f.) Herb. Syn: *B. longepedicellata* Pax	**013**
Bosica albitrunca (Burch.) Gilg & Ben.	**111**
Brachycorythis ovata Lindl.	**258**
Ceratandra grandiflora Eckl. ex Bauer	**259**
Brachylaena elliptica (Thunb.) DC.	**073**
Brackenridgea zanguebarica Oliver.	**255**
Bridelia cathartica Bertol. f.	**151**
Buddleja (L.) species	**222**
Bulbine frutescens (L.) Willd.	**061**
Bulbine latifolia (L. f.) Roem. & Schult.	**062**
Burchellia bubalina (L.f.) Sims Syn: *B. capensis* R.Br., *Lonicera bubalina* L.f.	**286**
Buxus macowanii Oliv.	**107**
Cadaba natalensis Sond.	**112**
Caesalpinia bondac (L.) Roxb.	**170**
Callilepis laureola DC.	**074**
Cannabis sativa L.	**109**
Canthium inerme (L.f.) Kuntze Syn: *C. ventosum* (L.) Kuntze, *Lycium inerme* L.f.	**287**
Capparis sepiaria L.	**113**
Capparis tomentosa Lam.	**114**
Carissa edulis Vahl Syn: *Azima pubescens* Suess.	**035**
Casearia gladiiformis Mast.	**191**
Cassine papillosa (Hochst.) Kuntze	**119**
Catha edulis (Vahl) Forssk. ex Endl.	**120**
Catunaregam species Syn: *C. spinosa* (Thunb.) Tirveng.	**288**
Cenia sericea DC.	**075**
Centella asiatica (L.) Urb.	**030**

Species	Code
Chamaecrista mimosoides (L.) Greene	**171**
Chenopodium ambrosiodes L.	**126**
Chironia krebsii Griseb.	**194**
Chlorophytum blepharophyllum Bak.	**063**
Chrysanthemoides monilifera (L.) T. Norl.	**076**
Cineraria aspera Thunb.	**077**
Cinnamomum camphora (L.) T. Ness & C.H. Eberm.	**219**
Cissampelos species	**241**
Clausena anis*ata* (Willd.) Hook. f. ex Benth.	**297**
Clematis villosa DC. subsp. *villosa* Syn: *Clematopsis scabiosaefolia* DC., *Clematopsis scabiosifolia* (DC.) Hutch. subsp. *stanleyi* Brummitt, *Clematopsis stanleyi* (Hook.) Hutch., *Clematopsis villosa* Hutch. subsp. *stanleyi* (Hook.) Kuntze	**281**
Clerodendrum species	**320**
Combretum adenogonium Steud. ex A. Rich. Syn *Combretum ternifolium* Engl. & Diels	**131**
Combretum microphyllum Klotzsch	**132**
Combretum molle R. Br. ex G. Don	**133**
Commelina africana L.	**135**
Conophytum species	**244**
Conyza scabrida DC.	**078**
Corchorus asplenifolius Burch. Syn: *C. serrifolius* Burch.	**318**
Corycium nigrescens Sond.	**260**
Cotyledon orbiculata L.	**138**
Crabbea hiruta Harv. Syn: *C. cirsioides* (Nees) Nees; *C. robusta* N.E.Br.	**001**
Crassula alba Forssk.	**139**
Crassula arborescens (Mill.) Willd.	**140**
Crinum species	**014**
Croton gratissimus Burch.	**152**
Croton sylva*ticus* Hoscht.	**153**

Species	Code
***Cucumis hir**sutus* Sond.	**142**
Cunonia capensis L.	**144**
Cussonia arborea A. Rich.	**045**
Cussonia longissima Hutch. & Dalz..	**046**
Cussonia paniculata Eckl & Zeyh..	**047**
Cussonia spicata Thunb..	**048**
Cyathea dregei Kunze	**145**
Cymbopogon nardus (L.) Rendle Syn: *C. afronardus* Stapf, *C. validus* (Stapf) Stapf ex Burtt Davy *validus* (Stapf.) Stapf. ex Burtt. Davy	**271**
Cynanchum obtusifolium L. f.	**050**
Cysticapnos pruinosa (Bernh.) Liden	**193**
Dalbergia obovata E. May.	**172**
Datura ferox L.	**311**
Datura stramonium L.	**312**
Desmodium barbatum (L.) Benth. Syn: *D. dimorphum* Welw. ex Baker var. *argyreum* Welw. ex Baker, *Nicolsonia barbata* (L.) DC. var. *argyrae* (Welw. ex Baker) Schindl.	**173**
Dianthus crenatus Thunb.	**116**
Dicoma anomala Sond.	**079**
Dicoma shinzii O. Hoffm.	**080**
Dioscorea diversiflora Griseb.	**146**
Dioscorea dregeana Baker	**147**
Diospyros lyciodes Desf.	**149**
Disa polygonoides Lindl.	**261**
Ekebergia capensis Sparrm.	**232**
Elephantorrhiza elephantina (Burch.) Skeels.	**174**
Eleutherine bulbosa (Miller.) Urban	**201**
Emex australis Steinh	**274**
Englerophytum magalismontanum Krause	**302**
Entada rheedii Spreng.	**175**
Entandrophragma spicatum (C. DC.) Sprague	**233**
Eriosema cordatum E. Mey.	**176**
Eriosema distinctum N.E. Br.	**177**
Eriosema salignum E. Mey.	**178**
Erythrophleum lasianthum Corbishley	**179**
Ethulia conyzoides L. f.	**081**
Euclea crispa (Thunb.) Guerke	**148**
***Eulophi**a* species	**262**
Euphorbia species	**154**
Exomis microphylla (Thunb.)	**127**
Fadogia species ***F. ancylantha*** Hiern	**289**
Faurea saligna Harv.	**278**
Ferraria glutinosa (Bak.) Rendle	**202**
Flueggea virosa (Roxb. ex Willd.) Voigt.	**155**
Galium capense Thunb.	**290**
Gardenia species	**291**
Gasteria croucheri (Hook.f.)	**064**
Gerrardina foliosa Oliv.	**192**
Gladiolus papilio Hook. f.	**203**
Gnidia kraussiana Meisn. Syn: *G. hoepfneriana* Gilg, *Lasiosiphon hoepfnerianus* Vatke ex Gilg, *Lasiosiphon kraussii* Meisn.	**316**
Gomphocarpus physocarpus E. Mey	**051**
Harpephyllum caffrum Bernh. Ex Krauss	**018**
Hartogiella schinoides	**121**
Harungana madagascariensis Lam. ex Poir.	**130**
Harveya huttonii Hiern.	**307**
Harveya speciosa Bernh. ex Krauss	**306**
Helichrysum aureonitens Sch. Bip.	**082**
Helichrysum odoratissimum (L.) Sweet	**084**
Helichrysum species	**083**
Helinus integrifolius (Lam.) Kuntze. Syn: *H. ovatus* E.Mey. ex Sond., *H. scandens* (Eckl. & Zeyh.) A.Rich.	**283**

Species	Code
Hemizygia bracteosa (Benth.) Briq.	**208**
Hermannia hyssopifolia L.	**315**
Heteromorpha trifoliata (Wendl.) Eckl. & Zeyh.	**031**
Hibiscus pusillus Thunb.	**229**
Hoslundia opposite Vahl	**209**
Hypericum perforatum L.	**129**
Hypoxis colchicifolia Bak.	**198**
Hypoxis hemerocallidea Fisch. & C.A. Mey	**199**
Ilex mitis (L.) Radlk. Syn: *I. capensis* Sond.	**0430**
Indigofera hilaris Eckl. & Zeyh.	**180**
Ipomoea species	**137**
Jatropha curcas L.	**156**
Kalanchoe brachyloba Welw. ex Britten	**141**
Khadi acutipetala (N.E. Br.) N.E. Br.	**245**
Kohautia amatymbica Eckl. & Zeyh. Syn: *Hedyotis amatymbica* (Eckl. & Zeyh.) Steud; *Oldenlandia amatymbica* (Eckl. & Zeyh.) Kuntze	**292**
Lannea discolor (Sond.) Engl.	**019**
Lannea schweinfurthii (Engl.) Engl.	**020**
Lantana rugosa Thunb.	**321**
Launaea nana (Bak.) Chiov.	**085**
Ledebouria cooperi (Hook. f.) Jessop Syn: *Scilla adlamii* Baker, *Scilla cinerascens* Van der Merwe, *Scilla cooperi* Hook.f., *Scilla galpinii* Baker, *Scilla glaucescens* Van der Merwe, *Scilla inandensis* Baker, *Scilla petiolata* Van der Merwe, *Scilla pusilla* Baker, *Scilla rogersii* Baker, *Scilla rupestris* Van der Merwe, *Scilla saturata* Baker	**196**
Leonotis leonurus (L.) R.Br.	**210**
Leucas martinicensis (Jacq.) R. Br.	**211**
Lichtensteinia interrupta (Thunb.) Sond. Syn: *L. kolbeana* Bolus, *L. pyrethrifolia* Cham. & Schltdl.	**032**
Lippia javanica (Burm. f.) Speng.	**322**
Lithospermum cinereum DC.	**104**
Lobelia decurrentifolia (Kuntze) K. Schum.	**221**
Lopholaena coriifolia (Sond.) Phill. & C.A. Sm.	**086**
Loranthus oleifolius (Wendl.) Cham. & Schlechtd.	**225**
Maerua angolensis DC.	**115**
Maesa lanceolata Forssk.	**252**
Malva parviflora L.	**230**
Margaritaria discoidea (Baill.) Webster	**157**
Markhamia obtusifolia (Bak.) Sprague	**101**
Maytenus heterphylla (Eckl. & Zeyh.) N.K.B. Robson	**123**
Maytenus senegalensis (Lam.) Excell	**122**
Melia azedarach L.	**234**
Melianthus comosus Vahl	**240**
Mentha aquatica L.	**212**
Mesembryanthemum species	**246**
Millettia grandis (E.Mey.) Skeels	**181**
Mimosa pudica L.	**182**
Mimosa pigra L.	
Momordica balsamina L	**143**
Monadenium lugardiae N.E. Br.	**158**
Monanthotaxis caffra (Sond.) Verdc. Syn: *Guatteria caffra* Sond., *Popowia caffra* (Sond.) Benth.	**026**
Mondia whitei (Hook. f.) Skeels Syn: *Chlorocodon whitei* Hook.f.	**265**
Moraea spathulata (L. f) Klatt	**204**
Myosotis afropalustris C.H. Wr.	**105**
Myrothamnus flabellifolius Welw.	**251**
Nenax microphylla (Sond.) Salter Syn: *Ambraria microphylla* Sond.	**293**
Newtonia hildebrandtii (Vatke) Torre	**183**

Species	Code
Nicotiana tabacum L.	**313**
Nuxia floribunda Benth.	**223**
Nylandtia spinosa (L.) Dumort. Syn: *Mundia spinosa* (L.) DC.	**272**
Nymania capensis (Thunb.) Lindb.	**237**
Nymphaea nouchali Burm. f.	**254**
Ocimim canum Sims	**213**
Ocotea bullata (Burch.) Baill.	**220**
Olea woodiana Knobl.	**256**
Oncinotis tenuiloba Stapf Syn: *O. inandensis* J.M.Wood & M.S.Evans	**036**
Oncosiphon piluliferum (L. f.) Källersjö	**087**
Oncosiphon suffruticosum (L.) Källersjö	**088**
Osyridicarpos schimperianus (Hochst. ex A. Rich.) A. DC.	**300**
Osyris lanceolata Hochst. & Steud.	**301**
Oxygonum species	**275**
Pachycarpus asperifolius Meisn.	**052**
Pachystigma pygmaeum (Schltr) Robyns Syn: *Vangueria pygmaea* Schltr.; *Vangueria setosa* Conrath	**294**
Pancratium tenuifolium Hochst. ex A.Rich Syn: *Chapmanolirion juttae* Dinter, *P. chapmannii* Harv.	**015**
Parinari capensis Harv	**128**
Pellaea calomelanos (Swartz) Link	**003**
Phygelius capensis E. Mey. ex Benth.	**308**
Phyllanthus reticulates Poir	**159**
Phytolacca species	**267**
Piper capense L. f.	**268**
Pittosporum viridiflorum Sims	**269**
Pleiocarpa pycnantha (K. Schum.) Stapf. Syn: *P. swynnertonii* S.Moore	**037**

Species	Code
Pleiospilos bolusii (Hook. f.) N.E. Br. Syn: *Mesembryanthemum bolusii* Hook.f., *P. barbarae* Karrer, *P. beaufortensis* L.Bolus	**247**
Pleurostylia capensis (Turcz.) Loes	**124**
Plumbago auriculata Lam.	**270**
Printzia pyrifolia Less.	**090**
Psoralea pinnata L.	**184**
Ptaeroxylon obliquum (Thunb.) Radlk. Syn: *P. utile* Eckl. & Zeyh.	**279**
Pterocelastrus rostratus Walp.	**125**
Pycnostachys urticifolia Hook	**214**
Pygmaeothamnus zeyheri (Sond.) Robyns Syn: *Pachystigma zeyheri* Sond.; *P. zeyheri* (Sond.) Robyns var. *oatesii* Robyns; *Vangueria zeyheri* (Sond.) Sond.	**295**
Rabiea albinota (Haw) N.E. Br.	**248**
Ranunculus species	**282**
Rapanea melanophloeos (L.) Mez	**253**
Raphionacme species	**266**
Rauvolfia caffra Sond. Syn: *R. natalensis* Sond.	**038**
Rhamnus prinoides L'Herit. Syn: *Celtis rhamnifolia* C.Presl	**284**
Rhoicissus tridentata (L.f.) Wild. Et R.B.Drumm	**326**
Rhus chirindensis Bak. f. (= *Searsia chirindensis* (Baker f.) Moffett)	**021**
Rhus natalensis Bernh. Ex krauss (= *Searsia natalensis* (Bernh. ex Krauss) F.A.Barkley)	**022**
Rhus pyroides Burch. (= *Searsia pyroides* (Burch.) Moffett)	**023**
Rhynchosia nervosa Benth. & Harv.	**185**
Rubus ludwigii Eckl. & Zeyh. Syn: *R. rhodacantha* E.Mey.	**285**
Ruta graveolens L.	**298**
Salvia chamelaeagnea Berg.	**215**

Species	Code
Scadoxus multiflorus (Martyn) Raf. Syn: *Haemanthus katharinae* Baker, *Haemanthus multiflorus* Martyn, *Haemanthus otaviensis* Dinter, *Haemanthus sacculus* E.Phillips	**016**
Scadoxus puniceus (L.) Friis & Nordal Syn: *Haemanthus magnificus* Herb., *Haemanthus natalensis* Pappe ex Hook., *Haemanthus puniceus* L. var. *puniceus*	**017**
Sceletium species	**249**
Schefflera umbillifera (Sond.) Baill.	**049**
Schizocarphus nervosus (Burch.) Van der Merwe Syn. *Ornithogalum nervosum* Burch., *S. acerosus* Van der Merwe, *S. gerrardii* (Baker) Van der Merwe, *S. rigidifolius* (Kunth) Van der Merwe, *Scilla gerrardii* Baker, ***Scilla nervosa*** (Burch.) Jessop***, Scilla*** *rigidifolia* Baker, *Scilla rigidifolia* Baker var. *acerosa* Van der Merwe, *Scilla rigidifolia* Baker var. *nervosa* Baker	**197**
Schotia brachypetala Sond.	**186**
Secamone gerrardii Harv. ex Benth.	**053**
Securidaca longipedunculata Fresen.	**273**
Senecio discodregeanus Hilliard & Burtt.	**091**
Senna didymobotrya (Fresenius) N.W. Irwin & R.C. Barneby Syn. *Cassia didymobotrya* Fresen.	**187**
Senna petersiana (Bolle) J.M. Lock Syn. *Cassia petersiana* Bolle	**188**
Sesamothamnus lugardii N.E. Br. ex Stapf	**264**
Sideroxylon inerme L.	**303**
Silene burchellii Otth	**117**
Silene capensis Ott. ex DC.	**118**
Siphonochilus aethiopicus (Schweinf.) B.L. Burtt	**327**
Sisyranthus huttoniae (S. Moore) S. Moore	**054**
Sisyranthus trichostomus K. Schum.	**055**
Solanecio angulatus (Vahl) C. Jeffrey	**092**
Sparaxis grandiflora (Delaroche) Ker-Gawl.	**205**
Sphedamnocarpus species	**227**
Stachys thunbergii Benth.	**216**
Stapelia gigantea N.E. Br. Syn: *S. cyclista* C.A.Lückh., *S. gigantea* N.E.Br. var. *pallida* E.Phillips, S. marlothii N.E.Br., *S. nobilis* N.E.Br., *S. youngii* N.E.Br.	**056**
Steganotaenia araliacea Hochst.	**033**
Stephania species	**242**
Strophanthus gerrardii Stapf	**039**
Strophanthus petersianus Klotzch Syn: *S. grandiflorus* (N.E.Br.) Gilg	**040**
Strophanthus speciosus (Ward & Harv.) Reber	**041**
Strychnos species	**224**
Sutera atropurpurea (Benth.) Hiern Syn *Lyperia atropurpureae* (Benth.)	**309**
Synaptolepis kirkii Oliv.	**317**
Syncolostemon parviflorus E. Mey. ex Benth	**217**
Tagetes minuta L.	**094**
Talinum species	**277**
Tarchonanthus camphorates L.	**095**
Tecomaria capensis	**102**
Tephrosia capensis (Jacq.) Pers.	**189**
Terminalia species	**134**
Thespesia acutiloba (Bak. f.) Exell & Mendonca	**231**
Thunbergia dregeana Ness	**002**
Tinnea zambesiaca Bak.	**218**
Tragia meyeriana Müll. Arg.	**160**
Trichodesma physaloides (Fenzl) A. DC.	**106**

Species	Code
Trichodiadema species	**250**
Tulbaghia alliaceae L. f.	**008**
Tulbaghia leucantha Bak Syn: *T. dieterlenii* E.Phillips	**009**
Tulbaghia violaceae Harv. Syn: *Omentaria cepacea* Salisb., *T. cepacea* L.f.	**010**
Turraea floribunda Hochst.	**235**
Turraea nilotica Kotschy & Peyr	**236**
Uvaria lucida Benth. Syn: *U. gazensis* Baker f., *U. virens* N.E.Br.	**027**
Valeriana capensis Thunb.	**319**
Vangueriopsis lanciflora (Hiern) Robyns Syn: *Canthium lanciflorum* Hiern; *Vangueria lateritia* Dinter	**296**
Vernonia adoensis Sch. Bip. ex Walp.	**096**
Vernonia amygdalina Del.	**097**
Vernonia neocorymbosa Hilliard	**098**
Vigna species	**190**
Viscum species	**325**
Vitellariopsis marginata (N.E. Br.)	**304**
Vitex rehmannii Guerke	**323**
Vitex wilmsii Guerke	**324**
Wahlenbergia grandiflora V. Brehm.	**108**
Warbergia salutaris (bertol. F.) Chiov.	**110**
Withania somnifera (L.) Dun Syn: *Physalis somnifera* L., *W. microphysalis* Suess	**314**
Wrightia natalensis Stapf	**042**
Xysmalobium undulatum (L.) Aiton.f.	**057**
Zanthoxylum capense (Thunb.) Harv. Syn *Fagara capensis* Thunb.	**299**

CHAPTER THREE

Antidepressants:

Screening for selective serotonin re-uptake inhibition (SSRI) activity

3.1. Introduction

Depressive disorders, including major depression and dysthymia, are significant and disabling illnesses. It is estimated that one in five individuals is affected by a mood disorder in his or her lifetime. The World Health Organization (WHO, 1999) estimates that major depression is the fourth most important cause worldwide of loss in disability-adjusted life years, and will be the second most important cause by 2020 (MURRAY and LOPEZ, 1996). Depression affects an estimated 121 million people worldwide.

In South Africa in 1990, the overall suicide rate was 17.2 per 100,000, which is slightly higher than that in the WHO report (1999). Initial estimates from the South African National Burden of Disease Study (2000) indicated suicide as the 10th out of 20 leading causes of mortality (BRADSHAW, GROENEWALD, LAUBSCHER, NANNAN, NOJILANA, NORMAN, PIETERSE, SCHNEIDER, 2003). South Africa has a history of traumatized citizens and is a society in transition. Suicidal behavior among the black population in South Africa appears to be on the increase (LOURENS and NASEEMA, 1998). South African, black youth do not only increasingly consider suicide as an option when they cannot cope, but act on it in certain cases as well, especially when they are under severe depression (MEEL, 2003). Depressive symptoms (64%) are highly prevalent immediately before suicide (HEILA, ISOMETSA, HENRIKSSON, HEIKKINEN, MARTTUNEN, LONNQVIST, 1997).

South Africa is in a HIV/AIDS epidemic of shattering dimensions (DORRINGTON, BOURNE, BRADSHAW and LAUBSCHER, 2001). Depression in the HIV positive individuals is significantly higher. Although depressive symptoms may not be strong enough to warrant a psychiatric diagnosis, a careful evaluation of risks is required (FUKUNISHI, MATSUMOTO, NEGISHI, HAYASHI, HOSAKA and MORIYA, 1997). Depressive symptoms and suicidal ideation are common amongst HIV positive patients, occurring at comparable or greater rates than those found in a variety of other medically ill populations (JUDD and MIJCH, 1996). In South Africa, as is the case in most Third World countries, there is a serious limitation for this care as treatment for severe mental disorders is not available in most primary health care settings.

Depression appears to have both a genetic and environmental basis. Twin studies suggest that about 25–30% of the variance is genetic (HENN, VOLLMAYR and SARTORIUS, 2004) and that environmental factors account for about 75% of the variance. The most important environmental factor is stress

(CHARNEY and MANJI, 2004). Psychiatric treatment of depression depends on our understanding of the pathophysiology of the disease and of the mechanisms by which drugs relieve symptoms of depression. Depressive illness was initially recognized as a biochemical phenomenon in the mid-sixties of the last century (SCHILDKRAUT, 1965). Since then the monoamine theory of depression became widely accepted. It briefly states that mental depression is due to deficiency of brain monoaminergic activity and that depression is treated by drugs that increase this activity (SCHILDKRAUT, 1965). Different mechanisms may increase the availability of brain monoamines. These include blocking the reuptake of the monoamine from the synapse, inhibiting the intraneuronal metabolism of the monoamine or blocking the presynaptic inhibitory auto- or hetero-receptors. Many recent reviews discussed the role of monoamines in depression (SVENSSON, 2000; HENSLER, 2002; 2003; BLIER and WARD, 2003; ELHWUEGI, 2004).

These reviews are largely written for specialists in the field and are partly covered in **Chapter 1** (page 13). Thus a simpler explanation with an emphasis on serotonin re-uptake is given in the following sections, with relevant background material, to enable adequate understanding of the concepts and findings of this research.

3.2. Monoamines and their role in depression

Considerable experimental and clinical evidence support the fundamental role of NA and 5-HT in the etiology of depression (ELHWUEGI, 2004). There are four classes of standard antidepressants, monoamine oxidase (subtype-A) inhibitors (MAOIs), tricyclic antidepressants (TCAs), serotonin re-uptake inhibitors (SSRIs) and noradrenaline (NA)–serotonin reuptake inhibitors. All these antidepressant drugs increase acutely the availability of these monoamines at the synapse (BALDESSARINI, 1989). During 2000 three out of five of the leading world wide CNS drugs were SSRI and the top five drugs all involved serotonin in their mechanism of action (**Table 3.2.1.**).

Table 3.2.1. Worldwide leading CNS products in 2000 (JONES and BLACKBURN, 2002).

Product	Generic name	Mechanism of action	Theraputic class	2000 sales (Million USD)
Prozac	Fluoxetine	SSRI	Antidepressant	2875
Seroxat	Paroxetine	SSRI	Antidepressant	2415
Zyprexa	Olanzapine	DA_2/5-HT_2/other	Antipsychotic	2391
Zoloft	Sertraline	SSRI	Antidepressant	2248
Risperidal	Risperidone	DA_2/5-HT_2/other	Antipsychotic	1707

It is well established that neurotransmitter transporters have roles in several neurological and psychiatric diseases. This is directly supported by several examples of naturally occurring mutations in the transporter genes that cause or increase the risk of developing certain diseases. Examples include

mutations in the gene encoding SERT that are associated with symptoms of obsessive–compulsive disorder, Asperger's syndrome, anorexia and autism (KILIC, MURPHY and RUDNICK, 2003). Furthermore, it is indirectly supported by the fact that the SLC6 family (SLC from solute carrier)of transporters, to which SERT belongs, is a target of several pharmaceutical compounds.

Monoamine transporters

The transmembrane transport of neurotransmitters is of primary importance for proper signaling between neurons. The transport processes are mediated by distinct classes of membrane transport protein that have key roles in controlling the neurotransmitter concentration in the synaptic cleft (GETHER, ANDERSEN, LARSSON and SCHOUSBOE, 2006). These transporters can be classed as intracellular vesicular transporters that are responsible for sequestering transmitters from the cytoplasm into synaptic vesicles, and plasma membrane transporters that are responsible for sequestering released transmitter from the extracellular space (**Figure 3.2.1.**). There are three subclasses of intracellular transporter: the vesicular amine transporters SLC18 gene family), the vesicular inhibitory amino acid transporter family (SLC32) and the vesicular glutamate transporters (SLC17 gene family).

There are two major subclasses of plasma membrane transporter: the high-affinity glutamate transporters (SLC1 gene family) and the Na^+/Cl^--coupled transporters (SLC6 gene family). The latter subclass is the largest and includes transporters of dopamine, serotonin, noradrenaline, glycine and GABA (**Figure 3.2.1.; Table 3.2.2.**).

Table 3.2.2. SLC6 gene family neurotransmitter transporters (after GETHER, ANDERSEN, LARSSON and SCHOUSBOE, 2006).

Transporter	Endogenous substrate	Synthetic substrates	Potent inhibitors	Therapeutic uses and potential
Dopamine transporter (DAT)	Dopamine; noradrenaline, adrenaline	Amphetamine; 1-methyl-4-phenylpyridinium (MPP)	WIN35428; GBR12909; benztropine; mazindol; nomifensine; RTI55; cocaine; Zn^{2+}	ADHD (amphetamines); Parkinson's disease
5-HT transporter (SERT or 5-HTT)	5-HT	4-methylene-dioxymeth-amphetamine (MDMA) ('ecstasy')	Citalopram; escitalopram; fluoxetine; paroxetine; sertraline; imipramine; cocaine; RTI55	Depression; anxiety; OCD
Noradrenaline transporter (NAT or NET)	Dopamine; noradrenaline, adrenaline	Amphetamine; MPP	Nisoxetine; nomifensine; nortriptyline; desipramine; mazindol	Depression
Glycine transporter 1 (GlyT1)	Glycine	-	(*R*)NFPS; NPTS; Org24598; Zn^{2+}	Schizophrenia? Psychosis? Dementia?

Transporter	Endogenous substrate	Synthetic substrates	Potent inhibitors	Therapeutic uses and potential
GlyT2	Glycine	-	ALX1393; ALX1405; Org25543	Anticonvulsant? Analgesic?
GABA transporter-1 (GAT-1)	GABA	-	Tiagabine; SKF89976A; THPO; exo-THPO	Epilepsy (tiagabine)
GAT-2 (equivalent to mouse GAT3)	GABA; β-Ala	-	-	unknown
GAT-3 (equivalent to mouse GAT4)	GABA; β-Ala	-	SNAP5114	unknown
GAT-2 (equivalent to BGT-1 or mouse GAT3)	GABA; betaine	-	EF1502; NNC052090; THPO; Zn^{2+}	Epilepsy

Abbreviations: ADHD, attention deficit hyperactivity disorder; exo-THPO, 4-amino-4,5,6,7-tetrahydrobenzo[d]isoxazol-3-ol; MDMA, 3,4-methylenedioxymethamphetamine; MPPC, 1-methyl-4-phenylpyridinium; NPTS, N-[3-phenyl-3-(40-(4-toluoyl)phenoxy)propyl]sarcosine; OCD, obsessive–compulsive disorder; (R)NFPS, N-[3-(40-fluorophenyl)- 3-(40-phenylphenoxy)propyl]sarcosine; THPO, 4,5,6,7-tetrahydroisoxazolo[4,5-c]pyridine-3-ol.

As mentioned previously the monoamine transporters are of therapeutic significance as targets of antidepressants. Classical tricyclic antidepressants target primarily NAT and/or SERT; selective 5-HT-reuptake inhibitors (SSRIs) are specific inhibitors of SERT, and 5-HT–noradrenaline-reuptake inhibitors (SNRIs) are active at both SERT and NAT (LEONARD, 1997; GOODNICK and GOLDSTEIN, 1998; HUMBLE, 2000; BRUNELLO, MENDLEWICZ, KASPER, LEONARD, MONTGOMERY, NELSON, PAYKEL, VERSIANI and RACAGNI, 2002; TORRES and CARON, 2003) (**Table 3.2.2**.).

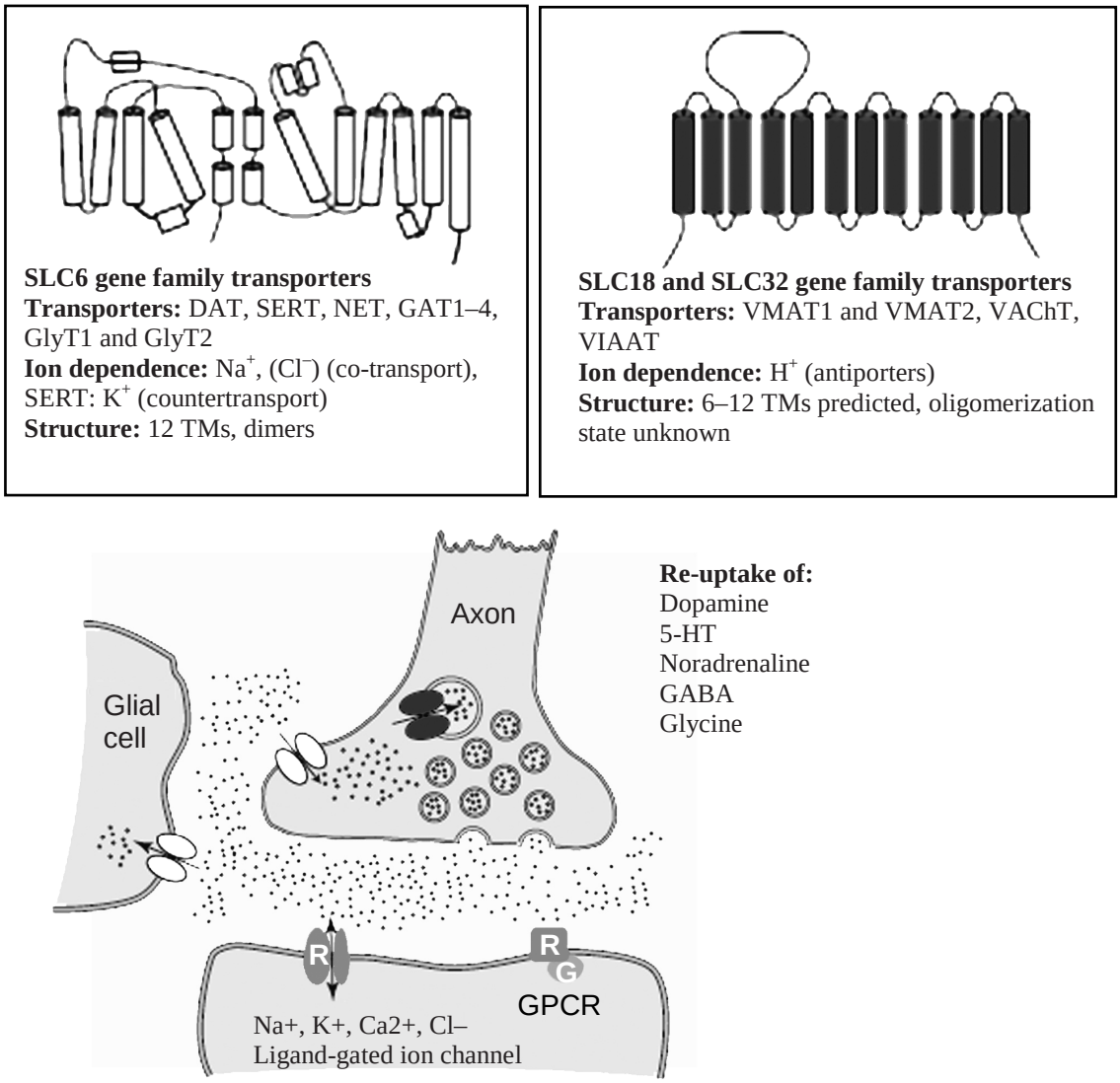


Figure 3.2.1. Monoamine re-uptake transporters.

In the presynaptic nerve terminals of dopamine-, 5-HT-, noradrenaline-, glycine- and GABA-containing synapses, vesicular transporters belonging to the SLC18 (vesicular monoamine transporter (VMAT)1 and VMAT2, and vesicular acetylcholine transporter (VAChT)) and SLC32 (vesicular inhibitory amino acid transporter (VIAAT)) gene families (black) sequester neurotransmitters into synaptic vesicles. Released neurotransmitter exerts its effects via ionotropic receptors (ligandgated ion channels) such as $GABA_A$ receptors, glycine receptors and $5\text{-}HT_3$ receptors (grey) or via G-protein-coupled receptors (GPCRs) such as dopamine receptors, adrenoceptors, 5-HT receptors and metabotropic $GABA_B$ receptors (with associated G protein in gray). The plasma membrane transporters responsible for removing neurotransmitter from the synaptic cleft belong to the SLC6 gene family (white) and are located in the membrane of the presynaptic neuron (DAT, SERT, NET, GlyT2, GAT-1 and GAT-2) or in the membrane of glial cells (GlyT1, GAT-1, GAT-2 and GAT-3) (after GETHER, ANDERSEN, LARSSON and SCHOUSBOE, 2006).

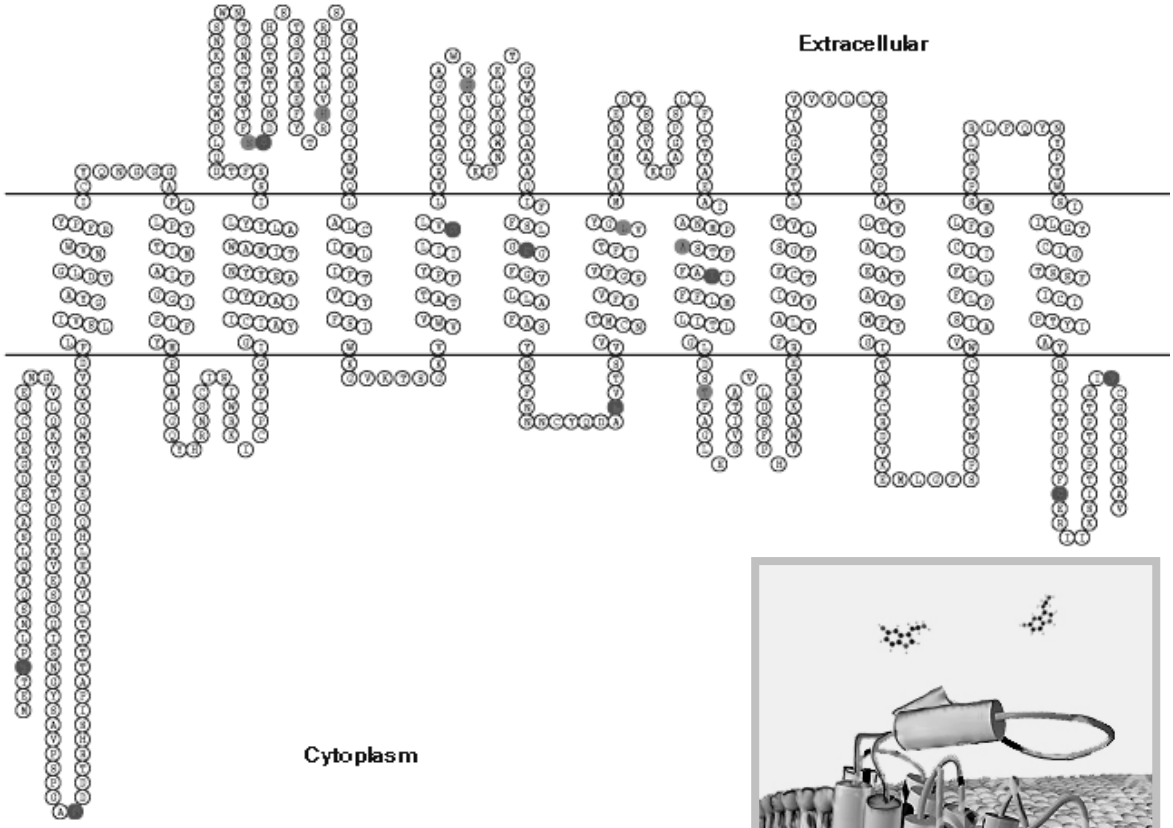
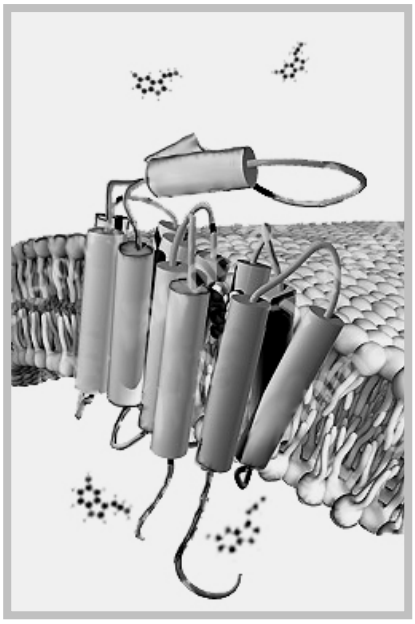


Figure 3.2.2. **The serotonin transporter (SERT)** resembles other biogenic amine transporters (*i.e.* noradrenaline and dopamine transporters). The protein's structure is composed of twelve transmembrane (TM) helices with an extracellular loop between TM helices 3 and 4. Both polypeptide termini are located within the cytoplasm and six putative phosphorylation sites (potential targets for protein kinase A and protein kinase C) exist in the same compartment. The areas important for selective serotonin (5-HT) affinity are localized within helices 1 through 3 and helices 8 through 12 (Adapted from LESCH, WOLOZIN, ESTLER, MURPHY, RIEDERER, 1993).



(**Insert**) A conceptualized structure of the molecule based on scientific findings. A transporter protein found in the plasma membrane of serotonergic neurons is responsible for re-uptake of the transmitter. The serotonin transporter is a carrier of serotonin molecules across the biological membrane. Transporters undergo conformational changes and move one or more molecules per "cycle", unlike channels that stay open or closed, thus allowing floods of molecules to move across membrane bilayers.

Primarily because of the lack of trustworthy structural models, there is still limited knowledge about the binding sites for antidepressants at monoamine transporters (for a recent review, see TORRES, GAINETDINOV and CARON, 2003). The monoamine transporters are also targets of widely abused compounds such as cocaine and amphetamine. Naturally occurring targets of monoamine transports are given in **Table 3.2.3**.

Table 3.2.3. Compounds known to interact with re-uptake of amine neurotransmitters into neurons and synaptic vesicles.
An important way neurotransmitters are removed from the synapse (synaptic cleft) involves energy-dependent (i.e. ATP-dependant) re-uptake into the cytosol of the releasing neuron. A major family of 12 transmembrane α-helix transporters co-transport amine neurotransmitters with Na^+ and Cl^-. Uptake of amine neurotransmitters from the neuronal cytosol into synaptic vesicles is achieved by vesicular monoamine transporters (VMAT1 and VMAT2) that sequester dopamine, adrenaline, noradrenaline and serotonin. The plant derived psychoactive drug cocaine inhibits dopamine, noradrenaline and serotonin re-uptake and hence is a stimulant. The synthetic compound fluoxetine (Prozac) inhibits serotonin re-uptake and hence is excitatory and antidepressant (POLYA, 2003).

Compound (class)	***Species* (Family)**	**Target (biological activity)**
Alkaloid		
Cocaine (+benzoyl-methylecgonine) (tropane)	*Erythroxylum coca*, *E. recurrens*, *E. steyermarckii*, *E.* spp. (Erythroxylaceae)	Dopamine transporter, noradrenaline transporter and serotonin transporter (topical anaesthetic, ophthalmic, CNS stimulant, through the elevation of synaptic dopamine, noradrenaline and serotonin)
O-Desmethylibogaine (= 12-Hydroxyibogamine) (indole)	Primary metabolite of ibogaine. *Tabernanthe iboga*, *Voacanga thouarsii* (Apocynaceae)	Vesicular dopamine transporter ligand, serotonin transporter ligand at cocaine and paroxetine sites
Ibogaine (= 12-Methoxyibogamine) (indole)	*Tabernanthe iboga*, *Voacanga thouarsii* (Apocynaceae)	Dopamine transporter (IC_{50} = 4 μM); Vesicular monoamine transporter; Serotonin transporter (IC_{50} = 0.6 μM) (elevates synaptic serotonin, currently used as anti-addictive)
Noribogaine (= 12-Hydroxyibogaine) (indole)	Metabolic product of ibogaine. *Tabernanthe iboga*, *Voacanga thouarsii* (Apocynaceae)	Dopamine transporter (IC_{50} = 4 μM); Serotonin transporter (IC_{50} = 40 nM) (elevates synaptic serotonin, anti-addictive, anticonvulsant, CNS stimulant, hallucinogen)
Rscinnamine (= Reserpinine) (indole)	*Rauwolfia* spp. (Apocynaceae)	Vesicular monoamine transporter (anti hypotensive, antipsychotic, tranquillizer)
Reserpine (indole)	*Rauwolfia* spp., *Catharanthus roseus*, *Vinca minor* (Apocynaceae)	Vesicular monoamine transporter, VMAT1, VMAT2 (anti hypotensive, carcinogen, tranquillizer, neuroleptic CNS depressant)
Phenolic		
Adhyperforin (phloroglucinol)	*Hypericum perforatum* (Hypericaceae)	Dopamine transporter, noradrenaline transporter and serotonin transporter (antidepressant)
Cannabidiol (phenolic)	*Cannabis sativa*, *Humulus lupulus* (Cannabaceae)	Dopamine transporter (K_d ~ 20 μM), noradrenaline transporter (K_d ~ 20 μM) and serotonin transporter (K_d ~ 20 μM), GABA transporter (K_d ~ 140 μM)
Hyperforin (phloroglucinol)	*Hypericum perforatum* (Hypericaceae)	Dopamine transporter and serotonin transporter (antidepressant)

Compound (class)	***Species* (Family)**	**Target (biological activity)**
Kavain (= Gonosan; Kawain) (pyrone)	*Piper methysticum* (Piperaceae)	Noradrenaline transporter
(+)-Methysticin (pyrone)	*Piper methysticum* (Piperaceae)	Noradrenaline transporter
Δ^1-Tetrahydrocannabinol (phenolic)	*Cannabis sativa* (Cannabaceae)	Dopamine transporter (K_d = 12 μM), noradrenaline transporter (K_d = 12-25 μM) and serotonin transporter (K_d = 12-25 μM), GABA transporter (K_d ~ 140 μM)
Δ^6-Tetrahydrocannabinol (phenolic)	*Cannabis sativa* (Cannabaceae)	Dopamine transporter (K_d ~ 20 μM), noradrenaline transporter (K_d ~ 20 μM) and serotonin transporter (K_d ~ 20 μM), GABA transporter (K_d ~ 140 μM)
Tyramine (=4-Hydroxyphenylalanine) (phenolic)	*Lophophora williamsii*, *Trichocereus pachanoi* (Cactaceae), *Hordeum vulgare*, *Lolium multiflorum* (Poaceae), *Citrus* spp. (Rutaceae), *Viscum album* (Viscaceae)	Dopamine transporter ligand
Other		
D-Cathinone (=(*S*)-2-Amino-1-phenyl-1-propanone) (phenylpropanoid)	*Catha edulis*, *Maytenus krukovii* (Celastraceae)	Dopamine transporter and serotonin transporter (anorexic, CNS stimulant, euphoriant)
Methcathinone (phenylpropanoid)	*Catha edulis*, *Maytenus krukovii* (Celastraceae)	Dopamine transporter and serotonin transporter (CNS stimulant)
Non-plant reference compound		
Citalopram (=Cipramil) (benzodioxol fluorophenyl piperidine)	Synthetic	Serotonin re-uptake inhibitor (antidepressant)
Fluoxetine (= Prozac) (trifluorophenoxy phenyl tertiary amine)	Synthetic	Serotonin re-uptake inhibitor (antidepressant)
Paroxetine (fluorophenyl isobenzofuran tertiary amine))	Synthetic	Serotonin re-uptake inhibitor (antidepressant)
Ritalin (= Methylphenidate)) (piperidine)	Synthetic	Dopamine transporter and serotonin transporter (elevates serotonin and dopamine, used to calm children with hyperactivity-attention deficit disorder)
Zimeldine (aryl piridinyl amine)	Synthetic	Serotonin re-uptake inhibitor (antidepressant)

3.3. Screening of southern African plants for SSRI activity

3.3.1. Introduction and Summary

Initially, seventy five extracts from 35 plant species (mostly indigenous) used in South African traditional medicine or taxonomically related to these were investigated for their affinity to the serotonin reuptake transport protein, making use of an *in vitro* serotonin reuptake transport protein binding assay (NIELSEN, SANDAGER, STAFFORD, VAN STADEN and JÄGER, 2004). Bioassay-guided fractionations of *Boophone disticha* (Amaryllidaceae) lead to the isolation and identification of two alkaloids, buphanadrine and buphanamine (SANDAGER, NIELSEN, STAFFORD, VAN STADEN and JÄGER, 2005). The activity of these alkaloids inspired further screening of several Amaryllidaceae alkaloids (ELGORASHI, STAFFORD, JÄGER and VAN STADEN, 2006) which does not form part of this thesis.

3.3.2. Materials and methods

Plant material

All plant material was selected on the basis of traditional uses described in **Chapter 2**. Material was collected from January to March 2003 in KwaZulu-Natal, South Africa (**Table 3.3.2.1.**). Material of *Gethyllis ciliaris* was obtained from Graham Duncan, from the Western Cape. Freshly collected plant parts were washed thoroughly with water and dried in an oven at 50 °C for 48-72 h. The dried material was ground to a fine powder and stored in plastic bottles in the dark until use. It should be noted that this practice has been changed due to the detection of contaminants (most notably phthalates), most-likely originating from the plastic bottles.

Table 3.3.2.1. Plant species, family, plant parts and voucher numbers of material collected for screening in the [^{3}H]-citalopram binding assay.

Family *Plant species*	**Voucher specimen**	**Plant parts investigated**
Agapanthaceae		
Agapanthus campanulatus F.M.Leight.	*Stafford 59 NU*	leaves, flowers and roots
Amaryllidaceae		
Boophone disticha (L.f.) Herb	*Stafford 53 NU*	leaves, roots and bulbs
Brunsvigia grandiflora Lindl.	*Stafford 10 NU*	leaves and bulbs
Gethyllis ciliaris L.f.	*Stafford 76 NU*	bulb
Apiaceae		
Alepidea natalensis Wood & Evans	*Stafford 61 NU*	leaves and roots
Apocynaceae		
Acokanthera oblongifolia (Hochst.) Codd	*Stafford 14 NU*	leaves
Stropharanthus speciosus (Ward et Harv.) Reber	*Stafford 94 NU*	leaves
Asclepiaceae *Gomphocarpus fruticosa* (L.) Aiton f. subsp. (=*Asclepias fruticosa* L.)	*Stafford 65 NU*	leaves
Xysmalobium undulatum (L.) Aiton.f.	*Stafford 95 NU*	aerial parts and roots
Asphodelaceae		
Bulbine frutescens (L.) Willd.	*Stafford 17 NU*	roots
Gasteria croucheri (Hook.f.) Baker	*Stafford 18 NU*	leaves
Asteraceae		
Artemisia afra Jacq. ex Willd.	*Stafford 63 NU*	leaves
Artemisia dracunculoides Pursh.	*Stafford 64 NU*	leaves

Family *Plant species*	Voucher specimen	Plant parts investigated
Campanulaceae		
Lobelia alata Labill.	*Stafford 83 NU*	leaves
Crassulaceae		
Cotyledon orbiculata L.	*Stafford 71 NU*	leaves
Fabaceae	*Stafford 27 NU*	leaves
Indigofera tristis E.Mey.		
Indigofera woodii Bolus	*Stafford 28 NU*	leaves
Hyperaceae		
Hypericum lanandii Choisy	*Stafford 79 NU*	leaves
Hypericum revolutum Vahl	*Stafford 80 NU*	leaves
Lamiaceae		
Hemizyga obermeyerae M.Ashby	*Stafford 78 NU*	leaves
Leonotis leonurus R.Br.	*Stafford 38 NU*	leaves
Mentha aquatica L.	*Stafford 84 NU*	leaves
Lauraceae		
Cinnamomum camphora (L.) Presl.	*Stafford 69 NU*	leaves
Malvaceae		
Malva parviflora L.	*Stafford 57 NU*	leaves
Oleaceae		
Olea africana (Mill) P.S. Green	*Stafford 87 NU*	bark
Periplocaceae		
Mondia whitei (Hook.f.) Skeels	*Stafford 43 NU*	leaves and flowers
Phytolaccaceae		
Phytolacca octandra L.	*Stafford 88 NU*	aerial parts
Piperaceae		
Piper capense L.	*Stafford 89 NU*	leaves and roots
Rosaceae		
Rubus ludwigii Eckl. et Zeyh.	*Stafford 44 NU*	roots
Rubiaceae		
Conostomium natalense (Hochst.) Bremek.	*Stafford 70 NU*	leaves and roots
Rutaceae		
Clausena anisata (Willd.) Hook.f.	*Stafford 47 NU*	leaves and bark
Zanthoxylum capense (Thunb.)Harv.	*Stafford 96 NU*	leaves
Scrophulariaceae		
Diclis reptans Benth	*Stafford 74 NU*	leaves
Solanaceae		
**Datura ferox* L.	*Stafford 72 NU*	leaves, flowers and seeds
**Datura stramonium* L.	*Stafford 73 NU*	leaves, seeds and seed pods

* exotic species

Preparation of plant extracts

Two grams of dried, grounded plant material were extracted in either 20 ml demineralized (distilled and de-ionized) water or 70% ethanol for 60 min in an ultra sonic bath. The extracts were filtered through Whatman No. 1 filter paper and then evaporated to dryness using rotary evaporators for the ethanolic extracts and freeze-dried in the case of aqueous extracts.

Bioassay-guided fractionation, isolation and identification of active constituents of Boophone disticha

Hundred g fresh leaves of *Boophone disticha* were extracted with 70% ethanol (3×500 ml). This was done by was done be 3x60 min periods in an ultrasound bath. The extract was fractionated on 150 g of Merck Silica gel 60 in a vacuum column. Five hundred milliliters of each of the following solvents were used as eluents: hexane; hexane:ethyl acetate 50:50; 25:75; ethyl acetate; ethyl acetate:methanol 90:10; 80:20; 70:30; 60:40; 50:50; 40:60; 30:70; 20:80; 10:90; 2×methanol; water. Active fractions from VLC (ethyl

acetate:methanol 90:10 and 80:20) where dissolved in 100 ml 70% ethanol, adjusted to pH 3 with 4% acetic acid and partitioned against diethyl ether (3×100 ml). The pH was adjusted to 10 with NaOH and the aqueous phase was partitioned against diethyl ether (3×100 ml), ethyl acetate (3×100 ml) and butanol (4×70 ml). The active fraction (diethyl ether from basic partition) was separated on preparative TLC using ethyl acetate: methanol:water (90:20:10) as mobile phase. The TLC plate was detected under UV $_{254/365\ nm}$ and a small part of the TLC plate was sprayed with Dragendorrf reagent to detect alkaloids. Five bands were scraped off the TLC plate, eluted with methanol and tested for activity. The isolated compounds were sent to Copenhagen where they were dissolved in $CDCl_3$ and the structures elucidated by ^{1}H-NMR and ^{13}C-NMR (100MHz for ^{13}C, 600MHz for ^{1}H) using a Bruker Advance 600.

Tissue preparation

All procedures were carried out at 0-4 °C. Whole rat brains, except cerebellum, were homogenised with an Ultra Turrax homogenizer in 1:10 (w/v) buffer (5 mM TRIS base, 150 mM NaCl and 20 mM EDTA, pH 7.5). The homogenate was centrifuged at 16,000×*g* for 10 min and the tissue membranes washed with 120 ml of the same buffer. The supernatant was discarded and the pellet was suspended in buffer (5mM TRIS base and 5 mM EDTA, pH 7.5), left to react for 20 min and then centrifuged at 16,000×*g* for 10 min. The supernatant was discarded and the pellet was washed with 120 ml buffer (50 mM TRIS base, 120 mM NaCl and 5 mM KCl, pH 7.5) and then centrifuged at 16,000×*g* for 10 min. The supernatant was discarded and the pellet finally suspended in 120 ml buffer (50 mM TRIS base, 120 mM NaCl and 5 mM KCl, pH 7.5). This homogenate was kept at −70 °C until use.

Validation of the serotonin re-uptake (^{3}H-citalopram binding) assay

In order to determine the optimum amount of [^{3}H]-citalopram for the bioassay and establish K_d, a curve of [^{3}H]-citalopram binding against ligand concentration was determined. [^{3}H]-citalopram binding to 50 µl rat brain homogenate (as prepared in previous section) was measured at ten concentrations (0, 0.084, 0.167, 0.25, 0.334, 0.501, 0.668, 0.835, 1.002, 1.169 and 1.336 mM). The various concentrations of [^{3}H]-citalopram (50 µl) were incubated with 50 µl rat brain tissue homogenate and 200 µl buffer (50 mM TRIS base, 120 mM NaCl and 5 mM KCl, pH 7.5) for 2 h at 23–26 °C and filtered through Advantec GC-50/25 glass fibre filters under vacuum. After 24 h the radioactivity of the filters containing protein bound [^{3}H]-citalopram was measured by liquid scintillation, using 5 ml Beckman Ready Value II^{TM} scintillation fluid. A double determination was done.

A curve of rat brain tissue homogenate volume versus [^{3}H]-citalopram binding was used to determine the optimum volume of tissue homogenate in the assay. Fifty µl 4 nM [^{3}H]-citalopram (final concentration in assay 0.67 nM) was incubated with 0, 25, 50, 75, 100, 125, 150, 175, 200, 225, and 250 µl tissue homogenate (as prepared in previous section) and made up to 300 µl with buffer (50 mM TRIS base, 120 mM NaCl and 5 mM KCl, pH 7.5) for 2 h at 23–26 °C. This was then filtered through Advantec GC-50/25 glass fibre filters under vacuum. After 24 h the radioactivity of the filters containing protein bound

[^{3}H]-citalopram was measured by liquid scintillation, using 5ml Beckman Ready Value II™ scintillation fluid. A double determination was done.

The effect of extraction solvent (ethanol) on the assay was determined using five concentrations of a similar range that would be used when testing plant extracts (7% to 0.0007 %). Again 50 µl 4nM [^{3}H]-citalopram was incubated for 2 h with 50 µl tissue homogenate and 200 µl ethanol solutions diluted with buffer to the above mentioned concentrations, then filtered through Advantec GC-50/25 glass fibre filters under vacuum. After 24 h the radioactivity of the filters containing protein bound [^{3}H]-citalopram was measured by liquid scintillation, using 5 ml Beckman Ready Value II™ scintillation fluid. A double determination was done.

[^{3}H]-Citalopram binding assay

The method described by PLENGE, MELLERUP and NIELSEN (1990) was used. Two hundred microlitre dilution of the extract (5, 1, 0.1, 0.01 and 0.001 mg/ml) in buffer (50 mM TRIS base, 120 mM NaCl and 5 mM KCl, pH 7.5) making a final concentration in the assay of 3.3, 0.7, 0.07, 0.007, and 0.0007 mg/ml were mixed with 50 µl 4nM [^{3}H]-citalopram and 50 µl rat brain tissue suspension in the listed order. For determination of unspecific binding, 200 µl of 1.5 µM paroxetine were mixed with 50 µl 4 nM [^{3}H]-citalopram and 50 µl tissue suspension. The total binding of [^{3}H]-citalopram was determined by mixing 200 µl buffer (50 mM TRIS base, 120 mM NaCl and 5 mM KCl, pH 7.5) with 50 µl of 4 nM [^{3}H]-citalopram and 50 µl tissue suspension. All samples were incubated for 2 h at 23–26 °C and filtered through Advantec GC-50/25 glass fibre filters under vacuum. After 24 h the radioactivity of the filters containing protein bound [^{3}H]-citalopram was measured by liquid scintillation, using 5 ml Beckman Ready Value II™ scintillation fluid. The assay was done in triplicate.

8-Hydroxy[^{3}H]DPAT binding assay

Two hundred microlitres of test solution were mixed with 50 µl of 8-hydroxy[^{3}H]DPAT and 50 µl of tissue suspension. Buspirone (1 µM) was used to determine unspecific binding. Subsequent procedures were as for the [^{3}H]-citalopram binding assay.

Estimation of IC_{50} values and K_i values

The IC_{50} values were calculated using GraFit 5 from Erithacus Software utilising a full four-parameter equation. The K_i values, which is independent of ligand concentration and thus more useful for inter-lab comparison, was calculated by $K_i = IC_{50}/(1 + [L]/K_d)$, where $[L]$ is the ligand concentration (here 0.75 nM) and K_d is 0.7 nM for citalopram.

3.3.3. Results

Validation of the serotonin re-uptake ([^{3}H]-Citalopram binding) assay

A greater concentration range (0.01 to 10 nM) of radiolabelled ligand was needed to achieve a sigmoidal curve. By extrapolation (dotted line **Figure 3.3.3.2.**) it was estimated that V_{max} for 50 µl rat brain tissue suspension was approximately 16 000 and K_d 0.6 nM. This is comparable to a K_d of (0.7 nM) reported by the [^{3}H]-citalopram supplier (Amersham).

The curve (**Figure 3.3.3.3.**) of rat brain tissue homogenate volume versus [^{3}H]-citalopram binding initially shows a linear relationship as the number of SERT increases (homogenate volume) as ligand ([^{3}H]-citalopram) remains constant. After 50 µl rat brain tissue suspension the graph flattens corresponding to a deficiency in [^{3}H]-citalopram. Thus, 0.67 nM [^{3}H]-citalopram should bind to all SERT proteins in 50 µl rat brain tissue suspension. The bound [^{3}H]-citalopram produced 5000 dpm with 50 µl rat brain tissue suspension. At the concentrations tested (7%-0.007%) ethanol had no observable effect on the bioassay.

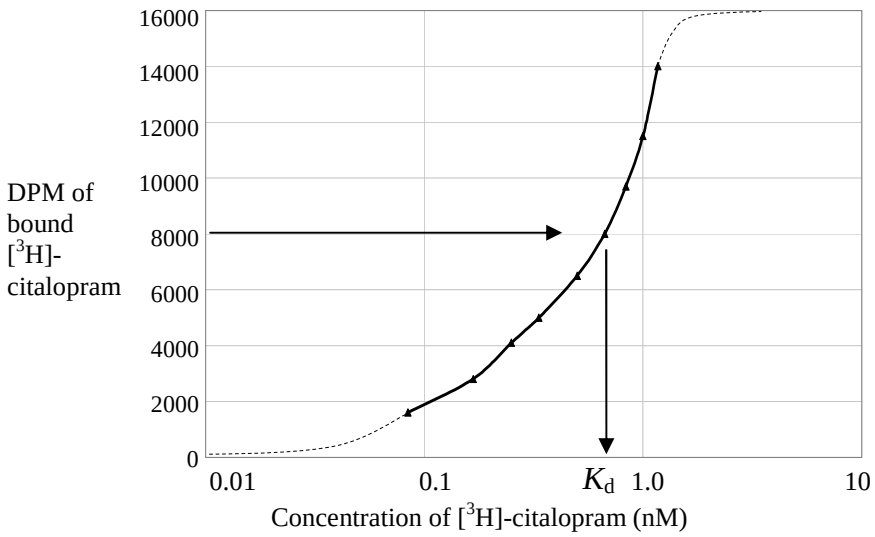


Figure 3.3.2.2. Ligand ([^{3}H]-citalopram) concentration verses binding curve showing V_{max} (±16000 dpm) and K_d (±0.6 nM).

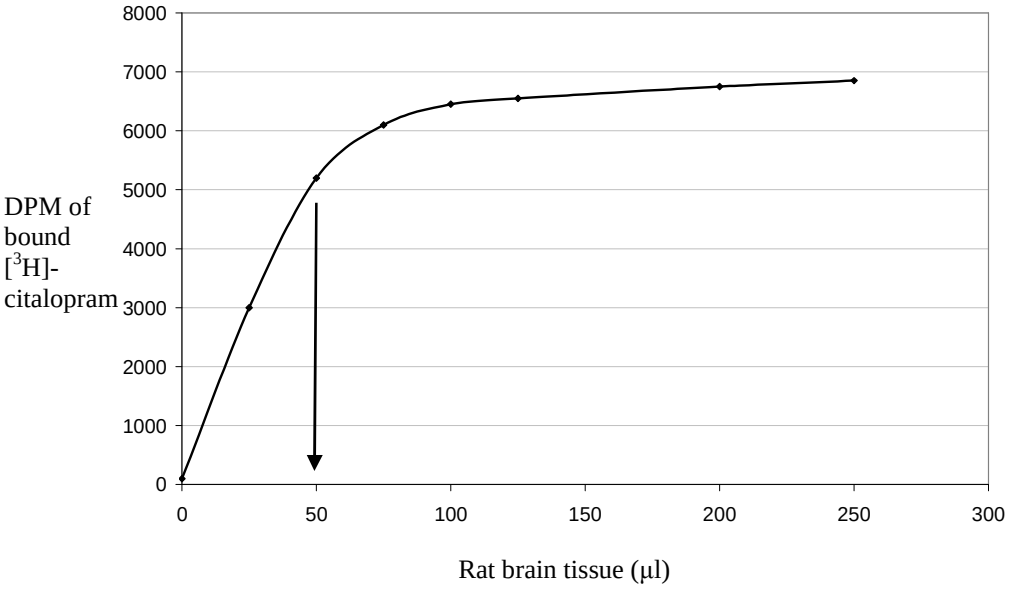


Figure 3.3.2.3. SERT (rat brain tissue) concentration verses [^{3}H]-citalopram binding curve.

Screening of South African plants for SSRI activity

Seventy five extracts from 35 plant species were selected for investigation in this study. Thirty seven of these extracts, derived from 15 plants showed some degree of affinity to the serotonin reuptake transport protein (**Table 3.3.3.1.**). Nine extracts from five plants had high affinity, characterized as high concentration-dependent inhibition with less than 50% [^{3}H]citalopram binding with the three strongest concentrations (5, 1, and 0.1 mgml^{-1}) and/or IC_{50} > 500 µgml^{-1} (highlighted in **bold, Table 3.3.3.1** and **Figures 3.3.3.1-3.**).

Table 3.3.3.1. Screening of plant extracts for affinity to the serotonin transporter protein

Plant species	Plant part	Extract[a]	Yield (mg)	[^{3}H]-citalopram binding in percent[b]					IC_{50} ± SE[c] (µg/ml)
				5 mg/ml	1 mg/ml	0.1 mg/ml	0.01 mg/ml	0.001 mg/ml	
Acokanthera oblongifolia	leaves	w	377	62	95	107	102	100	n.d. [d]
Agapanthus campanulatus	leaves	w	268	29	13	36	55	64	n.d.
		e	367	86	91	91	56	63	n.d.
	flowers	**w**	143	**7**	**10**	**24**	**43**	**62**	**4.7±1.1**
		e	193	19	69	84	73	85	n.d.
	roots	**w**	187	**9**	**23**	**78**	**97**	**92**	**298±79**
		e	286	97	119	93	115	109	n.d.
Alepidea natalensis	leaves	w	262	84	94	105	91	99	n.d.
	roots	w	121	117	115	98	115	126	n.d.
Artemisia afra	leaves	e	352	28	104	106	110	127	n.d.
Artemisia dracunculoides	leaves	w	308	69	96	106	116	121	n.d.
		e	244	22	85	107	110	103	n.d.
Asclepias fruticosa	leaves	w	337	66	87	92	102	97	n.d.
		e	294	45	99	103	101	101	n.d.
Boophone disticha	**leaves**	**w**	212	**12**	**14**	**42**	**86**	**83**	**67±29**
		e	256	**7**	**8**	**35**	**75**	**91**	**40±4**
	roots	w	91	79	69	127	126	133	n.d.
		e	101	**40**	**46**	**64**	**93**	**84**	**67±13**
	bulb	**w**	50	**34**	**14**	**39**	**75**	**85**	**23±21**
		e	307	61	36	83	90	74	n.d.
Brunsvigia grandiflora	leaves	w	265	16	46	82	79	98	n.d.
	outer bulb	w	142	53	79	109	111	107	n.d.
		e	463	31	52	80	96	96	n.d.
	inner bulb	w	216	41	72	97	108	94	n.d.
		e	415	47	65	110	106	94	n.d.
Bulbine frutescens	roots	w	135	38	17	28	7	24	n.d.
Cinnamomum camphora	leaves	w	225	83	101	102	109	110	n.d
Clausena anisata	leaves	w	156	62	101	125	125	140	n.d.
		e	132	63	73	103	130	129	n.d.
	bark	w	181	94	121	119	116	118	n.d.
		e	345	34	80	93	82	78	n.d.
Conostomium natalense	leaves	w	231	98	128	117	121	127	n.d.
		e	285	77	117	135	116	118	n.d.
	roots	e	206	53	101	120	119	102	n.d.
Cotyledon orbiculata	leaves	w	732	84	86	74	75	70	n.d.

Plant species	Plant part	Extract[a]	Yield (mg)	[^{3}H]-citalopram binding in percent[b]					IC_{50} ± SE[c] (µg/ml)
				5 mg/ml	1 mg/ml	0.1 mg/ml	0.01 mg/ml	0.001 mg/ml	
Datura ferox	leaves	w	148	24	18	80	71	99	n.d.
	flowers	w	151	20	15	75	74	79	n.d.
	seeds	**w**	126	**20**	**21**	**13**	**80**	**56**	**13±3**
Datura stramonium	leaves	w	422	14	57	112	123	123	n.d.
		e	329	20	57	110	114	94	n.d.
	seeds	w	94	28	62	90	119	112	n.d.
		e	96	56	45	84	105	102	n.d.
	seed pods	e	150	33	78	107	117	102	n.d.
Diclis reptans	leaves	w	300	100	107	111	109	108	n.d.
		e	461	70	99	98	108	101	n.d.
Gasteria croucheri	leaves	w	351	76	96	113	113	106	n.d.
		e	280	62	100	104	113	115	n.d
Gethyllis ciliaris	bulb	w	414	73	108	103	118	113	n.d.
		e	357	44	96	110	114	112	n.d.
Hemizyga obermeyerae	leaves	w	362	55	105	87	88	99	n.d.
Hypericum lanandii	leaves	w	178	127	105	109	105	92	n.d.
Hypericum revolutum	leaves	w	305	139	93	110	101	114	n.d.
Indigofera tristis	leaves	e	369	125	134	117	110	124	n.d.
Leonotis leonurus	leaves	e	318	24	91	131	131	117	n.d.
Lobelia alata	leaves	w	235	75	50	23	22	11	n.d.
Malva parviflora	leaves	w	202	39	62	70	92	94	n.d.
		e	272	6	53	85	92	95	n.d.
Mentha aquatica	leaves	e		67	57	124	134	114	n.d.
Mondia whitei	**leaves**	w	299	57	77	101	98	99	n.d.
		e	364	**4**	**21**	**61**	**83**	**89**	**39±34**
	flowers	w	388	41	82	103	106	107	n.d
Olea africana	bark	w	186	44	65	89	84	75	n.d.
		e	308	11	80	90	108	104	n.d.
Phytolacca octandra	leaves	w	244	67	104	102	106	133	n.d.
		e	35	53	58	92	105	95	n.d.
Piper capense	leaves	w	155	21	40	89	80	73	n.d.
		e	168	73	79	98	96	98	n.d.
	root	e	448	68	87	108	110	110	n.d.
Rubus ludwigii	roots	e	128	171	161	118	104	124	n.d.
Stropharanthus speciosus	leaves	w	339	35	56	78	68	73	n.d.

Plant species	Plant part	Extract[a]	Yield (mg)	[^{3}H]-citalopram binding in percent[b]					IC_{50} ± SE[c] (µg/ml)
				5 mg/ml	1 mg/ml	0.1 mg/ml	0.01 mg/ml	0.001 mg/ml	
Xysmalobium undulatum	**leaves**	w	332	40	61	73	88	52	n.d.
		e	332	**20**	**31**	**47**	**73**	**70**	**46±22**
	roots	w	179	51	68	81	117	114	n.d.
		e	165	76	93	107	82	111	n.d.
Zanthoxylum capense	leaves	w	182	35	81	106	76	96	n.d.
		e	242	10	47	89	110	112	n.d.

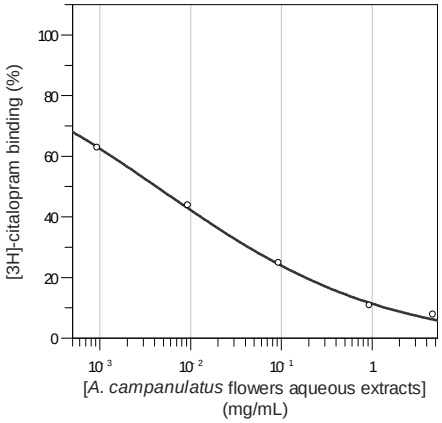
[a] solvents: w = deionized water; e = 70% ethanol

[b] [^{3}H]-citalopram binding in percent, the lower the percentage the more [^{3}H]-citalopram has been displace by the plant extract

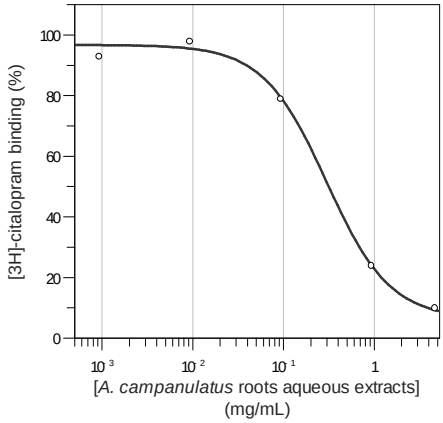
[c] IC_{50} (µgml^{-1}) and standard error, calculated using Grafit (© Erithacus Software Limited)

[d] n.d. = not detected, does not exhibit sufficient (IC^{50} < 500 µgml^{-1}) dose-dependant activity

Extracts from *Agapanthus campanulatus*, *Boophone disticha*, *Datura ferox*, *Mondia whitei* and *Xysmalobium undulatum* showed high affinity to the serotonin reuptake transport protein in the displacement assay utilized in this study.



Parameter	Value	Std. Error
Y Range	102.0905	3.5802
IC 50	0.0047	0.0011
Slope factor	0.3507	0.0313
Background	-2.1538	3.2053



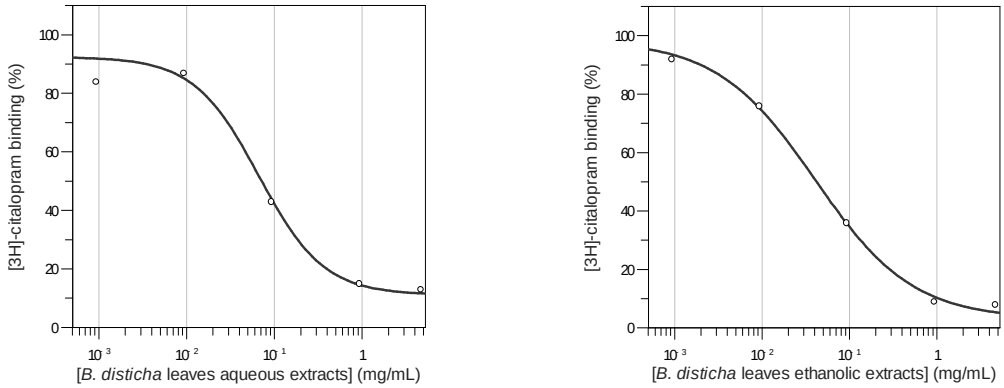
Parameter	Value	Std. Error
Y Range	90.3116	6.8339
IC 50	0.2979	0.0791
Slope factor	1.2407	0.2569
Background	6.4306	6.0489

Figure 3.3.3.1. IC_{50} determination of extracts of *Agapanthus campanulatus* which showed high affinity to the [^{3}H]-citalopram binding site.

Thirty eight extracts from 26 plants did not show any affinity for the serotonin transporter protein. Aqueous extracts of leaves, roots and flowers from *Agapanthus campanulatus* (Agapanthaceae) resulted in displacement of more than 60% transport protein bound [^{3}H]-citalopram at the three strongest concentrations. Aqueous extracts of flowers and roots of *Agapanthus campanulatus* showed good dose-dependent activity (**Figure 3.3.3.1.** above) with IC_{50} values of 4.7±1.1 μgml^{-1} and 298±79 μgml^{-1} respectively.

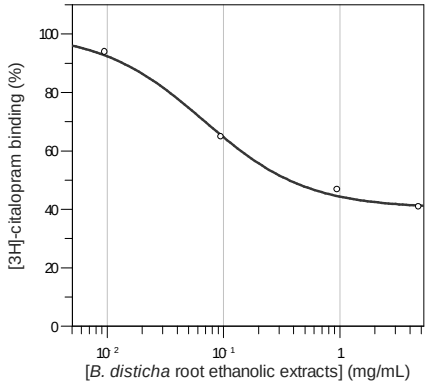
Aqueous and ethanolic extracts of the leaves and the aqueous extract of the bulbs from *Boophone disticha* (Amaryllidaceae) resulted in displacement of more than 50% of the bound [^{3}H]-citalopram at the three highest concentrations. The ethanolic extract of the leaf material (IC_{50} = 40±4 μgml^{-1}) and the aqueous extract of the bulb (IC_{50} = 23±21 μgml^{-1}) exhibited the best activity of the *B. disticha* extracts (**Figure 3.3.3.2**).

An aqueous extract from the seeds of exotic weed, *Datura ferox* displaced 80% of the bound [^{3}H]-citalopram at the three highest concentrations, and gave an IC_{50} value of 13±3 μgml^{-1} (**Figure 3.3.3.3**). *Xysmalobium undulatum* (ethanolic leaf extract) and *Mondia whitei* (ethanolic leaf extract) both displaced bound [^{3}H]-citalopram showing good affinity for the serotonin transporter protein (**Figure 3.3.3.3**), with IC_{50} values of 46±22 μgml^{-1} and 39±34 μgml^{-1}, respectively.

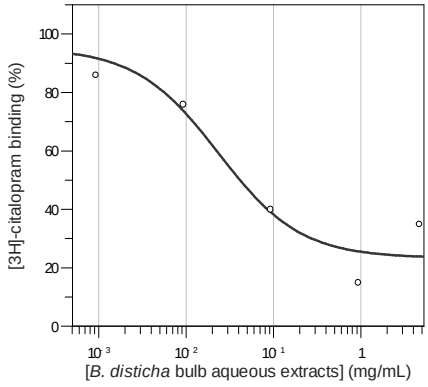


Parameter	Value	Std. Error
Y Range	81.2867	10.7220
IC50	0.0666	0.0294
Slope factor	1.1782	0.6018
Background	11.1311	7.4668

Parameter	Value	Std. Error
Y Range	95.4216	4.6641
IC50	0.0403	0.0080
Slope factor	0.7758	0.1034
Background	2.9870	3.3367

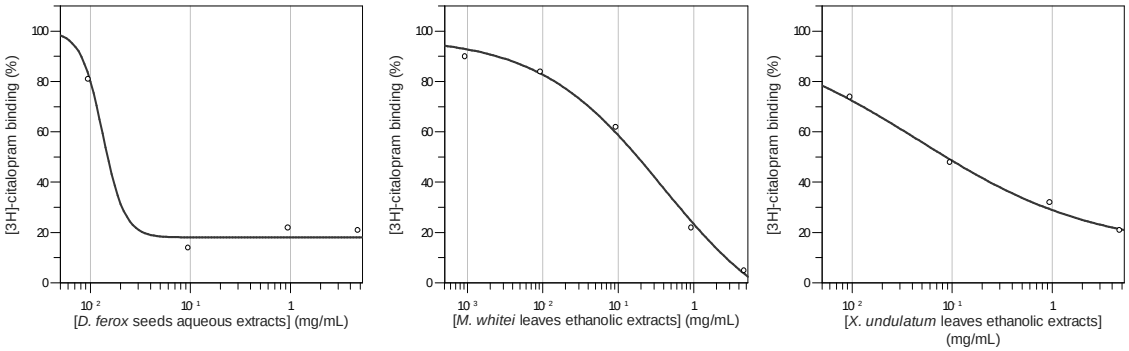


Parameter	Value	Std. Error
Y Range	59.9793	3.5953
IC50	0.0674	0.0134
Slope factor	0.9816	0.1830
Background	40.3878	2.3645



Parameter	Value	Std. Error
Y Range	71.9225	16.7210
IC50	0.0233	0.0209
Slope factor	0.9240	0.6393
Background	23.3178	11.0915

Figure 3.3.3.2. IC_{50} determinations of extracts of *Boophone disticha* which showed high affinity to the [^{3}H]-citalopram binding site.



Parameter	Value	Std. Error
Y Range	81.9316	6.3584
IC50	0.0133	0.0033
Slope factor	3.9728	3.2382
Background	18.0131	3.2413

Parameter	Value	Std. Error
Y Range	118.9299	23.3912
IC50	0.3885	0.3388
Slope factor	0.5319	0.1275
Background	-21.3967	21.6907

Parameter	Value	Std. Error
Y Range	86.8018	8.3063
IC50	0.0459	0.0220
Slope factor	0.4982	0.1104
Background	13.3246	7.5143

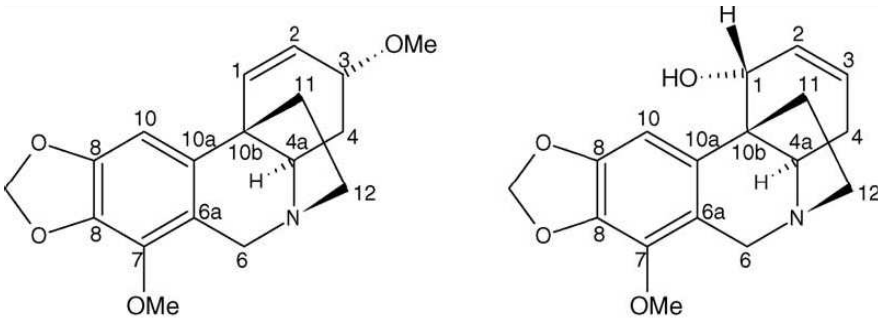
Figure 3.3.3.3. IC_{50} determinations of extracts of *Datura ferox, Mondia whitei* and *Xysmalobium undulataum* which showed high affinity to the [^{3}H]-citalopram binding site.

Bioassy-guided fractionation, isolation and identification of active constituents of B. disticha

NMR data for buphanamine are given in **Table 3.3.3.2.** below. Two compounds, buphanidrine (7.6 mg) and buphanamine (5.6 mg) (**Figure 3.3.3.4**), with activity on the SERT were isolated. Buphanidrine was identified by comparison with NMR data previously reported (VILADOMAT, CODINA, BASTIDA, SHAHEED and CAMPBELL, 1995). In the assay the IC_{50} values of buphanidrine (**22**) and buphanamine (**23**) were 274 and 1799 μM, respectively. The *K*i values of the two compounds were calculated to be 132 μM for buphanidrine and 868 μM for buphanamine. For citalopram the IC_{50} value was 1.3 nM, giving a *K*i value of 0.6 nM. Both buphanidrine and buphanamine bound to the $5HT_{1A}$ receptor with low affinity, the IC_{50} values were 1203 and 2975 μM, respectively.

Table 3.3.3.2. NMR data for buphanamine isolated from *Boophone disticha* (100MHz for ^{13}C, 600MHz for ^{1}H, $CDCl_3$)

Position	^{1}H	^{13}C	COSY (H→H)	NOESY (H→H)	HMBC (C→H)
1	4.70 d ($^{3}J_{1,2}$ = 5.6)	63.6	2	2, 10, 11 endo, 11exo	
2	6.05 dddd ($^{3}J_{2,3}$ = 10.0, $^{3}J_{2,1}$ = 5.6, $^{3}J_{2,4\beta}$ = 2.8, $^{3}J_{2,4\alpha}$ = 1.9)	125.4	1, 3, 4β	1, 3	1
3	5.86 ddd ($^{3}J_{3,2}$ = 10.0, $^{3}J_{3,4\alpha}$ = 4.6, $^{3}J_{3,4\beta}$ = 2.9)	127.0	2, 4α, 4β	2, 4α, 4β	1
4	α: 2.83 dddd ($^{2}J_{4\alpha,4\beta}$ = 19.6, $_{3}J_{4\alpha,4a}$ = 5.6, $^{3}J_{4\alpha,3}$ = 4.6, $^{4}J_{4\alpha,2}$ = 1.9) β: 2.21 ddt ($^{2}J_{4\beta,4\alpha}$ = 19.6, $^{3}J_{4\beta,4a}$ = 8.2, $^{3}J_{4\beta,3}$ = $^{4}J_{4\beta,2}$ = 2.8)	26.1	3, 4β, 4a 2, 3, 4α, 4a	3, 4β, 4a 3, 4α, 11exo, 12exo	
4a	3.79 t ($^{3}J_{4a,4\alpha}$ = $^{3}J_{4a,4\beta}$ = 8.2)	60.3	4α, 4β	4α, 6α	1, 3, 6β
6	α: 4.43 d ($^{2}J_{6\alpha,6\beta}$ = 16.7) β: 4.08 d ($^{2}J_{6\beta,6\alpha}$ = 16.7)	55.4	6β 6 α	6β, 4a 6α, 12endo	12endo
6a		113.3			10
7		140.7			β
8		134.0			10, 13
9		149.7			10, 13
10	6.61 s	98.3		1, 11endo	
10a		134.8			11exo
10b		49			1, 4α, 4β, 4a 6α, 6β, 10
11	endo: 1.99 dddd ($^{2}J_{11endo,11exo}$ = 12.3, $^{3}J_{11endo,12endo}$ = 8.7, $^{3}J_{11endo,12exo}$ = 3.0, $^{4}J_{11endo,4a}$ = 1.3) exo: 2.12 ddd ($^{2}J_{11exo,11endo}$ = 12.3, $^{3}J_{11exo,12exo}$ = 10.9, $^{3}J_{11exo,12endo}$ = 8.2)	36.9	11exo, 12endo, 12exo 11endo, 12endo, 12exo	1, 10, 11exo, 12 endo 1, 4β, 11endo, 12exo	1
12	endo: 2.99 ddd ($^{2}J_{12endo,12exo}$ = 13.2, $^{3}J_{12endo,1endo}$ = 8.7, $^{3}J_{12endo,11exo}$ = 8.2) exo: 3.91m	51.2	11exo, 11endo, 12exo 11exo, 11endo, 12 endo 9	6β, 11endo, 12exo 4β, 11exo, 12endo	6α, 6β
13	5.93 AB-system ($^{2}J_{A,B}$ = 1.5)	101.2			
14	4.01	59.3			



22 **23**

Figure 3.3.3.4. Structures of (**22**) buphanidrine and (**23**) buphanamine isolated from *Boophone. disticha*.

3.3.4. Discussion and conclusions

It is evident from **Table 3.2.1.** that before this study there were relatively few known monoamine re-uptake transporter inhibitors of natural origin. The few that are known are far less potent than the currently supported synthetic compounds.

Boophone disticha is used in traditional medicine for numerous purposes, e.g. hysteria in young women (GORDON, 1947). Sotho and Xhosa people are reported to use bulb scales as a narcotic (WATT and BREYER-BRANDWIJK, 1962). It is known as a toxic plant and alkaloids from the bulb are known to possess hallucinogenic properties (DE SMET, 1996; DU PLOOY, SWART and VAN HUYSTEEN, 2001). Structurally, buphanamine (**22**) and buphanadrine (**23**) have the benzo-1,3-dioxole moiety in common with the clinically used SSRI paroxetin, which could explain their affinity to the SERT. The traditional use and reported hallucinogenic effects obtained after accidental or purposeful overdosing with *Boophone disticha* extracts (DE SMET, 1996; VAN WYK, VAN HEERDEN and VAN OUDTSHOORN, 2002) indicate that the alkaloids reach the CNS. In the SERT assay the IC_{50} values of buphanidrine and buphanamine were 274 and 1799 μM, respectively. The K_i values of the two compounds were calculated to be 132 μM for buphanidrine and 868 μM for buphanamine. For citalopram the IC_{50} value was 1.3 nM, giving a K_i value of 0.6 nM. Both buphanidrine and buphanamine bound to the $5HT_{1A}$ receptor with low affinity, the IC_{50} values were 1203 and 2975 μM, respectively.

For the SSRIs, and most other types of antidepressant drugs, it takes 2-4 weeks for the therapeutic action to develop. A leading hypothesis of this delayed pharmacological action is desensitation of somatodendritic serotonin 1A autoreceptors ($5HT_{1A}$) in the midbrain raphe, which act by reducing neuronal firing (STAHL, 1998; HOLMES, YANG, MURPHY and CRAWLEY, 2002; HENSLER, 2002). A major goal of antidepressant development is to improve preceding drug classes with more rapid onset of antidepressant action and fewer unwanted side effects. Therapeutic agents acting both by inhibition of serotonin reuptake and by inhibiting the action of $5HT_{1A}$ autoreceptors might result in a more rapid onset of antidepressant action. The finding that buphanidrine and buphanamine had only slight

affinity to $5HT_{1A}$, eliminates the possibility of a dual-action system. Although the activities of buphanidrine and buphanamine on the serotonin transporter were lower than the activity of the clinically used SSRI citalopram, the activity supports the traditional use of *B. disticha* as a remedy for depression and anxiety.

Although not indigenous to South Africa *Datura* species are used by Zulu people as a hypnotic drug against hysterical fits and is also smoked for the relief of headaches (HUTCHINGS, SCOTT, LEWIS and CUNNINGHAM, 1996). The Vhavenda people use *Datura stramonium* for insanity (MABOGO, 1990). *Datura* species are known to contain tropane alkaloids such as hyoscyamine and hyoscine, the latter being a muscarinic receptor antagonist and a CNS depressant (RANG, DALE and RITTER, 1999). It is difficult at this stage to speculate which compounds are responsible for the observed SERT binding.

Xhosa people administer powdered roots of *Xysmalobium undulatum* as a snuff (inhaled through nostrils) to treat headaches and hysteria (HUTCHINGS, SCOTT, LEWIS and CUNNINGHAM, 1996). *X. undulatum* is sold as an herbal remedy under the name 'Uzara' in the West, particularly Germany where it is used as an intestinal smooth muscle relaxant. There is significant pharmacological evidence that $5\text{-}HT_3$ and $5\text{-}HT_4$ receptors of the submucosal sensory neurons represent promising targets for the treatment of functional gastro-intestinal diseases (HOUGHTON and WHORWELL, 1997; BERMAN, CHANG, SUYENOBU, CHANG, FITZGERALD, MANDELKERN, HAMM, VOGT and NALIBOFF, 2002), so the observed clinical effects could be due to serotonin receptor affinity of compounds in the plant extract.

3.4. Summary

Mental depression is due to deficiency of brain monoaminergic activity, in particular dopamine and serotonin. Different mechanisms may increase the availability of brain monoamines in order to treat depression. These include blocking the reuptake of the monoamine from the synapse, inhibiting the intra-neuronal metabolism of the monoamine or blocking the presynaptic inhibitory auto- or hetero-receptors.

Seventy six extracts from 35 plant species used in South African traditional medicine or taxonomically related species were investigated for their affinity to the serotonin reuptake transport protein. This was achieved using an *in vitro* assay that detects plant compounds that bind to the serotonin transporter protein of rat brain tissue, displacing [^{3}H]-citalopram. Aqueous and ethanolic extracts of various plant parts were screened and 45 extracts derived from 15 plant species showed affinity to the serotonin reuptake transport protein. The extracts of four plants were characterized as have high affinity for the SERT (more than 50% inhibition at 5, 1, and 0.5 mg/ml). Plant species with high affinity to the serotonin reuptake transport protein included *Agapanthus campanulatus*, *Boophone disticha*, *Datura ferox* and *Xysmalobium undulatum*. *Agapanthus campanulatus* yielded high activity in aqueous extracts from leaves and flowers. *Boophone disticha* showed high activity both in aqueous and ethanolic extracts of leaves and

bulbs. *Datura ferox* showed high activity in aqueous extracts from the seeds and *Xysmalobium undulatum* showed high activity in the ethanolic extract of the whole plant.

Two compounds, buphanadrine and buphanamine, were isolated by bioassay-guided fractionation from *Boophone disticha*. The IC_{50} values of buphanidrine and buphanamine were 274 μM (*K*i = 132 μM) and 1799 μM (*K*i = 868 μM), respectively. The two alkaloids were also tested for affinity to the $5HT_{1A}$ receptor, but only showed slight affinity.

The possibility of screening plants for MAO-A inhibition was also investigated but was abandoned due to low levels of MAO-A compared to MAO-B in rat liver tissue, for more details refer to **Chapter 5**.

The following publications relate to this chapter:

- N.D. Nielsen, M. Sandager, **G.I. Stafford**, J. van Staden and A.K. Jäger. 2004. Screening of indigenous plants from South Africa for affinity to the serotonin reuptake transport. *Journal of Ethnopharmacology* 94: 159-163.
- M. Sandager, N.D. Nielsen, **G.I. Stafford**, J. Staden and A.K. Jäger. 2005. Alkaloids from *Boophane disticha* with affinity to the serotonin transporter in rat brain. *Journal of Ethnopharmacology* 98: 367-370.

CHAPTER FOUR

Anti-epileptics and Anxiolytics:

Screening for sedative-like and antiepileptic activity in the benzodiazepine-$GABA_A$ receptor binding assay

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4.1. Introduction: the GABAergic system

γ-Aminobutyric acid GABA is a major inhibitory neurotransmitter in the vertebrate central nervous system. Depending on the brain region, approximately 20 to 50% of all central synapses use GABA as the transmitter (HALASY and SOMOGYI, 1993). GABA is a small amino acid derived from glutamate by glutamic acid decarboxylase. GABA activates three different receptor classes: $GABA_A$, $GABA_B$ and $GABA_C$ receptors (SIMMOMDS, 1983).

$GABA_A$ receptors are ligand-gated chloride ion channels (BORMANN, 1988; SILVILOTTI and NISTRI, 1991). These receptors are activated by GABA, muscimol and isoguvacine, and are inhibited by bicuculline, gabazine (SR95531) and (+) -β-hydrastine (WERMUTH and BIZIERE, 1986).

$GABA_B$ receptors are activated by GABA, (-)-baclofen, (+)-4-amino-3-(5-chloro-2-thienyl butanoic acid and 3-aminopropyl-(methyl)phosphinic acid (SKF 97541), and are inhibited by phaclofen, saclofen and 2-hydroxysaclofen (SEABROOK, HOWSON and LACEY, 1990). These receptors are known to be coupled to Ca^{2+} or K^+ channels via G-proteins so as to activate the second messenger systems within the cell (BORMANN, 1988).

$GABA_C$ receptors are derived from various isoforms of the ρ-subunit, and are directly associated with chloride ion channels. These receptors are activated by GABA and certain analogues of GABA such as *cis*-4-aminocrotonic acid (CACA) and *trans*-4-aminocrotonic acid (TACA), and are inhibited by imidazole-4-acetic acid and (1,2,5,6-tetrahydropyridin-4-yl)methylphosphinic acid (TPMPA) but are insensitive to bicuculline, barbiturates, benzodiazepines and baclofen (reviewed by MARTINEZ-TORRES, VAZQUEZ, PANICKER, MILEDI, 1998). It has been proposed that $GABA_C$ receptors should be classified as a specialized set of the $GABA_A$ receptors (see BARNARD, SKOLNICK, OLSEN, MÖHLER, SIEGHART, BIGGIO, BRAESTRUP, BATESON and LANGER, 1998).

The $GABA_A$ receptors are of great importance as they participate in the regulation of brain excitability, and many important drugs such as benzodiazepines, barbiturates, neurosteroids, ethanol, and some of the anticonvulsants and general anaesthetics interact with these receptors so as to bring about their pharmacological effects (MACDONALD and TWYMAN, 1992; MACDONALD and OLSEN, 1994).

Table 4.1. Summary of different GABA receptors (adapted from SILVILOTTI and NISTRI, 1991).

Receptors	$GABA_A$	$GABA_B$	$GABA_C$
Primary location	frontal cortex, cerebella granule cell layer, olfactory bulb, etc.	cerebella molecular layer, frontal cortex and thalamic nuclei	retina
Ion channels	ionotropic Cl^-	metabolic G-protein-coupled K^+	ionotropic Cl^-
Protein subunits	α_{1-6}, β_{1-4}, γ_{1-4}, δ, ε and π	R_{1a}, R_{1b}	ρ_{1-3}
Agonist	GABA Muscimol (hallucinogenic, spasmodic and toxic isolated from *Amanita muscaria* and *A. pantherina*) Isoguvacine (synthetic)	GABA (-)baclofen (synthetic skeletal muscle relaxant)	GABA CACA (*cis*-4-aminocrotonic acid) TACA
Antagonists	Bicuculine (antiseptic, convulsant and haemostatic isolated from *Adlumia fungosa, Corydalis incise, C. thalictrifolia* and *Hydrastis canadensis*) Picrotoxin (CNS stimulant, barbiturate antidote, insecticide and nematocide isolated from *Anamirta paniculata* and *Tinomiscium philippinense*)	Phaclofen Saclofen	3-APA (3-aminopropylphosphonic acid) and 3-APPA (3-aminopropylphosphinic acid)
Allosteric modulator	Benzodiazepines eg diazepam, barbiturates, neurosteroids etc	none	none
Receptor structure	Hetero-oligomeric pentamers, each subunit with 4 transmembrane domains	7 transmembrane domains couple with G-protein	Homo-oligomeric pentamers, each subunit with 4 transmembrane domains

4.2. The $GABA_A$ receptor

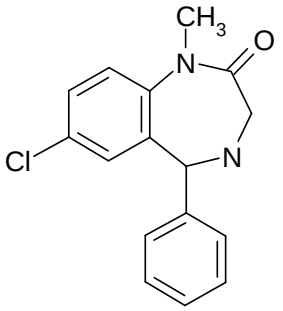
As with glycine, aspartate, glutamate, acetylcholine and 5-HT_3 receptors, the $GABA_A$ receptor belongs to the receptor family coupled to an ionophore which is an integral part of a transmembrane hetero-oligomeric protein. $GABA_A$ receptors are ligand-gated Cl^- channels. Its neurotransmitter GABA is a small amino acid derived from glutamate by glutamic acid decarboxylase. Activation of $GABA_A$ receptors by GABA results in an increase in neuronal membrane conductance for Cl^- from the prolonged openings of the ion channels, this influx of Cl^- causes a localized hyperpolarisition of the neuronal membrane, which counteracts the effects of depolarising stimuli and results in the inhibition of synaptic transmission (MACDONALD and TWYMAN, 1992).

The inhibitory action of GABA can be imitated by full agonists, such as muscimol, a natural product (**Tables 4.1. and 4.2.**) from the mushroom *Amanita muscaria.* It is also competitively antagonised by competitive antagonists, such as bicuculline, another natural product isolated from the plant *Dicentra cucullaria* and a variety of *Corydalis*, *Dicentra* and *Adlumia* species, and noncompetitively antagonised by picrotoxin which is an equimolar mixture of picrotoxinin and picrotin isolated from *Anamirta cocculus* and related poisonous plants.

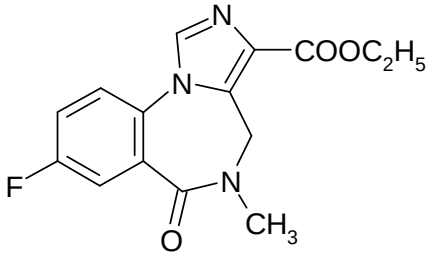
Structurally different compounds interact with the GABA binding site within the $GABA_A$ receptor complex as agonist, partial agonist and antagonist (**Table 4.1. and 4.2.**). Receptor antagonists have been shown to be essential tools for the characterisation of the physiological and pharmacological properties of receptors. The discovery of the convulsant alkaloid bicuculline as an antagonist of the inhibitory action of GABA in the CNS provided a vital pharmacological tool to investigate GABA-mediated inhibition.

The most notable phenomenon of $GABA_A$ receptors is that they possess a variety of allosteric binding sites (i.e. binding sites that are different from the neurotransmitter binding sites within the receptor complex) for several clinically important drugs. The most widely investigated allosteric modulatory sites are the benzodiazepine sites (LÜDDENS, KORPI and SEEBURG, 1995). Benzodiazepines were launched as clinical therapeutic agents in the early 1960s before GABA was considered as a neurotransmitter.

Benzodiazepine receptors are divided into two classes: central (receptors appeared in CNS) and peripheral receptors (peripheral tissues such as kidneys). Flumazenil (Ro 15-1788) only interacts with central receptors, while Ro 5-4868 (the 4'-chloro derivative of diazepam) is a selective ligand for peripheral receptors. Only the central benzodiazepine receptors are associated with $GABA_A$ receptors.



Diazepam (Central benzodiazepine receptor agonist)



Flumazenil (Ro 15-1788)

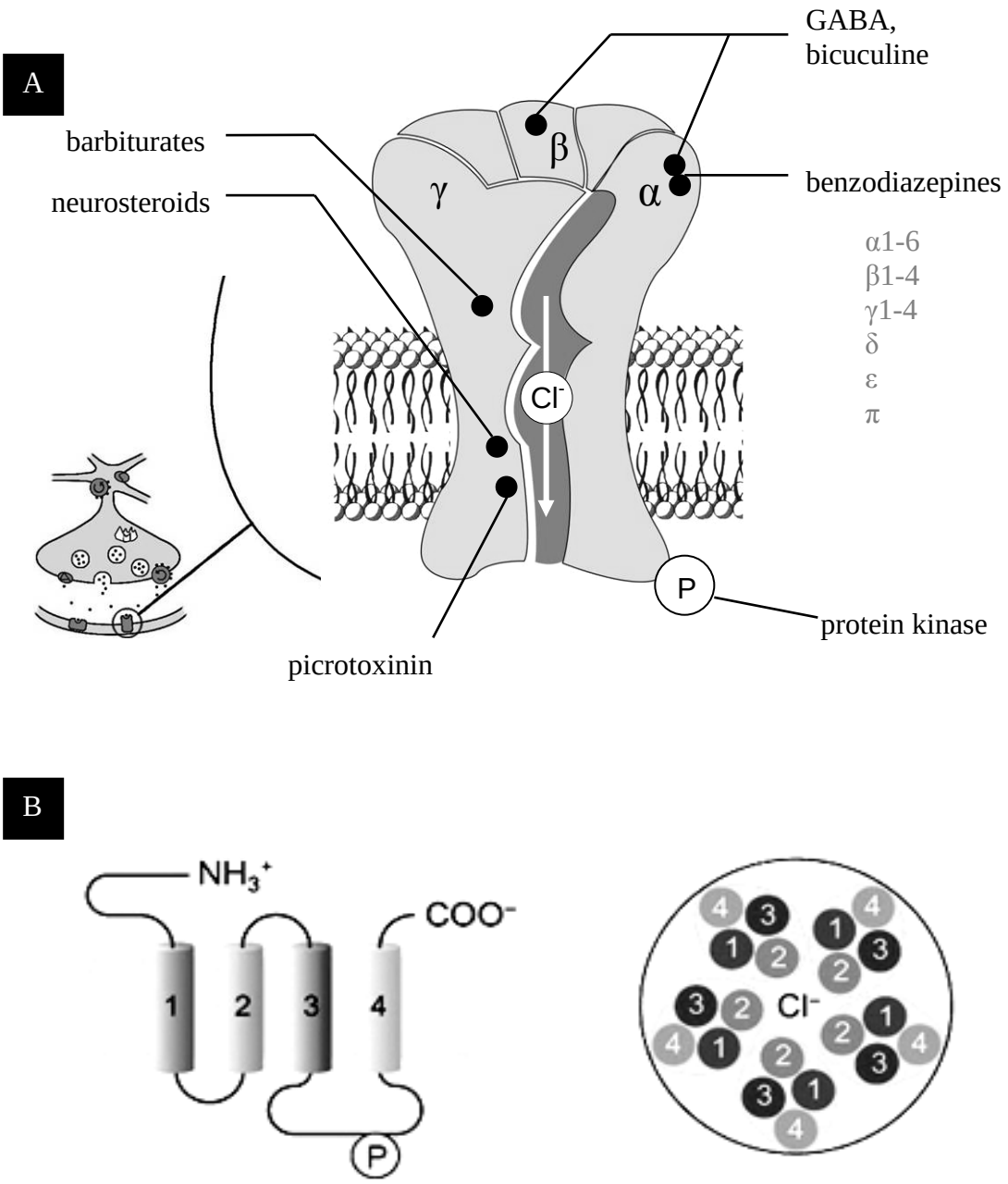


Figure 4.1. Schematic illustration of the $GABA_A$ receptor. [A] The $GABA_A$ receptor, which is a Cl^- pore, has binding sites for barbiturates, benzodiazepines and neurosteroids. The GABA responses are blocked competitively by bicuculline and non-competitively by picrotoxinin. The GABA (e.g. muscimol, bicuculline and 4-PIOL) and benzodiazepine (e.g. diazepam and flumazenil) recognition sites are known to be extracellular, whereas several other sites (e.g. barbiturates, neurosteroids, and alcohol) may be mostly at the transmembrane regions. Zn2+, La3+, picrotoxinin and TBPS most likely bind to sites in the channel proper. The vertebrate $GABA_A$ receptor is built from several subunits, some examples of which are listed on the right-hand side (in gray text). [B] Each subunit comprises four transmembrane domains.

4.3. $GABA_A$ receptors and neurological disease

Anticonvulsant and epilepsy treatment

A deficiency in GABA-nergic inhibitory synaptic transmission can potentially contribute to the neuronal epileptic activity and the spread of focal seizure activity (OLSEN, BUREAU, HOUSER, DELGADO-ESCUETA, RICHARDS and MOHLER, 1992). Compounds that elevate the synthesis, synaptic release, postsynaptic action of GABA can exhibit anticonvulsant activity. Many antiepileptic drugs exert their action by enhancing the brain GABA activity (e.g. benzodiazepines, barbiturates, vigabatrin) (EMILIEN and MALOTEAUX, 1998). The changes in GABA-nergic inhibition in human epilepsy are controversial. The functional GABA-nergic inhibition can be reduced, normal or slightly enhanced (MODY, 1998).

The prevalence of epilepsy in developing countries is generally higher than in developed countries (SANDER and SHORVON, 1996). A study conducted nearly a decade ago reported an increased risk of dying and a greater proportion of deaths that are epilepsy-related in Africa – as high as a six-fold increase in mortality in people with epilepsy. This is higher than the two- to three-fold increase reported in developed countries (CHRISTIANSON, ZWANE, MANGA, ROSEN, VENTER and KROMBERG, 2000; DIOP, HESDORFFER, LOGROSCINO and HAUSER, 2005). The reasons for this gap between the developed and the developing countries are not entirely clear but suggestions have been made that it might be due to social deprivation (SANDER, 2003). Interestingly, recent data suggest that people from socio-economically deprived backgrounds in developed countries are more likely to develop epilepsy (HEANEY, MACDONALD, EVERITT, STEVENSON, LEONARDI, WILKINSON and SANDER, 2002). A study from 2000 on the prevalence of epilepsy in a large rural community situated in the Northern Province in South Africa showed a lifetime prevalence in children as high as 73/1000 (CHRISTIANSON, ZWANE, MANGA, ROSEN, VENTER and KROMBERG, 2000).

Some infectious diseases might be a part of the explanation. Neurocysticercosis caused by *Taenia solium* (pork tapeworm) infections may trigger epileptic outburst (DEGIORGIO, PIETSCH-ESCUETA, TSANG, CORRAL-LEYVA, NG, MEDINA, ASTUDILLO, PADILLA, LEYVA, MARTINEZ, NOH, LEVINE, VILLASENOR and SORVILLO, 2005). A study on 578 epileptic patients in Pretoria showed neurocysticercosis in 28% of the patients (VAN AS and JOUBERT, 1991). HIV infection of the CNS or opportunistic infections caused by HIV might also trigger epileptic seizures (GARG, 1999; VISUDTIBHAN, VISUDHIPHAN and CHIEMCHANYA, 1999).

As mentioned in **Chapter 2** epilepsy is one of the illnesses that is viewed with a certain degree of fear and 'risk of contagious' effect due to the cultural attitudes and beliefs in South Africa. Studies conducted in townships showed that the parents of epileptic children believe that the disorder is caused by various parameters including bewitchment, fear or evil spirits (EASTMAN, 2005). It is also viewed as a shameful disorder and has severe social implications in African communities as it carries a stigma, a similar

problem exists with HIV. Sufferers are often shunned and discriminated against with respect to education, employment and marriage (ANDERMANN, 1995; BASKIND and BIRBECK, 2005).

This traditional African perception of epilepsy is also prominent in the treatment of the disorder where many people seek treatment by traditional healers and traditional medicine. There are also a large number of traditional treatments for epilepsy (see **Chapter 2**). A survey in 2000 showed, that 42.5% of epileptic children received traditional medicine alone or in combination with Western medicine whereas 34.6% received no treatment at all (CHRISTIANSON, ZWANE, MANGA, ROSEN, VENTER and KROMBERG, 2000).

A number of southern African plants have shown *in vivo* anticonvulsant activity against seizures produced in mice by pentylenetetrazole (PTZ), picrotoxin (PIC), bicuculline (BIC) and NMDA. However, most of the studies have been conducted on plant extracts and the active constituents are yet to be identified. Recently, OJEWOLE (2008c) reported on the anticonvulsant effect of *Searsia chirindensis* (Baker f.) Moffett (syn = *Rhus chirindensis* Baker f.) stem-bark aqueous extract in mice. *Searsia chirindensis* stembark aqueous extract (100–800mg/kg i.p.) significantly delayed the onset of, and antagonized PTZ induced seizures. The extract (100–800mg/kg i.p.) also profoundly antagonized PIC induced seizures, but only weakly antagonized BIC induced seizures.

Ojewole and his group have conducted several *in vivo* studies on extracts from South African medicinal plants including the fruit of *Tetrapleura tetraptera* (Schum.et Thonn.) Taub. (Fabaceae) (OJEWOLE, 2005), avocado leaf (*Persea americana* Mill. (Lauraceae)), (OJEWOLE and AMABEOKU, 2006), *Harpagophytum procumbens* D.C. ex Meisn. (Pedaliaceae) roots (MAHOMED and OJEWOLE, 2006), *Sutherlandia frutescens* (L.) R.Br. (variety *Incana* E.Mey.) (Fabaceae) shoots (OJEWOLE, 2008a) and *Hypoxis hemerocallidea* Fisch.Mey. & Ave-Lall. (Hypoxidaceae; Syn = *Hypoxis rooperii* T. Moore) corms (misleadingly called the ‘African Potato’) (OJEWOLE, 2008b). All these studies shared the interesting fact that the aqueous extracts of these various plants delayed and antagonized the onset of PTZ and PIC induced seizures while the effect on BIC induced seizures was weaker and only present in high doses.

Several Amaryllidaceae alkaloids isolated from *Crinum* and *Cyrthanthus* species were screened for potential activity in the $GABA_A$-benzodiazepine binding assay (ELGORASHI, STAFFORD, JÄGER and VAN STADEN, 2006). However, none of the tested alkaloids displayed any affinity to the binding site. Further plant derived compounds known to interact with the $GABA_A$-receptor are given in **Table 4.2**.

4.4. Screening of southern African plants for benzodiazepine-$GABA_A$ receptor binding activity

4.4.1. Introduction and Summary

Initially, the aqueous and ethanol extracts of 43 traditional South African plants medicinal used to treat epilepsy and convulsions, were tested in the $GABA_A$-benzodiazepine receptor binding assay (RISA, RISA, ADSERSEN, GAUGUIN, STAFFORD, VAN STADEN and JÄGER, 2004). A second screening of 46 ethanol extracts from 35 species, both indigenous and exotic that are traditionally used predominantly as sedatives or to treat various CNS-related ailments were tested in the $GABA_A$-benzodiazepine receptor-binding assay (STAFFORD, JÄGER and VAN STADEN, 2005). These investigations lead to the screening, isolation and identification of active compounds from *Searsia* species (previously *Rhus*) (SVENNINGSEN, DAMKJÆR MADSEN, LILJEFORS, STAFFORD, VAN STADEN and JÄGER, 2006) and *Mentha aquatica* (JÄGER, ALMQVIST, VANGSØE, STAFFORD, ADSERSEN and VAN STADEN, 2007).

Table 4.2. Compounds known to interact with ionotropic γ-aminobutyric acid (GABA) and benzodiazepine receptors.

Benzodiazepine receptors can be divided into two classes: "central" and "peripheral" receptors, referring to the receptors appearing in CNS and peripheral tissues such as the kidney, respectively. Flumazenil (Ro 15-1788) only interacts with the "central" receptors, while Ro 5-4868, the 4'-chloro derivative of diazepam, is a selective ligand for the "peripheral" receptors. Only the central BZD receptors are associated with $GABA_A$ receptors (after POLYA, 2003). Initially

Compound (class)	***Species* (Family)**	**Target (biological activity)**
Central $GABA_A$/benzodiazepine binding		
Alkaloid		
Delorazepam (benzodiazepine)	*Artemisia dracunculus* (Asteraceae); synthesised	Benzodiazepine receptor agonist (sedative, tranquilizer)
Diazepam (benzodiazepine)	Synthetic; but found in low quantities in germinating seeds of *Triticum aestivum* (Poaceae), *Solanum tuberosum* (Solanaceae)	Central benzodiazepine receptor agonist (IC_{50} = 18 nM ; K_d = 10 nM) (sedative, tranquilizer)
Harmaline (= 3,4-Dihydroharmine; Harmidine) (indole)	*Banisteria caapi* (Malpighiaceae), *Passiflora incarnate* (Passifloraceae), *Peganum harmala* (Zygophyllaceae)	Central benzodiazepine receptor agonist (Flunitrazepam displacement) (IC_{50} ~ 100 μM) (ataxic, excitatory, hallucinogenic, tremorigenic)
Harmalol (β-carboline, indole)	*Apocynum cannabium* (Apicynaceae), *Hippophae rhamoides* (Eleagnaceae), *Banisteria caapi* (Malpighiaceae), *Passiflora* spp. (Passifloraceae), *Peganum harmala* (Zygophyllaceae)	Central benzodiazepine receptor agonist
Harman (= Aribine; Loturine; 1-Methyl-β-carboline; Passiflorin) (β-carboline, indole)	*Cichorium intybus* (Asteraceae), *Eleagnus angustifolia* (Eleagnaceae), *Passiflora incarnate* (Passifloraceae), *Sickingia* (=*Arariba*) *rubra* (Rubiaceae), *Symplocos racemosa* (Symplocaceae), *Peganum harmala*, *Tribulus terrestris*, *Zygophyllum fabago* (Zygophyllaceae); smoke of tobacco *Nicotiana tabacum* (Solanaceae)	Central benzodiazepine receptor agonist (co-mutagenic, convulsant, cytoxic, genotoxic, motor depressant, DNA intercalator, vasorelaxant, sheep '*Tribulus* staggers')
Harmine[1] (=Banisterine; Leucoharmine; Telepathine; Yageine) (β-carboline, indole)	*Passiflora incarnata* (Passifloraceae), *Banisteria caapi* (Malpighaceae), *Peganum harmala*, *Tribulus terrestris* (Zygophyllaceae)	Central benzodiazepine receptor agonist
Lormetazepam (benzodiazepine)	Synthetic; found in low quantities in germinating tuber of *Solanum tuberosum* (Solanaceae)	Benzodiazepine receptor agonist (sedative, tranquilizer)

[1] Hallucinogen, used by Gestapo as a 'truth drug'.

Compound (class)	***Species* (Family)**	**Target (biological activity)**
Norharman (= β-Carboline) (β-Carboline, indole)	*Cichorium intybus* (Asteraceae), *Tribulus terrestris*, *Zygophyllum fabago* (Zygophyllaceae); smoke of tobacco *Nicotiana tabacum* (Solanaceae)	Central benzodiazepine receptor
Ricinine (dihydropyridine)	*Ricinus communis* (Euphorbiaceae)	Benzodiazepine receptor ligand (Flunitrazepam displacement) (convulsant, hypotensive, respiratory depressant, toxic)
Tabernanthine (= 13-Methoxyibogamine) (indole)	*Tabernanthe iboga*, *Conopharyngia* (*Tabernaemontana*) sp., *Stemmadenia* sp. (Apocynaceae)	Benzodiazepine receptor agonist (Flunitrazepam displacement) (IC_{50} = 150 μM)
Temazepam (benzodiazepine)	Synthetic; found in low quantities in *Artemisia dracunculus* (Asteraceae); *Solanum tuberosum* (Solanaceae)	Benzodiazepine receptor agonist (hypnotic, sedative)
Phenolic		
Amethoflavone (= 3',8"-Biapigenin) (biflavone)	*Viburnum prunifolium* (Caprifoliaceae), *Cycas revolute* (Cycadaceae), *Searsia* spp., (Anacardiaceae), *Ginkgo biloba* (Ginkgoaceae), *Hypericum* spp. (Hypericaeae), *Podocarpus montanus* (Podocarpaceae)	Central benzodiazepine receptor partial agonist (IC_{50} = 6-15 nM)
Apigenin (= 5,7,4'-Trihydroxyflavone) (flavone)	Widespread; Lamiaceae, Asteraceae; Apiaceae; Fabaceae	Central benzodiazepine receptor agonist (IC_{50} = 4 μM)
Baicalein (= 5,6,7-Trihydroxyflavone) (flavone)	*Scutellaria baicalensi*, *S.* spp. (Lamiaceae), *Plantago major* (Plantaginaceae)	Central benzodiazepine receptor ligand
Byakangelicol (furanocoumarin)	*Angelica dahurica*, *Ferula* spp. (Apiaceae), *Citrus limon* (Rutaceae)	Central benzodiazepine receptor ligand (Diazepam displacement) (IC_{50} = 12 μM)
2,5-Dihydroxy-7-methoxy-6,8-dimethylflavan-3-one (flavan-3-one)	*Leptospermim scoparium* (Myrtaceae)	$GABA_A$ receptor central benzodiazepine receptor ligand
5,7-Dimethoxyflavone (flavone)	*Leptospermim scoparium* (Myrtaceae)	Central benzodiazepine receptor ligand (Flunitrazepam displacement) (IC_{50} = 2 μM)
5,7-Dimethoxy-6-methylflavone (flavone)	*Leptospermim scoparium* (Myrtaceae)	Central benzodiazepine receptor ligand (Flunitrazepam displacement) (IC_{50} = 45 μM)
Dinatin (= Hispidulin; 6-Methoxy-5,7,4'-Trihydroxyflavone; Scutellarein 6-methyl ether) (flavone)	*Artemisia herba alba* (Asteraceae), peel of *Citrus sudachii* (Rutaceae), leaf of *Digitalis orientalis* and *D. purpurea* (Scrophulariaceae)	Central benzodiazepine receptor ligand (Diazepam displacement) (IC_{50} = 1 nM)

Compound (class)	***Species* (Family)**	**Target (biological activity)**
5,7-Dihydroxyflavone (= Chrysin) (flavone)	Widespread; *Daucus* (Apiaceae), *Spartium* (Fabaceae), *Scutellaria* (Lamiaceae), *Passiflora* (Passifloraceae), *Pinus* (Pinaceae), *Prunus* (Rosaceae), *Populus* (Salicaceae), *Escallonia* (Saxifragaceae) spp.	Central benzodiazepine receptor agonist (K_d = 3 μM) (non-amnestic anxiolytic)
5-Hydroxy-7-methoxy-6-methylflavone (flavone)	*Leptospermum scoparium* (Myrtaceae)	Central benzodiazepine receptor ligand (Flunitrazepam displacement) (IC_{50} = 3 μM)
5-Hydroxy-7-methoxy-6,8-dimethylflavone (flavone)	*Leptospermum scoparium* (Myrtaceae)	Central benzodiazepine receptor ligand (Flunitrazepam displacement) (IC_{50} = 40 μM)
1-Hydroxypinoresinol (lignan)	*Nothapodytes foetida* (Icacinaceae), *Valeriana officinalis* (Valerianaceae)	Central benzodiazepine receptor ligand
Imperatorin (furanocoumarin)	*Ammi majus, Pastinaca sativa* (Apiaceae), *Angelica dahurica* (Asteraceae)	Central benzodiazepine receptor ligand (Diazepam displacement) (IC_{50} = 8 μM)
Kaempferol 4'-*O*-methyl ether (flavonol)	*Pityrogramma* (fern) (Adiantaceae), *Baccharis* (Asteraceae), ***Prunus*** spp. (Rosaceae), *Linaria* spp. (Scrophulariaceae),	Benzodiazepine receptor ligand (K_d = 93 μM)
Oroxylin A (flavonol)	*Scutellaria baicalensis, S. galericulata* (Lamiaceae)	Central benzodiazepine receptor ligand (K_d = 15 μM)
Phellopterin (furanocoumarin)	*Angelica* spp., *A. dahurica, Ferula alliacae* (Apiaceae), *Citrus limon* (Rutaceae)	Central benzodiazepine receptor ligand (Diazepam displacement) (IC_{50} = 0.4 μM)
Skrofulein (=4',5-Dihydroxy-6,7-dimethoxyflavone) (flavone)	*Artemisia herba alba* (Asteraceae)	Central benzodiazepine receptor ligand (Diazepam displacement) (IC_{50} = 23 nM)
Skullcapflavone II (=5,1'-Dihydroxy-6,7,8,5'-tetramethoxyflavone) (flavone)	*Scutellaria baicalensis* (Lamiaceae)	Benzodiazepine receptor ligand (K_d = 0.4 μM)
Terpene		
Cryptotanshinone (diterpene quinine, tanshinone)	*Salvia miltorrhiza* (Lamiaceae) See Chapter 1 (pages 30-33) for additional related chemicals from *Salvia* spp.	Central benzodiazepine receptor partial agonist (Flunitrazepam competition) (IC_{50} = 2 μM) (tranquilizer)
Isocurcumenol (sesquiterpene)	*Cyperus rotundus* (Cyperaceae)	Central benzodiazepine agonist
Majonoside-R2 (triterpene saponin)	*Panax ginsing* (Araliaceae)	Central benzodiazepine agonist

Compound (class)	***Species* (Family)**	**Target (biological activity)**
Non-plant references		
Flumazenil (benzodiazepine)	Synthetic	Central benzodiazepine receptor agonist (tranquilizer)
Flunitrazepam (benzodiazepine)	Synthetic	Central benzodiazepine receptor agonist (K_d = 4 nM) (hypnotic, tranquilizer)
$GABA_A$ -receptor binding to sites other than the benzodiazepine site		
Alkaloid (+)-Bicuculline (phthalide isoquinoline)	*Adlumia fungosa, Corydalis incise, C. thalictrifolia, Dicentra cucullaria* (Papaveraceae), *Hydrastis canadensis* (Ranunculaceae)	$GABA_A$-receptor antagonist (IC_{50} = 20 μM) (antiseptic, convulsant, haemostatic)
Chelerythrine (benzophenanthridine)	*Chelidonium majus, Argemone, Bocconia, Eschscholzia, Glaucium, Sanguinaria* spp. (Papaveraceae), ***Zanthoxylum*** spp. (Rutaceae)	$GABA_A$-receptor ligand (IC_{50} = 25 μM)
Cocaine (=benzoylmethylecgonine) (tropane)	*Erythroxylum coca, E.* spp. (Erythroxylaceae)	$GABA_A$-receptor current block (IC_{50} = 130 μM)
Colchicine (tricyclic)	*Colchicum autumnale, C.* spp., ***Gloriosa superba*** (Liliaceae)	$GABA_A$-receptor antagonist (IC_{50} < 100 μM)
Corymine (indole)	*Hunteria zeylanica* (Apocynaceae)	$GABA_A$-receptor antagonist (IC_{50} < 100 μM)
Deformylcorymine (indole)	*Hunteria zeylanica* (Apocynaceae)	$GABA_A$-receptor (current inhibition)
N-Demethl-3-epi-dihydrocorymine (indole)	*Alstonia glaucescens* (Apocynaceae)	$GABA_A$-receptor antagonist (IC_{50} < 100 μM)
(+)-Hydrastine (phthalide isoquinoline)	*Berberis vulgaris, Mahonia aquifolium* (Berberidaceae), *Corydalis stricta* (Papaveraceae), *Hydrastis canadensis* (Ranunculaceae)	$GABA_A$-receptor antagonist (IC_{50} = 2 μM) GABA stimulated Diazepam binding (IC_{50} = 0.4 μM) (antiseptic, convulsant, haemostatic
Isocoryne (phthalide isoquinoline)	*Corydalis pseudoadunca* (Papaveraceae)	$GABA_A$-receptor inhibitors (blocks GABA induced current) (K_d = 1 μM)
Laudanosine (= Laudanine methyl ether) (benzylisoquinoline)	*Papaver somniferum* (Papaveraceae); metabolic product of synthetic skeletal muscle relaxant Atracurium	$GABA_A$-receptor antagonist (IC_{50} < 100 μM)

Compound (class)	***Species* (Family)**	**Target (biological activity)**
Protopine (= Biflorine; Corydalis C; Corydinine; Fumarine; Macleyine) (benzylisoquinoline)	*Chelidonium majus, Argemone, Bocconia, Corydalis, Eschscholzia, Glaucium, Macleaya, Papaver somniferum, Sanguinaria* spp. (Papaveraceae), *Fumaria officinalis* (Fumariaceae)	$GABA_A$-receptor ligand (Muscimol displacement) (sedative, relaxant)
Sanguinarine (= Pseudochelerythrine) (benzophenanthridine)	*Chelidonium majus, Argemone, Bocconia, Corydalis, Eschscholzia, Glaucium, Macleaya, Papaver somniferum, Sanguinaria* spp. (Papaveraceae), *Fumaria officinalis* (Fumariaceae), *Zanthoxyllum* (Rutaceae), *Pteridophyllum* spp. (Sapindaceae)	$GABA_A$-receptor ligand (Muscimol displacement) (IC_{50} = 39 μM)
Securinene (piperidinepyrroldine)	*Phyllanthus discoides, Securinega suffraticosa* (Euphorbiaceae), ***Securidaca longepedunculata*** (Fabaceae)	$GABA_A$-receptor antagonist
(+)-Tubocurarine (= curare active principle) bisbenzylisoquinoline)	*Chondrodendron tomentosum, C.* spp. (Menispermaceae)	$GABA_A$-receptor antagonist (skeletal muscle relaxant)
Phenolic		
Daidzein (= 4',7-Dihydroxyisoflavone) (isoflavone)	*Glycine max, Trifolium repens, Phaseolus, Psoralea, Pueraria, Sophora, Ulex, Vigna* spp. (Fabaceae)	($GABA_A$-receptor) (inactive as tyrosine kinase inhibitor cf. Genistein)
Desmethoxyyangonin (phenolic-derived dienolide lactone, kavapyrone)	*Piper methysticum* (Piperaceae)	Inactive as $GABA_A$-receptor modulatory agonist (cf. Yangonin and Kawain)
(+)-Dihydrokavain (= Dihydrogonosan; Dihydrokawain) (phenolic-derived lactone, kavapyrone)	*Piper methysticum* (Piperaceae)	$GABA_A$-receptor modulatory agonist; increases Bicuculline-binding (at IC_{50} = 10 μM); no binding to benzodiazepine receptor (anxiolytic)
(+)-Dihydromethysticin (phenolic-derived lactone, kavapyrone)	*Piper methysticum* (Piperaceae)	$GABA_A$-receptor modulatory agonist; increases Bicuculline-binding (at IC_{50} = 0.1 μM); no binding to benzodiazepine receptor (anxiolytic)
Genistein (= Genisteol; prunetol; Sophoricol; 4',5,7-trihydroxyisoflavone) (isoflavone)	Widespread: *Genista, Glycine, Phaseolus, Trifolium* spp. (Fabaceae), *Prunus* spp. (Roseaceae)	$GABA_A$-receptor (non-competitive antagonist)
Honokiol (lignan)	*Magnolia officinalis, M. obovata* (Magmoliaceae)	$GABA_A$-receptor (allosteric potentiating ligand) (IC_{50} = 8 μM) (anxiolytic, central depressant)
(+)-Kawain (= Gonosan; Kavain) (phenolic-derived lactone, kavapyrone)	*Piper methysticum* (Piperaceae)	$GABA_A$-receptor agonist (allosteric potentiating ligand) (at IC_{50} = 0.1 μM) no binding to benzodiazepine receptor (anxiolytic)
Magnolol (lignan)	*Sassafras randaiense* (Lauraceae); *Magnolia officinalis, M. obovata* (Magmoliaceae)	$GABA_A$-receptor (allosteric potentiating ligand) (IC_{50} = 6 μM) (anxiolytic, central depressant)

Compound (class)	***Species* (Family)**	**Target (biological activity)**
(+)-Methysticin (phenolic-derived lactone, kavapyrone)	*Piper methysticum* (Piperaceae)	$GABA_A$-receptor modulatory agonist (at IC_{50} = 0.1 μM) no binding to benzodiazepine receptor (anxiolytic)
Yangonin (phenolic-derived dienolide lactone, kavapyrone)	*Piper methysticum* (Piperaceae)	$GABA_A$-receptor modulatory agonist (at IC_{50} = 1 μM) no binding to benzodiazepine receptor (anxiolytic)
Terpene		
Anisatin (sesquiterpene lactone)	*Illicium anisatum* (Illiciaceae)	$GABA_A$-receptor non-competitive antagonist (IC_{50} = 0.4-1 μM); binds to Picrotoxinin site)
Carnosic acid (diterpene)	*Salvia officinalis* (Lamiaceae)	$GABA_A$-receptor chloride channel blocker; *tertiary*-butylbicyclophosphorothioate (TBPS) binding (IC_{50} = 33 μM)
Carnosol (abietane diterpene)	*Rosmarinus officinalis, Salvia officinalis* (Lamiaceae)	$GABA_A$-receptor chloride channel blocker; *tertiary*-butylbicyclophosphorothioate (TBPS) binding (IC_{50} = 57 μM)
Coriamyrtin (tutinolide sesquiterpene lactone)	*Coriaria japonica, C. myrtifolia* (Coriariaceae)	$GABA_A$-receptor antagonist (IC_{50} = 10-30 μM)
Dihydrotutin (tutinolide sesquiterpene lactone)	*Picrodendron baccatum* (Euphorbiaceae)	$GABA_A$-receptor non-competitive antagonist
Ginsenosides (triterpene saponin)	*Panax ginseng* (Araliaceae)	$GABA_A$-receptor ligand (Muscimol displacement)
Horminone (diterpene)	*Salvia deserta, Plectranthus hereroensis* (Lamiaceae)	$GABA_A$-receptor chloride current inhibition (IC_{50} = 10 μM)
Isohyenanchine (tutinolide sesquiterpene lactone)	*Picrodendron baccatum* (Euphorbiaceae)	$GABA_A$-receptor non-competitive antagonist
Picodendrins (tutinolide sesquiterpene lactone)	*Picrodendron baccatum* (Euphorbiaceae)	$GABA_A$-receptor non-competitive antagonist (IC_{50} = ±1 μM)
Picrotin (tutinolide sesquiterpene lactone)	*Anamirta paniculata* (= *A. cocculus; Menispermum occulus*), *Tinomiscium philippinense* (Menispermaceae)	$GABA_A$-receptor non-competitive antagonist (CNS-stimulant, barbiturate antidote)
Picrotoxin (= mixture of Picrotin and Pictotoxinin) (tutinolide sesquiterpene lactone)	*Anamirta paniculata* (= *A. cocculus; Menispermum occulus*), *Tinomiscium philippinense* (Menispermaceae)	$GABA_A$-receptor antagonist (IC_{50} = 0.2 μM); $GABA_A$-receptor chloride current inhibition (IC_{50} = 1 μM) (CNS-stimulant, barbiturate antidote)
Picrotoxinin (= Dehydropicrotin) (tutinolide sesquiterpene lactone)	*Salvia deserta* (Lamiaceae), *Anamirta paniculata* (= *A. cocculus; Menispermum occulus*), *Tinomiscium philippinense* (Menispermaceae)	$GABA_A$-receptor non-competitive antagonist (CNS-stimulant, barbiturate antidote)
Taxodione (abietane diterpenoid)	*Taxodium distichum* (Taxodiaceae)	$GABA_A$-receptor chloride current inhibition (IC_{50} = 100 μM)

Compound (class)	***Species* (Family)**	**Target (biological activity)**
α and β-Thujone (monoterpenes)	*Artemisia absinthium*, *Tanacetum vulgare* (Asteraceae), *Thuja occidentalis* (Cupressaceae), *Salvia* spp. (Lamiaceae)	$GABA_A$-receptor antagonist/negative modulator (Cl^- channel block) (convulsant, hallucinogenic, intoxicant) Reported neurotoxic agent of Absinthe liqueur.
Tutin (tutinolide sesquiterpene lactone)	*Coriaria thymifolia* (Coriariaceae), *Picrodendron baccatum* (Euphorbiaceae)	
Non-plant references		
Baclofen (= β-(aminomethyl)-4-chlorobenzenepropanoic acid) (aryl amine)	Synthetic	$GABA_B$-receptor antagonist (skeletal muscle relaxant)
Isoguvacine (= Piperidine-4-carboxylic acid) (piperidine)	Synthetic	$GABA_A$-receptor agonist
Muscimol (= 5-Aminomethyl-3-hydroxyisoxazole) (isoxazole)	*Amanita muscaria* (fly agaric), *A. pantheria* (Amanitaceae) Highly poisonous hallucinogenic mushrooms	$GABA_A$-receptor agonist
Pentobarbital (pyrimidine trione; barbiturate)	Synthetic	$GABA_A$-receptor agonist (anaesthetic, anticonvulsant)
Phenobarbital (= 5-Ethyl-5-phenylbarbituric acid; phenylbarbitone) (pyrimidine; barbiturate)	Synthetic	$GABA_A$-receptor agonist (anticonvulsant, hypnotic, sedative)

4.4.2. Materials and Methods

Plant materials

Plant species traditionally used to treat epilepsy and convulsions, were selected based on information in a database on plants used to treat mental diseases, constructed at the Research Centre for Plant Growth and Development, University of KwaZulu-Natal (see **Table 4.4.2.1.)**. The information in the database mainly originates from published literature. Plants were collected in KwaZulu-Natal, mostly from the University of KwaZulu-Natal Botanical Garden. Voucher specimens are deposited in the University of KwaZulu-Natal Herbarium. All plant materials were dried at 50 °C.

Table 4.4.2.1. Plant species, family, plant parts and voucher numbers of material collected for screening in the [^{3}H]-Ro 15-1788 (flumazenil) binding assays.

Family *Plant species*	**Voucher specimen**	**Plant parts investigated**
Agapanthaceae		
Agapanthus campanulatus F.M.Leight.	*Stafford 59 NU*	leaves, flowers and roots
Agapanthus praecox Willd.		leaves, stems, flowers and roots
Amaryllidaceae		
Brunsvigia grandiflora Lindl.	*Stafford 10 NU*	leaves and bulbs
Scadoxus puniceus (L.) Friis & I. Nordal	*Stafford 206 NU*	leaves and roots
Anacardaceae [South African *Rhus* recently reclassified as *Searsia*]		
Searsia chirindensis Bak.f.	*Stafford 11 NU*	leaves
Searsia dentata Thunb.	*Stafford 205 NU*	leaves
Searsia pyroides Burch. (syn. *Searsia tridentata*)	*Stafford 13 NU*	leaves
Searsia rehmanniana Engl.	*Stafford 12 NU*	leaves
Searsia pentheri A. Zahlbr.	*Stafford 204 NU*	leaves
Apiaceae		
Arctopus echinatus L.	*Stafford 211 NU*	whole plant
Apocynaceae		
Acokanthera oblongifolia (Hochst.) Codd	*Stafford 14 NU*	leaves
Araliacea		
Cussonia paniculata Eckl. & Zeyh.	*Stafford 15 NU*	leaves
Cussonia spicata Thunb.	*Stafford 16 NU*	leaves
Asclepiaceae		
Gomphocarpus physocarpus E. Mey.	*Stafford 65 NU*	leaves
Xysmalobium undulatum (L.) Aiton.f.	*Stafford 95 NU*	aerial parts and roots
Asphodelaceae		
Bulbine frutescens (L.) Willd.	*Stafford 17 NU*	leaves and roots
Gasteria croucheri (Hook.f.) Baker	*Stafford 18 NU*	leaves
Asteraceae		
Artemisia afra Jacq. ex Willd.	*Stafford 63 NU*	leaves
Berkheya bergiana Soderb.	*Stafford 208 NU*	leaves
Berkheya montana J.M. Wood & M.S. Evans	*Stafford 209 NU*	leaves
Berkheya rhapontica (D.C.) Hutchinson & Burtt Davy	*Stafford 210 NU*	leaves and roots
Helichrysum argyrolepis MacOwan	*Stafford 213 NU*	leaves
Helichrysum hesbaceum (Andrews) Sweet	*Stafford 214 NU*	leaves
Helichrysum nudifolium Less.	*Stafford 215 NU*	leaves
Helichrysum ruderale Hilliard & B.L. Burtt	*Stafford 216 NU*	leaves
Helichrysum rugulosum Less.	*Stafford 217 NU*	leaves
Helichrysum simillimum DC.	*Stafford 218 NU*	leaves
Helichrysum umbraculigerum Less.	*Stafford 219 NU*	leaves

Family *Plant species*	**Voucher specimen**	**Plant parts investigated**
Berkheya bergiana Soderb.	*Stafford 208 NU*	leaves
Celastraceae		
Catha edulis (Vahl) S. Endlicher	*Stafford 220 NU*	leaves
Combretaceae		
Combretum bracteosum Brandis ex Eng.	*Stafford 19 NU*	leaves and roots
Combretum imberbe Wawra.	*Stafford 20 NU*	leaves and roots
Crassulaceae		
Cotyledon orbiculata L.	*Stafford 71 NU*	leaves
Dioscoreaceae		
Dioscorea dregeana Baker	*Stafford 221 NU*	leaves
Euphorbaceae		
Antidesma venosum E. Mey. Ex Tul.	*Stafford 21 NU*	roots
Croton sylvaticus Schlecht.	*Stafford 122 NU*	bark
Jatropha panduaeifolia Andr.	*Stafford 22 NU*	leaves and root bark
Jatropha zeyheri Sond.	*Stafford 23 NU*	leaves
Fabaceae		
Acacia sieberiana DC.	*Stafford 123 NU*	bark
Acacia xanthophloea Benth.	*Stafford 124 NU*	bark
Bauhinia galpinii N.E. Br.	*Stafford 24 NU*	leaves
Bauhinia tomentosa L.	*Stafford 25 NU*	leaves
Dichrostachys cinera Miq.	*Stafford 26 NU*	leaves
Indigofera tristis E.Mey.	*Stafford 27 NU*	leaves
Indigofera woodii Bolus	*Stafford 28 NU*	leaves
Millettia grandis Skeels	*Stafford 125 NU*	leaves
Millettia sutherlandii Harv.	*Stafford 126 NU*	leaves
Schotia brachypetala Sond.	*McGaw 85 NU*	leaves
Senna didymobotrya (Fresenius) N.W. Irwin & R.C. Barneby	*Stafford 29 NU*	leaves
Senna petersiana (Bolle) J.M. Lock	*Stafford 30 NU*	leaves
Sutherlandia frutescens R. Br.	*No voucher available*	leaves
Flacoutiaceae		
Oncoba spinosa Forssk.	*Stafford 31 NU*	leaves and roots
Hypoxidaceae		
Hypoxis angustifolia Lam.	*Stafford 32 NU*	leaves and corm
Hypoxis colchicifolia Bak.	*Stafford 33 NU*	leaves and corm
Hypoxis hemerocallidea Fisch. & C.A. Mey.	*Stafford 34 NU*	leaves, corm and roots
Lamiaceae		
Hoslundia opposita Vahl	*Stafford 35 NU*	leaves
Leonotis dubra E. Mey.	*Stafford 36 NU*	leaves
Leonotis intermedia Lindl.	*Stafford 37 NU*	leaves
Leonotis leonurus R.Br.	*Stafford 38 NU*	leaves
Mentha aquatica L.	*Stafford 84 NU*	leaves
Mentha longifolia Huds.	*Stafford 127 NU*	leaves
Salvia chamelaeagnea Berg.	*Stafford 39 NU*	leaves
Lauraceae		
Cinnamomum camphora (L.) Presl.	*Stafford 69 NU*	leaves
Loganiaceae		
Buddleja saligna Willd.	*Stafford 40 NU*	leaves
Buddleja salviifolia Lam.	*Stafford 41 NU*	leaves
Malvaceae		
Malva parviflora L.	*Stafford 57 NU*	leaves
Meliaceae		
Ekebergia capensis Sparrm.	*Stafford 42 NU*	leaves and bark
Periplocaceae		
Mondia whitei (Hook.f.) Skeels	*Stafford 43 NU*	leaves and bark
Phytolaccaceae		
Phytolacca octandra L.	*Stafford 88 NU*	aerial parts
Piperaceae		
Piper capense L.	*Stafford 89 NU*	leaves and roots

Family *Plant species*	**Voucher specimen**	**Plant parts investigated**
Rosaceae		
Rubus ludwigii Eckl. et Zeyh.	*Stafford 44 NU*	leaves, fruit and roots
Rubus phoeniculacius Maxim	*Stafford 45 NU*	leaves, roots
Rubiaceae		
Catunaregam spinosa Thunb.	*Stafford 46 NU*	leaves and roots
Rutaceae		
Clausena anisata (Willd.) Hook.f.	*Stafford 47 NU*	leaves and bark
Ruta graveolens L.	*Stafford 48 NU*	leaves and stems
Zanthoxylum capense (Thunb.)Harv.	*Stafford 96 NU*	leaves
Solanaceae		
Datura ferox L.	*Stafford 72 NU*	leaves, flowers and seeds
Datura stramonium L.	*Stafford 73 NU*	leaves, seeds and seed pods
Verbenaceae		
Clerodendrum myricoides (Hochst.) Watke	*Stafford 50 NU*	leaves
Vitaceae		
Rhoicissus tomentosa (Lam.) Wild & Drum.	*Stafford 51 NU*	leaves
Rhoicissus tridentata (L.f.) Wild & Drum.	*Stafford 52 NU*	leaves

Extraction of plant materials for screening

Two grams of material were extracted with 20 ml ethanol for 60 min on an ultrasound bath, thereafter the extracts were filtered. The extracts were taken to dryness under reduced pressure. The residues were dissolved in ethanol, respectively, at 10mg/ml.

Isolation and identification of active constituents of Searsia pyroides (Rhus pyroides)

Grounded dry plant material (100 g) was extracted three times with 1000 ml of heptane for 60 min in an ultrasonic bath to remove non-active lipophilic compounds. The plant material was then dried on filter paper. The dried plant material was extracted with 3 x 1000 ml of 96 % ethanol for 60 min in an ultrasonic bath. The combined extracts were filtered and evaporated to dryness. The residue was redissolved in 1000 ml of ethyl acetate and subsequently partitioned against 3 x 1000 ml of water. The organic phase was evaporated to dryness.

The extracts were submitted to reversed-phase HPLC. A Phenomenex® LUNA C-18 column, 5 μm, 250 x 21 mm with a guard column (Phenomenex® LUNA C-18, 5 μm, 50 x 21 mm) was employed using Shimadzu LC-6A pumps, a Shimadzu SPD-M6A Photodiode Array UV-VIS detector and a Shimadzu SCL-6A system controller. HPLC fractionation was performed using a gradient of methanol and water (40-100 % methanol at t = 0-20 min and 100-40 % at t = 20-30 min). Both solvents contained 0.5 % (v/v) phosphoric acid (85 %). The flow-rate was 8 ml/min and detection was done at 200-400 nm. The fractions which eluted at 22.7 min (84 % methanol) and 25.4 min (68 % methanol) inhibited the ^{3}H-Ro 15-1788 (flumazenil) binding assay in a competitive manner and afforded agathisflavone **1** and amentoflavone **2**, NMR spectra were recorded on a Bruker Avance 400 MHz instrument. Apigenin - 4',5,7-trihydroxyflavon, ca. 95 %, was purchased from Sigma-Aldrich.

Isolation and identification of active constituents of Mentha aquatica

Aerial parts (>500 g dry weight) of *Mentha aquatica* L. (Lamiaceae) were collected at Cedara Agricultural College, Hilton, KwaZulu-Natal (29°32′S 30°17′E) in February 2006. A voucher specimen was deposited at the University of KwaZulu-Natal Herbarium, Pietermaritzburg (Voucher number, *Stafford* 84 NU). The material was dried in an oven at 50 ºC. For initial screening one g dried, powdered material was extracted with 10 ml petroleum ether, ethyl acetate, ethanol or water respectively for 30 min in an ultrasound bath. The extracts were filtered and taken to dryness under vacuum before being re-dissolved at 10 mg/ml in ethanol. Twenty g dried, powdered material was steam distilled with 500 ml distilled water for 4 h. The obtained essential oil was dissolved at 10 mg/ml in ethanol for initial screening.

Powdered dry leaf material (250 g) was steam distilled with 2500 ml distilled water for 2 h, yielding pale yellow oil. The oil was applied to a 80 g Silica 60 (0.040-0.063 mm) column (65 cm long, 2 cm diameter) and eluted with 100 ml of each combination of toluene:ethyl acetate (97.5:2.5; 95:5; 90:10; 85:15; 75:25; 50:50). Collected fractions were combined on basis of a TLC analysis, where the plate was eluted in toluene:ethyl acetate 93:7. The active fraction was subjected to GC-MS on an Agilent 6890N Network GC system coupled to a 5973 Network Mass Selective Detector. GC conditions: injector temperature: 150 ºC; temperature programme: start 50 ºC, 20 ºC/min to 300 ºC; column: HP5MS. A NIST library was used for comparison of MS data. The active fraction was also investigated by ^{13}C-NMR (Varian Mercury Plus 300 MHz; $CDCl_3$). Optical rotation was measured on a Perkin-Elmer 241 polarimeter.

For the isolation of active compound (naringenin) from *M. aquatica*, 15 g dried and powdered plant material (whole plant) was extracted with 150 ml ethanol for 30 min in an ultrasound bath. The extract was filtered, taken to dryness under vacuum, and then partitioned between ethyl acetate and water. The active ethyl acetate phase was subjected to solid-phase chromatography on a Bond Elut C2, eluted with water:methanol 3:1. This fraction was then applied to a prep TLC plate (Merck Silica gel 60, 0.5 mm thickness) and eluted in ethyl acetate:formic acid:glacial acetic acid:water (100:11:11:26). A strip at the side of the plate was sprayed with natural products - polyethylene glycol reagent. Bands were scraped off the plate and the silica eluted with ethanol. The active compound was subjected to ^{1}H-NMR (Varian Mercury Plus 300 MHz; $CDCl_3$) and optical rotation was determined on a Perkin-Elmer 241 polarimeter.

Rat tissue preparation

The tissue preparation was performed at 0–4 °C. Cerebral cortex from four rats was homogenised for 5 s in 20 ml of Tris–citrate (50 mM, pH 7.1) using an Ultra-Turrax. The suspension was centrifuged at 27 000 × g for 15 min, and the pellet was washed three times with buffer. The washed pellet was homogenised in 20 ml of buffer and the suspension was incubated in a water bath (37 °C) for 30 min to remove endogenous GABA. Then the suspension was centrifuged for 10 min at 27 000 × g. The final pellet was resuspended in 30 ml buffer and stored in 1 ml aliquots (in Eppendorf tubes) at −20 °C.

[^{3}H]-Ro 15-1788 (flumazenil) binding assay

The assay was carried out according to KAHNBERG, LAGER, ROSENBERG, SCHOUGAARD, CAMET, STERNER, NIELSEN, NIELSEN and LILJEFORS (2002). The membrane preparation was thawed and washed with 20 ml Tris–citrate (50 mM, pH 7.1, 0–4 °C). The suspension was centrifuged at 0–4 °C for 10 min at 27 000 × g. The pellet was resuspended in Tris–citrate (50 mM, pH 7.1, 2 mg original tissue per ml buffer), and then used for the binding assay. Membrane suspension (500 µl) was added to 25 µl test solution (plant extract/standard/blank) and 25 µl of flumazenil (Ro 15-1788, purchased from Perkin-Elmer Life Sciences) (0.5 nM, final concentration in assay), mixed and incubated for 40 min in an ice-bath (0–4 °C).

Non-specific binding was determined using clonazepam (1 µM, final concentration in assay) added to separate samples. After incubation 5 ml of ice-cold buffer were added to the samples and the mixture poured directly onto Adventic glass fibre filters (GC-50) under suction, and immediately washed with 5 ml of ice-cold buffer. The amount of radioactivity was determined by conventional liquid scintillation counting. Specific binding was calculated as total binding minus non-specific binding. All experiments were done in triplicate.

4.4.3. Results and Discussion

Forty three plants were initially selected for investigation as potential sources of antiepileptic compounds. A total of 116 extracts were tested in the ^{3}H-flumazenil binding assay. The binding of ^{3}H-flumazenil obtained at **five** concentrations of all plant extracts are shown in **Table 4.4.3.1** (page 163). In the second screening, 46 ethanolic extracts from 35 species were tested in the ^{3}H-flumazenil binding assay. The binding of ^{3}H-flumazenil obtained at **three** concentrations of all plant extracts are shown in **Table 4.4.3.2** (page 167)**.**

The most active extracts were the ethanolic leaf extracts of *Searsia dentata*, *Searsia rehmanniana*, *Hoslundia opposita* and the ethanolic corm extract of *Hypoxis colchicifolia*, which all showed good dose-dependent activity.

Out of the 46 extracts tested in the second screening, seven showed good dose dependant activity (**Table 4.4.3.2.**). These extracts were from *Arctopus echinatus*, *Artemisia afra*, *Helichrysum hesbaceum*, *Helichrysum ruderale*, *Helichrysum simillimum*, *Helichrysum umbraculigerum* and *Mentha aquatica* ethanolic leaf extracts. *Mentha aquatica* was investigated further as discussed below. Several plants showed moderate dose dependant activity. These plants include the ethanolic root extract of *Scadoxus puniceus*, the ethanolic leaf extracts of *Helichrysum rugulosum*, *Millettia grandis*, *Mentha longifolia*,

Malva parviflora, *Phytolacca octandra* and *Datura ferox*. The ethanolic extracts of *Acacia xanthophloea* and both leaf and tuber extracts of *Piper capense* exhibited moderate activity.

Various flavones, including rhamnetin, eupatlitin and bonazin have been isolated from the aerial parts of several *Artemisia* species (WOLLENWEBER and JAY, 1988). The observed anticonvulsant and sedative activity of *Artemisia dracunculus* L. (Tarragon) from Iran may be linked to the presence of monoterpenoids in the essential oil (SAYYAH, NADJAFNIA and KAMALINEJAD, 2004). Two flavonoids, hispidulin and cirsilineol, from *Artemisia herba-alba* Asso (used traditionally in Lebanon) with *in-vitro* $GABA_A$-benzodiazepine receptor activity were isolated by SALAH and JÄGER (2005). It is possible that these or similar compounds are responsible for the activity detected in *Artemisia afra*.

Table 4.4.3.1. Plant extracts initially screened for activity in the $GABA_A$-benzodiazepine receptor assay (RISA, RISA, ADSERSEN, GAUGUIN, STAFFORD, VAN STADEN and JÄGER, 2004).

Plant species	**Plant part**	**Extract[a]**	**[^{3}H]-Ro 15-1788 (flumazenil) binding in percent[b] at five concentrations**					**IC_{50} ± SE[c] (µg/ml)**
			10 mg/ml	**1 mg/ml**	**0.1 mg/ml**	**0.01 mg/ml**	**0.001 mg/ml**	
Amaryllidaceae								
Brunsvigia grandiflora	leaves	w	92±6	102±1	101±4	95±5	89±5	n.d.[d]
		e	38±9	84±5	105±1	108±2	106±3	n.d.
Anacardiaceae								
Searsia chirindensis	leaves	w	55±6	106±2	117±1	103±7	95±6	n.d.
		e	38±1	107±8	116±9	110±10	120±13	n.d.
	roots	w	63±2	106±5	101±6	89±4	102±4	n.d.
		e	30±2	92±5	122±15	85±6	112±12	n.d.
Searsia rehmanniana	**leaves**	w	53±2	101±6	96±1	99±10	94±2	n.d.
		e	**6±1**	**4±1**	**9±1**	**57±2**	**82±3**	**15.9±1.4**
Searsia dentata	**leaves**	w	50±1	88±2	96±4	107±6	110±8	n.d.
		e	**8±2**	**6±0**	**26±1**	**86±5**	**94±2**	**44.4±4.8**
Apocynaceae								
Acokanthera oblongifolia	leaves	w	61±6	71±4	67±10	102±5	110±2	n.d.
Araliaceae								
Cussonia paniculata	leaves	w	112±4	109±6	106±3	110±3	114±5	n.d.
		e	62±2	106±8	105±2	99±4	92±2	n.d.
Cussonia spicata	leaves	w	97±6	110±15	109±4	112±16	116±5	n.d.
		e	97±14	99±19	95±11	246±22	121±5	n.d.
Asphodelaceae								
Bulbine frutescens	leaves/roots	w	113±5	114±3	101±7	98±5	105±5	n.d.
		e	67±4	122±1	122±7	119±7	125±9	n.d.
Gasteria croucheri	leaves	w	99±3	94±6	91±4	96±9	92±4	n.d.
		e	55±4	85±2	91±3	107±2	95±5	n.d
	roots	w	124±5	122±1	122±7	119±7	125±9	n.d.
		e	66±0	101±2	111±6	107±2	109±2	n.d..
Combretaceae								
Combretum bracteosum	leaves	w	62±1	88±1	85±1	96±2	93±2	n.d.
		e	56±4	105±3	105±5	106±3	104±7	n.d.
	roots	w	53±3	81±3	103±7	105±5	107±4	n.d.
		e	38±3	85±1	98±4	100±4	106±2	n.d.
Combretum imberbe	leaves	w	53±3	80±1	98±6	93±2	95±1	n.d.
		e	13±0	86±2	99±2	105±4	101±7	n.d.
	roots	w	41±1	74±2	104±3	110±3	106±2	n.d.
		e	41±2	82±1	95±4	104±4	107±2	n.d.

Plant species	Plant part	Extract[a]	[^{3}H]-Ro 15-1788 (flumazenil) binding in percent[b] at five concentrations					IC_{50} ± SE[c] (µg/ml)
			10 mg/ml	1 mg/ml	0.1 mg/ml	0.01 mg/ml	0.001 mg/ml	
Euphorbiaceae								
Antidesma venosum	roots	w	63±3	97±9	107±15	112±24	96±2	n.d.
		e	66±1	134±3	126±15	117±3	98±10	n.d.
Jatropha panduaeifolia	leaves	w	114±1	116±4	104±2	97±7	111±3	n.d.
		e	56±7	101±5	101±2	100±2	100±2	n.d.
	root bark	w	92±2	107±1	112±2	107±5	105±4	n.d.
		e	28±1	89±6	102±5	109±6	102±3	n.d.
Jatropha zeyheri	leaves	w	122±4	103±23	102±4	88±13	109±8	n.d.
		e	79±3	120±1	101±9	105±4	120±5	n.d.
Fabaceae								
Bauhinia galpinii	leaves	w	72±4	117±10	136±32	109±23	127±9	n.d.
		e	27±2	93±16	104±3	111±8	96±5	n.d.
Bauhinia tomentosa	leaves	w	114±7	113±9	123±5	123±6	154±53	n.d.
		e	46±3	115±45	93±7	95±8	108±2	n.d.
Dichrostachys cinera	leaves	w	78±12	120±3	117±1	108±14	96±8	n.d.
		e	30±6	66±5	67±6	52±20	56±12	n.d.
Indigofera tristis	leaves	w	83±6	105±1	95±3	107±2	97±4	n.d.
		e	36±2	98±2	99±6	105±4	109±9	n.d.
Indigofera woodii	leaves	w	95±1	96±10	115±12	118±2	112±9	n.d.
		e	33±0	106±2	115±1	114±3	120±1	n.d.
Senna didymobotrya	leaves	w	90±1	90±3	91±2	91±4	94±1	n.d.
		e	28±1	79±2	91±5	95±4	-	n.d.
	roots	w	101±4	106±4	102±5	104±3	104±2	n.d.
		e	33±5	89±3	102±3	95±3	98±3	n.d.
Senna petersiana	leaves	w	75±3	88±3	96±8	94±2	88±2	n.d.
		e	24±3	78±2	92±4	95±5	95±3	n.d.
	roots	w	63±3	113±8	110±4	102±7	97±4	n.d.
		e	47±3	87±7	113±2	97±5	110±4	n.d.
Flacoutiaceae								
Oncoba spinosa	leaves	w	86± 6	80±33	99±9	102±7	103±5	n.d.
		e	46±1	89±2	94±7	96±2	82±2	n.d.
	roots	w	27±2	70±2	91±8	93±5	94±6	n.d.
		e	16±4	73±13	94±16	102±2	110±5	n.d.
Hypoxidaceae								
Hypoxis augustifolia	leaves	w	101±1	113±2	96±0	97±2	98±4	n.d.
		e	36±1	97±2	102±4	103±8	105±3	n.d.
	corm	w	85±1	90±1	98±3	103±6	98±4	n.d.
		e	38±4	79±8	96±1	104±0	104±3	n.d.

Plant species	Plant part	Extract[a]	[^{3}H]-Ro 15-1788 (flumazenil) binding in percent[b] at five concentrations					IC_{50} ± SE[c] (µg/ml)
			10 mg/ml	1 mg/ml	0.1 mg/ml	0.01 mg/ml	0.001 mg/ml	
Hypoxis colchicifolia	corm	w	95±6	104±9	107±4	107±5	104±8	n.d.
		e	23±3	77±1	83±6	88±3	91±3	n.d.
Hypoxis hemerocallidea	leaves	w	103±4	105±2	100±2	99±7	111±5	n.d.
		e	57±5	100±2	101±3	110±1	112±2	n.d.
	bark	w	68±5	107±3	111±4	108±9	98±9	n.d.
		e	36±0	89±3	111±3	130±2	109±4	n.d.
	roots	w	71±2	95±1	106±0	106±1	108±4	n.d.
		e	57±2	88±3	99±4	108±3	109±1	n.d.
Lamiaceae								
Hoslundia opposita	leaves	w	102±1	77±38	95±30	106±9	115±6	n.d.
		e	10±2	30±2	46±1	54±6	62±10	?
Leonotis dubra	leaves	w	128±7	110±7	111±5	111±8	109±8	n.d.
		e	38±9	81±8	104±8	106±3	112±1	n.d.
Leonotis intermedia	leaves	w	98±4	108±4	104±4	106±3	98±1	n.d.
		e	36±6	85±7	95±4	100±7	97±3	n.d.
Leonotis leonurus	leaves	w	66±4	89±3	115±2	108±6	107±4	n.d.
		e	35±7	97±6	88±5	91±3	102±9	n.d.
Salvia chanelaeagnea	leaves	w	84±3	95±3	-	95±18	116±10	n.d.
		e	28±3	93±11	139±10	132±11	155±19	n.d.
Loganiaceae								
Buddleja saligna	leaves	w	151±36	120±5	126±5	132±10	149±40	n.d.
		e	55±0	74±16	100±13	116±19	131±25	n.d.
Buddleja salviifolia	leaves	w	111±5	123±1	111±5	110±7	116±2	n.d.
		e	74±4	91±2	94±2	96±3	90±2	n.d.
Meliaceae								
Ekebergia capensis	leaves	w	96±3	115±4	120±7	121±5	96±8	n.d.
		e	27±3	55±20	36±7	41±10	51±4	?
	bark	w	69±3	103±3	110±4	102±5	109±4	n.d.
		e	29±2	60±3	63±8	42±3	76±30	?
Periplocaceae								
Mondia whitei	leaves	w	106±9	-	88±4	89±4	95±3	n.d.
		e	63±4	101±2	98±1	97±6	96±10	n.d
Rosaceae								
Rubus ludwigii	leaves	w	71±1	86±7	108±9	102±6	100±8	n.d.
		e	50±3	91±2	98±2	99±4	102±3	n.d.
	roots	w	79±3	100±6	105±10	101±2	102±2	n.d.
		e	65±2	104±2	106±2	106±2	103±4	n.d.
Rubus phoeniculacius	leaves	w	74±4	85±9	95±9	89±9	88±10	n.d.
		e	58±1	97±6	94±2	93±3	99±6	n.d.

Plant species	Plant part	Extract[a]	[^{3}H]-Ro 15-1788 (flumazenil) binding in percent[b] at five concentrations					IC_{50} ± SE[c] (µg/ml)
			10 mg/ml	1 mg/ml	0.1 mg/ml	0.01 mg/ml	0.001 mg/ml	
Rubiaceae								
Catunaregam spinosa	leave	w	108±7	134±14	147±20	151±28	104±7	n.d.
		e	54±1	111±2	87±6	113±10	115±3	n.d.
	roots	w	96±8	111±0	117±8	144±51	127±28	n.d.
		e	79±3	97±15	120±1	114±6	116±1	n.d.
Rutaceae								
Clausena anisata	**stem bark**	w	108±7	111±2	115±1	107±1	110±9	n.d.
		e	**18±1**	**54±2**	**91±3**	**91±4**	**87±1**	**1000±18**
	root bark	w	82±7	101±2	111±4	114±4	113±6	n.d.
		e	**46±1**	**67±2**	**93±3**	**104±1**	**103±7**	**670±164**
Ruta graveolens	leaves	w	87±5	114±10	113±9	112±6	124±7	n.d.
		e`	34±1	97±2	111±0	115±5	113±2	n.d.
Zanthoxylum capense	leaves	w	105±2	113±8	101±2	97±1	100±7	n.d.
		e	46±2	92±4	105±7	107±4	107±3	n.d.
Verbenaceae								
Clerodendrum myricoides	leaves	w	68±7	75±8	80±9	80±4	91±8	n.d.
		e	16±3	74±11	130±8	145±29	113±8	n.d.
Vitaceae								
Rhoicissus tomentosa	leaves	w	37±10	43±9	96±2	88±11	86±7	n.d.
		e	56±2	98±2	105±2	107±6	112±3	n.d.
Rhoicissus tridentata	leaves	w	91±2	95±3	99±4	98±4	102±4	n.d.
		e	61±3	104±1	94±4	102±5	103±1	n.d.

[a] solvents: w = deionized water; e = 70% ethanol

[b] [^{3}H]-Ro 15-1788 (flumazenil) binding in percent, the lower the percentage the more [^{3}H]-Ro 15-1788 (flumazenil) has been displace by the plant extract

[c] IC_{50} (µg/ml) and standard error, calculated using Grafit (© Erithacus Software Limited)

[d] n.d. = not detected, does not exhibit sufficient (IC^{50} < 1000 µg/ml) dose-dependant activity

Table 4.4.3.2. Percent binding of [^{3}H] Ro 15-1788 (flumazenil) to the $GABA_A$-benzodiazepine receptor in the presence of various concentrations of ethanolic extracts of individual plants used traditionally as sedatives or to treat various CNS-related ailments (STAFFORD, JÄGER and VAN STADEN, 2005).

Family	Plant part analysed	Percentage binding (±SE) at different plant extract concentrations in assay		
Species		0.455 mg/ml	0.046 mg/ml	0.005 mg/ml
Agapanthaceae				
Agapanthus campanulatus	Leaves	48.6±6.7	74.9±10.3	77.1±8.2
	Stem	47.5±4.6	71.0±1.9	76.5±1.8
	Root	60.9±3.7	78.4±4.2	85.1±5.3
Agapanthus praecox	Leaves	49.6±4.3	75.0±6.3	79.7±3.7
	Flowers	46.8±8.5	69.2±6.3	72.0±12.0
	Stem	70.7±7.0	82.1±9.1	79.6±5.9
	Root	40.2±2.2	72.2±3.3	80.4±4.2
Amaryllidaceae				
Scadoxus puniceus	Leaves	37.8±0.9	74.6±4.5	89.7±6.1
	Root	23.0±2.8	62.0±3.7	80.5±1.9
Apiaceae				
Arctopus echinatus	Whole plant	**0.0±0.1**	**18.9±1.0**	**60.2±2.6**
Asclepiadaceae				
Gomphocarpus physocarpus	Leaves	43.6±3.8	67.8±5.1	67.7±2.9
Xysmalobium undulatum	Leaves	52.5±2.6	80.2±1.1	84.4±0.8
	Root	72.6±5.1	84.9±0.9	86.2±4.5
Asteraceae				
Artemisia afra	Leaves	**5.0±0.9**	**45.3±3.5**	**81.77±4.2**
Berkheya bergiana	Leaves	39.4±3.1	69.7±4.0	77.8±6.2
Berkheya montana	Leaves	40.6±2.0	81.1±5.2	78.0±7.4
Berkheya rhapontica	Leaves	36.3±4.5	65.2±2.6	77.6±1.8
	Root	64.6±7.1	80.7±9.1	80.1±3.9
Helichrysum argyrolepis	Leaves	31.6±3.6	59.8±1.4	77.0±3.8
Helichrysum hesbaceum	Leaves	**12.9±0.4**	**44.4±2.4**	**67.3±3.3**
Helichrysum nudifolium	Leaves	43.1±2.1	71.2±2.6	74.7±3.2
Helichrysum ruderale	Leaves	**9.7±0.7**	**45.5±2.0**	**71.5±2.1**
Helichrysum rugulosum	Leaves	16.1±0.4	33.8±4.4	59.2±1.1
Helichrysum simillimum	Leaves	**13.3±1.8**	**41.0±2.0**	**61.3±3.3**
Helichrysum umbraculigerum	Leaves	**8.4±2.3**	**15.3±2.8**	**57.9±1.3**
Celastraceae				
Catha edulis	Leaves	24.3±1.9	53.3±1.2	63.2±1.0
Crassulaceae				
Cotyledon orbiculata	Leaves	45.8±5.7	78.9±6.8	95.7±9.4
Dioscoreaceae				
Dioscorea dregeana	Leaves	28.4±1.8	60.4±1.7	73.0±3.5
Euphorbiaceae				
Croton sylvaticus	bark	47.9±7.0	84.3±7.9	97.5±10.5
Fabaceae				
Acacia sieberiana	Bark	31.2±11.1	53.2±5.4	73.7±7.3
Acacia xanthophloea	Bark	18.4±2.4	22.8±1.0	55.6±6.5
Millettia grandis	Leaves	18.6±0.7	52.8±2.5	66.3±3.7
Millettia sutherlandii	Leaves	30.5±3.4	53.2±2.0	64.3±5.6
Schotia brachypetala	Leaves	28.8±2.6	51.5±3.0	73.2±2.3
Sutherlandia frutescens	Leaves	47.4±6.7	85.7±11.4	88.4±4.8
Lamiaceae				
Mentha aquatica	Leaves	**10.8±1.4**	**47.8±1.8**	**64.8±2.0**
Mentha longifolia	Leaves	20.8±2.0	53.1±2.0	67.1±3.8
Lauraceae				
Cinnamomum camphora	Leaves	23.8±2.7	63.1±3.2	80.0±5.3
Malvaceae				
Malva parviflora	Leaves	21.9±3.6	56.1±2.6	71.0±2.9
Phylolaccaceae				
Phytolacca octandra	Leaves	18.9±3.9	52.6±2.4	65.9±0.8

Family *Species*	Plant part analysed	Percentage binding (±SE) at different plant extract concentrations in assay 0.455 mg/ml	0.046 mg/ml	0.005 mg/ml
Piperaceae				
Piper capense	Leaves	15.9±1.3	57.1±3.2	83.9±2.2
	Tuber	22.6±3.0	52.9±3.1	65.4±3.9
Solanaceae				
Datura ferox	Leaves	21.7±3.1	68.7±4.2	93.7±10.0
	Seed	51.8±4.6	85.2±1.4	94.6±1.5
Datura stramonium				
	Leaves	42.1±6.9	78.8±2.9	99.9±9.8
	Seed	44.0±3.2	72.2±5.1	70.6±0.8

More than 14 species of *Helichrysum* are used in Zulu traditional medicine (HUTCHINGS, SCOTT, LEWIS, and CUNNINGHAM, 1996). Several species known as *imphepho* (zulu) are burned as ritual incenses at almost every traditional gathering, ritual or ceremony to invoke the goodwill of the ancestors. Healers burn these highly aromatic plants during consultations with patients again to invoke the goodwill of the ancestors and for guidance in diagnosis and treatment. The smoke is said to be helpful for insomnia (HUTCHINGS, SCOTT, LEWIS, and CUNNINGHAM, 1996). The smoke from *imphepho* is inhaled by healers to induce a trance. *Helichrysum* species contain flavonoids (VAN WYK, VAN OUDTSHOORN, and GERICKE, 1997), examples are helichrysetin, isolated from *H. odoratissimum* (L.) Less, and caespitin, a phloroglucinol derivative from *H. caespititium* (MATHEKGA, MEYER, HORN and DREWES, 2000).

Several southern African plants have shown *in vivo* anticonvulsant activity against seizures produced in mice by pentylenetetrazole, picrotoxin, bicuculline and *N*-methyl-DL-aspartic acid. However, the active constituents are yet to be identified. The plants include *Leonotis leonurus* (Lamiaceae) (BIENVENU, AMABEOKU, EAGLES, SCOTT and SPRINGFIELD, 2002) which is reported to have narcotic effects and is used as a substitute for *Cannabis* (WATT and BREYER-BRANDWIJK, 1962). The aqueous extracts of *L. leonurus* (400 mg/kg) protected against or delayed seizures induced by pentylenetetrazole, picrotoxin and *N*-methyl-dl-aspartic acid, but did not protect against bicuculline-induced seizures. In this study, the ethanol extracts of the three species of *Leonotis* had weak $GABA_A$-benzodiazepine receptor binding activity only at the highest concentration tested (10 mg/ml) (RISA, RISA, ADSERSEN, GAUGUIN, STAFFORD, VAN STADEN and JÄGER, 2004) but the aqueous extracts were not active, suggesting that the anticonvulsant mechanism is not via $GABA_A$-benzodiazepine receptor.

WATT (1967), one of the earliest researchers to recognize the potential of African plants in improving mental health, reported the use of *Cotyledon orbiculata* (Crassulaceae) leaves to treat epilepsy. Again, *in vivo* studies have demonstrated both aqueous and methanol extracts of *C. orbiculata* have anticonvulsant properties (moderate protection against pentylenetetrazole, bicuculline, picrotoxin and *N*-methyl-dl-aspartic induced seizures in mice) (AMABEOKU, GREEN and KABATENDE, 2007). However, in this

study the ethanolic extract did not show *in vitro* $GABA_A$-benzodiazepine receptor binding activity (STAFFORD, JÄGER and VAN STADEN, 2005).

Another study investigated a Northern Sotho remedy, *Sehlare sa Seebana*, for treatment of epilepsy. The recipe for this herbal remedy contains six plants, *Acrotome inflata*, *Aptosimum indivisum*, *Asparagus suaveolens*, *Barleria bolusii*, *Commiphora marlothii* and *Sesamum triphyllum*. Equal parts of the plants are placed in a red-hot clay pot and the patient inhales the smoke (JÄGER, MOHOTO, VAN HEERDEN and VILJOEN, 2005). Both aqueous and ethanol extracts of *Aptosimum indivisum* and *Asparagus suaveolens* and the aqueous extract of *Commiphora marlothii* showed good dose-dependent $GABA_A$-benzodiazepine receptor binding. Most of the plants have not been chemically investigated. Three metabolites: verbascoside, pinocembrinin 7-neohesperidoside and shanzhiside methyl ester were isolated from *A. indivisum*. *B. bolusii* contains verbascoside, which is known to inhibit the GABA receptor, but did not show much activity (DAELS-RAKOTOARISON, SEIDEL, GRESSIER, BRUNTE, TILLEQUIN, BAILLEUL, LUYCKX, DINE, CAZINI and CAZIN, 2000; JÄGER, MOHOTO, VAN HEERDEN and VILJOEN, 2005).

Isolation and identification of active compounds from Searsia pyroides (Searsia pyroides)

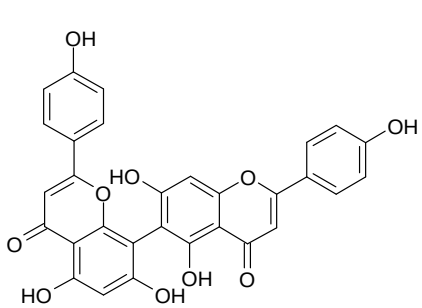
Two biflavonoids with activity in the ^{3}H-Ro 15-1788 (flumazenil) binding assay were isolated by HPLC fractionation of the ethanol extract of the leaves from *Searsia pyroides* (SVENNINGSEN, DAMKJÆR MADSEN, LILJEFORS, STAFFORD, VAN STADEN and JÄGER, 2006). The structures of the two biflavonoids were elucidated by NMR to be agathisflavone and amentoflavone. Agathisflavone and amentoflavone competitively inhibited the binding of ^{3}H-Ro 15-1788 with a K_i of 28 and 37 nM, respectively. Extracts of *Searsia dentata* and *Searsia pentheri* were not as active as the extract from *Searsia pyroides*; both were found to contain apigenin and agathisflavone.

Apigenin, agathisflavone and amentoflavone were also subjected to a pharmacophore model for ligands at the benzodiazepine receptor developed by ZHANG, KOEHLER, ZHANG and COOK (1995). The model has previously been validated and further developed to include flavones by DEKERMENDJIAN, KAHNBERG, WITT, STERNER, NIELSEN and LILJEFORS (1999) and KAHNBERG, LAGER, ROSENBERG, SCHOUGAARD, CAMET, STERNER, NIELSEN, NIELSEN and LILJEFORS (2002). Conformational analyses of the compounds were performed by using the Monte Carlo Multiple Minimum (MCMM) method (CHANG, GUIDA and STILL, 1989) and the MMFF94 force field (HALGREN, 1996) as implemented in the MacroModel program version 8 (MOHAMADI, RICHARDS, GUIDA, LISKAMP, LIPTON, CAUFIELD, CHANG, HENDRICKSON and STILL, 1990). Each compound was fitted into the model in a low energy conformation according to conformational analysis. Apigenin was fitted into the model in the same way as previously described for other flavone derivatives (KAHNBERG, LAGER, ROSENBERG, SCHOUGAARD, CAMET, STERNER, NIELSEN, NIELSEN and LILJEFORS, 2002). The low affinity of apiginin is most probably due to its inability to fill up the L2

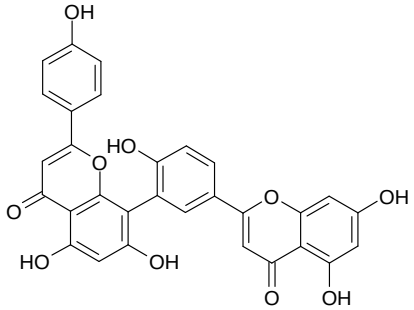
lipophilic pocket (**Figure 4.2. A**) which is important for high affinity binding of flavone derivatives (DEKERMENDJIAN, KAHNBERG, WITT, STERNER, NIELSEN and LILJEFORS, 1999).

In addition, the fit reveals a steric repulsive interaction at the S4 site (**Figure 4.2. A**). In the fitting of **1** and **2** it is assumed that one of the monomers in the biflavones binds to the receptor in the same way as apigenin (**Figure 4.2. A**). Thus the other monomer is regarded as a large substituent. Considering the steric constraints of the modelled binding site of the receptor shown in **Figure 4.2.** (the steric repulsive sites S1–S5 and the hydrogen bonding sites HI, H2, A2, A3) the only reasonable way to fit **1** and **2** into the model is to place these large substituents in the lipophilic area L3 or in the "subunit interface" area which is suggested to correspond to the interface between an α and a γ subunit in the $GABA_A$ receptor (KAHNBERG, LAGER, ROSENBERG, SCHOUGAARD, CAMET, STERNER, NIELSEN, NIELSEN and LILJEFORS, 2002). Compound **1** is proposed to bind to the receptor with its substituent in the lipophilic pocket (L3), between the steric repulsive site (S3) and the bi-functional hydrogen bond donor/acceptor site (H2/A3) (**Figure 4.2. B**). It should be noted that the L3 area according to the model of ZHANG, KOEHLER, ZHANG and COOK (1995) accommodates part of diazepam in its binding to the receptor. Compound **2** can be fitted into the model in two different ways; with the substituent in the lipophilic pocket (L3), as is the case for **1**, or in the "subunit interface" area. The latter offers a somewhat better space for larger substituents. It has previously been shown that this area in terms of the pharmacophore model can accommodate large substituents (KAHNBERG, LAGER, ROSENBERG, SCHOUGAARD, CAMET, STERNER, NIELSEN, NIELSEN and LILJEFORS, 2002). Thus, the binding of **2** to the receptor is proposed to take place with the substituent in the "subunit interface" area (**Figure 4.2. C**).

According to the pharmacophore modelling reported above, the significantly higher affinity of the biflavonoids **1** and **2** compared to that of apigenin is most probably due to affinity increasing interactions of one of the monomers (the substituent) with amino acid residues in the "subunit interface" area or the L3 lipophilic area. A similar increase in affinity due to large substituents proposed to be located in a subunit interface of the $GABA_A$ receptor was previously reported (FRØLUND, JORGENSEN, TAGMOSE, STENSBØL, VESTERGAARD, ENGBLOM, KRISTIANSEN, SANCHEZ, KROGSGAARD-LARSEN and LILJEFORS, 2002).



1
Agathisflavone



2
Amentoflavone

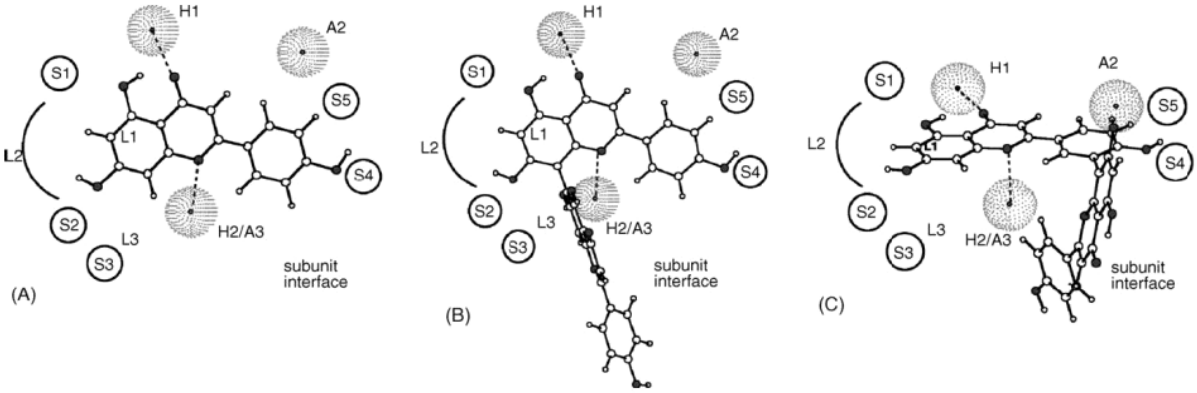


Figure 4.2. (A) Apigenin, (B) agathisflavone **1** and (C) amentoflavone **2** showing fit to the pharmacophore model.

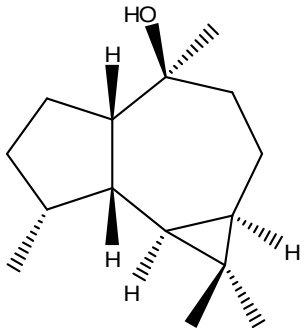
The binding of apigenin and amentoflavone to the flumazenil site has previously been reported (NIELSEN, FROKJAER and BRAESTRUP, 1988; VIOLA, WASOWSKI, LEVI DE STEIN, WOLFMAN, SILVERA, MEDINA and PALADINI, 1995) but apigenin showed no anticonvulsant properties *in vivo* (VIOLA, WASOWSKI, LEVI DE STEIN, WOLFMAN, SILVERA, MEDINA and PALADINI, 1995; AVALLONE, ZANOLI, PUIA, KLEINSCHNITZ, SCHREIER and BARALDI, 2000). Another group reported amentoflavone to be a relatively weak negative allosteric modulator of GABA action acting independently the flumazenil binding site (HANRAHAN, CHEBIB, DAVUCHERON, HALL and JOHNSTON, 2003). Thus, the use of these plants as anticonvulsive agents suggests involvement of a different neurotransmitter system.

Isolation and identification of active constituents of Mentha aquatica

In the initial screening the four solvent extracts and the essential oil were tested in the GABA-benzodiazepine assay. The results for displacement of the radioactive ligand indicated that water (77 %), ethanol extracts (77%) and the essential oil (64%) showed interesting displacement, whereas the displacement obtained with the ethyl acetate (46 %) and petroleum ether (31%) extracts were lower. This indicated that there were both polar and lipophilic active compounds in *M. aquatica*, and it was therefore decided to continue the isolation procedure using both the essential oil and ethanolic extracts.

The sesquiterpene viridiflorol (**3**) (yield: 45 mg from 15 g dried material) was isolated by bioassay-guided fractionation from the essential oil. The GC-MS analysis of the active compound from the essential oil indicated that the compound was ledol, however not with a good fit. There are three naturally occurring isomers, ledol, viridiflorol and globulol. The optical rotation of the isolated compound was measured to be $[\alpha]_{589} = 0.9$ º. (+/-)-Globulol has an optical rotation of 35-60º (WU, HUANG and CHEN, 1996), which eliminated the possibility that the isolated compound could be globulol. Viridiflorol and ledol both have an optical rotation of 2º (WU, HUANG and CHEN, 1996), so this technique could not be used to differentiate between these two isomers. ^{13}C-NMR (C-1: 58.07; C-2: 25.72; C-3: 29.00; C-4: 38.38; C-5:

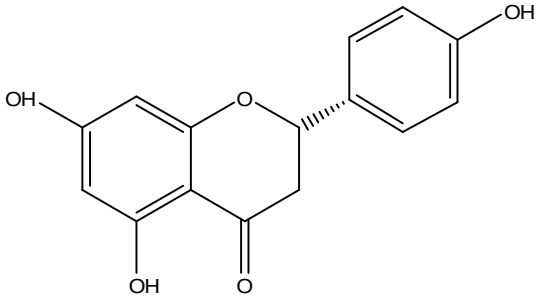
39.63; C-6: 22.21; C-7: 28.48; C-8: 18.77) with comparison to data from BOMBARDA, RAHARIVELOMANANA, RAMANOELINA, FAURE, BIANCHINI and GAYDOU (2001), who synthesized the two isomers, unequivocally determined the structure to be viridiflorol (**3, below**). Viridiflorol has previously been detected in *M. aquatica* (UMEMOTO, ARAI, NII and FURUKAWA, 1994; ESMAEILI, RUSTAIYAN, MASOUDI and NADRI, 2006).



3

Viridiflorol

Bioassay-guided isolation of the ethanol extract lead to the isolation of the flavanone naringenin (**4)**. The 1H-NMR data (H-3_{eq}: 2.76, 1H, dd; H-3_{ax}: 3.10, 1H, dd; H-2: 5.34, 1H, dd; H-6: 5.97, 1H, d; H-8: 5.99, 1H, d; H-3', H-5': 6.9, 2H, dd; H-2', H-6': 6.9, 2H, dd) in comparison with previously published data (DU, JERZ and WINTERHALTER, 2004) determined the structure to be naringenin. An optical rotation $[\alpha]^{25}_{589}$ = -20.7 (EtOH) was determined, which correspond to (*S*)-naringenin (**4**) (GIORGIO, PARRINELLO, CACCAMESE and ROSINI, 2004).



4

Naringenin

The IC_{50} values in the GABA-benzodiazepine assay were determined from dose-response curves (**Figure 4.3**.) to be 0.19 M for viridiflorol and 0.0026 M for (*S*)-naringenin, giving a K_i-value of 2 mM for (*S*)-naringenin. Compared to the clinically used benzodiazepine, diazepam, which had an IC_{50}-value of 0.1 µM, the two compounds isolated in this study were not very active. In a study on structure-activity relationship of flavonoids binding to the GABA-benzodiazepine site naringenin had a K_i -value of 770 nM, about 100 times less active than diazepam with a K_i -value of 8.5 nM, but it was not stated which stereoisomer of naringenin was tested (DEKERMENDJIAN, KAHNBERG, WITT, STERNER, NIELSEN and LILJEFORS, 1999).

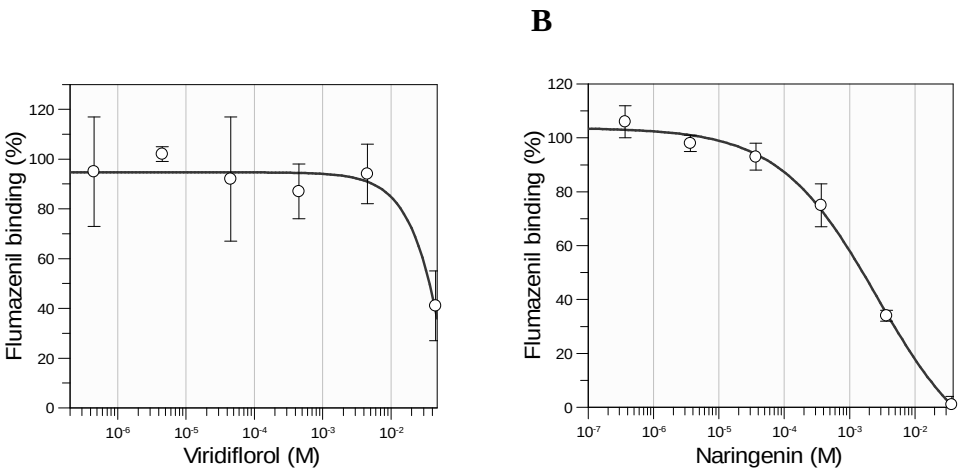


Figure 4.3. IC_{50} determinations for (A) viridiflorol and (B) (*S*)-naringenin in the flumazenil binding assay.

4.4.4. Conclusions

None of the isolated compounds exhibit high enough affinity to the GABA-benzodiazepine site to be candidates for drug development, but the displayed activity validates their use in traditional medicine. Those that did not exhibit activity in this investigation may act on a different mechanism related to their traditional use.

Often plant extracts and compounds active in the flumazenil assay are not active *in vivo* suggesting that these compounds may not be able to pass the blood brain barrier (BBB). In the search for new antiepileptic and anticonvulsant compounds with effect on the $GABA_A$ receptor, it is essential that the compounds are able to pass the BBB. Therefore it is important to confirm this activity in an *in vivo* animal model or functional assay such as the cortical wedge assay. Naringenin has been shown to pass the BBB (YOUDIM, QAISER, BEGLEY, RICE-EVANS and ABBOTT, 2004), which means that it can exercise an effect on the CNS.

The following publications relate to this chapter:

- J. Risa, A. Risa, A. Adsersen, B. Gauguin, **G.I. Stafford**, J. van Staden and A.K. Jäger. 2004. Screening of plants used in southern Africa for epilepsy and convulsions in the $GABA_A$-benzodiazepine receptor assay. *Journal of Ethnopharmacology*, 93: 177-182.
- **G.I. Stafford**, A.K. Jäger and J. van Staden. 2005. Activity of traditional South African sedative and potentially CNS-acting plants in the GABA-benzodiazepine receptor assay. *Journal of Ethnopharmacology*, 100: 210-215
- A.B. Svenningsen, K. Damkjær Madsen, T. Liljefors, **G.I. Stafford**, J. van Staden and A.K. Jäger. 2006. Biflavones from *Searsia* species with affinity for the $GABA_A$/benzodiazepine. *Journal of Ethnopharmacology*, 103: 276-280.
- A.K. Jäger, J.P. Almqvist, S.A.K. Vangsøe, **G.I. Stafford**, A. Adsersen, J. Van Staden. 2007. *Mentha aquatica* in the $GABA_A$-benzodiazepine receptor assay. *South African Journal of Botany*, 73: 518-521.

CHAPTER FIVE

Age-related, dementia and debilitative mental disorders:

Screening South African medicinal plants for monoamine oxidase B inhibitors (MAO-B) to treat Parkinson's disease

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5.1. Introduction

The increasing average life expectancy is leading to major demographic changes worldwide. CNS disorders associated with old age, such as Alzheimer's (AD) and Parkinson's disease (PD), and other 'senile' dementia will have dramatic societal and economic impact in the next decades (WORLD HEALTH REPORT, 1998). In the absence of any disease (e.g. AD, hardening of arteries in the brain) a person's mental abilities will remain mostly intact throughout life. Many people maintain a mentally functional and active life well into their nineties. Therefore the term 'senile dementia' is somewhat misleading, since getting old in itself does not cause significant or debilitating decline. Disease impairs the mind, not simply aging (SPINELLA, 2005). Alzheimer's disease is by far the most frequent cause of dementia, increasing in prevalence from less than 1 % below the age of 60 to more than 40 % above the age of 85 (LINDEBOOM and WEINSTEIN, 2004). Parkinson's disease (PD) affects one in every 100 persons above the age of 65 years; it is the second most common neurodegenerative (DE RIJK, LAUNER, BERGER, BRETELER, DARTIGUES, BALDERESCHI, FRATIGLIONI, LOBO, MARTINEZ-LAGE, TRENKWALDER and HOFMAN, 2000).

The prevalence of AD among indigenous South African subjects is not known. Local neurologists and old-age psychiatrists rarely encounter the disorder (DE VILLIERS and LOUW, 1996). Cultural attitudes in Africa surrounding dementia may have contributed to this apparent low prevalence. One suggestion is that dementia sufferers may not survive for long. They may die quickly, from conditions such as pneumonia or diarrhoea due to the decreasing status of the olderly in the developing world (CHANDRA, GANGULI and RATCLIFF, 1994; LEVKOFF, MACARTHUR and BUCKNALL, 1995). Another explanation is that dementia, like other kinds of mental illness, remains a stigmatised condition, and as such may be hidden from researchers (INEICHEN, 2000). Given that rural communities still have difficulties in accessing tertiary health care and that memory impairment may be accepted as part of normal ageing, patients do not readily interface with clinicians (DE VILLIERS and LOUW, 1996). The demented may not even be seen as ill (DE VILLIERS and LOUW, 1996).

PD occurs worldwide, but the prevalence and incidence appear to exhibit substantial geographic and ethnic variability, with generally lower rates reported in Africa (OKUBADEJO, BOWER, ROCCA and MARAGANORE, 2006). Much of the variation has been attributed to methodological differences of studies, but genetic and environmental diversity may be contributory (OKUBADEJO, 2008).

The HIV epidemic is further complicated by the development of a subcortical dementing illness known as AIDS dementia complex (ADC) (GUILLEMIN and BREW, 2007). Highly active antiretroviral therapy (HAART) has effectively lengthened HIV infected patients' life expectancy; indeed some are approaching an age where the risk of Alzheimer's disease (AD) is starting to become significant. Furthermore, many such patients have hyperlipidemia, which increases the risk of AD. Consequently, it has been predicted, by GUILLEMIN and BREW (2007) that HIV infected patients are at an increased risk of AD or ADC.

5.2. Aims

> "*Given the potential of plants which have not yet been investigated …, many different avenues need to be taken for the discovery of novel therapeutic agents. Action needs to be taken quickly, notably against diseases for which there is not yet an effective remedy: AIDS, multiple sclerosis, Parkinson's disease, Alzheimer disease and certain cancers*" (HOSTETTMANN, MARSTON, NDJOKO and WOLFENDER, 2000).

Despite the relatively small number of reported African herbal treatments for age-related CNS disorders (NEUWINGER, 2000), an attempt was made to investigate southern African plants for monoamine oxidase activity (subtype B) based on a lose association with their traditional usage and taxonomic links to other active species.

Recently, MAO-B inhibitors have been included in the treatment of anxiety disorders, AD and PD (YAMADA and YASUHARA, 2004). An inexpensive peroxidase-linked photometric assay for the detection of MAO inhibitors was developed from earlier methods of HOLT, SHARMAN, BAKER and PALCIC (1997) and SCHMIDT, LI, SCHUBERT, HUANG, STOYANOVA and HAMBURGER (2003). This enabled the initial screening for MAO inhibitory activity of South African plants with CNS-related traditional usage and the isolation of some of the active compounds.

Summary

Development of the peroxidase-linked photometric assay and the subsequent determination of monoamine oxidase inhibitory activity by southern African medicinal plants were conducted in collaboration with P.D. Pedersen, from the Department of Medicinal Chemistry, The Faculty of Pharmaceutical Sciences, University of Copenhagen (STAFFORD, PEDERSEN, JÄGER and VAN STADEN, 2007). The isolation of the MAO-inhibitor naringenin from *Mentha aquatica* L. was achieved together with H.T. Olsen from the Department of Medicinal Chemistry, The Faculty of Pharmaceutical Sciences, University of Copenhagen (OLSEN, STAFFORD, VAN STADEN, CHRISTENSEN, JÄGER, 2008).

Table 5.1. Compounds known to inhibit enzymes involved in neurotransmitter degradation.
Neurotransmitters are removed by translocation into vesicles (re-uptake) or destroyed in enzyme-catalysed reactions. A variety of plant derived compounds inhibit acetylcholinesterase (AChE) and there is considerable interest in AChE inhibitors as potential therapies for cognition inhancement and for Alzheimer's disease. Organophosphorous compounds alkylate an active sit, serine, on AChE and the AChE inhibition by this mechanism is the basis for the use of such compounds as insecticides. Catecholamines can be oxidised (via monoamine oxidase , MAO) or methylated (via catechol-*O*-methyltransferase, COMT). (Adapted from POLYA, 2003).

Compound (class)	***Species* (Family)**	**Enzyme inhibited (biological activity) in vivo effects**
Acetylcholinesterase (AChE), Butyryl-cholinesterase (BChE)		
Alkaloid Berberine (=umbellatine) (protoberberine isoquinoline)	*Coelocline* (Annonaceae), *Berveris, Hydrastis, Mahonia, Nandina* (Berberidaceae), *Archangelica* (Menispermaceae), *Argremone, Chelidonium, Corydalis* (Papaveraceae), *Coptis, Thalictrum* (Ranunculaceae), *Evodia, Toddalia, Zanthoxylum* (Rutaceae) spp.	AChE ligand (IC_{50} = 167 μM) BChE ligand (IC_{50} = 56 μM)
Cassaine (diterpene alkaloid)	*Erythophleum guineense, E. suaveolens* bark (Fabaceae)	AChE ligand (IC_{50} < 550 μM)
α-Chaconine (triterpene, steroidal alkaloid)	*Notholirion hyacinthinum, Veratrum stenophyllum* (Lilaceae), *Solanum tuberosum, S. choacoense, S. nigrum* (Solanaceae)	BChE ligand (at physiological postprandial (potato meal) serum levels) teratogen and toxic
Coumingine (alkaloid)	*Erythrophleum* sp. (Fabaceae)	AChE ligand (IC_{50} < 550 μM)
Dehydroevodiamine (indole)	*Evodia rutaecarpa* (Rutaceae)	AChE ligand (IC_{50} = 38 μM) antiamnesic
Deoxypeganine (= deoxyvasicine) (quinazoline quinoline)	*Peganum harmala. P. nigellastrum* (Zygophyllaceae)	AChE cholinergic
Faleoconitine (norditerpene alkaloid)	*Aconitum falconeri* (Ranunculaceae)	AChE
Galanthamine (= Galantamine; lycoremine; Reminyl) (galanthaman Amaryllidaceae alkaloid)	*Galanthus woronii* (snowdrop), *Crinum, Galanthus, Hippeastrum, Hymenocallis, Leucojum, Lycoris, Narcissus, Pancratium, Ungernia* spp. (Amaryllidacae)	AChE (nACh-R allosteric modulator) analgesic, clinical cognitive enhancer for Alzheimer's disease, reverses amnesia from scopolamine, insecticide, neuroleotic
(-)-Huperzine A (carbobicyclic pyridinone)	*Huperzia serrata* (moss), *Lycopodium selago* (club moss, Lycopodiaceae)	AChE (K_d = 5 nM) BChE cholinergic – causes stomach cramps, dizziness, slurred speech, toxic, atropine antidote

Compound (class)	***Species* (Family)**	**Enzyme inhibited (biological activity) in vivo effects**
(-)-Huperzine B (carbobicyclic pyridinone)	*Huperzia serrata* (moss), *Lycopodium selago* (club moss, Lycopodiaceae)	AChE cholinergic – Alzheimer's disease symptomatic treatment
N-(*p*-hydroxyphenethyl) actinidine (monoterpene alkaloid)	*Valeriana officinalis* (Valerianaceae)	AChE
(+)-Nepapakistamine A (steroidal alkaloid)	*Sarcococca coriacea*	AChE
Palmatine (= calystigine) (benzophenanthridine isoquinoline)	*Berberis, Mahonia* spp. (Berberidaceae), *Jateorrhiza palmate* (Menispermaceae), *Corydalis* (Papaveracea), *Coptis* (Ranunculaceae) spp.	AChE ligand (IC_{50} = 125 μM) BChE ligand (IC_{50} = 426 μM)
Papaverine (benzylisoquinoline)	*Rauwolfia serpentine* (Annonaceae), *Papaver bractaetum, P. serpentine, P somniferum* (Papaveraceae)	AChE antitussive, smooth muscle relaxant, spasmolytic, vasodilator
Peganine (= linarine; vasicine) (quinazoline quinoline)	*Adhatoda vasica, Justica adhtoda* (Acanthaceae), *Lunaria* spp. (Cruciferae), *Sida cordifolia* (Malvaceae), *Peganum harmala* (Zygophyllaceae)	AChE abortefacient, bronchodilatory, cholinergic, hypotensive, respiratory stimulant
Physostigmine (= eserine; physosterine; physostol) (indole)	*Hippomane mancinella* (Euphorbiaceae), *Physostigma veneosum* (Fabaceae)	AChE, BChE (carbamoylates active site Serine) Alzheimer's disease treatment especially amyloid plaque- & tangle- associated ChE over expression, organophosphate poison antidote, toxic
Physovenine (indole)	*Physostigma veneosum* (Fabaceae)	AChE parasympathetic, toxic
Protopine (= biflorine; corydalis C; corydinine; fumarine; macleyine) (benzylisoquinoline)	*Fumaria officinalis* (Fumariaceae), *Argemone mexicana, Corydalis ternate, Papaver somniferum* (Papaveraceae)	AChE (IC_{50} = 50 μM) Alzheimer's disease treatment, sedative, smooth muscle relaxant
Pseudaconitine (norditerpene alkaloid)	*Aconitum falconeri, A. ferox, A. spictatum* (Ranunculaceae)	AChE (nACh-R) Anticholergic, cardiac and respiratory depressant, hypotensive, toxic
Sanguinarine (= pseudochelerythrine) (benzophenanthridine)	*Chelidonium majus, Dicentra spectabilis, D. Peregrina, Papaver somniferum, Sanguinaria canadensis* (Papaveraceae), *Fumaria officinalis* (Fumariaceae), *Zanthoxylum* spp. (Rutaceae), *Pteridophyllum* spp. (Sapindaceae)	AChE ligand (IC_{50} = 11 μM) BChE ligand (IC_{50} = 17 μM)

Compound (class)	***Species* (Family)**	**Enzyme inhibited (biological activity) in vivo effects**
α-Solanine (= solatunine) (triterpene, steroidal alkaloid)	*Lycopersicon esculentum*, *Capsicum* spp., *Solanum tuberosum*, *S. nigrum* (Solanaceae)	BChE (at physiological postprandial (potato meal) serum levels) causes coma, diarrhea, hallucination, vomiting, insecticide, teratogen, toxic
Ungiminorine (Amaryllidaceae alkaloid)	*Narcissus* sp. (Amaryllidaceae)	AChE
(-)-Vaganine D (steroidal alkaloid)	*Sarcococca coriacea* (Buxaceae)	AChE
Phenolic		
Resorcinolic lipids (phenolic esters)	*Triticum aestivum* (Poaceae)	Membrane AChE (IC_{50} = 18-90 μM)
Terpene		
1,8-Cineole (= cajeputol; eucalyptol) (momoterpene)	*Artemisia maritima* (Asteraceae), *Salvia lavandulaefolia* (Lamiaceae), *Eucalyptus globulus*, *E.* spp., *Melaleuca leucadendron* (Myrtaceae), *Alpina*, *Curcuma* (Zingiberaceae)	AChE (IC_{50} = 670 μM)
α-Pinene (= 2-Pinene) (momoterpene)	*Juniper macropoda* (Cupressaceae), *Mentha*, *Salvia* spp. (Lamiaceae), *Eucalyptus globulus* (Myrtaceae), *Pinus palestris*, *P. walliciana*, *P.* spp. (Pinaceae), *Citrus* spp. (Rutaceae)	AChE (IC_{50} = 630 μM)
Ursolic acid (=malol; malolic acid; micromerol; prunol; urson) (triterpene)	Widespread	AChE (IC_{50} = 6 pM)
Other		
Solanum CPI (= potato carboxypeptase inhibitors) (5 kDa protein)	*Solanum tuberosum* (potato) (Solanaceae)	*in vivo* AChE, BChE especially AD amyloid plaque and tangle-associated
Non-plant reference compound		
Rivastigmine (carbamate)	synthetic	AChE (carbamoylates pseudoirreversibly – forms carbamoyl ester with active site Serine) Clinical cognition enhancer for Alzheimer's disease
Sarin (= isopropoxy-methylphosphoryl fluoride) (organophosphate)	synthetic	AChE (forms phosphoryl ester with active site Serine Chemical warfare agent

Compound (class)	***Species* (Family)**	**Enzyme inhibited (biological activity) in vivo effects**
Monoamine oxidase (MAO)		
Alkaloid		
Cinchonaminone (=[3'*R*,4'*S*]-2-(2-ethenyl-4-piperidinyl)-1-(4-quinolinyl)-1-propanol (piperidinyl quinoline)	*Cinchona succirubra* (Rubiaceae)	MAO (IC_{50} = 32 μM)
Cinchonaminone (=[*1S*, 3'*R*,4'*R*]-3-(3-ethenyl-4-piperidinyl)-1-(4-quinolinyl)-1-propanol (piperidinyl quinoline)	*Cinchona succirubra* (Rubiaceae)	MAO (IC_{50} = 12 μM)
Harmaline (= 3,4-dihydroharmine; harmidine) (indole, carboline)	*Passiflora incarnata* (Passifloraceae), *Banisteria caapi, Banisteriopsis caapi* (Malpighiaceae), *Peganum harmala* (Zygophyllaceae)	MAO-A hallucinogenic
Harman (= aribine;loturine;1-methyl-β-carboline; passiflorin) (β-carboline, indole)	*Phaseolus vulgaris* (Fabaceae), *Passiflora edulis, P. incarnate* (Passifloraceae), *Singickia rubra* (Rubiaceae), *Symplocos racemosa* (Symplocaceae), *Peganum harmala, Tribulus terrestris, Zygophyllum fabago* (Zygophyllaceae)	MAO-A (IC_{50} = 0.5 μM; K_d = 5 nM) MAO-B (IC_{50} = 5 μM) antidepressant, co-mutagenic, convulsant, hypotensive, motor depressant – sheep 'tribulus stagers'
Harmine (=banisterine; leucoharmine;telepathine; yageine) (β-carboline, indole)	*Passiflora incarnate* (Passifloraceae), *Banisteria caapi* (Malpighiaceae), *Peganum harmala, Tribulus terrestris* (Zygophyllaceae)	MAO-A (IC_{50} = 2 nM) CNS stimulant, hallucinogenic Used as a WW2 Nazi Gastapo 'truth drug'
2-Methoxytetrahydro-β-carboline (= 2-methoxy-tetrahydronorharman) (β-carboline, indole)	*Palicourea marcgravii* (Rubiaceae)	n MAO-A (IC_{50} = 1 μM)
2-Methoxytetrahydro-β-carboline (= 2-methyl-tetrahydronorharman) (β-carboline, indole)	*Palicourea marcgravii* (Rubiaceae)	MAO-A
Norharman (=β-carboline) (β-carboline, indole)	*Cichorium intybus* (Asteraceae), *Tribulus terrestris, Zygophyllum fabago* (Zygophyllaceae); tobacco smoke [ex *Nicotiana tabacum* (Solanaceae)]	MAO-A (weak) Benzodiazepine receptor 'Tribulus staggers' in sheep
Quinine (quinoline)	*Cinchona officinalis, C. succirubra, C.* spp. , *Remijia pedunculata* (Rubiaceae)	MAO-A (IC_{50} = 16 μM)

Compound (class)	***Species* (Family)**	**Enzyme inhibited (biological activity) in vivo effects**
Tetrahydroharmine (β-carboline, indole)	*Banisteria caapi* (liana), *Banisteriopsis caapi* (ayahuasca) Malpighiaceae)	MAO
1,2,3,4-Tetrahydroisoquinoline 1-cyano adduct (isoquinoline)	Tobacco smoke [ex *Nicotiana tabacum* (Solanaceae)]	MAO-A (K_d ~ 30 nM) tobacco smoke inhibits MAO and has a protective effect against Parkinson's disease
1,2,3,4-Tetrahydroisoquinoline *N*-(1'-cyanoethyl), *N*-(1'-cyanopropyl) & *N*-(1'-cyanobutyl) adducts (isoquinoline)	Tobacco smoke [ex *Nicotiana tabacum* (Solanaceae)]	MAO-A (K_d ~ 30 nM) tobacco smoke inhibits MAO and has a protective effect against Parkinson's disease
Tryptamine (=3-(2-aminoethyl) indole) (indole)	*Cucumis sativus* (Cucurbitaceae), *Mucuna pruriens*, *Piptadenia peregrina*, *Prosopis juliflora* (Fabaceae), *Hordeum vulgare*, *Zea mays* (Poaceae), *Lycopersicon esculentum*, *Nicotiana tabacum*, *Solanum melongena*, *S. tuberosum* (Solanaceae)	Precursor of tetrahydro-β-carboline which has MAO-A (IC_{50} = 5 μM) and MAO-B (IC_{50} ~ 50 μM)
Phenolic		
Apigenin (= 5,7,4'- trihydroxyflavone) (flavone)	Widespread; Lamiaceae, Asteraceae; Apiaceae; Fabaceae *Apium*, *Daucus* (Apiaceae), *Mentha* (Lamiaceae), ferns (leaf surface)	MAO-A (IC_{50} = 1; 8 μM) MAO-B
Chrysin (= 5,7-dihydroxyflavone) (flavone)	*Daucus* (Apiaceae), *Pinus* spp. (Pinaceae), *Populus* spp. (Salicaeae), *Escallonia* spp. (Saxifragaceae)	MAO-A (IC_{50} = 2 μM) MAO-B
Confluentic acid (depside, aryl ester)	*Himatanthus sucuuba* (Apocynaceae)	MAO-B (IC_{50} = 0.2 μM)
Desmethoxyyangonin (pyrone, phenolic derivative)	*Piper methysticium* (Piperaceae)	MAO-B (IC_{50} = 0.3 μM)
(+/-)-Dihydrokavain (=dihydrokawain) (pyrone, phenolic derivative)	*Piper methysticium* (Piperaceae)	MAO-B
(+/-)-Dihydromethysticin (pyrone, phenolic derivative)	*Piper methysticium* (Piperaceae)	MAO-B
(-)-Epicatechin (2*R*,3*R*)-5,7,3',4'-tetrahydroxyflavan-3-ol) (flavan-3-ol)	Widespread; *Aesculus californica* (Hippocastanaceae), *Pterocarpus* spp. (Fabaceae), *Podocarpus nagi* (Podocarpaceae), *Camellia sinensis* (Theaceae)	MAO- A (IC_{50} > 25 μM)

Compound (class)	***Species* (Family)**	**Enzyme inhibited (biological activity) in vivo effects**
Isopsoralen (furocoumarin)	*Psoralea corylifolia* (Fabaceae)	MAO- A (IC_{50} = 9 μM; K_d = 7 μM) MAO- B (IC_{50} = 13 μM; K_d = 11 μM)
Kaempferol (=3,5,7,4'-tetrahydroxyflavone) (flavonol)	Widespread as aglycone and glycosides	MAO-A (IC_{50} = 0.7 μM) MAO-B
(+/-)-Kavain (= gonosan; kawain) (pyrone, phenolic derivative)	*Piper methysticium* (Piperaceae)	MAO-B
Lemuninol A (naphthalene dimmer)	*Diospyros* sp. (Ebenaceae)	MAO (IC_{50} = 12-25 μM)
Lemuninol B (naphthalene dimmer)	*Diospyros* sp. (Ebenaceae)	MAO (IC_{50} > 62 μM)
Lemuninol C (naphthalene dimmer)	*Diospyros* sp. (Ebenaceae)	MAO (IC_{50} > 60 μM)
Malvidin 3-glycoside (anthocyanin)	*Malva sylvestris* (Malvaceae), *Ligustrum vulgare* (Oleaceae), *Vitis vinifera* (Vitaceae)	MAO-A (IC_{50} > 25 μM) mauve colour
3-Methyl-8-methoxy-1,4-naphthoquinone (naphthoquinone)	*Diospyros* sp. (Ebenaceae)	MAO (IC_{50} > 108 μM)
2'-*O*-methylperlatolic acid (depside, aryl ester)	*Himatanthus sucuuba* (Apocynaceae)	MAO-B (IC_{50} = 81 μM)
N-methyltyrame (phenolic amine)	*Palicourea marcgravii* (Rubiaceae)	MAO-A (competitive substrate)
(+/-)-Methysticin (pyrone, phenolic derivative)	*Piper methysticium* (Piperaceae)	MAO-B (K_d = 1 μM) spasmolytic
Myristicin (phenylpropane)	*Apium graveolens, Daucus carota, Levisticum scoticum, Pastinaca saliva, Petroselinum crispum* (Apiaceae), *Cinnamomum glanduliferum* (Lauraceae), *Orthodon* spp. (Lamiaceae), *Myristica fragans* (Myristicaceae, nutmeg oil)	MAO psychotropic
Pelargonidin 3,5-di-*O*-glucoside (= pelargonin) Anthocyanin)	*Commiphora muhul* (Burseraceae), *Pelargonium zonale* (Geraniaceae), *Gladiolus* sp. (Iridaceae)	MAO-A (IC_{50} > 25 μM) red colour
Psoralen (= ficusin) (furocoumarin)	*Pastinaca sativa, Petroselinium crispum* (Apiaceae), *Coronilla glauca, Psoralea corylifolia, P.* spp. (Fabaceae), *Ficus carica* (Moraceae), *Phebalium argenteum, Xanthoxylum flavum* (Rutaceae)	MAO- A (IC_{50} = 15 μM; K_d = 14 μM) MAO- B (IC_{50} = 62 μM; K_d = 58 μM)

Compound (class)	***Species* (Family)**	**Enzyme inhibited (biological activity) in vivo effects**
trans-Resveratrol (= 3,5,4'-trihydroxystilbene) (stilbene)	*Nothofagus* (Fagaceae), *Cassia, Intsia, Trifolium* (Fabaceae), *Veratum* (Liliaceae), *Eucalyptus* (Myrtaceae), *Pinus* (Pinaceae), *Artocarpus, Morus* (Moraceae), *Polygonium* (Polygonaceae), *Vitis* (Vitaceae) spp.	MAO- A (IC_{50} = 27 μM; K_d = 47 μM)
Tyramine (= 4-hydroxyphenethylamine) (phenolic amine)	*Lophophora williamsi, Trichocereus pachanoi* (Cactaceae), *Hordeum vulgare, Lolium multiflorum* (Poaceae), *Palicourea marcgravii* (Rubiaceae), *Citrus* spp. (Rutaceae), *Viscum album* (Viscaceae)	Precursor of *N*-methyltyramine and tetrahydro-β-carboline Substrate of both MAO-A and B sympathomimetic
Veraphenol (stilbene)	*Veratrum taliense* (Liliaceae)	MAO- A (IC_{50} = 38 μM; K_d = 36 μM)
Yangonin (pyrone, phenolic derivative)	*Piper methysticum* (Piperaceae)	MAO-B
Other		
[2-naphthylamine] (naphthalene amine)	*Nicotiana tabacum* (Solanaceae) cigarette smoke	MAO-A (K_d = 52 μM) MAO-B (K_d = 40 μM)
Non-plant reference compound		
Pargyline	Synthetic	MAO-A antihypertensive
Clorgyline	Synthetic	Irreversibly inhibits MAO-A *in vivo*
Deprenyl	Synthetic	MAO-B clinical Alzheimer's and Parkinson' s disease treatment

5.3. Screening of southern African plants for monoamine oxidase B (MAO-B) inhibitors

5.3.1. Introduction

Monoamine oxidases (MAOs) are flavoenzymes that catalyze the oxidative deamineation of primary, secondary and some tertiary amines to imines, which further hydrolyse to the corresponding inactive aldehydes (BINDA, HUBÁLEK, LI, HERZIG, STERLING, EDMONDSON, MATTEVI, 2004; EDMONDSON, BINDA and MATTEVI, 2004). The overall oxidation reaction proceeds as follows:

$$RCH_2NH_2 + FAD + O_2 + H_2O \rightarrow RCHO + FADH_2 + H_20_2 + NH_3$$

Flavin adenine dinucleotide (FAD) is a redox coenzyme involved in several important reactions in metabolism. FAD can exist in two different redox states and its biochemical role usually involves changing between these two states. FAD can be reduced to $FADH_2$, whereby it accepts two hydrogen atoms.

Monoamine oxidase (MAO) is an enzyme present in the outer-mitochondrial membrane of neuronal and non-neuronal cells. Two isoforms of MAO are recognized, commonly referred to as MAO-A and MAO-B. The enzymes have two main functions in humans; they regulate the free intra-neural concentration and storage of noradrenalin and serotonin; they also inactivate, by oxidative deamination, endogenous and ingested amines (e.g. tyramine) (RANG, DALE and RITTER, 2003). They have different substrate preference (**Table 5.3.1**), inhibitor specificity, and tissue distribution (YAMADA and YASUHARA, 2004). MAO-A preferentially deaminates serotonin, noradrenaline, and adrenaline. In the human brain about 75% of MAO is of the B subtype (SAURA MARTI, KETTLER, DA PRADA and RICHARDS, 1990). MAO-B deaminates dopamine, β-phenylethylamine (PEA), and benzylamine.

Table 5.3.1. Substrate preferences for subtypes of MAO (FOLEY, GERLACH, YOUDIM and RIEDERER, 2000).

MAO Subtype	Substrate preference
MAO-A	Serotonin Noradrenalin Adrenaline Octopamine
MAO-A and MAO-B	Dopamine Tyramine Tryptamine Kynuramine 3-Methoxytyramine
MAO-B	β-Phenethylene Benzylamine Methylhistamine *N*-Acetylputrescine *n*-Phenylamine Octylamine Milacemide 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine

Inhibitors of MAO cause an increase in the amount of these amines stored and released from the nerve terminals, thus increasing the monoaminergic activity. Inhibition of MAO-A predominantly affects neurotransmitters considered to be important in depression and anxiety disorders. MAO-B inhibitors would increase the basal dopamine levels in the nigrostriatal dopaminergic input pathway. Selegiline is the only selective and irreversible MAO-B inhibitor with marketing approval in many countries (BODKIN and AMSTERDAM, 2002; YAMADA and YASUHARA, 2004). More recently, MAO-B inhibitors have been included in the treatment of anxiety disorders, Alzheimer's disease and Parkinson's disease (YAMADA and YASUHARA, 2004). MAO-B inhibition also has neuroprotective effects, since the oxidation step catalyzed by MAO-B yields reactive hydrogen peroxide as a by-product of amine turnover, the generated hydrogen peroxide and other reactive oxygen species may cause deterioration in neuronal function or eventually lead to neuronal death (YAMADA and YASUHARA, 2004).

The pharmaceutical potential of MAO inhibitors, in particular MAO-B inhibitors, has lead to the search for novel active compounds. Several MAO inhibitors from botanicals are described in **Table 5.2.** Apart from *Hypericum perforatum* which contains hypericin reported to show MAO inhibitory activity (BUTTERWECK, NAHRSTEDT, EVANS, HUFEISEN, RAUSER, SAVAGE, POPADAK, ERNSBERGER and ROTH, 2002), several herbal remedies have been investigated. Recent discoveries of specific MAO-A inhibitory activity of traditionally used herbal remedies include *Acorus gramineus* (TAO, IRIE, LI and KEUNG, 2005), *Rhazya stricta* (ALI, BASHIR, TANIRA, MEDVEDEV, JARRETT, SANDLER and GLOVER, 1998), *Zanthoxylum schinifolium* (JO, HOUNG, BAE, LEE and KIM, 2002) and *Kaempferia galangal* (HUONG, DAT, MINH, KANG and KIM, 2002). MAO-A inhibitory activity has been reported in *Arisaema amurense, Lilium brownie, Lycium chinense* (LIN, HOU, YEN and LEE, 2003), *Gentiana lutea* (HARAGUCHI, TANAKA, KABBASH, FUJIOKA, ISHIZU and YAGI, 2004), *Uncaria rhynchophylla* (LIN, HOU, YEN and LEE, 2003; HOU, LIN, CHEN and LEE, 2005).

Parkinson's disease treatment with MAO-B inhibitors

Parkinson's disease (PD) or *Paralysis Agitans* was first documented in 1817 by Dr. James Parkinson as the "shaking palsy" that afflicted his gardener (PARKINSON, 1817, CRITCHLEY, 1955). Affecting one in every 100 persons above the age of 65 years, it is the second most common neurodegenerative disease after Alzheimer's disease (DE RIJK, LAUNER, BERGER, BRETELER, DARTIGUES, BALDERESCHI, FRATIGLIONI, LOBO, MARTINEZ-LAGE, TRENKWALDER and HOFMAN, 2000) and the most common neurologically based movement disorder, clinically diagnosed by the presence of bradykinesia, postural instability, resting tremor and rigidity.

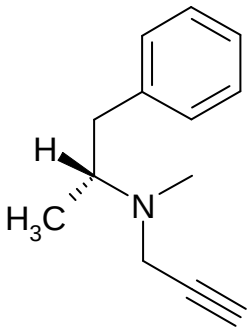
PD is characterized neuropathologically by the presence of intracytoplasmic inclusions from protein aggregates called Lewy bodies (LBs) (BURKE, 1998) and the depletion of pigmented dopamine-containing neurons in the region known as the substantia nigra pars compacta (FORNO, 1996). LBs consist of a heterogeneous mixture of proteins and lipids. Approximately 80% of dopaminergic neurons

in the susbtantia nigra are already irreversibly destroyed when the first symptoms of PD become significantly visible. To date PD remains an incurable disease. The currently available pharmacological and non-pharmacological treatments are able to offer only symptomatic relief for patients (KATZUNG, 2001). Symptoms can be managed with several different drugs most of which either boost the levels of dopamine in the brain or mimic the effects of dopamine.

Because dopamine is preferentially deaminated by MAO-B in the human brain, MAO-B inhibitors should increase the basal central dopamine levels in patients with Parkinson's disease. The MAO-B inhibitor selegiline was developed for the treatment of Parkinson's disease based on this hypothesis (KNOLL, 2000). MAO-B inhibition also has neuroprotective effects. Since the oxidation step catalyzed by MAO-B yields reactive hydrogen peroxide as a by-product of amine turnover, the generated hydrogen peroxide and other reactive oxygen species may cause deterioration in neuronal function or eventually lead to neuronal death. MAO inhibitors reduce oxidant stress by limiting the formation of this reactive species and, hence, may contribute to the control of the aging process (BLANDINI and GREENAMYRE, 1999; KNOLL, 2000). The findings that selegiline prevents MPTP-induced Parkinsonism stimulated an interest in anti-oxidative therapy as a means of retarding the progression of Parkinson's disease (SHOULSON, 1998).

Similarly, the use of MAO-B inhibitors in the treatment of AD has been suggested to suppress the neurodegenerative processes that occur, primarily by the reduced oxidant stress. The enzymatic activity of MAO-B increases with age, and is particularly high around senile plaques (YAMADA and YASUHARA, 2004).

The selective inhibitor of MAO-B, (R)-deprenyl (selegeline), is already in use in dopamine replacement therapy in PD. Currently treatment with deprenyl occurs in combination with L-DOPA, assuming that the MAO-B inhibitor reduces the metabolism of dopamine delaying the need for L-DOPA therapy. A reduction in apotic cell death due to oxidative-stress is another positive effect reported in treatment with deprenyl. A disadvantage of the treatment is that (R)-deprenyl is metabolized to (R)-ethamphetamine which has vasopressor properties (VLOK, MALAN, CASTAGNOLI, BERGH, and PETZER, 2006).



(R)-deprenyl

Due to the disadvantage of the side effects associated with the traditional non-selective irreversible MAOIs ('cheese effect') coupled with the potential therapeutic value of new inhibitors that are reversible and selective towards either MAO-A or MAO-B, the search for new MAO inhibitors is validated (KONG, CHENG and TAN, 2001; 2004; VLOK, MALAN, CASTAGNOLI, BERGH, and PETZER, 2006).

Detection of MAO activity

When developing a bioassay the target must be relevant to the disease of interest. Typical targets of *in vitro* assays can be receptors as was the case in previous Chapters or enzymes and cell line models. It is further important that the assay gives a quantifiable response and that the assay includes a negative control (ensure not false positive reaction) as well as a positive control (ensure the desired reaction is working) (HOUGHTON, 2000).

There are several different assays for detection of MAO-I described in the literature (**Table 5.3.2.**). They each have their advantages and disadvantages. Generally it is important that the assay is reproducible, quick, and possesses a low risk for false positive or false negative results. The sensitivity of the method is also important. Additionally, and most importantly in this case, would be the running cost and the availability of equipment required to run the assay. Radiolabelled compounds are too expensive, fluorometric and HPLC detection equipment was not available leaving only the spectrophotometric option.

Since hydrogen peroxide is a product formed during deamination of almost all amine oxidase substrates, it represents an ideal target for a quantitative amine oxidase assay, and although generally less sensitive than other detection methods, spectrophotometry perhaps represents that technique which is most widely accessible to all laboratories.

Table 5.3.2. Methods for the detection of MAO activity.

Assay	Detection method	Substrate(s)	References
Peroxidase-linked spectrophotometric assay	Spectrophotometric determination of quinoneimine dye (nm). Hydrogen peroxide produced during the oxidative deamination of monoamines in turn oxidises 4-aminoantipyrine in the presence of peroxidase. The oxidised 4-aminoantipyrine condenses with vanillic acid to give a red quinoneimine dye.	tyramine	HOLT et al., 1997; SCHMIDT et al., 2003
Spectrophotometric determination of kynuramine consumption	Spectrophotometric determination of oxidative deamination of kynuramine by MAO.	kynuramine	WEISSBACH et al., 1960; SCHMIDT et al., 2003

Assay	Detection method	Substrate(s)	References
4-hydroxyquinoline fluorometric detection	The fluorescence of 4-hydroxyquinoline, which is formed from kynuramine by MAO, is measured at an excitation wavelength of 315 nm and an emission wavelength of 380 nm, using a fluorescence spectrometer	kynuramine	KRAML, 1965; SATOH and YAMAZAKI, 1989; LEE et al., 1999; HARAGUCHI et al., 2004.
Amplex-red fluorometric assay	In the presence of peroxidase, the H_2O_2 drives the oxidation of Amplex Red forming Resorufin, the fluorescent product. The Resorufin fluorescence is measured using an excitation wavelength of 550 nm and an emission wavelength of 585 nm. This reaction has been frequently employed for measurements of low concentrations of H_2O_2 in biological samples.	5-HT for MAO-A and benzylamine for MAO-B assay	ZHOU and PANCHUK-VOLOSHINA, 1997
Radiolabelled substrate assay	The MAO activity was determined radiometrically by using [^{3}H]-5-HT creatinine sulfate (final radioactivity 5 mCi/mmol) and [^{14}C]-PEA hydrochloride (final radioactivity 20 mCi/mmol) as the selective substrates of MAO-Aand MAO-B, respectively. The inhibitors used in this study were added right before initiation of the reaction by adding substrates.	[^{14}C] 5-HT for MAO-A assay and [^{14}C] β-PEA for MAO-B assay	WURTMAN and AXELROD, 1963; TIPTON,1985; LYLES and CALLINGHAM, 1982; EGASHIRA et al., 1999; KONG et al., 2001; KONG et al., 2004.
Rapid fluorimetric assay for monoamine oxidase utilizing HPLC	This method utilizes high pressure liquid chromatography with fluorescence excitation at 280 nm and detection at 330 nm at pH 5.0 of indoleacetic acid and 5-hydroxyindoleacetic acid, the deaminated products of two substrates for MAO, tryptamine, and serotonin, respectively. The assay allows for the complete separation of metabolites from either of the two substrates. The method has been used to determine MAO activity in the frontal cortex and caudate nucleus of rat brain using tryptamine and serotonin as substrates.	tryptamine for MAO-A, and serotonin (5-HT) for MAO-B	KOBES et al., 1980
HPLC with electrochemical detection	The end product dihydroxyphenylacetic acid (DOPAC) is measured by electrochemical detection.	dopamine	HUSSEINI et al., 1995
HPLC-based bioactivity profiling using human recombinant monoamine oxidase	This assay involves the combination of human recombinant MAO-A, expressed as GST-fusion protein in yeast, with the kinetic measurement of the conversion kynuramine to 4-hydroxyquinoline.	kynuramine	DITTMANN et al., 2004

Assay	Detection method	Substrate(s)	References
On-line radiochemical assay for monoamine oxidase utilizing HPLC	The method is based on the separation and quantitation of ^{14}C-labeled assay products by high-performance liquid chromatography, which is interfaced directly into a flow-through radioactivity detector. This allows on-line quantization of the radioactive compounds with picomole sensitivity. The method makes possible the complete separation and detection of the deaminated products of monoamine oxidase A and B substrates benzylamine and 5-hydroxytryptamine, respectively. This assay has been applied to the measurement of monoamine oxidase A and B activities in rat brain.	benzylamine and 5-HT	NISSINEN et al., 1984

A sensitive colorimetric assay was developed by SZUTOWICZ, KOBESAND and ORSULAK (1984) to be used for the determination of MAO activities. However, this peroxidase-coupled method, which uses 2,2'-azino-bis(3- ethylbenzthiazoline-6-sulfonic acid) (ABTS) as the chromogen, suffers from the drawback that ABTS causes substantial inhibition of MAO at concentrations present in the assay. Furthermore, the chromogen is insoluble and absorbs only weakly at the relatively neutral pH conditions necessary for MAO activity. As a result, while this reagent is extremely useful in the discontinuous assay described by the authors, it is unsuitable for use in continuous measurement protocols. A peroxidase-linked colorimetric assay, described originally by YAMADA, ISOBE, TANI and HIROMI (1979), where 4-aminoantipyrine acts as the proton donor in the peroxidase reaction and then condenses with 2,4-dichlorophenol to form a red quinoneimine dye was therefore considered. The absorbance, measured at 495 nm, is directly proportional to the amount of hydrogen peroxide formed during amine metabolism. While this protocol has proved suitable for the assay of most, if not all enzymes classified as EC 1.4.3.6, HOLT, SHARMAN, BAKER and PALCIC (1997) found that no color formation was evident when homogenates of, or mitochondria purified from, rat liver or brain were incubated with MAO substrates. This was overcome by replacing 2,4-dichlorophenol with vanillic acid (HOLT, SHARMAN, BAKER and PALCIC, 1997), resulting in a rapid quantitative spectrophotometric assay (**Figure 5.3.2**).

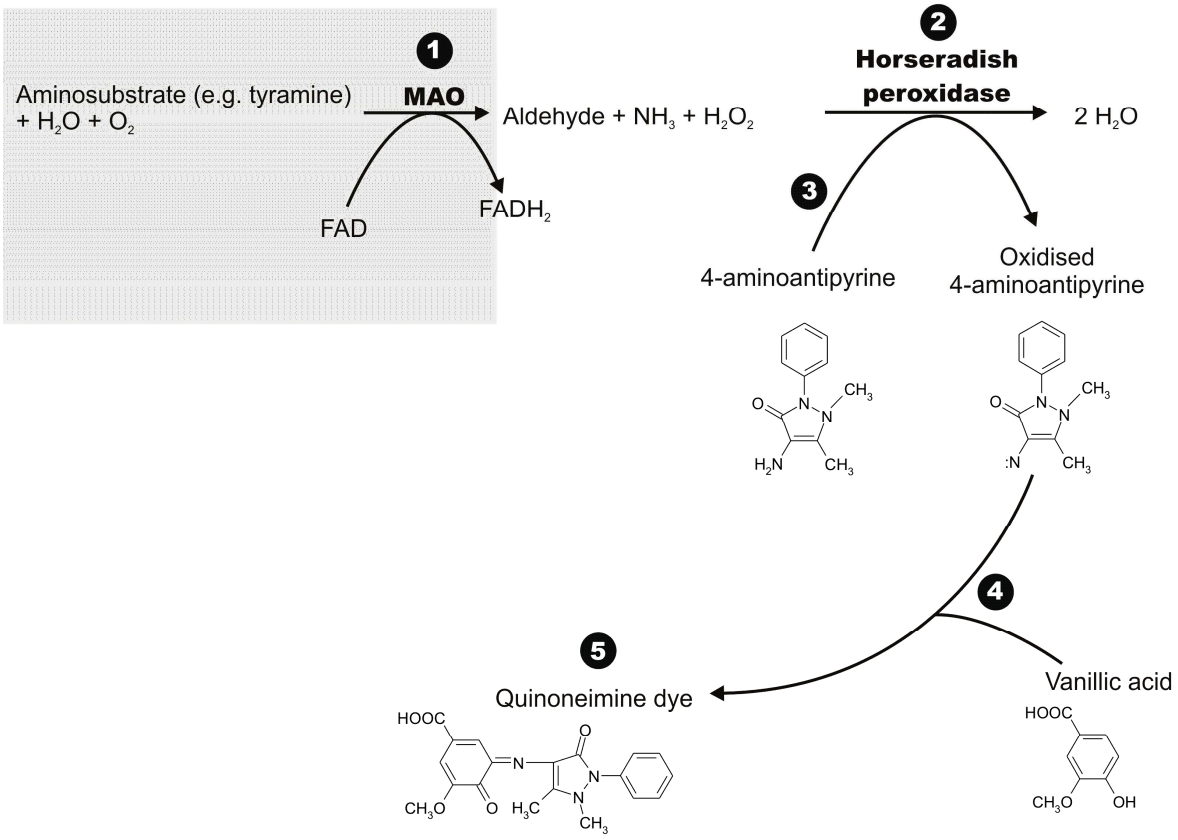


Figure 5.3.2. Scheme for the continuous peroxidase-linked photometric monoamine oxidase (MAO) inhibitor bioassay. (1) The inhibition of MAO isolated from rat liver tissue, which catalyze the oxidative deamination of monoamines (e.g. tyramine) to aldehydes. The hydrogen peroxide produced by this rate determining step oxidises 4-aminoantipyrine (3) in the presence of peroxidase (2). The oxidised 4-aminoantipyrine condenses with vanillic acid (4) to give a red quinoneimine dye (5). The production of the quinoneimine dye was detected at 490 nm by a microplate reader.

The continuous peroxidase-linked photometric monoamine oxidase (MAO) inhibitor bioassay for the detection of MAO inhibitory activity of plant extracts was developed and validated together with P.D. Pedersen, Department of Medicinal Chemistry, The Faculty of Pharmaceutical Sciences, University of Copenhagen (PEDERSEN, 2005).

Aims

More than 120 *Helichrysum* species are found in KwaZulu-Natal and many are used in traditional remedies and cultural ceremonies (HUTCHINGS, SCOTT, LEWIS and CUNNINGHAM, 1996). The most frequent use is as *imphepho*, where the leaves and stems are burned as incense to 'invoke the goodwill of ancestral spirits' (HUTCHINGS, SCOTT, LEWIS and CUNNINGHAM, 1996). Some species are used by *izangoma* (traditional healer) to induce trances (HUTCHINGS, SCOTT, LEWIS and CUNNINGHAM, 1996) and dressings for infected wounds (WATT and BREYER-BRANDWIJK, 1962). *H. nudifolium* (icholocholo, in Xhoza and Zulu) a common species, is also used in infusions (teas) to treat colds and steam from infusions poured on hot stones is inhaled to treat bad dreams and fever (WATT and BREYER-BRANDWIJK, 1962). These apparent CNS-related uses prompted the investigation in the MAO inhibition of extracts of several *Helichrysum* species. The success of this screen lead to a further screening of other South African plants with reported CNS-related medicinal uses. Good MAO inhibitory

activity was detected in *Mentha aquatica*, which prompted the bioassay-guided fraction, isolation and identifaction of an active compound.

5.3.2. Materials and Methods

Plant materials

Seven *Helichrysum* species (Asteraceae) were collected in KwaZulu-Natal, South Africa from September 2004 to January 2005 for intial screening in the peroxidase-linked photometric assay. Voucher specimens are deposited in the University of KwaZulu-Natal Herbarium (**Table 5.3.2.1**). Plant material was oven-dried at 50 °C for two days.

Table 5.3.2.1. *Helichrysum* species investigated, including voucher numbers and part used.

Species	**Voucher Number**	**Plant part tested**
Helichrysum argyrolepis MacOwan	Stafford 213 NU	Leaf
Helichrysum herbaceum (Andrews) Sweet	Stafford 214 NU	Whole plant
Helichrysum nudifolium Less.	Stafford 215 NU	Leaf
Helichrysum ruderale Hilliard & B.L. Burtt	Stafford 216 NU	Whole plant
Helichrysum rugulosum Less.	Stafford 217 NU	Whole plant
Helichrysum simillimum DC.	Stafford 218 NU	Whole plant
Helichrysum umbraculigerum Less.	Stafford 219 NU	Whole plant

Other plant species traditionally used as sedatives or to treat various CNS-related ailments, were selected based on the literature relating to their use in the treatment of mental diseases. Plants were collected in KwaZulu-Natal, South Africa. Voucher specimens are deposited in the University of KwaZulu-Natal Herbarium (**Table 5.3.2.2**). Plant material was dried at 50 ºC for a maximum of two days.

Table .5.3.2.2. Plant species, herbarium voucher numbers and plant part investigated.

Plant name	**Family**	**Voucher specimen**	**Plant part tested**
Agapanthus campanulatus F.M.Leighton	Alliaceae	*Stafford 59 NU*	Bulb and root
Agapanthus praecox Willd.	Alliaceae	*Stafford 212 NU*	Bulb and root
Boophane disticha (L.f.) Herb	Amaryllidaceae	*Stafford 53 NU*	Leaf
Buddleja salvifolia L.	Loganiaceae	*Stafford 41 NU*	Leaf
Cinnamomum camphora (L.) T.Ness & C.H.Eberm.	Lauraceae	*Stafford 69 NU*	Leaf
Clausena anisata (Willd.) Hook.f.	Rutaceae	*Stafford 47 NU*	Leaf
Datura ferox L.	Solanaceae	*Stafford 72 NU*	Seeds
Datura stramonium L.	Solanaceae	*Stafford 73 NU*	Seeds
Dioscorea dregeana Baker	Dioscoreaceae	*Stafford 221 NU*	Leaf
Gasteria croucheri (Hook.f.) Baker	Aloaceae	*Stafford 18 NU*	Root
Gomphocarpus physocarpus E.Mey	Asclepiadaceae	*Stafford 207 NU*	Leaf

Plant name	Family	Voucher specimen	Plant part tested
Hypoxis hemerocallidea Fisch. & C.A. Mey	Hypoxidaceae	*Stafford 34 NU*	Bulb
Leonotis leonurus (L.) R.Br.	Lamiaceae	*Stafford 38 NU*	Leaf
Mentha aquatica L.	Lamiaceae	*Stafford 84 NU*	Leaf
Millettia grandis (E.Mey.) Skeels	Fabaceae	*Stafford 125 NU*	Leaf
Rhoicissus tridentata (L.f.) Wild. et R.B.Drumm	Vitaceae	*Stafford 13 NU*	Leaf
Ruta graveolens L.	Rutaceae	*Stafford 48 NU*	Leaf
Scadoxus puniceus (L.) Friis & I. Nordal	Amaryllidaceae	*Stafford 206 NU*	Root
Schotia brachypetala Sond.	Fabaceae	*MacGaw 85 NU*	Bark
Xysmalobium undulatum (L.) Aiton.f.	Asclepiadaceae	*Stafford 95 NU*	Root

Aerial parts of *Mentha aquatica* for bulk extraction and isolation of active constituent were collected at Cedara (29°32′S 30°17′E), KwaZulu-Natal, South Africa. A voucher specimen is deposited in the Bews Herbarium, University of KwaZulu-Natal, Pietermaritzburg (Stafford NU 84).

Preparations of extracts for screening

Two g of material were extracted three times with 20 ml solvent (water, 70% ethanol, ethyl acetate and petroleum ether) for 60 min in an ultrasound bath. The extracts were then filtered under vacuum through Whatman No 1 filter paper. The filtered extracts were taken to dryness under reduced pressure at 40°C. The residues were re-dissolved in DMSO respectively at 36 mg/ml when required, to be diluted further in the assay with potassium phosphate buffer (0.2M, pH 7.6) to seven final concentrations of 1, 0.5, 0.25, 0.1, 0.01, 0.001 and 0.0001 mg/ml respectively.

Isolation and structure elucidation of naringenin from M. aquatica

*Extraction***:** 2 g of dried, ground plant material were extracted twice either with 20 ml water, 70% ethanol, acetone, butanol, dichloromethane or petroleum ether in an ultrasound bath. For isolation, 500 g dried, ground plant material was defatted with petroleum ether. After drying, the plant material was extracted with 70% ethanol.

Fractionation on VLC: The 70 % ethanol extract (25.71 g) was fractionated on 200 g of Merck Silica Gel 60 in a vacuum liquid column. 400 ml of each of the following solvent mixtures were used; petroleum ether: ethyl acetate; 100:0, 80:20, 60:40, 40:60, 20:80, 0:100 and ethyl acetate: ethanol 80:20, 60:40, 40:60, 20:80, 0:100. The column was then washed with 400 ml methanol and 400 ml water. Active fractions (petroleum ether: ethyl acetate; 20:80, 0:100 and ethyl acetate: ethanol; 80:20) were combined.

Preparative TLC systems: The combined active fractions were loaded on preparative TLC plates (Merck Silica Gel 60, 0.25 mm thickness) developed in hexane: ethyl acetate 3:2. The plates were divided into bands and the silica scraped off. The silica was eluted using ethyl acetate, and the eluate passed through a

Celite column to remove silica, before being assayed for MAO inhibition. An active band with R_f-value 0.35 was loaded on a TLC plate, which was developed in hexane: ethyl acetate 1:2. The active band (80 mg) on this plate had an R_f-value of 0.78.

Stucture elucidation: ^{1}H, ^{13}C and ^{13}C-DEPT NMR spectra were recorded using a Gemini Varian NMR instrument. Optical rotation was determined on a Perkin-Elmer 241 polarimeter.

Preparation of MAO-rich rat liver homogenate

MAO was partially purified by isolation of mitochondria from rat liver homogenates according to HOLT, SHARMAN, BAKER and PALCIC (1997). Briefly, male Wistar rats (280-300g), were euthanased by carbon monoxide and livers dissected out, washed in ice-cold potassium phosphate buffer (0.2 M, pH 7.6), and stored at -70 °C until required. Liver tissue (5 g) was homogenized 1:40 (w/v) in 0.3 M sucrose. Following centrifugation at 1000 x *g* for 10 min the supernatant was further centrifuged at 10 000*g* for 30 min to obtain a crude mitochondrial pellet. The pellet was resuspended in 4 ml of 0.3 M sucrose and was layered onto 40 ml of 1.2 M sucrose. A mitochondrial pellet was obtained by centrifugation at 53 000 x *g* for 2 h. Following a single wash in potassium phosphate buffer; mitochondria were suspended in 40 ml buffer. Total protein concentration was measured by the method of BRADFORD (1976) and adjusted with phosphate buffer (0.2 M; pH 7.6) to 0.2 mg protein per ml, after which aliquots of 1 ml were stored at -70 °C until required.

Monoamineoxidase peroxidase linked assay

The continuous peroxidase-linked photometric assay was carried out in the 96-well microtiter format modified from HOLT, SHARMAN, BAKER and PALCIC (1997) and SCHMIDT, LI, SCHUBERT, HUANG, STOYANOVA and HAMBURGER (2003) (**Figure 5.3.2**). Plant extracts (water, 70% ethanol, ethyl acetate and petroleum ether) were re-dissolved to 36 mg/ml with DMSO. Plant extracts were ten-fold serially diluted with potassium phosphate buffer (0.2 M, pH 7.6) and 40 µl of each dilution was placed in 96-well microplates to give final concentrations from 6 to 0.0006 mg/ml. Distilled water was use as a negative control. Each test well contained 120 µl substrate (2.5 mM tyramine in potassium phosphate buffer), 40 µl chromogenic solution (1 mM vanillic acid, 0.5 mM 4-aminoantipyrine, 4 U/ml peroxidase in potassium phosphate buffer), 40 µl enzyme (rat liver homogenate) and 40 µl of sample. Background wells contained potassium phosphate buffer (0.2 M, pH 7.6) in place of enzyme (rat liver homogenate). To test for specific MAO-B activity the rat liver homogenate was pre-incubated (37 °C; 30 min) with 50 µM clorgyline (selective MAO-A-I) to total block MAO-A activity. Reactions were followed at 490 nm using a microplate-reader. Absorbancy readings were taken every 5 min over a period of 40 min. Plates were incubated between readings at 37 °C. Percent inhibition was calculated from the slope of the absorbancy/time plot (test well reading minus background reading), relative to negative controls (distilled water) serving for measurement of 0% inhibition plots. IC_{50} concentrations were calculated using Grafit 5© (Erithacus Software Limited). Assays were done in triplicate. For

determination of MAO-A/B selectivity, pure enzymes (Sigma) were used at 8 U/ml in place of rat liver homogenate. Clorgyline and deprenyl were used as selective inhibitors.

5.5.3. Results and Discussion

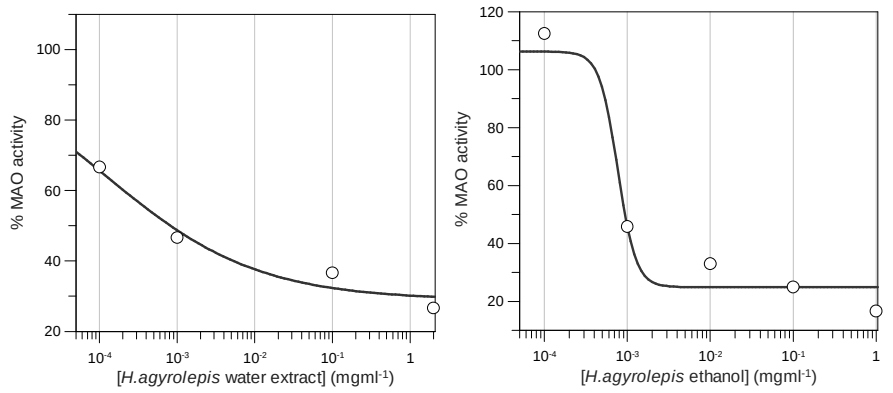
MAO-I activity of aqueous and ethanolic extract of seven *Helichrysum* species using a peroxidase-linked photometric assay are shown in **Table 5.3.3.1** and **Figure 5.3.3.1.** Clorgyline and Selegiline (L-deprenyl) are irreversible inactivators of MAO which have been used extensively because of their selectivity for MAO-A and MAO-B respectively. They were included in the assays as positive controls. Although MAO-A and MAO-B are expressed in most mammalian tissues, marked species differences in both tissue distribution and substrate specificities have been described. In the rat liver it is thought that MAO-B is predominant (INOUE, CASTAGNOLI, MABIC, IGARASHI and CASTAGNOLI, 1999). Seven species were assayed for MAO inhibitory activity using a peroxidase-linked photometric assay. *H. agyrolepis* (water extract IC_{50} = 0.1 µg/ml; ethanol extract 0.8 µg/ml), *H. umbraculigerum* (water extract IC_{50} = 2.4 µg/ml; ethanol extract 3.1 µg/ml) were the most active species, although all species tested exhibited varying degrees of MAO inhibitory activity. These active species showed good dose-dependant activity (**Figure 5.3.3.1.**). It is possible that the active compound(s) has been extracted by both water and 70% ethanol; in both plants the aqueous extract was more active. This validates the use of these plants in infusions as it is traditionally administered, but further research is required to determine if the smoke derived from these plants has any biological activity.

Table 5.3.3.1. Determination of MAO-I activity of aqueous and ethanolic extracts of seven *Helichrysum* species using a peroxidase-linked photometric assay (IC_{50} values expressed in µg/ml). Values are the mean of results obtained from three assays.

Plant extract	**IC_{50} (µg/ml) [b]**
H.agyrolepis, water	**0.1±0.009**
H.agyrolepis, ethanol	**0.8±0.3**
H.herbaceum, water	57.3±84.1
H.herbaceum, ethanol	108.5±126
H.nudifolium, water	n.d.
H.nudifolium, ethanol	7.3±4.8
H.ruderale, water	22.9±82
H.ruderale, ethanol	3.3±5
H.rugulosum, water	155.2±335
H.rugulosum, ethanol	17.2±34
H.simillimum, water	23.0±14
H.simillimum, ethanol	n.d.
H.umbraculigerum, water	**2.4±1.8**
H.umbraculigerum, ethanol	**3.1±0.1**
Clorgyline (selective MAO-A inhibitor)	31±10 nM
Selegiline (selective MAO-B inhibitor)	111±68 nM
Clorgyline + Selegiline (1:1)	39±2 nM

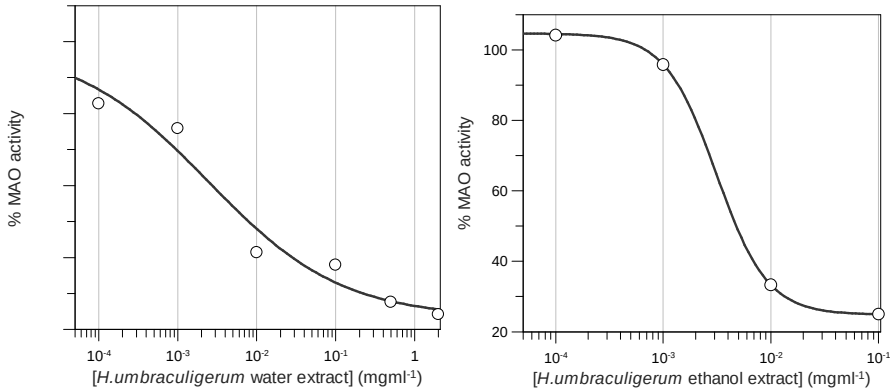
[a] IC_{50} and standard error calculated using Grafit 5 (© Erithacus Software Limited). Extract concentration in (µg/ml) and standard reference drugs in nM.

MAO-I activity has not be shown in *Helichrysum* species, but two active compounds have been isolated from other Asteraceae. Norharman (=β-carboline) an indole alkaloid from *Cichorium intybus* is a week MAO-A inhibitor (POLYA, 2003). Apigenin, a flavone which occurs in *Erigeron annuus* and *Matricaria chamomilla* amongst other non-Asteraceae species, especially the Apiaceae, is a MAO-A inhibitor (IC_{50} = 8 µM) (POLYA, 2003). In the previous Chapter it was demonstrated that *H. hesbaceum*, *H. ruderale*, *H. simillimum* and *H. umbraculigerum* (ethanolic leaf extracts) possessed good dose dependant *in vitro* $GABA_A$-benzodiazepine receptor binding activity (STAFFORD, JÄGER and VAN STADEN, 2005).



Parameter	Value	Std. Error
Y Range	71.2184	8.8891
IC 50	0.0001	9.34502e-005
Slope factor	0.4414	0.2540
Background	28.9063	6.4943

Parameter	Value	Std. Error
Y Range	81.3296	8.4279
IC 50	0.0008	0.0003
Slope factor	3.9736	5.5410
Background	24.9206	5.2686



Parameter	Value	Std. Error
Y Range	75.7282	10.5309
IC 50	0.0024	0.0018
Slope factor	0.5138	0.1832
Background	23.2340	7.3084

Parameter	Value	Std. Error
Y Range	79.8087	0.6181
IC 50	0.0031	0.0001
Slope factor	1.8290	0.0484
Background	24.8540	0.5025

Figure 5.3.3.1. IC_{50} determinations of *H. argyrolepis* (upper) water extracts, ethanol extracts and *H. umbraculigerum* (lower) water extracts, ethanol extracts.

Table 5.3.3.2 shows the IC_{50} values for MAO inhibitory activity of South African medicinal plants. IC_{50} concentrations and standard error were calculated using Grafit 5© (Erithacus Software Limited). The standard error (SE) calculated is related to closeness of fit of the activity at each concentration of extract to the sigmoidal IC_{50} graph. Therefore a high SE is an indication of poor dose-dependant activity. The non-polar extracts of *Ruta graveolens* leaf material exhibited the best MAO inhibitory activity (ethyl acetate extract = IC_{50} 5±1 µg/ml; petroleum ether extract = 3±1 µg/ml) and specific MAO-B inhibition (ethyl acetate extract = IC_{50} 7.±6 µg/ml; petroleum ether extract = 3±1 µg/ml) (**Figure 5.3.3.2**). *Schotia brachypetala*, *Mentha aquatica* and *Gasteria croucheri* also exhibited good MAO-B inhibition activity.

Table 5.3.3.2. MAO inhibitory activity of South African medicinal plants.

FAMILY *Species*	Plant Part	Extract	Non selective MAO inhibition IC_{50} (µg/ml) [a]	Selective MAO-B inhibition IC_{50} (µg/ml) [ab]
ALLIACEAE				
Agapanthus campanulatus	bulb	water	nd	nt
		ethanol	nd	nt
	root	water	nd	nt
		ethanol	nd	nt
Agapanthus praecox	bulb	water	nd	nt
		ethanol	218±141	nt
	root	water	nd	nt
		ethanol	nd	nt
AMARYLLIDACEAE				
Scadoxus puniceus	root/bulb	water	853±596	nt
	root/bulb	ethanol	406±411	344±242
ALOACEAE				
Gasteria croucheri	root	ethanol	72±38	nt
ASCLEPIDACEAE				
Gomphocarpus physocarpus	leaf	ethanol	1040±680	199±153
Xysmalobium undulatum	rhizome	ethyl acetate	849±110	nt
DIOSCOREACEAE				
Dioscorea dregeana	leaf	ethanol	108±119	nd
FABACEAE				
Schotia brachypetala	bark	water	5±5	nd
	bark	ethanol	44±15	nd
HYPOXIDACEAE				
Hypoxis hemerocallidea	bulb	ethanol	53±27	nt
	bulb	ethyl acetate	25±5	nt
LAMIACEAE				
Leonotis leonurus	leaf	water	1110±147	345±399
	leaf	ethanol	63±12	nt
Mentha aquatica	leaf	water	23±5	101±21
	leaf	ethanol	24±36	68±42
LAURACEAE				
Cinnamomum camphora	leaf	water	156±33	nt
LOGANIACEAE				
Buddleja salvifolia	leaf	water	47±22	nt
		ethanol	8±1	nt
		ethyl acetate	12±2	nt
RUTACEAE				
Clausena anisata	leaf	ethanol	45±42	nt
Ruta graveolens	leaf	water	267±262	1436±909
	leaf	ethanol	18.5±1.5	35±56
	leaf	ethyl acetate	5±1	7±6
	leaf	petroleum ether	3±1	3±1

FAMILY *Species*	Plant Part	Extract	Non selective MAO inhibition IC_{50} (µg/ml) [a]	Selective MAO-B inhibition IC_{50} (µg/ml) [ab]
SOLANACEAE				
Datura stramonium	seeds	water	4136±2195	nd
VITACEAE				
Rhoicissus tridentata	leaf	water	595±915	nt
	leaf	ethanol	864±1000	nt
STANDARDS				
Clorgyline (selective MAO-A inhibitor)			31±10 nM	
Selegiline (R-deprenyl) (selective MAO-B inhibitor)			111±68 nM	
Clorgyline + Selegiline (1:1)			39±2 nM	

[a] IC_{50} and standard error calculated using Grafit 5 (© Erithacus Software Limited). Extract concentration in (µg/ml) and standard reference drugs in nM. [b] activity not detected – nd, extract not tested – nt

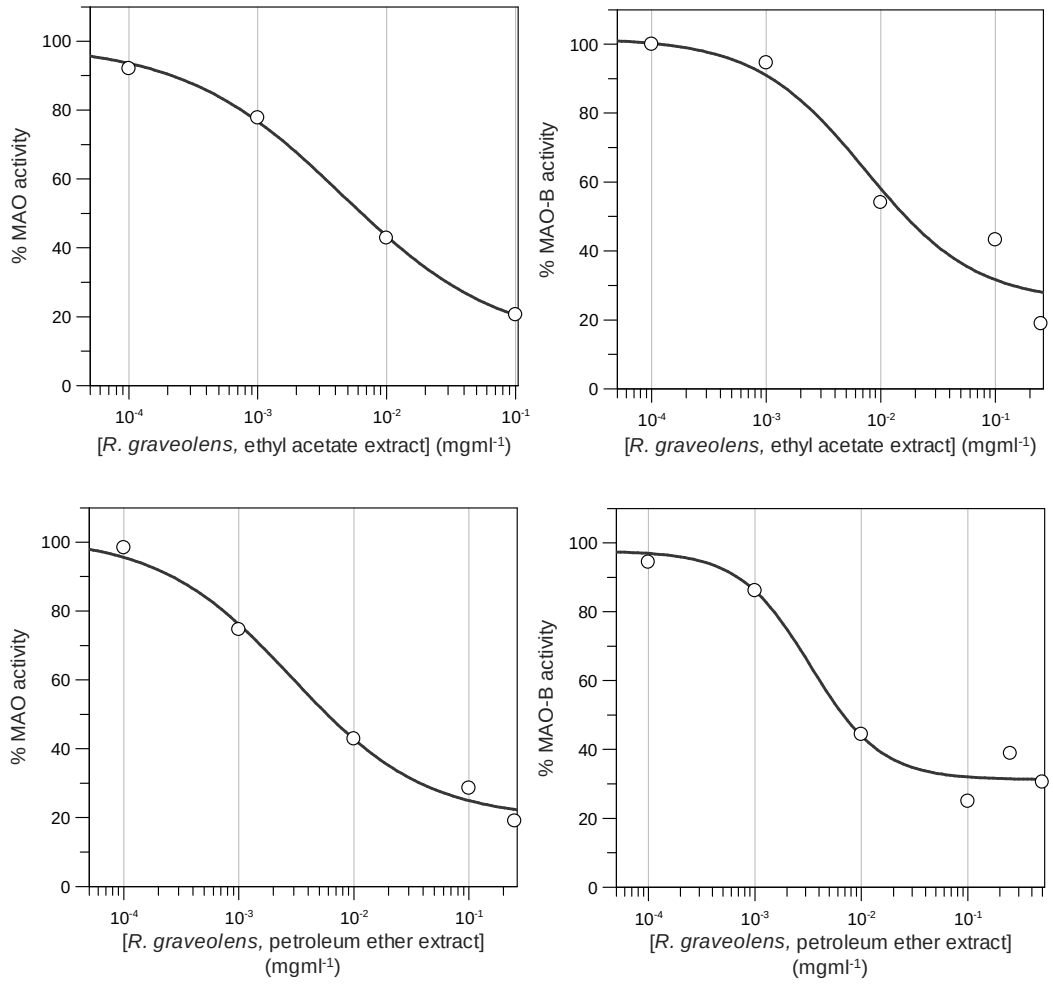


Figure 5.3.3.2. IC_{50} determinations of *Ruta graveolens* MAO activity (left) ethyl acetate (upper; IC_{50} = 5±1 mg/ml) and petroleum ether extracts (lower; IC_{50} = 3±1 mg/ml), and MAO-B activity (right) ethyl acetate (upper; IC_{50} = 7±6 mg/ml) and petroleum ether extracts (lower; IC_{50} = 3±1 mg/ml).

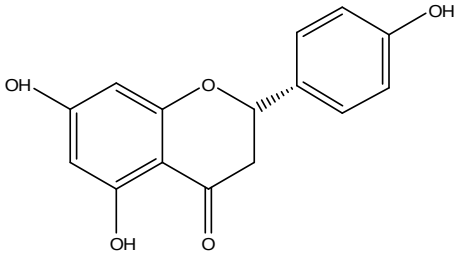
Since ancient times, *R. graveolens* (garden rue) has been an important plant in the European pharmacopoeia (SAN MIGUEL, 2003). Its medicinal value is due to the numerous secondary metabolites it contains like furocoumarins, furoquinolines and acridone alkaloids. Recently, the methanol, petroleum ether, ethyl acetate and water–methanol extracts of *R. graveolens* were found to possess antimicrobial and

cytotoxic activities (IVANOVA, MIKHOVAA, NAJDENSKIB, TSVETKOVAB and KOSTOVA, 2005). Amongst furocoumarins, bergapten has been used for decades for the treatment of various skin diseases such as vitiligo and psoriasis (SONG and TAPLEY, 1979). Further studies are required to determine the chemical(s) involved in the MAO inhibition. *Mentha aquatica* is known to contain flavones and flavanone derivatives (BURZANSKA-HERMANN, RZADKOWSKA-BODALSKA and OLECHNOWICZ-STEPIEN, 1977) which may be responsible for the observed activity.

Tyramine is a substrate for both MAO-A and MAO-B. An important characteristic of traditional MAOIs, such as tranylcypromine and phenelzine, is their lack of selectivity for MAO isoenzymes. By inhibiting both these compounds the metabolism of ingested exogenous tyramine often results in the accumulation of tyramine. This has the potential to precipitate a dangerous hypertensive crisis, known as the 'cheese effect' (YAMADA and YASUHARA, 2004). There are very few known specific MAO-B inhibitors and it is hoped that such novel compounds can be isolated and identified from the active plants highlighted in this investigation.

Isolation and structure elucidation of naringenin from M. aquatica

The 70 % ethanol extract of *M. aquatica* was the most active in the MAO-inhibitory bioassay (IC_{50} = 40 µg/ml). Naringenin was isolated from a 70 % ethanol extract by bioassay-guided isolation utilizing VLC and preparative TLC (**Figure 5.3.3**).



Naringenin

The 70 % ethanol extract was the most active in the MAO-inhibitory bioassay. Naringenin was isolated from a 70 % ethanol extract by bioassay-guided isolation utilizing VLC and TLC and identified on basis of ^{1}H, ^{13}C and ^{13}C-DEPT NMR, by comparison with data reported previously (DU, JERZ, and WINTERHALTER, 2004). Values from ^{1}H-analysis: 2,70 δ(H-3_{eq}), 3,10 δ (H-3_{ax}), 3,31 δ (Me-d), 5,34 δ (H-2), 5,88 δ (H-6) 5,90 δ (H-8) 6,82 δ (H-3`,H-5`), 7,31 δ (H-2`-H-6`). Values from ^{13}C analysis: 43,8(C-3), 48,99(MeOH-d_4), 80,2(C-2), 96,0(C-8), 96,8(C-6) 103,1(C-10) 116,1(C-3`-C-5`) 128,9(C-2`-C-6`) 130,8(C-1`), 158,7(C-4`) 164,6(C-9), 165,2(C-5), 168,0(C-7), 197,5(C-4). An optical rotation $[\alpha]_{589}^{25}$ = -20.7 (ethanol) was determined, which correspond to (*S*)-naringenin (GIORGIO, PARRINELLO, CACCAMESE and ROSINI, 2004).

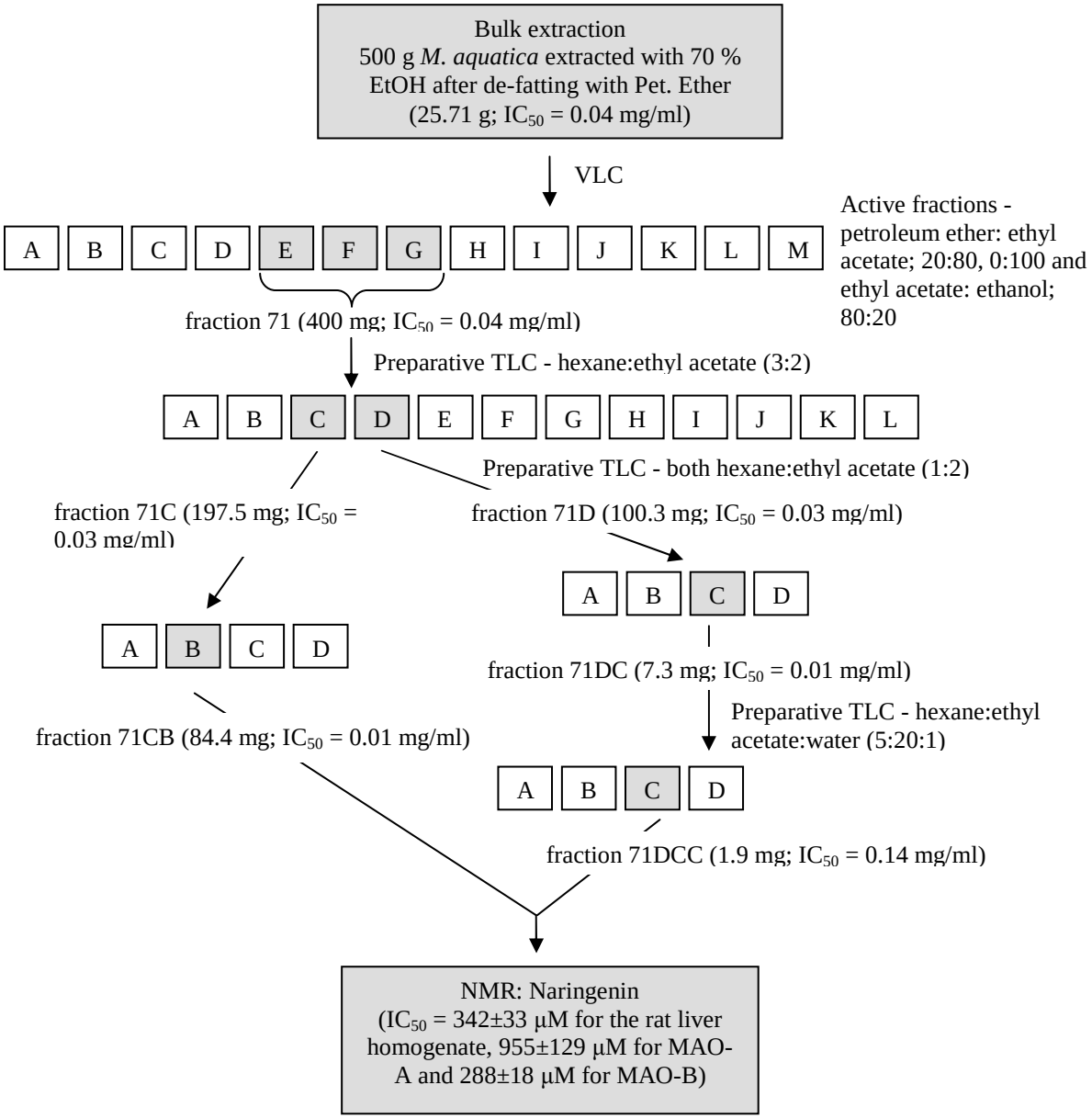


Figure 5.3.3.3. Flow chart showing bioassay-guided fractionation and isolation of naringenin from *M. aquatica*.

The IC_{50} values for MAO inhibition by naringenin were 342±33 μM for the rat liver mitochondrial fraction, 955±129 μM for MAO-A and 288±18 μM for MAO-B. This reflects that the rat liver mitochondrial fraction mainly contains MAO-B, and the bioassay-guided isolation therefore must be viewed as an isolation for MAO-B inhibitors The IC_{50} values for clorgyline (MAO-A inhibitor) and deprenyl (MAO-B inhibitor) were found to be 0.3±0.05 nM and 105±10 nM respectively.

A number of flavonoids have previously been shown to have MAO inhibitory activity. Four flavonoids, quercitrin, isoquercitrin, rutin and quercetin isolated from *Melastoma candicum* exhibited MAO-B inhibition with IC_{50}-values of 19.06, 11.64, 3.89, 10.89 μM, respectively, in an assay where deprenyl had an IC_{50}-value of 19.06 μM (LEE, LIN, SHEN, YANG, YEN and HOU, 2001). However, it has also been reported that quercetin is a selective MAO-A inhibitor with an IC_{50}-value of 0.01μM for MAO-A and 20

μM for MAO-B (CHIMENTI, COTTIGLIA, BONSIGNORE, CASU, CASU, FLORIS, SECCI, BOLASCO, CHIMENTI, GRANESE, BEFANI, TURINI, ALCARO, ORTUSO, TROMBETTA, LOIZZO and CUARINO, 2006). (+)-Catechin and (-)-epicatecin have been shown to inhibit MAO-B with IC_{50}–values of 088.6 μM and 58.9 μM in an assay where deprenyl had an IC_{50}-value of 0.31 μM (HOU, LIN, CHEN and LEE, 2005). Two flavonoids isolated from *Sophora flavescens*, formononetin with IC_{50}-values of 21.2 μM for MAO-A and 11 μM for MAO-B and kushenol F with IC_{50}-values of 103.7 μM for MAO-A and 63.1 μM for MAO-B, had a slight selectivity for MAO-B (HWANG, LEE, HONG, LEE, LEE, HWANG and RO, 2005). 5-Hydroxyflavanone isolated from *Sinofranchetia chinensis* also exhibited better inhibitory activity towards MAO-B with IC_{50}-values of 39.6 μM for MAO-A and 3.8 μM for MAO-B (HARAGUCHI, TANAKA, KABBASH, FUJIOKA, ISHIZU and YAGI, 2004). It is difficult to compare the IC_{50}-values obtained for the flavonoids, as assay conditions vary between studies, and not all studies give values for standard compounds.

It is been considered doubtful whether plant constituents, especially flavonoids, are able to reach the brain. Naringenin has been shown to pass the blood-brain barrier (YOUDIM, QAISER, BEGLEY, RICE-EVANS and ABBOTT, 2004), which means that it can exercise an effect on the CNS. Naringenin has been shown to have many effects outside the CNS, such as being a cancer chemopreventive agent, a mutagenic inhibitor, a genotoxicology inhibitor, and having antioxidant, hypocholesterolic, antibacterial, antiviral, antiallergic, antiangionetic, apopotic and cytostatic activity (HODEK, TREFIL and STIBOROVA, 2002). This non-selectivity might limit the compound's clinical use.

5.3.4. Conclusions

The quantitative spectrophotometric assay has proven to be an inexpensive method for the detection of potential MAO inhibitory activity. It has also lead to the bioassay-guided fractionation and isolation of an active compound. The selectivity of the compound was successfully determined using pure MAO-A and MAO-B. Further research is required to determine the active constituents of *Helichrysum agyrolepis*, *H. umbraculigerum*, *Ruta graveolens*, *Schotia brachypetala*, *Mentha aquatica* and *Gasteria croucheri*. The success of traditional medicines is often attributed to the 'placebo effect' (WEISS, 1988), rather than through active principles producing predictable physiological responses, some of these findings support the latter and may lead to the discovery of novel MAO inhibitors.

Galanthamine, also referred to as Galantamine (Reminyl® - Johnson & Johnson), is approved in many European countries for the treatment of Alzheimer's disease (SRAMEK, FRACKIEWICS and CUTLER, 2000). This alkaloid was first isolated from the European snowdrop (*Galanthus* spp., most notably *G. woronowii*), but is also obtained from *Narcissus* spp., *Leucojum* spp. (esp. *L. aestivium*) and many other Amaryllidaceae spp. as well as synthetically (HEINRICH and TEOH, 2004). The long acting, selective, reversible, and competitive acetylcholinesterase inhibitory properties of galanthamine have inspired the

search for other AChE inhibitors from the family Amaryllidaceae (RISA, RISA, ADSERSEN, STAFFORD, VAN STADEN and JÄGER, 2004).

Aqueous and ethanol extracts of five plants used in southern Africa to treat memory loss; *Malva parviflora* L. (leaves), *Boophone disticha* (L.f.) Herb. (leaves and bulbs), *Albizia adianthifolia* (Schumach.) W. Wright (stem bark), *Albizia suluensis* Gerstner (root bark) and *Crinum moorei* Hook.f. (bulbs) were investigated for AChE inhibitory activity (RISA, RISA, ADSERSEN, STAFFORD, VAN STADEN and JÄGER, 2004) using an assay on thin layer chromatography (TLC) based on Ellman's reaction (ELLMAN, COURTNEY, ANDRES and FEATHERSTONE, 1961).

Promising resultswere obtained with Amaryllidaceae bulbs of *Boophone disticha* and *Crinum moorei*. Aqueous and ethanol extracts of *Crinum moorei* and *Boophone disticha* also showed AChE inhibiting activity in the TLC assay. KwaZulu-Natal grasslands are particularly rich in Amaryllidaceae, with over 20 species from nine genera used in Zulu medicine (HUTCHINGS, SCOTT, LEWIS and CUNNINGHAM, 1996). Several alkaloids, isolated and identified by Dr E.E. Elgorashi from South African Amaryllidaceae, including *Crinum moorei* Hook.f., *Crinum macowanii* Baker, *Crinum bulbispermum* (Burm.f.) Milne-Redh. & Schweick. and *Cyrtanthus falcatus* R.A.Dyer, were recently screening to determine their AChE inhibitory activity (ELGORASHI, STAFFORD and VAN STADEN, 2004; ELGORASHI, MALAN, STAFFORD and VAN STADEN, 2006). Differences in AChE inhibitory activity could be related to structural differences, in particular different ring types. Lycorine-type alkaloids were the most active against AChE with 1-*O*-acetyllycorine (IC_{50} = 0.96µM) exhibiting inhibitory activity comparable to that of galanthamine. The inhibitory activity of the alkaloids lycorine and 1,2 di-*O*-acetyllycorine was 100-times less potent than that of 1-*O*-acetyllycorine (ELGORASHI, STAFFORD and VAN STADEN, 2004).

These findings support earlier reports claiming the inhibitory activity of AChE by lycorine-type alkaloids (LÓPEZ, BASTIDA, VILADOMAT and CODINA, 2002). LÓPEZ, BASTIDA, VILADOMAT and CODINA (2002) suggested that the aromatic ring C that gives a certain planarity to those molecules could explain the higher activity of assoanine and oxoassoanine compared to other lycorine-type alkaloids. *Crinum glaucum* and *Crinum jagus* (Amaryllidaceae) have been used in Nigeria by traditional healers for memory loss and other CNS-related ailments associated with aging. HOUGHTON, AGBEDAHUNSI and ADEGBULUGBE (2004) isolated alkaloids from the plants and tested their AChE inhibitory properties. Several reviews have been published on plants used through the World to treat age-related dementia (HOWESand HOUGHTON, 2003; HOWES, PERRY and HOUGHTON, 2003; HOUGHTON, 2005 BARBOSA FILHO, MEDEIROS, DINIZ, BATISTA, ATHAYDE-FILHO, SILVA et al., 2006; HOUGHTON, REN and HOWES, 2006; HOSTETTMANN, BORLOZ, URBAIN and MARSTON, 2006; ADAMS, GMÜNDER and HAMBURGER, 2007; MUKHERJEE, KUMAR, MAL and HOUGHTON, 2007). Some of the know ACHE inhibitors isolated from plants are given in **Table 5.2**.

AChE is also the target of drugs designed to combat neuromuscular disorders, such as myasthenia gravis and glaucoma (TAYLOR, 2001).

The following publications relate to this chapter:

- A. Risa, J. Risa, A. Adsersen, **G.I. Stafford**, J. van Staden and A.K. Jäger. Acetylcholinesterase inhibitory activity of plants used as memory-enhancers in traditional. *South African Journal of Botany* 70, 664-666.
- **G.I. Stafford**, P.D. Pedersen, A.K. Jäger and J. van Staden. Monoamine oxidase inhibition by southern African traditional medicinal plants. *South African Journal of Botany* 73, 384-390
- H.T. Olsen, **G.I. Stafford**, J. van Staden, S.B. Christensen, A.K. Jäger. Isolation of the MAO-inhibitor naringenin from *Mentha aquatica* L. *Journal of Ethnopharmacology* 117, 500-502.
- **G.I. Stafford**, P.D. Pedersen, J.C. Chukwujekwu, A.K. Jäger and J. van Staden. *Helichrysums*: antibacterial and monoamine oxidase inhibitory activity of South African summer-rainfall species. *In preparation*.

CHAPTER SIX

Summary and Conclusions:
Implications and future research areas

6.1. Summary of main findings

This thesis has two main objectives: firstly, to bring together a comprehensive and detailed record of psychotropic plants used in southern Africa by indigenous peoples for medicinal or cultural purposes; and secondly, this research attempts to determine the validity and rationale of the use of these plants by screening them in various biological assays for psychotropic activity. The first object was achieved with an annotated list that was compiled from available ethnobotanical literature of plants traditionally used for central nervous system-related purposes. It contains more than 330 species, from 94 families, which are currently used or have been used for cultural, medicinal and recreational purposes related to the central nervous system.

The second aim was partially achieved with 320 extracts from nearly 100 species being screened in one or more of four bioassays. The assays were selected to detect potentially useful neurological activity for the treatment of mental illness. The plant extracts to be screened were selected based on their traditional use. Twenty-three Amaryllidaceae alkaloids were screened for inhibition of the serotonin reuptake transporter protein (SERT); $GABA_A$-benzodiazepine receptor binding and inhibition of acetylcholinesterase. Three flavonoids and a sesquiterpene with affinity to the benzodiazepine site were isolated using bioassay-guided fractionation. This study is the first comprehensive investigation of the CNS activity of South African plants.

6.1.1. Southern African plants with CNS-related activity

When an attempt is made to classify plants used in African traditional medicine according to their use in treating ailments that are defined by western concepts difficulties inevitably arise. An important causal factor considered in African traditional medicine is the status of relations existing between the ‘unwell’ individual and other human beings, both the living, and the deceased. Thus, philosophy, religion, psychiatry, physiology and biology, are all essential elements within the practice of African traditional medicine. It is therefore difficult to tease apart the biomedically important plants from those that are culturally important or are used for religious purposes, for example those plants used to counteract, or protect patients from ‘witchcraft’ or ‘sorcery’. Several factors need to be considered when working with such plants. Firstly, almost all of the literature available on African medicinal plants has been documented by people who do not belong to the cultural or even language group that they are reporting on. One needs

to be aware that it is possible that in the translation and interpretation of the plant use, biases dictated by the recorders own world-view and beliefs may have occurred. Where possible the method of preparation and mode of administration should also be considered. It is import to determine if the plant material is likely to have an effect on the user's physiology. If the plant material is not ingested, inhaled, taken as an enema, applied topically or applied to scarification or cuts in the skin then it is unlikely to have a biomedical mode of action.

An annotated list compiled from available ethnobotanical literature of plants traditionally used for central nervous system-related purposes is provided (**Table 2.2.**, page 68). It contains more than 330 species, from 94 families, which are currently used or have been used for cultural, medicinal and recreational purposes related to the central nervous system (CNS). Where available, information pertaining to local names and their meaning, plant part used, preparation method, dosage, route of administration, known and potentially active constituents are included. The Zulu botanical names were looked at in some detail and a strong correlation between the traditional use and the name was observed.

A large proportion of the plants are used to treat mental disorders such as madness, nervous disorders and various types of hysteria. Often the specific details (i.e. symptoms) of these ailments are not given and therefore they can not be classified further. Nearly 150 plant species from 63 families are used to treat sympyoms of epilepsy and convulsions, possibly reflecting the severity of this problem in the population. The families most often represented are Fabaceae (15 species), Asteraceae (13 species) and Lamiaceae (9 species). Over 40 plant species from 26 families are used for ailments that could be described as being similar to depression. The number of plants used for dementia and age-related mental problems is lower, with only 15 species from 7 families recorded (STAFFORD, PEDERSEN, VAN STADEN and JÄGER, 2008). This could be due to a previous demographic situation, where traditional healers less frequently encountered very old patients.

What follows are brief summaries of the main findings of the research contained in this thesis. This is concluded with a summary table (**Table 6.1**) of the screening results of all four assays employed in this thesis.

6.1.2. Southern African plants with antidepressant activity

Seventy five extracts from 34 indigenous plant species used in South African traditional medicine or taxonomically related to these were investigated for their affinity to the serotonin reuptake transport protein, making use of an *in vitro* [^{3}H]-citalopram serotonin reuptake transport protein binding assay (NIELSEN, SANDAGER, STAFFORD, VAN STADEN and JÄGER, 2004). Aqueous and 70% ethanolic extracts of various plant parts were screened and 45 extracts derived from 15 plant species showed affinity. The affinity of 12 extracts from four plants was characterized as high (more than 50%

inhibition at 5, 1, and 0.5 mg/ml). Plant species with high affinity to the serotonin reuptake transport protein included *Agapanthus campanulatus*, *Boophone disticha*, *Datura ferox* and *Xysmalobium undulatum*. *Agapanthus campanulatus* yielded high activity in aqueous extracts from leaves and flowers. *B. disticha* showed high activity both in aqueous and ethanolic extracts of leaves and bulbs. *D. ferox* showed high activity in aqueous extracts from the seeds and *X. undulatum* showed high activity in the ethanolic extract of the whole plant.

Two compounds, buphanadrine and buphanamine, were isolated by bioassay-guided fractionation on vacuum-liquid-chromatography and preparative thin-layer-chromatography from *B. disticha* (SANDAGER, NIELSEN, STAFFORD, VAN STADEN. and JÄGER, 2005). The structures of the compounds were determined by ^{1}H and ^{13}C NMR. Fractions were tested for affinity to the serotonin transporter in a binding assay. The IC_{50} values of buphanidrine and buphanamine were 274 μM (K_i = 132 μM) and 1799 μM (K_i = 868 μM), respectively. These two alkaloids were also tested for affinity to the $5HT_{1A}$ receptor, but only showed slight affinity. The affinity of these two alkaloids to the serotonin transporter prompted the screening of a further 20 alkaloids isolated from other South African Amaryllidaceae, *Crinum moorei*, *C. bulbispermum*, *C. macowanii*, and *Cyrtanthus falcatus* (ELGORASHI, STAFFORD, JÄGER and VAN STADEN, 2006). The majority of the Amaryllidaceae alkaloids assayed that showed affinity to the serotonin transporter were crinine-type alkaloids. However, the most active was cherylline, a cherylline-type alkaloid (IC_{50} = 3.4 μM, K_i = 1.6) (ELGORASHI, STAFFORD, JÄGER and VAN STADEN, 2006).

Further studies have been performed by a collegue on the ethanolic extracts from *Agapanthus campanulatus*, *Boophone disticha*, *Mondia whitei* and *Xysmalobium undulatum* for functional inhibition (semi-*in vivo* assay) of SERT, noradrenalin uptake (NAT) and dopamine uptake (DAT) using COS-7 cells expressing hSERT, hNAT or hDAT (PEDERSEN, SZEWCZYK, STACHOWICZ, WIERONSKA, ANDERSEN, STAFFORD, VAN STADEN, PILC JÄGER, 2008). Extracts from *Agapanthus campanulatus*, *Boophone disticha* and *Mondia whitei* showed an effect in the functional assays. Ethanolic extracts from *Agapanthus campanulatus*, *Boophone disticha*, *Mondia whitei* and *Xysmalobium undulatum*, were investigated for *in vivo* antidepressant-like effects in three animal models for depression (PEDERSEN, SZEWCZYK, STACHOWICZ, WIERONSKA, ANDERSEN, STAFFORD, VAN STADEN, PILC JÄGER, 2008). The assays employed were forced swim test in both mice and rats and the tail suspension test in mice. All four extracts exhibited antidepressant-like effects in the animal models in various degrees.

6.1.3. Southern African plants with antiepileptic and anxiolytic activity

Aqueous and ethanol extracts of 43 plants that are traditionally used to treat against epilepsy and convulsions were initially tested in the $GABA_A$-benzodiazepine receptor binding assay, where the binding of ^{3}H-Ro 15-1788 (flumazenil) to the benzodiazepine site is measured. Out of the 118 extracts tested, one aqueous and 18 ethanol extracts showed activity (RISA, RISA, ADSERSEN, GAUGUIN, STAFFORD, VAN STADEN and JÄGER, 2004). The most active extracts in this initial screening were the ethanolic leaf extracts of *Searsia pyroides*, *Searsia rehmanniana*, *Hoslundia opposita* and the ethanolic corm extract of *Hypoxis colchicifolia*. All of these showed good dose-dependent activity. A further forty-six ethanol extracts from another 35 species, both indigenous and exotic that are traditionally used predominantly as sedatives or to treat various CNS-related ailments were tested in the $GABA_A$-benzodiazepine receptor-binding assay (STAFFORD, JÄGER and VAN STADEN, 2005). Out of the 46 extracts tested, seven have shown good activity and 10 with moderate activity. The most active extracts were the ethanolic leaf extracts of *Arctopus echinatus*, *Artemisa afra*, four *Helichrysum* species and *Mentha aquatica.* Again all these extracts exhibited good dose-dependent activity.

Two biflavonoids with activity in the ^{3}H-Ro 15-1788 (flumazenil) binding assay were isolated by high pressure liquid chromatography fractionation of the ethanol extract of the leaves from *Searsia pyroides*. The structures of the two biflavonoids were elucidated by nuclear magnetic resonance spectroscopy to be agathisflavone and amentoflavone (SVENNINGSEN, MADSEN, LILJEFORS, STAFFORD, VAN STADEN and JÄGER, 2006). Agathisflavone and amentoflavone competitively inhibited the binding of ^{3}H-Ro 15-1788 with a K_i of 28 and 37 nM, respectively. Extracts of *Searsia dentata* and *Searsia pentheri* were not as active as the extract from *Searsia pyroides*; both were found to contain apigenin and agathisflavone. The monomer apigenin, agathisflavone and amentoflavone were fitted into a pharmacophore model for ligands binding to the $GABA_A$ receptor benzodiazepine site. This reflected the affinities of the compounds in the [^{3}H]-flumazenil binding assay.

Further functional characterization of the *Searsia* extracts showed inhibitory effects on spontaneous epileptiform discharges in mouse cortical slices (PEDERSEN, VESTERGAARD, STAFFORD, VAN STADEN and JÄGER, 2008). Interestingly, the effect was not caused by the previous isolated flavonoids. The extracts contained *N*-methyl-d-aspartic acid (NMDA) receptor antagonists, which might explain the effect of the plants reported by the traditional healers. However, these findings need be confirmed by *in vivo* anticonvulsive studies before any conclusions can be made.

Mentha aquatica, a mint that is found in Europe and Africa, is used in Zulu traditional medicine for spiritual purposes. The ethanolic leaf extract exhibited a strong affinity to the GABA-benzodiazepine

receptor. Viridiflorol from the essential oil and (*S*)-naringenin from an ethanolic extract were isolated by bioassay-guided fractionation using binding to the GABA-benzodiazepine site. Viridiflorol had an IC_{50} of 0.19 M and (*S*)-naringenin of 0.0026 M (JÄGER, ALMQVIST, VANGSØE, STAFFORD, ADSERSEN and VAN STADEN, 2007).

6.1.4. Southern African plants useful in the treatment of Alzheimer's and Parkinson's diseases

Twenty plants used in Zulu traditional medicine for several CNS-related ailments were screened for MAO inhibition and specific MAO-B inhibition activity (STAFFORD, PEDERSEN, JÄGER and VAN STADEN, 2007). MAO-B inhibitors are currently employed in the treatment of neurodegenerative related illnesses such as Parkinson's and Alzheimer's diseases. A photometric peroxidase linked assay was used to determine the inhibition of the oxidative deamination of tyramine by MAO isolated from rat liver. *Ruta graveolens* exhibited the best MAO inhibitory activity (ethyl acetate leaf extract = IC_{50} 5 ± 1 μg/ml, petroleum ether extract = 3 ± 1 μg/ml) and specific MAO-B inhibition (ethyl acetate leaf extract = IC_{50} 7 ± 6 μg/ml petroleum ether extract = 3 ± 1 μg/ml). *Schotia brachypetala*, *Mentha aquatica* and *Gasteria croucheri* also exhibited good MAO-B inhibition activity.

Six extracts of varying polarity of *Mentha aquatica* were tested in a photometric peroxidase linked MAO bioassay (OLSEN, STAFFORD, VAN STADEN, CHRISTENSEN and JÄGER, 2008). The 70% ethanol extract had highest inhibitory activity. (*S*)-Naringenin was isolated from the extract by bioassay guided fractionation on VLC and preparative TLC. The structure of the compound was determined by ^{1}H, ^{13}C and ^{13}C-DEPT NMR and optical rotation. The IC_{50} values for MAO inhibition by naringenin were 342 ± 33 μM for the rat liver mitochondrial fraction, 955 ± 129 μM for MAO-A and 288 ± 18 μM for MAO-B respectively.

Further studies on twenty-three Amaryllidaceae alkaloids having several different ring types were evaluated for their acetylcholinesterase enzyme inhibitory activity. The alkaloid 1-*O*-acetyllycorine (IC_{50} = 0.96 ± 0.04) showed significant AChE inhibitory activity (ELGORASHI, STAFFORD and VAN STADEN, 2004).

6.2. General conclusions

A total of 96 species from 35 families were investigated for biological activity in this study. From seven of these species, 25 pure compounds were screened. The main findings of this study are summarised in **Table 6.1**. Interesting activity (both SSRI and AChEI activity) was observed from members of the Amaryllidaceae, in particular the Amaryllidaceae alkaloids. These findings together with several studies conducted throughout the world suggest that this family has the potential to provide more useful and

novel biologically active compounds. These results also illustrate that using ethnobotanical knowledge as a guide in deciding which southern African plants to screen for the treatment of age-related CNS ailments is perhaps not necessarily the best option, due to the relatively small number of such traditional treatments. Taxonomic based decisions, such as screening southern African Amaryllidaceae for AChE inhibitors, on the knowledge that European genera have given promising candidates, may be more successful. This study provided one alkaloid, 1-*O*-acetyllycorine from *Crinum moorei*, which has shown activity comparable to that exhibited by galanthamine. Perhaps further studies on other Amaryllidaceae genera, such as *Apodolirion*, *Brunsvigia*, *Cyrtanthus*, *Gethyllis*, *Haemanthus* and *Strumaria* may yield novel alkaloids with promising activity.

Steroidal alkaloids of members of the Buxaceae, specifically *Sarcococca* and *Buxus* species, have shown anti-cholinesterase activities (CHOUDHARY, SALMA, SHEHNAZ, ASAAD, ABDUL, ATTA-UR-RAHMAN, MASOOD, 2003; CHOUDHARY, PRASAD, AHMAD, ROSA, ATTA-UR-RAHMAN, 2005). There are only two representatives of Buxaceae in South Africa, namely *Buxus macowanii*, which is used to treat madness, and *B. natalensis.* Niether of these species have been investigated for cholinesterase inhibitory activity.

Two genera from the Anacardiaceae, *Searsia* (Syn = *Rhus*) and *Lannea* have been reported to have anticonvulsant activity. The active constituents, notably flavonoids, of several *Searsia* species have been demonstrated. The leaves of *Lannea discolor* are used by the Luvale people of Zambia to treat fits (WATT and BREYER-BRANDWIJK, 1962). The Zulu people use root infusions (***isiganganyane***) as a wash for convulsions in South Africa. *Lannea schweinfurthii* roots are used by unspecified ethnic groups in South Africa as a sedative snuff (VAN WYK and GERICKE, 2000). The powdered root is sprinkled on food and used as a sedative in Venda people (ARNOLD and GULUMIAN, 1984). These species, and perhaps other members of the Anacardiaceae, should be investigated further for their ability to suppress the CNS through the GABA system.

Figure 1.5.1. (page 40) shows the important elements of neuronal signal transduction and the potential targets of compounds that have an effect on the CNS. Three targets are employed in this thesis: (1) the inhibition of the serotonin reuptake transporter protein (SERT); (2) $GABA_A$- benzodiazepine receptor binding; and (3) inhibition of catabolic enzymes (e.g. acetylcholinesterase and monoamine oxidase). There are, however, many other targets within the central nervous system that were not investigated in this study. For example, the effect of plant extracts and isolated compounds were tested on one monoamine re-uptake system, namely the serotonin re-uptake system. This system is indeed important in the treatment of depression although other aspects such as the noradrenaline transporter (NET) remain to be investigated. The NET is another important cellular binding site for clinically used antidepressants, and in particular dual serotonin-noradrenaline re-uptake inhibitors (SNRI). Thus, although some plants

did not exhibit any activity in the bioassays employed in this study, they may exert their effect on the CNS through other biological pathways.

When used by the traditional healers, the medicinal plants are often combined in complex mixtures. Thus the potential number of neuroactive compounds in the pool of ingredients is increased, which makes the identification of active components very difficult. This also raises the question of the potential for synergistic relationships between the numerous compounds present in such mixtures. Much of the ethnobotanical literature on the southern African plants is several decades or more old. African traditional medicinal knowledge is not static, and is constantly being added to, while some practices are being lost. Field studies comprising interviews with traditional healers are needed to gain a better understanding of which plants are currently valued and to update the body of knowledge that has been accumulated so far.

This research has highlighted some the difficulties of studying medicinal plants in assays for CNS activity. Although this project has made a substantial contribution to our understanding of South African traditional medicine's effect on some systems within the CNS, still very few compounds have been isolated and characterized. Most CNS studies on African plants are conducted on crude extracts and in animal systems where the mechanism of action might involve several different neurotransmitter systems. The lack of selectivity, the need for functional assays (i.e. not as specific as *in vitro* assays but more informative than *in vivo* assays) and the mismatch between *in vitro* and *in vivo* findings makes it a challenging task to examine plants for potential new drugs.

Overall, the findings reported in these and subsequent studies yield creditability to some of the practices used in South African traditional medicine in the treatment of mental illness. They support the idea that there are several culturally and medicinally important psychoactive plants in southern Africa similar to the numbers observed in the New World. Knowledge of the biological activities and active constituents of plants utilised in traditional medicine opens up a possibility for development of standardized products, which would help secure more reliable medication for patients.

Table 6.1. Summary of the biological activity of plant extracts and isolated compounds screened in the four bioassays for CNS activity (+++ good activity; ++ moderate activity, + mild activity detected; - no activity detected). Data take from research documented in this thesis, as well as ELGORASHI, STAFFORDand VAN STADEN (2004); ELGORASHI, STAFFORD, JÄGER and VAN STADEN (2006) and STAFFORD, PEDERSEN, VAN STADEN and JÄGER (2008)

Family	**Biological activity**			
Plant species	**SSRI**	**GABA$_A$**	**AChE**	**MAO**
compound				
Agapanthaceae				
Agapanthus campanulatus	+	-		-
Agapanthus praecox		-		+
Amaryllidaceae				
Boophone disticha	+		+	
buphanidrine	+	-		
buphanamine	-	-		
Brunsvigia grandiflora	-	-		
Crinum bulbispermum			+	
3-*O*-acetylhamayne	-	-	-	
crinamine	-	-	-	
6-hydroxycrinamine	-	-	+	
8α-ethoxyprecriwelline	-	-	-	
N-desmethyl-8α-ethoxypretazettine	-	-	+	
N-desmethyl-8β-ethoxypretazettine	-	-	+	
Crinum macowanii			+	
hamayne	-	-	-	
lycorine	-	-	+	
Crinum moorei			+	
crinine	+	-	+	
epibuphanisine	+	-	-	
powelline	+	-		
epivittatine	+	-	+	
1-*epi*-deacetylbowdenisine	-			
1-*O*-acetyllycorine	+	-	+++	
cherylline	++	-	+	
Cyrtanthus falcatus			+	
maritidine	+	-		
O-methylmaritidine	+	-		
papyramine	-	-		
tazettine	+	-	-	
Gethyllis ciliaris	-			
Scadoxus puniceus		-		-
Anacardaceae				
[Southern African *Rhus* was reclassified as *Searsia*]				
Searsia chirindensis		-		
Searsia dentata		+		
Searsia pyroides (syn. *Rhus tridentata*)		++		
apigenin		+		
agathisflavone		++		
amentoflavone		++		
Searsia rehmanniana		+		
Searsia pentheri		+		

Family	**Biological activity**			
Plant species compound	**SSRI**	**GABA$_A$**	**AChE**	**MAO**
Apiaceae				
Alepidea natalensis	-			
Arctopus echinatus		++		
Apocynaceae				
Acokanthera oblongifolia	-	-		
Stropharanthus speciosus	-			
Araliacea				
Cussonia paniculata		-		
Cussonia spicata		-		
Asclepiaceae				
Gomphocarpus physocarpus	-	-		+
Xysmalobium undulatum	+	-		-
Asphodelaceae				
Bulbine frutescens	-	-		
Gasteria croucheri	-	-		+
Asteraceae				
Artemisia afra	-	+		
Artemisia dracunculoides	-			
Berkheya bergiana		-		
Berkheya montana		-		
Berkheya rhapontica		-		
Helichrysum argyrolepis		-		+
Helichrysum hesbaceum		+		-
Helichrysum nudifolium		-		+
Helichrysum ruderale		+		+
Helichrysum rugulosum		-		+
Helichrysum simillimum		+		-
Helichrysum umbraculigerum		+		+
Campanulaceae				
Lobelia alata	-			
Celastraceae				
Catha edulis		-		
Combretaceae				
Combretum bracteosum		-		
Combretum imberbe		-		
Crassulaceae				
Cotyledon orbiculata	-	-		
Dioscoreaceae				
Dioscorea dregeana		-		+
Euphorbaceae				
Antidesma venosum		-		
Croton sylvaticus		-		
Jatropha panduaeifolia		-		
Jatropha zeyheri		-		
Fabaceae				
Acacia sieberiana		-		
Acacia xanthophloea		+		
Bauhinia galpinii		-		
Bauhinia tomentosa		-		
Dichrostachys cinera.		-		
Indigofera tristis	-	-		
Indigofera woodii		-		
Millettia grandis		-		

Family	**Biological activity**			
Plant species compound	**SSRI**	**GABA$_A$**	**AChE**	**MAO**
Millettia sutherlandii		-		
Schotia brachypetala		-		+
Senna didymobotrya		-		
Senna petersiana		-		
Sutherlandia frutescens		-		
Flacoutiaceae				
Oncoba spinosa		-		
Hyperaceae				
Hypericum lanandii	-			
Hypericum revolutum	-			
Hypoxidaceae				
Hypoxis angustifolia		-		
Hypoxis colchicifolia		-		
Hypoxis hemerocallidea		-		+
Lamiaceae				
Hemizyga obermeyerae	-			
Hoslandia opposita		+		
Leonotis dubra		-		
Leonotis intermedia		-		
Leonotis leonurus	-	-		+
Mentha aquatica	-	+		+
naringenin		+		+
Mentha longifolia		-		
Salvia chamelaeagnea		-		
Lauraceae				
Cinnamomum camphora	-	-		-
Loganiaceae				
Buddleja saligna		-		
Buddleja salviifolia		-		+
Malvaceae				
Malva parviflora	-	-		
Meliaceae				
Ekebergia capensis		+		
Oleaceae				
Olea africana	-			
Periplocaceae				
Mondia whitei	+	-		
Phytolaccaceae				
Phytolacca octandra	+	+		
Piperaceae				
Piper capense	-	+		
Rosaceae				
Rubus ludwigii	-	-		
Rubus phoeniculacius		-		
Rubiaceae				
Catunaregam spinosa		-		
Conostomium natalense	-			
Rutaceae				
Clausena anisata	-	+		+
Ruta graveolens		-		+
Zanthoxylum capense	-	-		
Scrophulariaceae				
Diclis reptans	-			

Family *Plant species* compound	**Biological activity**			
	SSRI	**$GABA_A$**	**AChE**	**MAO**
Solanaceae				
Datura ferox	-	-		
Datura stramonium	-	-		-
Verbenaceae				
Clerodendrum myricoides		-		
Vitaceae				
Rhoicissus tomentosa		-		
Rhoicissus tridentata		-		-

LITERATURE CITED

ABDOOL KARIM, S.S., ZIQUBU-PAGE, T.T. and ARENDSE, R., **1994**. Bridging the Gap: Potential for a health care partnership between African traditional healers and biomedical personnel in South Africa. *South African Medical Journal* **84**: 1-16.

ADAMS, M., GMÜNDER, F. and HAMBURGER, M., **2007**. Plants traditionally used in age related brain disorders - A survey of ethnobotanical literature. *Journal of Ethnopharmacology* **113**: 363-381.

ADESINA, S.K., **1982**. Studies on some plants used as anticonvulsants in Amerindian and African traditional medicine. *Fitoterapia* **5-6**: 147-162.

AHUJA, M., **1965**. Medicinal Plants of Saharanpur: Survey of Medicinal Plants. Dharmpal Vidyalankar Publishers, Saharanpur.

ALI, B.H., BASHIR, A.K., TANIRA, M.O.M., MEDVEDEV, A.E., JARRETT, N., SANDLER, M. and GLOVER, V., **1998**. Effect of extract of *Rhazya stricta*, a traditional medicinal plant, on rat brain tribulin. *Pharmacology Biochemical Behaviour* **59**: 671-675.

ALLEN, O.N. and ALLEN, E.K., **1981**. *The Leguminosae*. Winconsin, University of Winsconsin Press.

AL-MOTARREB, A., BAKER, K., and BROADLEY, K.J., **2002**. Khat: pharmacological and medical aspects and its social use in Yemen. *Phytotherapy Research* **16**: 403-413.

AMABEOKU, G.J., GREEN, I. and KABATENDE, J., **2007**. Anticonvulsant activity of *Cotyledon orbiculata* L. (Crassulaceae) leaf extract in mice. *Journal of Ethnopharmacology* **112**: 101-107.

ANDERMANN, L., **1995**. Epilepsy in developing countries. *Transcultural Psychiatric Research Review* **32**: 351–84.

ARNOLD, H–J. and GULUMIAN, M., **1984**. Pharmacoepia of traditional medicine in Venda. *Journal of Ethnopharmacology* **12**: 35-74.

ARNOLD, T.H. and DE WET, B.C., **1993**. *Plants of Southern Africa: Names and Distribution. Memoirs of the Botanical Survey of South Africa* No. **62**. Pretoria, National Botanical Institute.

ASAKURA, M. and TSUKAMOTO, T., **1985**. Antidepressant drugs and central monoaminergic receptors. *Yakubutsu, Seishin, kodo* **5**: 303-319.

ASHFORTH, A., **2005**. *Muthi*, Medicine and Witchcraft: Regulating 'African Science' in Post-Apartheid South Africa? *Social Dynamics* **31**: 211-242

ASHTON, E.H., **1943**. Medicine, magic and sorcery among the Southern Sotho**.** *Communications from the School of African Studies Cape Town, New Series* **10**: 1–32.

ASHTON, H., **1992**. *Brain Function and Psychotropic Drugs*. Oxford University Press, Oxford.

ASHWELL, A., **1994**. *Getting to know Kirstenbosch National Botanical Garden. Indigenous Healing Plants of the Herb and Fragrance Garden*. Claremont, National Botanical Institute.

ATTA-UR-RAHMAN, FEROZ F., NAEEM I., ZAHEER-UL-HAQ, NAWAZ S.A., KHAN N., KHAN M.R. and CHOUDHARY M.I., **2004**. New pregnane-type steroidal alkaloids from *Sarcococca saligna* and their cholinesterase inhibitory activity. *Steroids* **69**: 735-741.

ATTA-UR-RAHMAN, PARVEEN, S., KHALID, A., FAROOQ, A. and CHOUDHARY, M.I., 2001. Acetyl and butyrylcholinesterase-inhibiting triterpenoid alkaloids from *Buxus papillosa*, *Phytochemistry* **58**: 963-968.

AVALLONE, R., ZANOLI, P., PUIA, G., KLEINSCHNITZ, M., SCHREIER, P. and BARALDI, M., **2000**. Pharmacological profile of apigenin, a flavonoid isolated from *Matricaria chamomilla*. *Biochemical Pharmacology* **59**: 1387-1394.

AXELROD, J., WHITBY, R. and HERTTIG, G., **1961**. Effect of psychotropic drugs on the uptake of tritiated noradrenaline by tissues. *Science* **133**: 383–384.

AYENSU, E.S., **1978**. *Medicinal Plants of West Africa*. Algonac, Michigan, Reference Publications Inc.

BAI, D.L., TANG, X.C. and HE, X.C., **2000**. Huperzine A, a potential therapeutic agent for treatment of Alzheimer's disease. *Current Medicinal Chemistry* **7**: 355-374.

BALDESSARINI, R.J., **1989**. Current status of antidepressants: clinical pharmacology and therapy. *Journal of Clinical Psychiatry* **50**: 117-126.

BALICK, M.J. and COX, P.A., **1996**. *Plants, People and Culture: The Science of Ethnobotany*. The Scientific American Library, New York.

BALICK, M.J., **1990**. *Ethnobotany and the Identification of Therapeutic Agents from the Rainforest.* In: *Bioactive Compounds from Plants*, ed. Chadwick, D.J. and Marsh, J. Ciba Foundation Symposium no. 154. J. Wiley and Sons, Chichester, pp. 22-29.

BALLY, P.R.O., **1938**. Heil- und Giftpflanzen der Eingeborenen von Tanganyika. Repertorium specierum novarum regni vegetabilis (edited by F. Fedde). Beiheft 102. Im Selbstverlage, Dahlem.

BARBOSA FILHO, J.M., MEDEIROS, K.C.P., DINIZ, M. DE F.F.M., BATISTA, L.M., ATHAYDE-FILHO, P.F., SILVA, M.S. et al., **2006**. Natural products inhibitors of the enzyme acetylcholinesterase. *Revista Brasileira de Farmacognosia* **16**: 258-285.

BARNARD, E.A., SKOLNICK, P., OLSEN, R.W., MÖHLER, H., SIEGHART, W., BIGGIO, G., BRAESTRUP, C., BATESON, A.N. and LANGER, S.Z., **1998**. International Union of Pharmacology. XV. Subtypes of gamma-aminobutyric acid-A receptors: classification on the basis of subunit structure and receptor function. *Pharmacology Reviews* **50**: 291-313.

BARONE, J.J. and ROBERTS, H.R., **1996**. Caffeine consumption. *Food and Chemical Toxicology* **34**: 119-129.

BASKIND, R. and BIRBECK, G.L., **2005**. Epilepsy-associated stigma in sub-Saharan Africa: The social landscape of a disease. *Epilepsy and Behavior* **7**: 68-73.

BATTEN, A. and BOKELMANN, A., **1966**. *Wild Flowers of the Eastern Cape*. Cape Town, Books of Africa.

BERGER, M., **2006**. Traditional medicine: clear and present danger? *South Africa Journal of Science* **102**: 178-179.

BERGLUND, A., **1976**. *Zulu thought patterns and symbolism*. London: C. Hurst and Co.

BERMAN, S., CHANG, L., SUYENOBU, B. et al., **2002**. Condition-specific deactivation of brain regions by 5-HT(3) receptor antagonist Alosetron. *Gastroenterology* **123**: 969-977.

BERMAN, S.M., CHANG, L., SUYENOBU, B., DERBYSHIRE, S.W., STAINS, J., FITZGERALD, L., MANDELKERN, M., HAMM, L., VOGT, B., NALIBOFF, B.D. and MAYER, E.A., **2002**. Condition specific deactivation of brain regions by 5-HT_3 receptor antagonist Alosetron. *Gastroenterology* **123**: 969-977.

BIENVENU, E., AMABEOKU, G.J., EAGLES, P.K., SCOTT, G. and SPRINGFIELD, E.P., **2002**. Anticonvulsant activity of aqueous extract of *Leonotis leonurus*. *Phytomedicine* **9**: 217-223.

BIGHAM, A.K., MUNRO, T.A., RIZZACASA, M.A., and ROBINS-BROWNE, R.M., **2003**. Divinatorins A-C, New Neoclerodane Diterpenoids from the Controlled Sage *Salvia divinorum*. *Journal of Natural Products* **66**: 1242-1244.

BINDA, C.; HUBÁLEK, F.; LI, M.; HERZIG, Y.; STERLING, J.; EDMONDSON, D. E. and MATTEVI, A., **2004**. Crystal structures of monoamine oxidase B in complex with four inhibitors of the N-propargylaminoindan class. *Journal of Medicinal Chemistry* **47**: 1767-1774

BLANDINI, F. and GREENAMYRE, J.T., **1999**. Protective and symptomatic strategies for therapy of Parkinson's disease. *Drugs of Today* **35**: 473-483.

BLIER, P. and WARD, N.M., **2003**. Is there a role for 5-HT1A agonists in the treatment of depression? *Biological Psychiatry* **53**: 193-203.

BODKIN, J.A. and AMSTERDAM, J.D., **2002**. Transdermal selegiline in major depression: a double-blind, placebo-controlled, parallel-group study in outpatients. *American Journal of Psychiatry* **159**: 1869-1875.

BOEHM, S. and KUBISTA, H., **2002**. Fine tuning of sympathetic transmitter release via ionotrpoic and metabotropic presynaptic receptors. *Pharmacological Reviews* **54**: 43–99.

BOLOFO, R.N. and JOHNSON, C.T., **1988**. The Identification of "Isicakathi" and its medicinal use in Transkei. *Bothalia* **18**: 125–130.

BOMBARDA, I. RAHARIVELOMANANA, P. RAMANOELINA, P.A.R. FAURE, R. BIANCHINI J.-P. and GAYDOU, E.M., **2001**. Spectrometric identifications of sesquiterpene alcohols from niaouli (*Melaleuca quinquenervia*) essential oil. *Analytica Chimica Acta* **447**: 113–123.

BOOIJ, L., VAN DER DOES, W., BENKELFAT, C., BREMNER, J.D., COWEN, P.J., FAVA, M., GILLIN, C., LEYTON, M., MOORE, P., SMITH, K.A. and VAN DER KLOOT, W.A., **2002**. Predictors of mood response to acute tryptophan depletion. A reanalysis. *Neuropsychopharmacology* **27**: 852-861.

BORMANN, J. and FEIGENSPAN, A., **1995**. $GABA_C$ receptors. *Trends in Neuroscences* **18**: 515- 519.

BORMANN, J., **1988**. Electrophysiology of $GABA_A$ and $GABA_B$ receptor subtypes. *Trends in Neuroscience* **11**: 112-116.

BOSKER, F.J., KLOMPMAKERS, A.A. and WESTENBERG, H.G.M., **1997**. Postsynaptic 5-HT_{1A} receptors mediate 5-hydroxytryptamine release in the amygdale through a feedback to the caudal linear raphe. *European Journal of Pharmacology* **333**: 147–157.

BOULOS, L., **1983**. *Medicinal Plants of North Africa*. Algonac, Michigan, Reference Publications Inc.

BOWERY, N.G., **1993**. GABA receptor pharmacology. *Annual Review in Pharmacology and Toxicology* **33**: 109–147.

BRADFORD, M.M., **1976**. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein–dye binding. *Analytical Biochemistry* **72**: 248-254.

BRADSHAW, D., GROENEWALD, P., LAUBSCHER, R., NANNAN, N., NOJILANA, B., NORMAN, R., PIETERSE, D. and SCHNEIDER, M., **2003**. Initial estimates from South African National Burden of Disease Study, 2000. Medical Research Policy Brief , 1-4.

BRANDT, H.D., OSUCH, E.; MATHIBE, L. and TSIPA, P., **1995**. Plants associated with accidental poisoned patients presenting at Ga-Rankuwa Hospital, Pretoria. *Suid-Arikaanse Tydskrif vir NatuurWetenskap* **91**: 57-59.

BRAUN, K., **1912**. Alkoholische Getra¨nke der Neger in Deutsch-Ost-Afrika. *Der Pflanzer* **8**: 219–229.

BRAUN, K., **1925**. Pflanzen aus Deutsch-Ostafrika, ihre Namen und Verwendung bei den Eingeborenen. *Archiv für Pharmazie* (Berlin) **263**: 123–138.

BRINKHAUS, B., LINDNER, M., SCHUPPAN, D. and HAHN, E.G., **2000**. Chemical, pharmacological and clinical profile of the East Asian medical plant *Centella asiatica*. *Phytomedicine* **7**: 427–448.

BROSTER, J., **1981**. *Amagqirha, Religion, Magic and Medicine in Transkei*. Cape Town, Via Africa.

BROSTER, J., **1982**. *Amagqirha.* Via Afica, Cape Town.

BROWN, J.H. and TAYLOR, P., **2001**. *Muscarinic Receptor Agonists and Antagonists*. In: Hardman, J.G., Limbird, L.E., Gilman, A.G. (Eds.), *Goodman and Gillman's The Pharmacological Basis of Therapeutics*, (tenth edR) McGraw-Hill, New York, pp. 155– 174.

BRUNELLO, N., MENDLEWICZ, J., KASPER, S., LEONARD, B., MONTGOMERY, S., NELSON, J., PAYKEL, E., VERSIANI, M. and RACAGNI, G., **2002**. The role of noradrenaline and selective noradrenaline reuptake inhibition in depression. *European Neuropsychopharmacology* **12**: 461–475.

BRYANT, A.T., **1966**. *Zulu Medicine and Medicine Men*. Cape Town, Struik.

BUHRMANN, M., **1984**. *Living in two worlds: Communication between a white healer and her black counterparts*, Chiron Publications, Wilmette, Chiron.

BURKE, R.E., **1998**. In: Koliatos, V.E., Rattan, R.R. (Eds.), *Parkinson's Disease, Cell Death and Disease of the Nervous System*. Humana Press Inc., Totowa, NJ, pp. 459–475.

BURZANSKAHERMANN, Z., RZADKOWSKABODALSKA, H. and OLECHNOWICZSTEPIEN, W., **1977**. Isolation and identification of flavonoid compounds of *Mentha aquatica* L.herb. *Rocznika Chemii* **51**: 701–709.

BUTTERWECK, V., **2003**. Mechanism of action of St John's wort in depression: what is known? *CNS Drugs* **17**: 539-562.

BUTTERWECK, V., JURGENLIEMK, G., NAHRSTEDT, A., and WINTERHOFF, H., **2000**. Flavonoids from Hypericum perforatum show antidepressant activity in the forced swimming test. *Planta Medica* **66**: 3–6.

BUTTERWECK, V., NAHRSTEDT, A., EVANS, J., HUFEISEN, S., RAUSER, L., SAVAGE, J., POPADAK, B., ERNSBERGER, P., and ROTH, B. L., **2002**. *In vitro* receptor screening of pure constituents of St. John's wort reveals novel interactions with a number of GPCRs. *Psychopharmacology* (Berl) **162:** 193– 202.

BUTTERWECK, V., PETEREIT, F., WINTERHOFF, H., and NAHRSTEDT, A., **1998**. Solubilized hypericin and pseudohypericin from Hypericum perforatum exert antidepressant activity in the forced swimming test. *Planta Medica* **64**, 291-294.

BUTTERWECK, V., WALL, A., LIEFLANDER-WULF, U., WINTERHOFF, H., and NAHRSTEDT, A., **1997**. Effects of the total extract and fractions of Hypericum perforatum in animal assays for antidepressant activity. *Pharmacopsychiatry* **30**: 117-124.

CALLAWAY, H., 1991. *The initiation of a Zulu diviner*. In Peek, P.M. (Ed.). *African Divination Systems: Ways of Knowing*. Bloomington and Indianapolis, Indiana University Press.

CARDINAL, R.N., PARKINSON, J.A., HALL, J. and EVERITT, B.J., **2002**. Emotion and motivation: the role of the amygdala, ventral striatum, and prefrontal cortex. *Neuroscience and Biobehavioral Reviews* **26**: 321–352.

CARLINI, E. A., **2003**. Plants and the central nervous system. *Pharmacology, Biochemistry and Behavior* **75**: 501–512.

CARLSON, N.R., **1988**. *Foundations of physiological psychology*. Boston, MA, Allyn & Bacon.

CAWSTON, F.G., **1933**. Native medicine in Natal. *South African Medical Journal* **10**: 370–371.

CHANDRA, V., GANGULI, M. and RATCLIFF, G., **1994**. Studies of the epidemiology of dementia: comparisons between developed and developing countries. *Aging Clinical Experimental Research*. **6**: 307-321.

CHANG, W.C. GUIDA W.C. and STILL, J., **1989**. An internal coordinate Monte Carlo method for searching conformational space. *Journal of American Chemistry Society* **111:** 4379–4386.

CHARNEY, D.S. and MANJI, H.K. **2004**. Life stress, genes, and depression: multiple pathways lead to increased risk and new opportunities for intervention. *Science STKE* 225

CHATTERJEE, T.K., CHAKARBOTTY, A. and PATHAK, M., **1992**, Effect of plant extract *Centella asiatica* (Linn.) on cold restraint stress ulcer in rats. *Indian Journal of Experimental Biology* **30**: 889-891.

CHAVUNDUKA, G. L., **2004**. Jimmy and Busi's interview with Prof. Chavunduka, Chairman of Zimbabwe National Traditional Healers Association, ZINATHA'.

CHEVALLIER, A., **1996**. *The Encyclopedia of Medicinal Plants*. London, Dorling Kindersley.

CHHABRA, S.C., UISO, F.C. and MSHIU, E.N., **1984**. Phytochemical screening of Tanzanian medicinal plants. I. *Journal of Ethnopharmacology* **11**: 157–179.

CHIEJ, R., **1984**. Encyclopaedia of medicinal plants. MacDonald Publishers.

CHIMENTI, F., COTTIGLIA, F., BONSIGNORE, L., CASU, L., CASU, M., FLORIS, C., SECCI, D., BOLASCO, A., CHIMENTI, P., GRANESE, A., BEFANI, O., TURINI, P., ALCARO, S., ORTUSO, F., TROMBETTA, G., LOIZZO, A. and CUARINO, I., **2006**. Quercetin as the active

principle of *Hypericum hircinum* exerts a selective inhibitory activity against MAO-A: Extraction, biological analysis, and computational study. *Journal of Natural Products* **69**: 945-949.

CHINEMANA, F., DRUMMOND, R.B., MAVI, S. and DE ZOYSA, I. **1985**. Indigenous plant remedies in Zimbabwe. *Journal of Ethnopharmacology* **14**: 159–172.

CHOUDHARY, M.I., PRASAD, D.K., AHMAD N.S., ROSA, R. And ATTA-UR-RAHMAN, **2005**. Cholinesterase inhibitory pregnane-type steroidal alkaloids from *Sarcococca hookeriana*. *Steroids* **70**: 295-303.

CHOUDHARY, M.I., SALMA, S., SHEHNAZ, P., ASAAD, K. ABDUL, M.A.S., ATTA-UR-RAHMAN and MASOOD, P., **2003**. New triterpenoid alkaloid cholinesterase inhibitors from *Buxus hyrcana*. *Journal of Natural Products* **66**: 739-742.

CHRISTIANSON, A.L., ZWANE, M.E., MANGA, P., ROSEN, E., VENTER, A. and KROMBERG, J.G., **2000**. Epilepsy in rural South African children–prevalence, associated disability and management. *South African Medical Journal* **90**: 262–66.

COATES-PALGRAVE, K., **2002**. *Trees of southern Africa*, edn 3. Struik, Cape Town.

COOPER, J.R., BLOOM, F.E. and ROTH, R.H. **1982**. *Biochemical Basis of Neuropharmacology*. Oxford University Press, New York.

COURT, W.E., **1983**. Alkaloid distribution in some African *Rauvolfia* species. *Planta Medica* **48**: 228–233.

COX, P.A.. and BALICK, M.J., **1994**. The ethnobotanical approach to drug discovery. *Scientific American* **270**: 60–65.

CRITCHLEY, M., **1955**. *James Parkinson*. Macmillan and Co Ltd., New York, USA.

CROUCH, N.R., SMITH, G.F. and SMITH, M.T., **2000**. *Haseltonia*, **7**: 30-36.

CUNNINGHAM, A.B., **1988**. *An Investigation of the Herbal Medicine Trade in Natal*. Investigational Report 29. Pietermaritzburg, Institute for Natural Resources.

CURZON, G., **1990**. How reserpine and chlorpromazine act: the impact of key discoveries on the history of psychopharmacology. *Trends in Pharmacological Sciences* **11**: 61–63.

DAELS-RAKOTOARISON, D.A., SEIDEL, V., GRESSIER, B., BRUNTE, C., TILLEQUIN, F., BAILLEUL, F., LUYCKX, M., DINE, T., CAZINI, M. and CAZIN, J.C., **2000**. Neurosedative and antioxidant activities of phenylpropanoids from Ballota nigra. *Arzneimittel Forschung* **50**: 16–23.

DAHLSTRÖM, A. and FUXE, K., **1962**. Evidence for the existence of monoamine containing neurons in the central nervous system: I. Demonstration of monamines in the cell bodies of brain stem neurons. *Acta Physiologica Scandinavica* **232**: S1–S55.

DALY, J.W., **1993**. Mechanism of action of caffeine. In: Garattini, S. (Ed.), Caffeine, Coffee and Health. Raven Press, New York, pp. 97–150.

DALZIEL, J.M., **1937**. *The Useful Plants of West Tropical Africa*. London, Crown Agents for the Colonies.

DAVAL, J.-L., WERCK, M. C., NEHLIG, A. and PEREIRA DE VASCONCELOS, A., **1991**. Quantitative autoradiograpic study of the postnatal development of adenosine A_1 receptors and their coupling to G proteins in the rat brain. *Neuroscience* **40**: 841-851.

DE JAGER, E.J., **1963**. Notes on the magical charms of the Cape Nguni tribes. *Fort Hare Papers* **2**: 293–309.

DE RIJK, M.C., LAUNER, L.J., BERGER, K., BRETELER, M.M., DARTIGUES, J.F., BALDERESCHI, M., FRATIGLIONI, L., LOBO, A., MARTINEZ-LAGE, J., TRENKWALDER, C. and HOFMAN, A., **2000**. Prevalence of Parkinson's disease in Europe: a collaborative study of population-based cohorts. *Neurology* **54**: S21-S23.

DE SIO, F., **1970**. Notes on the research of ibogaine, used as a doping factor. *Medicina Dello Sport* **23**: 362-363.

DE SMET, P.A.G.M., **1990**. *Drugs Used in Non-Orthodox Medicine*. In: Dukes, M.N.G., Beeley, L. (Eds.), *Side Effects of Drugs*, Annual 14. Elsevier, Amsterdam, pp. 429–451.

DE SMET, P.A.G.M., **1996**. Some ethnopharmacological notes on African hallucinogens. *Journal of Ethnopharmacology* **50**: 141–146.

DE SMET, P.A.G.M., **1998**. Traditional pharmacology and medicine in Africa Ethnopharmacological themes in sub-Saharan art objects and utensils. *Journal of Ethnopharmacology* **63**: 1–179.

DE VILLIERS, C. and LOUW, S.J., **1996**. Determining the Prevalence of Alzheimer's Disease in elderly South Africans. *South African Medical Journal* **86**: 135-136.

DEBELLA, A., KUNERT, O., SCHMID, M.G., MICHL, G., BUCAR, F., ABEBE, D. and HASLINGER, E., **2000**. A diterpene, a flavonol glycoside, and a phytosterol glycoside from *Securidaca longipedunculata* and *Entada abyssinica*. *Monatshefte fuer Chemie* **131**, 401–408.

DEGIORGIO, C., PIETSCH-ESCUETA, S., TSANG, V., CORRAL-LEYVA, G., NG, L., MEDINA, M. T., ASTUDILLO, S., PADILLA, N., LEYVA, P., MARTINEZ, L., NOH, J., LEVINE, M., VILLASENOR, R. and SORVILLO, F., **2005**. Sero-prevalence of *Taenia solium* Cysticercosis and *Taenia solium* Taeniasis in California, USA. *Acta Neurologica Scandinavica*, **111**: 84-88.

DEKERMENDJIAN, K. KAHNBERG, P. WITT, M.R. STERNER, O. NIELSEN M. and LILJEFORS, T., **1999**. Structure-activity relationships and molecular modeling analysis of flavonoids binding to the benzodiazepine site of the rat brain $GABA_A$ receptor complex. *Journal of Medicinal Chemistry* **42**: 4343–4350.

DELGADO, P.L., MILLER, H.L., SALMON, R.M., LICINIO, J., KRYSTAL, J.H., MORENO, F.A., HENINGER, G.R. and CHARNEY, D.S., **1999**. Tryptophan-depletion challenge in depressed patients treated with desipramine or fluoxetine: implication for the role of serotonin in the mechanism of antidepressant action. *Biological Psychiatry* **46**: 212-220.

DENCKER, S.J., **2000**. The need for new and better antidepressants: reboxetine a new option. *Acta Psychiatrica Scandinavica* **101**: S6–S11.

DENT, G.R. and NYEMBEZI, C.L.S., **1999**. Scholar's *Zulu Dictionary; English-Zulu, Zulu-English*. Shuter & Shooter (Pty) Ltd

DESAI, R.V. and RUPAWALA, E.N., **1966**. Antifertility activity of the steroidal oil derived of the seed of *Abrus precatorius* L. *Indian Journal of Pharmacology* **29**: 235–237.

DIOP, A.G., HESDORFFER, D.C., LOGROSCINO, G. and HAUSER, W.A., **200**5. Epilepsy and Mortality in Africa: A Review of the Literature. *Epilepsia* **46**: 33–35.

DITTMANN, K., RIESE, U. and HAMBURGER, M., **2004**. HPLC-based bioactivity profiling of plant extracts: a kinetic assay for the identification of monoamine oxidase-A inhibitors using human recombinant monoamine-A. *Phytochemistry* **65**:2885-2891.

DOBKIN DE RIOS, M., **1986**. Enigma of drug-induced altered states of consciousness among the !Kung Bushmen of the Kalahari desert. *Journal of Ethnopharmacology* **15**: 297–304.

DOHADWALLA, A.N., **1985**. Natural product pharmacology: strategies in search of leads for new drug designs. *Trends in Pharmacological Sciences* **6**: 49–53.

DOKE, C.M. and VILAKAZI, B.W., **1972**. *Zulu–English Dictionary*. 2nd edn. Johannesburg, Witwatersrand University Press.

DOLD, T., COCKS, M. And KRALO, P., **1999**. *IQilika*: Mesemb beer of the Eastern Cape. *Aloe* **36**: 52–54.

DORNAN, S.S., **1924**. Witchcraft. *The South African Quarterly Journal* **6/7**: 3–13.

DORRINGTON, R., BOURNE, D., BRADSHAW, D. and LAUBSCHER, R., **2001**. Technical *Report on the Impact of HIV/AIDS on Adult Mortality in South Africa*. Cape Town: Centre for Actuarial Research, Medical Research Council and the Actuarial Society of South Africa.

DOUGLAS, M., 1987. Sorcery accusations unleashed: The Lele revisited, 1987. *Africa* **69**: 177-193.

DRAFT NATIONAL POLICY on ATMSA**, 2008.** The Draft National Policy on African Traditional Medicine in South Africa. *Government Gazette* **No. 31271**: 2-27.

DU PLOOY, W.J., SWART, L. and VAN HUYSTEEN, G.W., **2001**. Poisoning with *Boophane disticha*: a forensic case. *Human and Experimental Toxicology* **20**: 277–278.

DU, Q., JERZ, G. and WINTERHALTER, P., **2004**. Preparation of three flavonoids from the bark of Salix alba by high-speed countercurrent chromatographic separation. *Journal of Liquid Chromatography and Related Technologies* **27**: 3257–3264.

DUKE, J., **1985**. *Handbook of Medicinal Herbs*. Boca Raton, Florida, CRC Press.

DURLACH-MISTELI, C. and VAN REE, J.M., **1992**. Dopamine and melatonin in the nucleus accumbens may be implicated in the mode of action of antidepressant drugs. *European Journal of Pharmacology* **217**: 15–21.

EASTMAN, R., **2005**. Epilepsy in South Africa. *Acta Neurolica Scandinavica* **112**: 8–11.

EDMONDSON, D.E., BINDA, C. and MATTEVI, A., **2004**. The FAD binding sites of human monoamine oxidases A and B. *NeuroToxicology* **25**: 63-72.

EGASHIRA, T., TAKAYAMA, F. and YAMANAKA, Y., **1999**. The inhibition of monoamine oxidase activity by various antidepressants: differences found in various mammalian species. *Japanese Journal of Pharmacology* **81**, 115–121.

EICHHORN, A., **1911**. Beitra¨ge zur Kenntnis der Waschambaa. Nach hinterlassenen Aufzeichnungen von A. Karasek. *Baessler-Archiv* **1:** 155–222.

EISCH, A.J., BARROT, M., SCHAD, C.A., SELF, D.W. and NESTLER E.J., **2000**. Opiates inhibit neurogenesis in the adult rat hippocampus. *Proceedings of the National Academy of Science of the United States of America* **97**: 7579–7584.

ELGORASHI, E.E. and VAN STADEN, J., **2003**. Alkaloids from *Cyrtanthus falcatus*. *South African Journal of Botany* **69**: 593-594.

ELGORASHI, E.E., DREWES, S.E. and VAN STADEN, J., **1999**. Alkaloids from *Crinum bulbispermum*. *Phytochemistry* **53**: 533-536.

ELGORASHI, E.E., DREWES, S.E. and VAN STADEN, J., **2001a**. Alkaloids from *Crinum moorei*. *Phytochemistry* **56**: 637-640.

ELGORASHI, E.E., DREWES, S.E. and VAN STADEN, J., **2001b**. Alkaloids from *Crinum macowanii*. *Biochemical and Systematic Ecololgy* **29**: 749-750.

ELGORASHI, E.E., MALAN, S.F., STAFFORD, G.I. and VAN STADEN, J., **2006**. Quantitative structure–activity relationship studies on acetylcholinesterase enzyme inhibitory effects of Amaryllidaceae alkaloids. *South African Journal of Botany* **72**, 224-231.

ELGORASHI, E.E., STAFFORD, G.I. and VAN STADEN, J., **2004**. Acetylcholinesterase enzyme inhibitory effects of Amaryllidaceae alkaloids. *Planta Medica* **70**, 260– 262.

ELGORASHI, E.E., STAFFORD, G.I., JÄGER, A.K. and VAN STADEN, J., **2006**. Inhibition of [^{3}H]citalopram binding to the rat brain serotonin transporter by Amaryllidaceae alkaloids. *Planta Medica* **72**, 470-473.

ELHWUEGI, A.S., **2004**. Central monoamines and their role in major depression. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* **28**: 435-451.

ELIADE, M., **1987**. Shamanism: An Overview. In Eliade, M. (Ed.). *The Encyclopedia of Religion*. New York, Macmillan Publishing Company.

ELLMAN, G.L., COURTNEY, D., ANDIES, V. and FEATHERSTONE, R.M. **1961**. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology* **7**: 88-95.

ELSOHLY, H.N. and SLADE, D., **2005**. Chemical constituents of marijuana: The complex mixture of natural cannabinoids. *Life Sciences* **78**: 539-548.

ELSOHLY, H.N., BOEREN, E.G., TURNER, C.E. and ELSOHLY, M.A., **1984**. *Constituents of Cannabis sativa* L. XXIIII: *Cannabitetrol, a new polyhydroxylated cannabinoid*. In: Agurell, S., Dewey, W.L., Willette, R.E. (Eds.), *The Cannabinoids: Chemical, Pharmacologic, and Therapeutic Aspects*. Academic Press, New York, pp. 89– 96.

ELSOHLY, M.A., **2002**. *Chemical constituents of cannabis*. In: Grotenhermen, F., Russo, E.B., (Eds.). *Cannabis and cannabinoids: pharmacology, toxicology, and therapeutic potential*. New York' Haworth Integrative Healing Press p. 27– 36.

EMBODEN, W., **1972**. *Narcotic Plants*. London, Studio Vista.

EMILIEN, G. and MALOTEAUX, J.M., **1998**. Pharmacological management of epilepsy. Mechanism of action, pharmacokinetic drug interactions, and new drug discovery possibilities. *International Journal of Clinical Pharmacology and Therapy* **36**: 181-194.

ENGELBRECHT, J.A., **1936**. *The Korana*. Cape Town, Maskew Miller.

ERIKSSON, E., **2000**. Antidepressant drugs: does it matter if they inhibit the reuptake of noradrenaline or serotonin? *Acta Psychiatrica Scandinavica* **101**: S12– S17.

ERNST, E., **1995**. St. John's wort as antidepressive therapy. *Fortschr Medicine***113**:354-355.

ESMAEILI, A., RUSTAIYAN, A., MASOUDI, S. and NADRI, K., **2006**. Composition of the essential oils of *Mentha aquatica* L. and Nepeta meyeri Benth. From Iran. *Journal of Essential Oil Research* **18**: 263–265.

FARNSWORTH, N.R. and MORRIS, R.W., **1976**. Higher plants: the sleeping giant of drug development, *American Journal of Pharmacology* **147**: 46-52.

FARNSWORTH, N.R., AKERELE, O., BINGEL, A.S., SOEJARTO, D.D. and GUO, Z.G., **1985**. Medicinal plants in therapy. *Bulletin of the WHO* **63**: 83-97.

FATTORUSSO, V. and RITTER, O., **1967**. *Dictionnaire de Pharmacologie Clinique*. Masson, Paris.

FELIX, **1931**. Native medicines. *South African Outlook* **61** (723): 156–157.

FENNELL, C.W., LIGHT, M.E., SPARG, S.G., STAFFORD, G.I. and VAN STADEN, J., **2004**. Assessing African medicinal plants for efficacy and safety: agricultural and storage practices. *Journal of Ethnopharmacology* **95**: 113–121.

FENNELL, C.W., LINDSEY, K.L., MCGAW, L.J., SPARG, S.G., STAFFORD, G.I., ELGORASHI, E.E., GRACE, O.M. and VAN STADEN, J., **2004**.AssessingAfrican medicinal plants for efficacy and safety: pharmacological screening and toxicology. *Journal of Ethnopharmacology* **94**: 205–217.

FERRE, S., VON EULER, G., JOHANSSON, B., FREDHOLM, B.B. and FUXE, K., **1991**. Stimulation of high-affinity adenosine A2 receptors decreases the affinity of dopamine D2 receptors in rat striatal membranes. *Proceedings of the National Academy of Science U S A.* **88**: 7238–7241.

FOLEY, P., GERLACH, M., YOUDIM, M.B.H. and RIEDERER, P. **2000**. MAO-B inhibitors: multiple roles in the therapy of neurodegenerative disorders. *Parkinson Related Disorders* **6**: 25–47.

FORMUKONG, E.A. EVANS, A.T. and EVANS, F.J., **1989**. The medicinal uses of *Cannabis* and it constituents. *Phytotherapy Research* **3**: 219-231

FORNO, L.S. **1996**. Neuropathology of Parkinson's disease. *Journal of Neuropathology & Experimental Neurology* **55**: 259-272.

FOURIE, T.G., SWART, I. and SNYCKERS, F.O. **1992**. Folk Medicine: a viable starting point for pharmaceutical research. *South African Journal of Science* **88**: 190-192.

FREDHOLM, B.B., **1995**. Adenosine, adenosine receptors and the actions of caffeine. *Pharmacology and Toxicology* **76**:93-101.

FRØLUND, B., JORGENSEN, A.T., TAGMOSE, L., STENSBØL, T.B., VESTERGAARD, H.T., ENGBLOM, C., KRISTIANSEN, U., SANCHEZ, C., KROGSGAARD-LARSEN, P. and

LILJEFORS, T., **2002**. A novel class of potent 4-arylalkyl substituted 3-isoxazolol GABA-A antagonists: synthesis, pharmacology and molecular modeling. *Journal of Medicinal Chemistry* **45**: 2454–2468.

FUKUNISHI, I., MATSUMOTO, T., NEGISHI, M., HAYASHI, M., HOSAKA, T. and MORIYA, J., **1997**. Somatic complaints associated with depressive symptoms in HIV-positive patients. *Psychotherapy and Psychosomatics* **66**: 248–251.

FULLER, R.W. **1995**. Serotonin uptake inhibitors: uses in clinical therapy and in laboratory research. *Progress in Drug Research* **45**: 167-204.

FULLER, R.W., **1980**. Pharmacology of central serotonin neurons. *Annual Review of Pharmacology and. Toxicology* **20**: 111-127.

GADDUM, J.H. and HAMEED, K.A., **1954**. Drugs which antagonize 5-hydroxytryptamine. *British Journal of Clinical Pharmacology* **9**: 240–248.

GARG, R.K., **1999**. HIV infection and seizures. *Postgraduate Medical Journal* **75**: 387–390.

GARRETT, B.E. and GRIFFITHS, R.R., **1997.** The role of dopamine in the behavioral effects of caffeine in animals and humans. *Pharmacology, Biochemistry and Behaviour* **57**: 533-541.

GARRETT, B.E. and HOLTZMAN, S.G., **1995**, Does adenosine receptor blockade mediate caffeine-induced rotational behavior?, *Journal of Pharmacology and Experimental Therapeutics*. **274**: 207-214.

GELFAND, M., MAVI, S., DRUMMOND, R.B. and NDEMERA, B., **1985**. The Traditional Medical Practitioner in Zimbabwe. Mambo Press, Zimbabwe

GERICKE, N. P.and VAN WYK, B-E., **2001**. United States Patent 6,288,104: *Pharmaceutical compositions containing mesembrine and related compounds*. Inventors: Nigel Peter Gericke and Ben-Erik Van Wyk, Assignee: African Natural Health CC.

GERMISHUIZEN, G. and MEYER, N.L. (Eds.), **2003**. Plants of southern Africa: an annotated checklist. *Strelitzia* **14**. National Botanical Institute, Pretoria.

GERSTNER, J., **1939**. A preliminary checklist of Zulu names of plants with short notes. *Bantu Studies* **13**: (1) 49–64, (2) 131–149, (4) 307–326.

GERSTNER, J., **1941**. A preliminary checklist of Zulu names of plants with short notes. *Bantu Studies* **15**: (3) 277–301, (4) 369–383.

GETHER, U., ANDERSEN, P.H., LARSSON, O.M. and SCHOUSBOE, A., **2006**. Neurotransmitter transporters: molecular function of important drug targets, *Trends in Pharmacology Science* **27:** 375–383.

GIBBS, R. D., **1974**. *Chemotaxonomy of Flowering Plants*. Vol. I. Constituents. Montreal.

GILANI, A.H., **1998**. Novel developments from natural products in cardiovascular research. *Phytotherapy Research* **12**: S66–S69.

GILANI, A.H., SHAH, A.B., GHAYUR, M.N. and MAJEED, K., **2005**. Pharmacological basis for the use of turmeric in gastrointestinal and respiratory disorders. *Life Sciences* **76**: 3089-3105.

GILBERT, R.M., **1976**. *Caffeine as a Drug Of Abuse*. In: Gibbins RJ, Israel Y, Kalant H, Popham RE, Schmidt W, Smart RG, editors. Research *Advances in Alcohol and Drug Problems*, vol. 3. New York: Wiley, pp. 49-77.

GILGES, S.W., **1953**. Trees in South Africa 5, 17. *The Occasional Papers of the Rhodes Livingstone Museum Number 11.*

GILGES, S.W., **1955**. Some African poison plants and medicines of Northern Rhodesia. *The Occasional Papers of the Rhodes Livingstone Museum Number 11.*

GINGRICH, J.A. and CARON, M.G., **1993**. Recent advances in the molecular biology of dopamine receptors. *Annual Reviews in Neuroscience* **16**: 299– 321.

GIORGIO, E., PARRINELLO, N., CACCAMESE, S. and ROSINI, C., **2004**. Non-empirical assignment of the absolute configuration of (−)-naringenin, by coupling the exciton analysis of the circular dichroismspectrumand the ab initio calculation of the optical rotatory power. *Organic Biomolecular Chemistry* **2**: 3602–3607.

GITHENS, T.S., **1949**. *Drug Plants of Africa*. African Handbooks. University of Pennsylvania Press, Philadelphia, USA.

GITHINJI, C. and KOKWARO, J.O., **1993**. Ethnomedicinal study of major species in the family Labiatae from Kenya. *Journal of Ethnopharmacology* **39**, 197-203.

GLENNON, R. A., TITLER, M., and MCKENNEY, J. D., **1984**. Evidence for 5-HT_2 involvement in the mechanism of action of hallucinogenic agents. *Life Sciences* **35**: 2505-2511.

GLUCKMAN, M., **1956**. *Custom and conflict in Africa*. Glencoe, IL: Free Press.

GOBBI, M., MOIA, M., PIRONA, L., MORIZZONI, P. and MENNINI, T., **2001**. *In vitro* binding studies with two *Hypericum perforatum* extracts–hyperforin, hypericin and biapigenin–on 5-HT6, 5-HT7, GABA(A)/benzodiazepine, sigma, NPY-Y1/Y2 receptors and dopamine transporters. *Pharmacopsychiatry* **34:** S45–S48.

GOODNICK, P.J. and GOLDSTEIN, B.J., **1998**. Selective serotonin reuptake inhibitors in affective disorders: II. Efficacy and quality of life. *Journal of Psychopharmacology* **12**: S21– 54.

GOODWIN, F.K. and BUNNEY Jr., W.E., **1971**. Depressions following reserpine: a re-evaluation. *Seminars in Psychiatry* **3**: 435-448.

GORDON, M.M., **1947**. *The concept of the sub-culture and its application*. In K. Gelder and S. Thornton (eds) *The Subcultures Reader*. London: Routledge, 1997.

GREEN, E.C., **1994**. *AIDS and STDs in Africa*. Boulder, CO: Westview Press.

GRINSPOON, L. and BAKALAR, J.B., **1997**. *Psychedelic Drugs Reconsidered*, 2nd ed. The Lindesmith Center, New York.

GROPPETTI, A., CERESOLI, G., MANDELLI, V. and PARENTI, M., **1990**. Role of opiates in striatal D-1 dopamine receptor supersensitivity induced by chronic L-dopa treatment. *Journal of Pharmacology and Experimental Therapeutics* **253**: 950-956.

GUILLEMIN, G.J. and BREW, B.J., **2007**. Chronic HIV infection leads to an Alzheimer's disease like illness. Involvement of the kynurenine pathway. *International Congress Series* **1304**: 324-334.

GUMEDE, M.V., **1990**. *Traditional healers*. Johannesburg: Skotaville.

GURIB-FAKIM, A., SEWRAJ, M., GUEHO, J. and DULLOO, E., **1993**. Medical ethnobotany of some weeds of Mauritius and Rodrigues. *Journal of Ethnopharmacology* **39**: 175–185.

HABIB, M.S. and COURT, W.E., **1974**. Estimation of the alkaloids of *Rauwolfia caffra. Planta Medica* **25**: 261-266.

HADDJERI, N., BLIER, P. and DE MONTIGNY, C., **1996**. Effect of the alpha-2 adrenoceptor antagonist mirtazapine on the 5-hydroxytryptamine system in the rat brain. *Journal of Pharmacology and Experimental Therapeutics* **277**: 861–871.

HAERDI, F., **1964**. Die Eingeborenen-Heiplipflazen des Ulanga-Distriktes Tangajikas (Ostafrika). *Acta Tropica Supplementum* **8**: 1–278.

HALASY, K. and SOMOGYI, P., **1993**. Distribution of GABA-ergic synapses and their targets in the dentate gyrus of rat: a quantitative immuno-electron microscopic analysis. *J. Hirnforsch* **34**: 299-308.

HALGREN, T.A., **1996**. Merck molecular force field. I. Basis, form, scope, parametrization, and performance of MMFF94. *Journal of Computational Chemistry* **17**: 490–519.

HAMMOND-TOOKE, W.D., **1989**. *Rituals and Medicines*. A.D. Donker (PTY) LTD., Johannesburg. ISBN 0 86852 110 8.

HANRAHAN, J.R., CHEBIB, M., DAVUCHERON, N.M., HALL, B.J. and JOHNSTON, G.A.R., **200**3. Semisynthetic preparation of amentoflavone: a negative modulator at $GABA_A$ receptors. *Bioorganic and Medicinal Chemistry Letters* **13**: 2281-2284.

HARAGUCHI, H., TANAKA, Y., KABBASH, A., FUJIOKA, T., ISHIZU, T. and YAGI, A., **2004**. Monoamine oxidase inhibitors from *Gentiana lutea*. *Phytochemistry* **65**: 2255-2260.

HARRISON, P., **1998**. Herbal medicine takes root in Germany. *Canadian Medical Association Journal* **158**: 637-639.

HEANEY, D.C., MACDONALD, B.K., EVERITT, A., STEVENSON, S., LEONARDI, G.S., WILKINSON, P. and SANDER, J.W., **2002**. Socioeconomic variation in incidence of epilepsy: prospective community based study in south east England. *British Medical Journal* **325**: 1013-1016.

HEDBERG, I. and STAUGARD, F., **1989.** *Traditional Medicinal Plants: Traditional Medicine in Botswana*. Gabarone, Ipeleng.

HEDBERG, I., HEDBERG, O., MADATI, P.J., MSHIGENI, K.E., MSHIU, E.N. and SAMUELSSON, G., **1982**. Inventory of plants used in traditional medicine in Tanzania. Plants of the families Acanthaceae–Cucurbitaceae. *Journal of Ethnopharmacology* **6**: 29–60.

HEFFTER, A., **1896**. Ueber Cacteenalkaloide, *Ber. Deutsch. Chem. Ges.*, **29**: 216-227.

HEILA, H., ISOMETSA, E.T., HENRIKSSON, M.M., HEIKKINEN, M.E., MARTTUNEN, M.J., LONNQVIST, J.K., **1997**. Suicide and schizophrenia: a nationwide psychological autopsy study on age- and sex-specific clinical characteristics of 92 suicide victims with schizophrenia. *American Journal of Psychiatry* **154**; 1235–1242.

HEINRICH, M. and TEOH, H.L., **2004**. Galanthamine from snowdrop—the development of a modern drug against Alzheimer's disease from local Caucasian knowledge. *Journal of Ethnopharmacology* **92**: 147–162.

HENN, F., VOLLMAYR, B. and SARTORIUS, A., **2004**. Mechanisms of depression: the role of neurogenesis. *Drug Discovery Today: Disease Mechanisms* **1**: 407-411.

HENSLER, J.G., **2002**. Differential regulation of 5-HT_{1A} receptor-G protein interactions in brain following chronic antidepressant administration. *Neuropsychopharmacology* **26**: 565-573.

HENSLER, J.G., **2003**. Regulations of 5-HT_{1A} receptor function in brain following agonist or antidepressant administration. *Life Sciences* **72**: 1665-1682.

HEWSON, M.G., **1998**. Traditional healers in southern Africa. *Annals of Internal Medicine* **128**: 1029–1034

HIRST, M., **1990**. The healers art: Cape Nguni diviners in the township of Grahamstown. PhD (Anthropology) thesis, Rhodes University, Grahamstown.

HIRST, M., **1997a**. The utilization of *Catha edulis* in the household economy of Xhosa farm inhabitants of the Bolo reserve, eastern Cape. *Journal of Contemporary African Studies* **15**: 119–143.

HIRST, M., **1997b**. A river of metaphors: interpreting the Xhosa diviner's myth. In McAllister, P. (Ed.) *Culture and the Commonplace: Anthropological Essays in Honour of David Hammond-Tooke.* Johannesburg, Witwatersrand University Press.

HIRST, M., **2005**. Dreams and medicines: the perspective of Xhosa diviners and novices in the Eastern Cape. *South - Indo-Pacific Journal of Phenomenology* **5**: 1-22.

HODEK, P., TREFIL, P. and STIBOROVA, M., **2002**. Flavonoids-potent and versatile biologically acrivity compounds interacting with cytochromes P450. *Chemico-Biological Interactions* **139**: 1-21.

HOFMANN, A., **1982**. Ololiuqui: a sacred drug of the Aztecs. *Pharmacology International* **3**: 216-217.

HOLMES, A., YANG, R.J., MURPHY, D.L. and CRAWLEY, J.N., **2002**. Evaluation of antidepressant-related behavioral responses in mice lacking the serotonin transporter. *Neuropsychopharmacology* **27:** 914–923.

HOLT, A., SHARMAN, D.F., BAKER, G.B. and PALCIC, M.M. **1997**. A Contionous Spectrophotometric Assay for Monoamine Oxidase and Related Enzymes in Tissue Homogenates. *Analytical Biochemistry* **244**: 384-392

HORTON, R., **1976**. *Rationality* (Wilson, B. R., ed.), Blackwell pp. 132–171.

HORTON, R., **1993**. *Patterns of Thought in Africa and the West*, Cambridge: Cambridge University Press.

HOSTETTMANN, K., BORLOZ, A., URBAIN, A. and MARSTON, A., **2006**. Natural Product Inhibitors of Acetylcholinesterase. *Current Organic Chemistry* **10**: 825-847.

HOSTETTMANN, K., MARSTON, A., NDJOKO, K. and WOLFENDER, J-L. **2000**. The Potential of African Plants as a Source of Drugs. *Current Organic Chemistry* **4**: 973-1010.

HOU, W.-C., LIN, R.-D., CHEN, C.-T. and LEE, M.-H. **2005**. Monoamine oxidase B (MAO-B) inhibition by active principles from *Uncaria rhynchophylla*. *Journal of Ethnopharmacology* **100**: 216-220.

HOUGHTON, L.A. and WHORWELL P.J., **1997**. Opening the doors of perception in irritable bowel syndrome. *Gut* **41**: 567–8.

HOUGHTON, P.J., **2000**. Use of Small Scale Bioassays in the Discovery of Novel Drugs from Natural Sources. *Phytotheraphy Research* **14**: 419-423.

HOUGHTON, P.J., **2005.** *Use of Medicinal Plants in CNS Disorders*. In: *Handbook of medicinal plants* Eds Z. Yaniv and U. Bachrach. Food Products Press, The Harworth Medical Press. New York pp 353.

HOUGHTON, P.J., AGBEDAHUNSI, J.M. and ADEGBULUGBE, A., **2004**. Choline esterase inhibitory properties of alkaloids from two Nigerian *Crinum* species. *Phytochemistry* **65**: 2893–2896.

HOUGHTON, P.J., REN, Y. and HOWES, M.-J., **2006**. Acetylcholinesterase inhibitors from plants and fungi. *Natural Product Reports* **23**: 181–199.

HOWELL, L.L., **1993**. Comparative effects of caffeine and selective phosphodiesterase inhibitors on respiration and behavior in rhesus monkeys. *Journal of Pharmacology and Experimental Therapy* **266**: 894–903.

HOWES, M.J. and HOUGHTON, P.J., **2003**. Plants used in Chinese and Indian traditional medicine for improvement of memory and cognitive function. *Pharmacology Biochemistry and Behavior* **75**: 513–527.

HOWES, M.R., PERRY, N.S.L. and HOUGHTON, P.J., **2003**. Plants with traditional uses and activities, relevant to the management of Alzheimer's disease and other cognitive disorders. *Phytotherapy Research* **17**: 1–18.

HOWLETT, A.C., BIDAUT-RUSSELL, M., DEVANE, W.A., MELVIN, L.S., JOHNSON, M.R., and HERKENHAM, M., **1990**. The cannabinoid receptor: biochemical, anatomical and behavioral characterization. *Trends in Neuroscience* **13**: 420-423.

HULME, M.M., **1954**. *Wild Flowers of Natal*. Shuter and Shooter, Pietermaritzburg.

HUMBLE, M., **2000**. Noradrenaline and serotonin reuptake inhibition as clinical principles: a review of antidepressant efficacy. *Acta Psychiatrica Scandinavica* **101**: S28-S36.

HUONG, D.T.L., DAT, N.T., MINH, C.V., KANG, J.-S. and KIM, Y.H., **2002**. Monoamine oxidase inhibitors from *Aquilaria agallocha*. *Natural Product Sciences* **8**: 30-33.

HUSSEINI, H., MITROVIC, V. and SCHLEPPER, M., **1995**. Determination of monoamine oxidase B activity by high performance liquid chromatography with electrochemical detection. *Journal of Chromatograph B* **672**: 138-142

HUTCHINGS, A. and VAN STADEN, J., **1994**. Plants used for stress-related ailments in traditional Zulu, Xhosa and Sotho medicine. Part 1: Plants used for headaches. *Journal of Ethnopharmacology* **43**: 89–124.

HUTCHINGS, A., **1989**. Observations on plant usage in Xhosa and Zulu medicine. *Bothalia* **19**: 225–235.

HUTCHINGS, A., SCOTT, A.H., LEWIS, G. and CUNNINGHAM, A., **1996**. *Zulu Medicinal Plants. An Inventory*. Pietermaritzburg, University of Natal Press.

HWANG, J.S., LEE, S.A., HONG, S.S., LEE, K.S., LEE, M.K., HWANG, B.Y. and RO, J.S., **2005**. Monoamine oxidase inhibitory components from the roots of *Sophora flavescens*. *Archives of Pharmaceutical Research* **28**: 190-194.

IHEZUE, U.H. and OKPARA, E., **1989**. Psychiatric Disorders of old age in Enugu, Nigeria. *Acta Neurologica Scandinavica* **79**: 332-337.

INEICHEN, B., **2000**. The epidemiology of dementia in Africa: a review. *Social Science and Medicine* **50**: 1673-1677.

INGSTAD, B., **1989**. 'Healer, Witch, Prophet, or Modern Health Worker?: The Changing Role of *Ngaka Ya Setswana* (Botswana)', in A. Jacobson-Widding and D.Westerlund (eds), *Culture, experience, and Pluralism: Essays on African ideas of Illness and Healing*, Uppsala, Sweden: University of Uppsala Press.

INGSTAD, B., **1990**. 'The Cultural Construction of AIDS and Its Consequences for Prevention in Botswana', *Medical Anthropology Quarterly* **4**: 28-40.

INOUE, H., CASTAGNOLI, K., VAN DER SCHYF, C., PHANE MABIC, S., GARASHI, K.I. and CASTAGNOLI, N. JR., **1999**. Species-Dependent Differences in Monoamine Oxidase A and B-catalyzed oxidation of various c4 substituted 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridinyl derivatives. *The Journal of Pharmacology and Experimental Therapeutics* **291**: 856-864.

IRVINE, F.R., **1961**. *Woody Plants of Ghana*. London, Oxford University Press.

IVANOVA, A., MIKHOVAA, B., NAJDENSKIB, H., TSVETKOVAB, I. and KOSTOVA, I., **2005**. Antimicrobial and cytotoxic activity of *Ruta graveolens*. *Fitoterapia* **76**: 344-347.

IVERSON, L.L., **1991**. *The Uptake and Storage of Noradrenaline in Sympathetic Nerves* Cambridge Univ. Press, London, England.

IWU, M.M., **1993**. *Handbook of African Medicinal Plants.* Florida, CRC Press.

JACOBSSON, L., **2002**. Traditional treatment of mental and psychosomatic disorders in Ethiopia. *International Congress Series* **1241**: 265–269.

JACOT GUILLARMOD, A., **1971**. *Flora of Lesotho*. Lehre, J. Cramer.

JAFFE, J.H. and MARTIN, W.R., **1975**. Narcotic analgesics and antagonists. In: L.S. Goodman and A. Gilman (Eds.), *The Pharmacological Basis of Therapeutics*, 5th edn., McMillan Publishing Co., pp. 245-283.

JÄGER, A.K., ALMQVIST, J.P., VANGSØE, S.A.K., STAFFORD, G.I., ADSERSEN, A. and VAN STADEN, J., **2007**. Compounds from *Mentha aquatica* with affinity to the GABA-benzodiazepine receptor. *South African Journal of Botany* **73**: 518–521.

JÄGER, A.K., MOHOTO, S.P., VAN HEERDEN, F.R. and VILJOEN, A.M., **2005**. Activity of a traditional South African epilepsy remedy in the GABA-benzodiazepine receptor assay. *Journal of Ethnopharmacology* **96**: 603–606.

JASPERSEN-SCHIB, R., **1970**. Tbxische Amaryllidaceen. *Pharmaceutica Acta Helvetiae* **45**: 424-433.

JENKINS, M.D., **1987**. (Ed.). *Madagascar: an Environmental Profile*. Gland, Switzerland, IUCN.

JERKOVIC, I. and MASTELIC, J., **2001**. Composition of free and glycosidically bound volatiles of *Mentha aquatica* L. *Croatica Chemica Acta* **74**: 431–439.

JO, Y.S., HOUNG, D.T.L., BAE, K., LEE, M.K. and KIM, Y.H., **2002**. Monoamine oxidase inhibitory coumarin from *Zanthoxylum schinifolium*. *Planta Medica* **68**: 84–85.

JOHNSTON, T.F., **1972**. *Datura fastuosa* L. Its use in Tsonga girl's initiation. *Economic Botany* **26**: 340–351.

JONES, B.J. and BLACKBURN, T.P., **2002**. The medical benefit of 5-HT research. Pharmacology, *Biochemistry and Behaviour* **71**: 555–568.

JUDD, F.K. and MIJCH, A.M., **1996**. Depressive symptoms in patients with HIV infection. *Australian and New Zealand Psychiatry* **30**: 104–109.

JUNOD, H.A., **1962**. *The Life of a South African Tribe*. 2nd edn. New York, University Books Inc. 2 vols.

KAHNBERG, P., LAGER, E., ROSENBERG, C., SCHOUGAARD, J., CAMET, L., STERNER, O., NIELSEN, E.O., NIELSEN, M., and LILJEFORS, T., **2002**. Refinement and Evaluation of a Pharmacophore Model for Flavone Derivatives Binding to the Benzodiazepine Site of the $GABA_A$ Receptor. *Journal of Medicinal Chemistry* **45**: 4188-4201.

KALSNER, S., **2000**. The question of feedback at the somatodendritic region and antidepressant drug action. *Brain Research Bulletin* **6**: 467– 73.

KANDEL, E.R. and SCHWARTZ, J.H., **1985**. *Principles of neural science*, 2nd ed. New York, NY, Elsevier.

KATZ, R., **1982**. *Boiling Energy. Community Healing among the Kalahari !Kung*. Cambridge, MA, Harvard University Press.

KATZUNG, B.G., **2001**. *Pharmacological Management of Parkinsonism and Other Movement Disorders*. In: *Basic and Clinical Pharmacology*, 8th ed. Lange Medical Books, McGraw Hill Companies, Inc.

KENNEDY, J., **1985**. Coca Exotica: The Illustrated Story of Cocaine, Fairleight Dickenson University Press, Cornwall Books.

KERHARO J. and ADAM J.G., **1974**. La Pharmacopée Sénégalaise Traditionnelle: Plantes médicinales et toxiques. Paris.

KHALID, A., HAQ, Z.U., GHAYUR, M.N., FEROZ, F., RAHMAN, A.U., GILANI, A.H. and CHOUDHARY, M.I., **2004**. Cholinesterase inhibitory and spasmolytic potential of steroidal alkaloids. *Journal of Steroid Biochemistry and Molecular Biology* **92**: 477–484.

KHAN, M.A., **1986**. Studies in the alkaloids of *Rauwolfia caffra* Sonder. *Natural Product Chemistry, Proceedings of the International Symposium, Pakistan-U.S.A.* from *Chemical Abstracts* CA106(19):153050y.

KILIC, F., MURPHY, D.L., and RUDNICK, G., **2003**. A human serotonin transporter mutation causes constitutive activation of transport activity. *Molecular Pharmacology* **64**: 440–446.

KIM, S.R., HWANG, S.Y., JANG, Y.P., PARK, M.J., MARKELONIS, G.J., OH, T.H. and KIM, Y.C., **1999**. Protopine from *Corydalis ternate* has anticholinesterase and antiamnestic activities. *Planta Medica* **65**: 218-221.

KLEBER, E., OBRY, T., HIPPELI, S., SCHNEIDER ,W. and ELSTNER, E.F., **1999**. Biochemical activities of extracts from Hypericum perforatum L. 1st Communication: inhibition of dopamine-beta-hydroxylase. *Arzneimittelforschung* **49**:106-109.

KLEINMAN, A., EISENBERG, L., and GOOD, B., **1978**. Culture, Illness, and Care. *Annals of Internal Medicine* **88**: 251-259.

KLING, H., **1923**. *Die Sieketrooster*. Cape Town, Van de Sandt de Villiers.

KNOLL, J., **2000**. (-)Deprenyl (Selegiline): past, present and future. *Neurobiology (Bp)* **8**: 179-199.

KOBES, R.D., WYATT, R.J. and NECKERS, L.M., **1980**. A sensitive and rapid fluorimetric assay for monoamine-oxidase utilizing high-pressure liquid-chromatography. *Journal of Pharmacological Methods*. **3**: 305-310.

KOKWARO, J.O., **1976**. *Medicinal Plants of East Africa*. Kampala, East African Literature Bureau.

KONG, L.D., CHENG, C.H.K. and TAN, R.X., **2004**. Inhibition of MAO A and B by some plant-derived alkaloids, phenols and anthraquinones. *Journal of Ethnopharmacology* **91**: 351–355

KONG, L.D., CHENG, C.H.K. and TAN, R.X., **2001**. Monoamine Oxidase Inhibitors from Rhizoma of *Coptis chinensis*. *Planta Medica* **67**: 74-76.

KOORBANALLY, N., MULHOLLAND, D.A. and CROUCH, N., **2000**. Alkaloids and triterpenoids from *Ammocharis coranica* (Amaryllidaceae), *Phytochemistry* **54**: 93-97.

KOPIN, I.J., **1985**. Catecholamine metabolism: basic aspects and clinical significance. *Pharmacological Reviews* **37:** 333–364.

KRAML, M., **1965**. A rapid microfluorimetric determination of monoamine oxidase. *Biochemical Pharmacology* **14**: 1684-1686.

KRIEG, M., **1964**. Green Medicine, Rand McNally and Co., Chicago.

KRIGE, E.J., **1940**. Medicine, magic and religion of the Lovedu. D.Litt. thesis, University of the Witwatersrand.

KWAZULU-NATAL HEALTH ACT, 2000. Gazette No: 5560. http://www.kznhealth.gov.za/healthact.pdf.

LAIDLER, P.W. **1928**. The magic medicine of the Hottentots. *South African Journal of Science* **25**: 433.

LAING, R.D., **1965**. *The Divided Self: An Existential Study in Sanity and Madness.* Tavistock Publications, London.

LAL, J. and GUPTA, P.C., **1973**. Physcion and phytosterol from the roots of *Cassia occidentalis*. *Phytoche* **12**: 1186, from Chemical Abstracts. *CA* **79**: 50727.

LAMBO, E., **1982**. Basic health services programme: The Nigerian experience. *Vikalpa* **7**: 119-128.

LAMBRECHT, I., **1998**. *A psychological study of shamanic trance states in South African shamanism*. PhD (Psychology) thesis, University of the Witwatersrand, Johannesburg.

LANGER, S.Z., **1980**. Presynaptic regulation of the release of catecholamines. *Pharmacology Review* **32**: 337–362.

LAST, M. and CHAVUNDUKA G., **1986**. *The Professionalisation of African Medicine*, Manchester: Manchester University Press (International African Institute).

LAYDEVANT, F., **1932**. Religious or sacred plants of Basutoland. *Bantu Studies* **6**: 65–69.

LAYDEVANT, F., **1939**. Initiation du medecin-sorcier en Basutoland. *Anneli latern* **3**: 99–139.

LEE, M.H., LIN, R.D., SHEN, L.Y., YANG, L.L., YEN, K.Y. and HOU, W.C., **2001**. Monoamine oxidase B and free radical scavenging activities of natural flavonoids in *Melastoma candidum* D. Don. *Journal of Agriculture and Food Chemistry* **49**: 5551-5555.

LEE, R., **1979**. *The !Kung San: Men, Women and Work in a Foraging Society*. Cambridge, England, Cambridge University Press.

LEE, S.S., KAI, M. and LEE, M.K., **1999**. Effects of natural isoquinoline alkaloids on monoamine oxidase activity in mouse brain: inhibition by berberine and palmatine. *Medicinal Science Research* **27**: 749-751.

LEMA, W.J., BLANKENSHIP, J.W., and MALONE, M.H., **1986**. Prostaglandin synthetase inhibition by alkaloids of *Heimia salicifolia*. *Journal of Ethnopharmacology* **15**: 161-167.

LEONARD, B.E., **1997**. Noradrenaline in basic models of depression. *European Neuropsychopharmacology* **7**: S11-S16.

LESCH, K.P., WOLOZIN, B.L., ESTLER, H.C., MURPHY, D.L., and RIEDERER, P., **1993**. Isolation of a cDNA encoding the human brain serotonin transporter. *Journal of Neural Transmission. General Section.* **91**: 67–72.

LESTER, H.A., CAO, Y. and MAGER, S., **1996**. Listening to neurotransmitter transporters. *Neuron* **17**: 807–810.

LEVKOFF, S.E., MACARTHUR, I.W. and BUCKNALL, J., **1995**. Elderly Mental Health in the Developing World. *Social Science Medicine* **41**: 983-1003.

LEWIN, L., **1924**. *Phantastica: Narcotic and Stimulating Drugs*. Park Street Press, Rochester.

LEWIS, W.H. and ELVIN-LEWIS, M.P.F., **1977**. *Medical Botany: Plants Affecting Man's Health*. Wiley, Canada, pp. 284–295.

LIDDELL, C., BARRETT, L. and BYDAWELL, M., **2005**. Indigenous representations of illness and AIDS in Sub-Saharan Africa. *Social Science and Medicine* **60**: 691–700.

LIENGME, C.A., **1981**. Plants used by the Tsonga people of Gazankulu. *Bothalia* **13**: 501–518.

LIN, R.-D., HOU, W.C., YEN, K.Y. and LEE, M.H., 2003. Inhibition of monoamine oxidase B (MAO-B) by Chinese herbal medicines. *Phytomedicine* **10**: 650-656.

LINDE, K. and MULROW, C.D., **2003**. St John's wort for depression. *Cochrane Database Systematic Reviews* **2**, CD000448.

LINDE, K., RAMIREZ, G., MULROW, C.D., PAULS, A., WEIDENHAMMER, W. and MELCHART D., **1996**. St John's wort for depression--an overview and meta-analysis of randomised clinical trials. *British Medical Journal* **313**: 253-258.

LINDEBOOM, J., and WEINSTEIN, H., **2004**. Neuropsychology of cognitive ageing, minimal cognitive impairment, Alzheimer's disease, and vascular cognitive impairment. *European Journal of Pharmacology* **490**: 83–86

LLINAS, R.R., **1977**. Details depolarization-release coupling systems in neurons. *Neuroscience Research Program Bulletin* **15**: 555– 687.

LOEB, E.M., KOCH, C. and LOEB, E.M.K., **1956**. Kuanyama Ambo magic. Part 6. Medicinal, cosmetical and charm flora and fauna. *Journal of American Folklore* **69**: 147–174.

LÓPEZ, S., BASTIDA, J., VILADOMAT, F. and CODINA ,C., **2002**. Acetylcholinestrase inhibitory activity of some Amaryllidaceae alkaloids and *Narcissus* extracts. *Life Sciences* **71**: 2521-2529.

LOURENS, S. and NASEEMA, B.M., **1998**. Department of Medically Applied Psychology, Faculty of Medicine, University of Natal, South Africa. Cited in Meel, B.L., 2003. Determinants of suicide in the Transkei sub-region of South Africa. *Journal of Clinical Forensic Medicine* **10**: 153–157.

LÜDDENS, H., KORPI, E.R. and SEEBURG, P.H., **1995**. $GABA_A$/benzodiazepine receptor heterogeneity: neurophysiological implications. *Neuropharmacology* **34**: 245–254.

LUNA, L.E., **1984**. The concept of plants as teachers among four mestizo shamans of Iquitos, North Eastern Peru. *Journal of Ethnopharmacology* **11**: 135–156.

LUO, L., SUN, Q., MAO, Y. Y., LU, Y. H. and TAN, R. X., **2004**. Inhibitory effects of flavonoids from *Hypericum perforatum* on nitric oxide synthase. *Journal of Ethnopharmacology* **93**: 221-225.

LYLES, G.A. and CALLINGHAM, B.A. **1982**. *In vitro* and *in vivo* inhibition by benserazide of clorgyline-resistant amine oxidase in rat cardiovascular tissues. *Biochemical Pharmacology* **31**: 1417-1424.

MABOGO, D.E.N., 1990. *The ethnobotany of the Vhavenda*. MSc thesis, University of Pretoria.

MACDONALD, R.L. and OLSEN, R.W., **1994**. $GABA_A$ receptor channels. *Annual Reviews in Neuroscience* **17**: 569-602.

MACDONALD, R.L. and TWYMAN, R.E., **1992**. Kinetic properties and regulation of $GABA_A$ receptor channels, In Narahashi, T. (ed.): *Ion Channels*. New York: Plenum Press, Vol 3, pp 315-343.

MADATI, P.J., KAYANI, M.J., PAZI, H.A.M. and NYAMGENDA, A.F.D., **1977**. Alkaloids of *Rauvolfia caffra* Sond. I. Phytochemical and toxicological studies. *Planta Medica* **32**, 258–267.

MADAUS, G., **1979**. Lehrbuch der biologischen Heilmittel. Band II. Georg Olms Verlag, Hildesheim.

MAGEE, A.R., VAN WYK, B.E. and VAN VUUREN, S.F., **2007**. Ethnobotany and antimicrobial activity of sieketroos (*Arctopus* species). *South African Journal of Botany* **73**: 159–162.

MAHOMED, I.M. and OJEWOLE, J.A.O., **2006**. Anticonvulsant activity of *Harpagophytum procumbens* DC [Pedaliaceae] secondary root aqueous extract in mice. *Brain Research Bulletin* **69**: 57–62.

MAKANJU, O.O.A., **1983**. Behavioral and anticonvulsant effects of an aqueous extract from the roots of *Clausena anisata* Rutaceae. *International Journal of Crude Drug Research* **21**: 29–32.

MAKRIYANNIS, A. and RAPAKA, R.S., **1990**. The molecular basis of cannabinoid activity, *Life Sciences* **47**: 2173-2184.

MANANA, C.J., **1968**. *Umongo Wolwazi. African Medical Herbs.* Pinetown Printers, Mayville, Durban.

MARTINDALE, W., **1958**. *The Extra Pharmacopeia*. 24th edn. London, Pharmaceutical Press.

MASSON, J., SAGNE, C., HAMON, M. and EL MESTIKAWY, S., **1999**. Neurotransmitter transporters in the central nervous system. *Pharmacology Reviews*. **51**: 439–464.

MATHEKGA, A.D.M., MEYER, J.J.M., HORN, M.M., and DREWES, S.E., **2000**. An acylated phloroglucinol with antimicrobial properties from *Helichrysum caespititium, Phytochemistry* **53**: 93-96.

MAYR, F., **1907**. The Zulu kaffirs of Natal. *Anthropos* **2**: 302–399.

McKENNA, D.J., **1996**. Plant hallucinogens: springboards for psychotherapeutic drug discovery. *Behaviour and Brain Research* **73**: 109–116.

MECHOULAM, R. and GAONI, Y., **1967**. Recent advances in the chemistry of hashish. *Fortschr Chem Org Naturst* **25**: 175-213.

MEDICAL, DENTAL AND SUPPLEMENTARY HEALTH SERVICES PROFESSION ACT, 1974. No. 18 of 1995: Medical, Dental and Supplementary Health Service Professions Amendment Act, 1995. www.doh.gov.za/docs/legislation/acts/1995/act95-018.html.

MEEL, B.L., **2003**.Suicide among former mineworkers in the sub region of Transkei, South Africa: case reports. *Archives of Suicide Research* **7**: 1-6.

MERCK, 1989. *The Merck Index.* 11th edn. Rahway, Merck.

MEYER, J.J.M., AFOLAYAN, A.J., TAYLOR, M.B. and ENGELBRECHT, L., **1996**. Inhibition of herpes simplex virus type 1 by aqueous extracts from shoots of *Helichrysum qureonites. Journal of Ethnopharmacology* **52**: 41-43.

MILLAN, M.J., NEWMAN-TANCREDI, A., BROCCO, M., GOBERT, A., LEJEUNE, F., AUDINOT, V., RIVET, J.M., SCHREIBER, R., DEKEYNE, A., SPEDDING, M., NICOLAS, J.P. and PEGLION, J.L., **1998**. S 18126 ([2-[4-(2,3-dihydrobenzo[1,4]dioxin-6-yl)piperazin-1-yl methyl]indan-2-yl]), a potent, selective and competitive antagonist at dopamine D4 receptors: an in vitro and in vivo comparison with L 745,870 (3-(4-[4-chlorophenyl]piperazin-1-yl)methyl-1H-pyrrolo[2, 3b]pyridine) and raclopride. *Journal of Pharmacology and Experimental Therapy* **287**: 167-186.

MILLER, H.I., DELGADO, P.L., SALOMON, R.M., BERMAN, R., KRYSTAL, J.H., HENINGER, G.R. and CHARNEY, D.S., **1996**. Clinical and biochemical effects of catecholamine depletion on antidepressant-induced remission of depression. *Archives of General Psychiatry* **53**: 117-128.

MILLS, S. and BONE, K., **2000**. *Principles of herbal pharmacology*. In: *Principles and Practice of Phytotherapy – Modern Herbal Medicine*. Edinburgh: Churchill Livingstone.

MILNE, F.J., PINKNEY-ATKINSON, V.J., **2004**. Hypertension guideline 2003 update. *South African Medical Journal* **94**: 209–225.

MODY, I., **1998**. Ion channels in epilepsy. *International Reviews in Neurobiology* **42**:199-226.

MOHAGHEGHZADEH, A., FARIDIA, P., SHAMS-ARDAKANIB, M. and GHASEM, Y., **2006**. Medicinal smokes. *Journal of Ethnopharmacology* **108**: 161–184.

MOHAMADI, F., RICHARDS, N.G.J., GUIDA, W.C., LISKAMP, R., LIPTON, M., CAUFIELD, C., CHANG, G., HENDRICKSON, T. and STILL, W.C., **1990**. MacroModel—an integrated software system for modeling organic and bioorganic molecules using molecular mechanics. *Journal of Computational Chemistry* **11**: 440–467 (The program is available from Schrodinger Inc., 1500 S.W. First Avenue, Suite 1180, Portland, OR 97201-5815).

MONNIG, H.O., **1967**. *The Pedi*. Pretoria, J.L. van Schaik Publishers.

MORENO, F.A., GELENBERG, A.J., HENINGER, G.R., POTTER, R.L., MCKNIGHT, K.M., ALLEN, J., PHILLIPS, A.P. and DELGADO, P.L. **1999**. Tryptophan depletion and depressive vulnerability. *Biol. Psychiatry* **46:** 498-505.

MORGAN, W.T.W., **1981**. Ethnobotany of the Turkana: Use of plants by a pastoral people and their livestock in Kenya. *Economic Botany* **35**: 96–130.

MORRIS, K., **2001**. Treating HIV in South Africa – a tale of two systems. *The Lancet* **357**: 1190.

MUJUMDAR, A.M., KAPADI, A.H. and PENDSE, G.S., **1979**. Chemistry and pharmacology of betel nut, *Areca catechu. Linnean Journal of Plantation Crops* **7**: 69-92.

MUKHERJEE, P.K., KUMAR, V. MAL, M. and HOUGHTON, P.J., **2007**. Acetylcholinesterase inhibitors from plants. *Phytomedicine* **14**: 289–300.

MULLER, W.E., **2003**. Current St. John's wort research from mode of action to clinical efficacy. *Pharmacological Research* **47**: 101-109.

MUNRO, T.A. and RIZZACASA, M.A., **2003**. Salvinorins D-F, new neoclerodane diterpenoids from *Salvia divinorum*, and an improved method for the isolation of salvinorin A. *Journal of Natural Products* **66**: 703-705.

MURRAY, C.J. and LOPEZ, A.D., **1996**. The Global Burden of Disease: a comprehensive assessment of mortality and disability from diseases, injuries and risk factors in 1990 and projected to 2020. Vol.1, Global Burden of Disease and Injury series. Boston: Harvard School of Public Health.

MYSLIVECEK, J. and TROJAN, S., **2000**. Mechanisms of G-protein coupled receptor regulation. *Sbornik Lek.* **101**: 205-213.

NASSER, A.M.A.G. and COURT, W.E., **1984**. Stem bark alkaloids of *Rauvolfia caffra*. *Journal of Ethnopharmacology* **11**: 99-117.

NATIONAL DRUG INTELLIGENCE CENTER, **2003**. Information bulletin: *Salvia divinorum. Microgram* **XXXVI**: 122-125.

NEHLIG, A and DEBRY, G., **1994**. Caffeine and sports activity: a review. *International Journal of Sports Medicine* **15**: 215-223.

NELSON, N., **1998**. The family of Na^+/Cl^- neurotransmitter transporters. *Journal of Neurochemistry* **71**: 1785-1803.

NENCINI, P., AHMED, A.M. and ELMI, A.S. **1986**. Subjective effects of khat chewing in humans. *Drug Alcohol Depend* **18**: 97-105.

NEUWINGER, H.D., **2000**. *African Traditional Medicine. A Dictionary of Plant Use and Applications*. Medpharm GmbH Scientific Publishers, Stuttgart, Germany.

NGUBANE, H., **1977**. *Body and mind in Zulu medicine: An ethnography of health and disease in Nyuswa-Zulu thought and practice*. London: Academic Press.

NGWENYA, M.A., KOOPMAN, A. and WILLIAMS, R., **2003**. *Zulu Botanical Knowledge*: *An Introduction.* National Botanical Institute, Durban

NICHOLS, D., **2004**. Hallucinogens. *Journal of Pharmacology and Experimental Therapeutics* **101:** 131-181.

NICKERSON, M. and COLLIER, B., **1975**. Drugs inhibiting adrenergic systems and structures innervated by them. In: L.S. Goodman and A. Gilman (Eds.), *The Pharmacological Basis of Therapeutics*, 5th edn., McMillan Publishing Co., pp. 533-564.

NICOLETTI, F., MEEK, J., IADAROLA, M. J., CHUANG, D. -M., ROTH, B. L., and COSTA, E., **1986**. Coupling of inositol phospholipid metabolism with excitatory amino acid recognition sites in rat hippocampus. *Journal of Neurochemistry* **46**: 40-46.

NIELSEN, M., FROKJAER, S. and BRAESTRUP, C., **1988**. High affinity of the naturally occurring biflavonoid, amentoflavone, to brain benzodiazepine receptors in vitro. Biochemical Pharmacology **37**: 3285-3287.

NIELSEN, N.D., SANDAGER, M., STAFFORD, G.I., VAN STADEN, J. and JÄGER, A.K., **2004**. Screening of indigenous plants from South Africa for affinity to the serotonin reuptake transport protein. Journal of Ethnopharmacology **94**: 159–163.

NISSINEN, E., **1984**. Determination of monoamine oxidase B activity by high-performance. liquid chromatography. *Journal of Chromatograph* **309**: 156-159.

NJUS, D., KELLEY, P.M. and HARDABEK, G.J., **1986**. Bioenergetics of secretory vesicles. *Biochimica et Biophysica Acta* **853**: 237-265.

NORTON, S. A., **1998**. Betel: consumption and consequences. *Journal of the American Academy of Dermatology* **38**: 81-88.

NWOSU, M.O., **1999**. Herbs for mental disorders. *Fitoterapia* **70**: 58–63.

NYAZEMA, N.Z., **1984**. Poisoning due to traditional remedies. *Central African Journal of Medicine* **30**, 80-83:

OJEWOLE, J.A.O. and AMABEOKU, G.J., **2006**. Anticonvulsant Effect of *Persea americana* Mill (Lauraceae) (Avocado) Leaf Aqueous Extract in Mice. *Phytotherapy Research* **20**: 696–700.

OJEWOLE, J.A.O., **2005**. Analgesic and Anticonvulsant Properties of *Tetrapleura tetraptera* (Taub) (Fabaceae) Fruit Aqueous Extract in Mice. *Phytotherapy Research* **19**: 1023–1029.

OJEWOLE, J.A.O., **2008a**. Anticonvulsant property of *Sutherlandia frutescens* R. BR. (variety Incana E. MEY.) [Fabaceae] shoot aqueous extract. *Brain Research Bulletin* **75**: 126–132.

OJEWOLE, J.A.O., **2008b**. Anticonvulsant Activity of *Hypoxis hemerocallidea* Fisch. and C. A. Mey. (Hypoxidaceae) Corm ('African Potato') Aqueous Extract in Mice. *Phytotherapy Research* **22**: 91–96.

OJEWOLE, J.A.O., **2008c**. Anticonvulsant effect of *Rhus chirindensis* (Baker F.) (Anacardiaceae) stem-bark aqueous extract in mice. *Journal of Ethnopharmacology* **117**: 130-135.

OKPAKO, D.T., **1999**. Traditional African medicine: theory and pharmacology explored. *Trends in Pharmacological Sciences* **20**: 482-485.

OKUBADEJO, N.U., **2008**. An analysis of genetic studies of Parkinson's disease in Africa. *Parkinsonism and Related Disorders* **14**: 177–182.

OKUBADEJO, N.U., BOWER, J.H., ROCCA, W.A. and MARAGANORE, D.M., **2006**. Parkinson's disease in Africa: a systematic review of epidemiologic and genetic studies. *Movement Disorders* **21**: 2150–2156.

OLIVER-BEVER, B., **1986**. *Medicinal Plants in Tropical West Africa*. London, Cambridge University Press.

OLSEN, H.T., STAFFORD, G.I., VAN STADEN, J., CHRISTENSEN, S.B. and JÄGER, A.K., **2008**. Isolation of the MAO-inhibitor naringenin from *Mentha aquatica* L. *Journal of Ethnopharmacology*, **117**: 500-502.

OLSEN, R.W., BUREAU, M., HOUSER, C.R., DELGADO-ESCUETA, A.V., RICHARDS, J.G. and MOHLER, H., 1992. GABA/benzodiazepine receptors in human focal epilepsy. *Epilepsy Research* **8**: 383-91.

OMINO, E.A. and KOKWARO, J.O., **1993**. Ethnobotany of Apocynaceae species in Kenya. *Journal of Ethnopharmacology* **40**: 167–180.

ONGINI, E. and FREDHOLM, B.B., **1996**. Pharmacology of adenosine A_2A receptors, *Trends in Pharmacological Sciences* **17:**, 364-372.

ORTEGA, A., BLOUNT, J.F. and MANCHAND, P.S., **1982**. Salvinorin, a new *trans-neoclerodane* diterpene from *Salvia divinorum* (Labiatae). *Journal of the Chemical Society, Perkin Transactions I: Organic and Bio-Organic Chemistry* 2505-2508.

OTT, J., **1996**. *Salvia divinorum* Epling and Jativa. *Eleusis* **4**: 31–39.

PALMER, E. and PITMAN, N., **1972**. *Trees of Southern Africa*. Cape Town, Balkema. 3 vols.

PAMMEL, L.H., **1911**. *Manual of Poisonous Plants*. Cedar Rapids, Iowa, The Torch Press.

PAPPE, L., **1847**. *A list of South African Indigenous Plants Used as Remedies by the Colonists of the Cape of Good Hope*. Cape Town, G.J. Pike.

PAPPE, L., **1857**. *Florae Capensis Medicae Prodromus*. 2nd edn. Cape Town, W. Brittain.

PARKINSON, J., **1817**. *An Essay on the Shaking Palsy*. Sherwood Neely & Jones, London.

PARLE, J. **2003**. Witchcraft or Madness? The Amandiki of Zululand, 1894-1914 *Journal of Southern African Studies* **29**: 105-132.

PATEL, V., MUSARA, T., MARAMBA, P. and BUTAU, T., **1995**. Concepts of mental illness and medical pluralism in Harare. *Psychological Medicine* **25**: 485-493.

PEDERSEN, P.D., **2005**. *Implementering og validering af bioassay til detektering af mooaminoxidase (MAO) inhibitorer i plantemateriale*. MSc Thesis, Danmarks Farmaceutiske Universitet.

PEDERSEN, M.E., SZEWCZYK, B., STACHOWICZ, K., WIERONSKA, J., ANDERSEN, J., STAFFORD, G.I., VAN STADEN, J., PILC, A. and JÄGER, A.K. **2008**. Effects of South African traditional medicine in animal models for depression. *Journal of Ethnopharmacology* **119**: 542–548.

PEDERSEN, M.E., VESTERGAARD, H.T., STAFFORD, G.I., VAN STADEN, J. and JÄGER, A.K. **2008**. The effect of extracts of Searsia species on epileptiform activity in slices of the mouse cerebral cortex. *Journal of Ethnopharmacology* **119**: 538–541.

PERNET, R. and MEYER, G., **1957**. Pharmacopée de Madagascar, Publications de l'Institut de Recherche Scientifique, Tananarive-Tsimbazaza.

PERRY, N.S.L., HOUGHTON, P.J., THEOBALD, A.E., JENNER, P. and PERRY, E.K., **2000**. *In-vitro* inhibition of human erythrocyte acetylcholine esterase by *Salvia lavandulaefolia* essential oil and constituent terpenes. *Journal of Pharmacy and Pharmacology* **52**: 895–902.

PERT, C. B., and SNYDER, S. H., **1973**. Opiate receptor: demonstration in nervous tissue. *Science* **179:** 1011-1014.

PHILLIP, M., KOHNEN, R. and HILLER, K.O., **1999.** *Hypericum* extract versus impramine or placebo in patients with moderate depression: Randomized multicenter study of treatment for eight weeks. *British Medical Journal* **319**: 1534–1538.

PHILLIPS, E.P., **1917**. A contribution to the flora of the Leribe Plateau and environs. *Annals of the South African Museum* **16**.

PICKWELL, S.M., SCHIMELPFENIG, S. and PALINKAS, L.A., **1994**. "Betelmania": betel quid chewing by Cambodian women in the United States and its potential health effects. *Western Journal of Medicine* **160**: 326-330.

PILLSBURY, B., **1982**. Policy and Evaluation Perspectives on Traditional Health Practitioners in National Health Care Systems', *Social Science and Medicine* **16**: 1825-1834.

PINEL, J.P.J., **1990**. *Biopsychology*. Boston, MA Allyn & Bacon.

PINÊYRO, G. and BLIER, P., **1999**. Autoregulation of serotonin neurons: role in antidepressant drug action. *Pharmacological Reviews* **51**: 533–591.

PLENGE, P., MELLERUP, E.T. and NIELSEN, M., **1990**. Inhibitory and regulatory binding sites on the rat brain serotonin transporter: molecular weight of the [3H]paroxetine and [3H]citalopram binding proteins. *European Journal of Pharmacology Molecular Pharmacology* **189**: 129-134.

POLYA, G., **2003**. *Biochemical Targets of Plant Bioactive compounds. A pharmacological reference guide to sites of action and biological effects*. CRC Press.

POOLEY, E., **1993**. *The Complete Field Guide to Trees of Natal, Zululand and Transkei*. Flora Publications Trust, Natal Herbarium, Durban, KwaZulu-Natal.

POOLEY, E., **2005**. *A field guide to wild flowers , KwaZulu-Natal and the eastern region*. Natal Flora Publications Trust, Natal Herbarium.

POPIK, P., LAYER, R.T. and SKOLNICK P., **1995**. 100 years of Ibogaine: neurochemical and pharmacological actions of a putative anti-addictive drug. *Pharmacological Reviews* **47**: 235-53.

POTTER, H., **1932**. *Potter's Cyclopedia of Botanical Drugs and Preparations*, 4th edn. London, Potter and Carke.

PUJOL, J., 1990. *NaturAfrica: The Herbalist Handbook*. Jean Pujol Natural Healers Foundation, Durban.

RAFFAUF, R.F., **1970**. A *Handbook of Alkaloids and Alkaloid-containing Plants*. Wiley Interscience, New York.

RANG, H.P. and DALE, M.M., **1987**. *Pharmacology*. 1st Edition, Churchill Livingston, London, UK.

RANG, H.P., DALE, M.M. and RITTER, J.M., **1996**. *Pharmacology*. 3rd Edition, Churchill Livingston, London UK.

RAY, A.B., CHATTOPADIJAY, R.M., TRIPATHI, R.P., GAMBIR, S.S. and DAS, P.K., **1979**. Isolation and pharmacological action of epistephanine, an alkaloid of *Stephania hernandifolia*. *Planta Medica* **35**: 167-173.

RIEDEL, W.J., KLAASSEN, T. and SCHMITT, J.A., **2002**. Tryptophan, mood, and cognitive function. *Brain, Behavior, and Immunity* **16**: 581-589.

RISA, A., RISA, J., ADSERSEN, A., STAFFORD, G.I., VAN STADEN, J. and JÄGER, A.K., **2004**. Acetylcholinesterase inhibitory activity of plants used as memory-enhancers in traditional South African medicine. *South African Journal of Botany* **70:** 664-666.

RISA, J., RISA, A., ADSERSEN, A., GAUGUIN, B., STAFFORD, G.I., VAN STADEN, J. and JÄGER, A.K., **2004**. Screening of plants used in southern Africa for epilepsy and convulsions in the $GABA_A$-benzodiazepine receptor assay. *Journal of Ethnopharmacology* **93**: 177–182.

ROBBINS, T.W. and EVERITT, B.J., **1996**. Neurobehavioural mechanisms of reward and motivation. *Current Opinion in Neurobiology* **6**: 228-236.

ROBERTS, M., **1990**. *Indigenous Healing Plants*. Halfway House, Southern Book Publishers.

ROBINSON, T.E. and KOLB, B., **1999.** Alterations in the morphology of dendrites and dendritic spines in the nucleus accumbens and prefrontal cortex following repeated treatment with amphetamine or cocaine. *European Journal of Neuroscience* **11**: 1598-1604.

RODIN, R.J., **1985**. The Ethnobotany of the Kwanyama Ovambos. *Monographs in Systematic Botany from the Missouri Botanical Garden* **9**.

RON, B.H., WILLIS, C.A., BONE, K., and MORGAN, M., **2000**. Herbal products: active constituents, mode of action and quality control. *Nutrition Research Reviews* **13**: 47–77.

ROTH, B.L., BANER, K., WESTKAEMPER, R., SIEBERT, D., RICE, K.C., STEINBERG, S., ERNSBERGER, P. and ROTHMAN, R.B., **2002**. Salvinorin A: A potent naturally occurring non-

nitrogenous n-opioid selective agonist. *Proceedings of the National Academy of Sciences of the USA* **99(18)**: 11934-11939.

ROTH, B.L., LOPEZ, E., BEISCHEL, S., WESTKAEMPER, R.B. and EVANS, J.M., **2004**. Screening the receptorome to discover the molecular targets for plant-derived psychoactive compounds: a novel approach for CNS drug discovery. *Pharmacology and Therapeutics* **102:** 99–110.

ROTHMALER, W., **1944**. Pteriodophyten studien I. *Feddes Repertorium Specierum Novarum* **54**, 55–82.

ROTHMAN, R. B., VU, N., PARTILLA, J. S., ROTH, B. L., HUFEISEN, S. J., COMPTON-TOTH, B. A., BIRKES, J., YOUNG, R., and GLENNON, R. A., **2003**. In vitro characterization of ephedrine-related stereoisomers at biogenic amine transporters and the receptorome reveals selective actions as norepinephrine transporter substrates. *Journal of Pharmacology and Experimental Therapy* **307**: 138-145.

RUSSO, E.B., **2001**. Hemp for headache: an in-depth historical and scientific review of cannabis in migraine treatment. *Journal of Cannabis Therapeutics* **1**: 21– 92.

RUSSO, E.B., **2002**. Cannabis Treatments in Obstetrics and Gynecology: A Historical Review. *Journal of Cannabis Therapeutics* **2**: 5-35.

SALAH, S.M. and JÄGER, A.K., **2005**. Screening of traditionally used Lebanese herbs for neurological activities, *Journal of Ethnopharmacology* **97**: 145-149.

SAN MIGUEL, E., **2003**. Rue (*Ruta* L., Rutaceae) in traditional Spain: frequency and distribution of its medicinal and symbolic applications. *Economic Botany* **57**: 231-244.

SANCHEZ-RAMOS, J. and MASH, D., **1994**. Ibogaine research update: phase I human study. *Multidisciplinary Association for Psychedelic Studies Bulletin* **4**: 11.

SANDAGER, M., NIELSEN, N.D., STAFFORD, G.I., VAN STADEN, J. and JÄGER, A.K., **2005**. Alkaloids from *Boophane disticha* with affinity to the serotonin transporter in rat brain. *Journal of Ethnopharmacology* **98**: 367-370.

SANDER, J.W. and SHORVON S.D., 1996. Epidemiology of the epilepsies. *Journal of Neurology, Neurosurgery and Psychiatry* **65**: 433-443.

SANDER, J.W., **2003**. The epidemiology of epilepsy revisited. Current Opinon in Neurology **16**: 165-170.

SATOH, Y. and YAMAZAKI, M., **1989**. Studies on the monoamine oxidase inhibitory potency of TL-1, isolated from a fungus, *Talaromyces luteus*. *Chemical and Pharmacology Bulletin* **37**: 206–207.

SAURA MARTI, J., KETTLER, R., DA PRADA, M. and RICHARDS, J.G., **1990**. Molecular neuroanatomy of MAO-A and MAO-B. *Journal of Neural Transmission* **32**: 49-53.

SAVAGE, M.T.D., **1985.** Health. In Basic needs in rural areas. *South African National Scientific Programmes Report* **116**: 49-66.

SAWYERR, H., **1978**. *Themes in African Social and Political Thought* (Otite, O., ed.), Fourth Dimension Publishers, p. 73.

SAYYAH, M., NADJAFNIA, L. and KAMALINEJAD, M., **2004**. Anticonvulsant activity and chemical composition of *Artemisia dracunculus* L. essential oil. *Journal of Ethnopharmacology* **94**: 283-287.

SCHILDKRAUT, J.J., **1965**. The catecholamine hypothesis of affective disorders: a review of supporting evidence. *American Journal of Psychiatry* **122**: 509-522.

SCHIMMEL and CO., **1909**. Buchu leaf oil. *Journal of the Chemical Society* **96**: 313.

SCHMIDT, K., LI, Z., SCHUBERT, B., HUANG, B., STOYANOVA, S. and HAMBURGER, M., **2003**. Screening of entomopathogenic Deuteromycetec for activities on targets involved in degenerative diseases of the central nervous system. *Journal of Ethnopharmacology* **89**: 251-260.

SCHOLTZ, H.J., PHILLIPS, V.M. and KNOBEL, G.J., **1997**. Muti or ritual murder. *Forensic Science International* **87**: 117-123.

SCHRADER, E. ; MEIER, B. and BRATTSTROM, A., **1998**. *Hypericum* treatment of mild-moderate depression in a placebo-controlled study. A prospective, double-blind, randomized, placebo-controlled, multicentre study. *Human psychopharmacology* **13**: 163-169.

SCHULTES, R.E. and HOFMANN, A., **1992**. *Plants of the Gods. Their Sacred, Healing and Hallucinogenic Powers*. Rochester, Vermont, Healing Arts Press.

SCHULTES, R.E. and RAFFAUF, R.F., **1990**. *The Healing Forest.* Oregon, Dioscorides Press.

SCHULTES, R.E., **1967**. *The place of ethnobotany in the ethnopharmacologic search for psychotomimetic drugs*. In: *Ethnopharmacologic Search for Psychoactive Drugs*, ed. Efron, D. Washington, D.C. U.S. Govt. Printing Office.

SCHULTES, R.E., **1981**. *Einführung in die Botanik der wichtigsten psychotropen Pflanzen*. In: Völger, G., von Welck, K., Legnaro, A. (Eds.), *Rausch und Realität—Drogen im Kulturvergleich.* Teil 1, Rautenstrauch-Joest-Museum für Völkerkunde, Köln, pp. 28–41.

SCHULZ, V., **2002**. Clinical trials with *Hypericum* extracts in patients with depression-Results, comparisons, conclusions for therapy with antidepressant drugs. *Phytomedicine* **9**: 468-474.

SEABROOK, G.R., HOWSON, W. and LACEY, M.G., **1990**. Electrophysiology characterization of potent agonists and antagonists at pre- and postsynaptic GABA receptors on neurones in rat brain slices, *British Journal of Pharmacology* **101**: 949–957.

SEEDAT, H.A. and VAN WYK, C.W., **1988**. Betel-nut chewing and submucous fibrosis in Durban. *South African Medical Journal* **74**: 568-571.

SHIH, J.C., **1994**. Molecular basis of human MAO A and B. *Neuropsychopharmacology* **4**: 1-7.

SHONE, D.K. and DRUMMOND, R.B., **1965**. Poisonous plants of Rhodesia. *Rhodesian Journal of Agriculture* **62**: 7–64.

SHOULSON, I., **1998**. DATATOP: a decade of neuroprotective inquiry. Parkinson Study Group. Deprenyl And Tocopherol Antioxidative Therapy Of Parkinsonism. *Annals of Neurology* **44**: 160–166.

SIEBERT, D.J., **1994**. *Salvia divinorum* and salvinorin A: new pharmacologic findings. *Journal of Ethnopharmacology* **43**: 53–56.

SILVERSTONE, T. and TURNER, P., **1988**. *Drug Treatment In Psychiatry*. 4th edn. Routledge, London.

SILVILOTTI, L. and NISTRI, A., **1991**. GABA receptor mechanisms in the central nervous system. *Progress in Neurobiology* **36**:35–92.

SIMMEN, U., BOBIRNAC, I., ULLMER, C., LUBBERT, H., BERGER BUTER, K., SCHAFFNER, W. and SCHOEFFTER, P., **2003**. Antagonist effect of pseudohypericin at CRF1 receptors. *Eurpean Journal of Pharmacology* **458**: 251–256.

SIMMEN, U., BURKARD, W., BERGER, K., SCHAFFNER, W., and LUNDSTROM, K., **1999**. Extracts and constituents of *Hypericum perforatum* inhibit the binding of various ligands to recombinant receptors expressed with the Semliki Forest virus system. *Journal of Receptor Signal Transduction Research* **19**: 59–74.

SIMMEN, U., HIGELIN, J., BERGER-BUTER, K., SCHAFFNER, W., and LUNDSTROM, K., **2001**. Neurochemical studies with St. John's wort in vitro. *Pharmacopsychiatry* **34**: S137-S142.

SIMMONDS, M.A., **1983**. Multiple GABA receptors and associated regulatory sites. *Trends in Neurosciences* **6**:279-295.

SIMON, C. and LAMLA, M., **1991**. Merging pharmacopoeia: understanding the historical origins of incorporative pharmacopoeial processes among Xhosa healers in Southern Africa. *Journal of Ethnopharmacology* **33**: 237–242.

SINGH, Y.N., and SINGH, N.N., **2002**. Therapeutic potential of kava in the treatment of anxiety disorders. *CNS Drugs* **16**: 731-743.

SKLAIR-TAVRON, L., XING SHI, W., LANE, S.B., HARRIS, H.W., BUNNEY, B.S. and NESTLER, E.J., **1996**. Chronic morphine induces visible changes in the morphology of mesolimbic dopamine neurons. *Proceedings of the National Academy of Science of the United States of America*, **93**: 11202–11207.

SMITH, A., **1888**. *A Contribution to South African Materia Medica*. 2nd edn. Lovedale, South Africa, Lovedale Press.

SMITH, A., **1895**. *A Contribution Tto South African Materia Medica, Chiefly from Plants in Use Among the Natives*. Juta, Cape Town.

SMITH, M.T, FIELD, C.R, CROUCH, N.R and HIRST M., **1998**. The Distribution of Mesembrine Alkaloids in Selected Taxa of the Mesembryanthemaceae and their Modification in the *Sceletium* Derived 'Kougoed'. *Pharmaceutical Biology* **36**: 173-179.

SMITH, M.T., CROUCH, N.R., GERICKE, N. and HIRST, M., **1996**. Psychoactive constituents of the genus *Sceletium* N.E.Br. and other Mesembryanthemaceae: a review. *Journal of Ethnopharmacology* **50**: 119-130.

SNYDER, S.H., KATIMS, J.J., ANNAU, Z., BRUNS, R.F., and DALY, J.W., **1981**. Adenosine receptors and behavioral actions of methylxanthines. *Proceedings of the National Academy of Science USA* **78**: 3260-3264.

SOBIECKI, J.F., **2002**. A preliminary inventory of plants used for psychoactive purposes in southern Africa healing traditions. *Transactions of the Royal Society of South Africa* **57**: 1–24.

SOLECKI, R.S., **1975**. Shanidar IV, a Neanderthal flower burial in northern Iraq. *Science* **190**: 880–881.

SONDERS, M.S., ZHU, S.J., ZAHNISER, N.R., KAVANAUGH, M.P. and AMARA, S.G., **1997**. Multiple ionic conductances of the human dopamine transporter: the actions of dopamine and psychostimulants. *Journal of Neuroscience* **17**: 960–974.

SONG, P.S. and TAPLEY, K.J., **1979**. Photochemistry and photobiology of psoralens. *Photochemistry and Photobiology* **29**, 1177-1197.

SPINELLA, M., **2005**. Concise Handbook of Psychoactive Herbs. Medicinal Herbs for Treating Psychological and Neurological Problems. The Harworth Press, Binghanton, NY.

SRAMEK, J.J., FRACKIEWICS, E.J. and CUTLER, N.R., **2000**. Review of the acetylcholinesterase inhibitor galanthamine. *Expert Opinion on Investigational Drugs* **9**: 2393–2402.

STAFFORD, G.I., JÄGER, A.K. and 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**: 210–215.

STAFFORD, G.I., PEDERSEN, M.E., VAN STADEN, J. and JÄGER, A.K., **2008**. Review on plants with CNS-effects used in traditional South African medicine against mental diseases. *Journal of Ethnopharmacology* **119**: 513–537

STAFFORD, G.I., PEDERSEN, P.D., JÄGER, A.K. and VAN STADEN, J., **2007**. Monoamine oxidase inhibition by southern African traditional medicinal plants. *South African Journal of Botany* **73**, 384–390.

STAHL, S.M., **1998**. Basic psychopharmacology of antidepressants, part 1: Antidepressants have seven distinct mechanisms of action. *The Journal of Clinical Psychiatry* **59**: 5-14.

STARKE, K., TAUBE, H.D. and BOROWSKI, E., **1977**. Pre- and postsynaptic receptors in catecholaminergic transmission. *Naunyn-Schmiedebergs Archives of Pharmacology* **297**: S43-S44.

STASTNY, J., KONSTANTINIDIS, A., SCHWARZ, M.J., ROSENTHAL, N.E., VITOUCH, O., KASPER, S. and NEUMEISTER, A., **2003**. Effects of tryptophan depletion and catecholamine depletion on immune parameters in patients with seasonal affective disorder in remission with light therapy. *Biological Psychiatry* **53(4)**: 332-337.

STOLL, M., **1967**. Essential oils. In: *Kirk-Othmer Encyclopedia of Chemical Technology*, 2nd. edn., Vol. 14.

SUPPRESSION OF WITCHCRAFT ACT, 1957.
http://www.legalb.co.za/SANatTxt/1957_000/1957_003_000-Act-v19570222asunamended.html

SVENNINGSEN, A.B., MADSEN, K.D., LILJEFORS, T., STAFFORD, G.I., VAN STADEN, J. and JÄGER, A.K., **2006**. Biflavones from *Rhus* species with affinity for the GABAA/benzodiazepine receptor. *Journal of Ethnopharmacology* **103**: 276-280.

SVENSSON, T.H., **2000**. Brain noradrenaline and the mechanisms of action of antidepressant drugs. *Acta Psychiatrica Scandinavica* **402**: 18-27.

SWIFT, C.R. and ASUNI, T., **1975**. *Mental Health and Disease in Africa*. Churchill Livingston, Edinburgh.

SZUTOWICZ, A., KOBES, R. D. and ORSULAK, P. J., **1984**. Colorimetric assay for monoamine oxidase in tissues using peroxidase and 2,29-azinodi(3-ethylbenzthiazoline-6-sulfonic acid). *Analytical Biochemistry***138**: 86-94.

TABUTI, J.R.S., LYE, K.A. and DHILLION, S.S., **2003**. Traditional herbal drugs of Bulamogi, Uganda: plants, use and administration. *Journal of Ethnopharmacology* **88:** 19-44.

TAO, G., IRIE, Y., LI, D.-J. and KEUNG, W.M., **2005**. Eugenol and its structural analogs inhibit monoamine oxidase A and exhibit antidepressant-like activity. *Bioorganic and Medicinal Chemistry* **13**: 4777-4788.

TAYLOR, P., **2001**. *Anticholinesterase agents*. In: Hardman, J.G., Limbird, L.E., Gilman, A.G. (Eds.), *Goodman and Gilman's The Pharmaco logical Basis of Therapeutics*, 10th ed. McGraw Hill, New York, pp. 175–191.

TAYLOR, R.F.H., AL-JARAD, N., JOHN, L.M.E., CONROY, D.M. and BARNES, N.C., **1992**. Betel-nut chewing and asthma. *Lancet* **339**: 1134.

TEICHLER, G.H., 1971. Notes on the Botswana Pharmacopoea. *Botswana Notes and Records* **3**: 8–11.

THIEDE, H.-M. and WALPER, A., **1994**. Inhibition of MAO and COMT by *Hypericum* Extracts and Hypericin. *Journal of Geriatric Psychiatry and Neurology* **7**: S54-S56.

THPA, **2004.** *The Traditional Health Practitioners Bill*, 2003. Government Gazette no 24751,14 April, CapeTown: South Africa. http://www.polity.org.za/pdf/TradHeal-ractAct35.pdf

TIPTON, K.F., **1985**. Determination of monoamine oxidase. *Methods and Findings in Experimental and Clinical Pharmacology* **7**: 361-367.

TORRES, G.E. and CARON, M.G., **2003**. Centre stage for the serotonin transporter: a gain–of–function polymorphism in persons with obsessive–compulsive disorder. *Molecular Pharmacology* **64**: 196–198.

TREASE, G.E. and EVANS, W.C., **1983**. *Pharmacognosy.* 12th edn. London, Bailliere Tindall.

TSEY, K., **1997**. Traditional medicine in contemporary Ghana: a public policy analysis. *Social Science and Medicine* **45**: 1065-1074.

TURNER, C.E., ELSOHLY, M.A. and BOEREN, E.G., **1980**. Constituents of *Cannabis sativa* L. XVII. A review of the natural constituents. *Journal of Natural Products* **43**: 169-234.

TYRER, P.J., **1982**. *Drugs in Psychiatric Practice*. Butterworth, London.

UMEMOTO, K., ARAI, T., NII, H. and FURUKAWA, K., **1994**. Chemical constituents of wild mints. 20. Essential oil of self-pollinated plants of *Mentha aquatic* with sesquiterpene alcohols as major components. *Nippon Nogei Kagakukaishi* **68**: 1567–1570.

UNGERSTEDT, U., **1971**. On the anatomy, pharmacology and function of the nigrostriatal dopamine system. *Acta Physiologia Scandanavia* **367**: 1-48

VÁLDES III, L.J., **1983**. *The Pharmacognosy of Salvia divinorum (Epling and Jativa-M): an Investigation of Ska Maria Pastora*. University of Michigan, Ann Arbor, pp. 1– 237.

VÁLDES, L. J. III., BUTLER, W.M., HAFFIELD, G.M., PAUL, A.G. and KOREEDA, M., **1984**. Divinorin A, a psychotropic terpenoid, and divinorin B from the hallucinogenic mint *Salvia divinorum*. *Journal of Organic Chemistry* **49**: 4716-4720.

VALDES, L.J. III, **1994.** *Salvia divinorum* and the unique diterpene hallucinogen, Salvinorin (divinorin) A. *Journal of Psychoactive Drugs* **26**: 277-283.

VALDÉS, L.J. III, DÍAZ, J., PAUL, A.G., **1983**. Ethnopharmacology of ska María Pastora (*Salvia divinorum*, Epling and Játiva-M.) *Journal of Ethnopharmacology* **7**: 287-312.

VÁLDES, L.J. III., DIAZ J.L. and ARA G. P., **1983**.Ethnopharmacology of Ska Maria Pastora *(Salvia divinorum* Epling and Jativa-M.). *Journal of Ethnopharmacology* **7**: 287-312.

VÁLDES, L.J. III., HATFIELD, G.M., PAUL, A.G. and KOLEEDA M., **1987**. Studies of *Salvia divinorum* (Lamiaceae), an hallucinogenic mint from the Sierra Mazateca in Oaxaca, Central Mexico. *Economic Botany* **41**: 283-291.

VAN AS, A.D. and JOUBERT, J., **1991**. Neurocysticercosis in 578 black epileptic patients. *South African Medical Journal* **80**: 327–8.

VAN DER WALT, S.J. and STEYN, D.G., **1940**. Recent investigations into the toxicity of known and unknown poisonous plants of the union of South Africa. X. *Onderstepoort Journal of Veterinary Science* **15**: 261–277.

VAN HEERDEN, F.R., VILJOEN, A. and MOHOTO SP., **2002**. A phytochemical investigation of *Craterocapsa tarsodes*, a plant used for treatment of epilepsy by the Nothern Sotho people of South Africa. *South African Journal of Botany* **68**: 77-79.

VAN WYK, B.-E., VAN HEERDEN, F.R. and VAN OUDTSHOORN, B., **2002**. Poisonous Plants of South Africa. Briza Publications, Pretoria.

VAN WYK, B-E. and GERICKE, N., **2000**. *People's Plants.* Briza Publications, Pretoria.

VAN WYK, B-E., VAN OUDTSHOORN, B. and GERICKE, N., **1997**. *Medicinal Plants of South Africa.* Briza Publications, Pretoria.

VEALE, D.J.H., **1987**. Poisonous plants in South African homes and gardens. *South African Pharmacology Journal* **54**: 45-50.

VEALE, D.J.H., FURMAN, K.I. and OLIVER, D.W., **1992**. South African traditional herbal medicines used during pregnancy and childbirth. *Journal of Ethnopharmacology* **27**: 341–346.

VENTER, F. and VENTER, J.A., **1996**. *Making the Most of Indigenous Trees*. Pretoria, Briza Publications.

VERDCOURT, B. and TRUMP, E.C., **1969**. Common Poisonous Plants of East Africa. Collins, London.

VERTULANI, J. and SULSER, F., **1975**. Action of various antidepressant treatments reduces reactivity of noradrenergic cyclic AMP-generating system in limbic forebrain. *Nature* **257**: 495-496.

VILADOMAT, F., BASTIDA, J., CODINA, C., NAIR, J. and CAMPBELL, W.E., **1997**. Alkaloids of the South African Amaryllidaceae. *Recent Research Developments in Phytochemistry* **1**: 131–171.

VILADOMAT, F., CODINA, C., BASTIDA, J., SHAHEED, M. and CAMPBELL, W.E., **1995.** Further alkaloids from *Brunsvigia josphinae*, *Phytochemistry* **40:** 961–965.

VIOLA, H., WASOWSKI, C., LEVI DE STEIN, M., WOLFMAN, C., SILVERA, R., MEDINA, A.E. and PALADINI, A.C., **1995**. Apigenin, a component of *Matricaria recutita* flowers, is a central benzodiazepine receptors-ligand with anxiolytic effects. *Plant Medica* **61**: 213-216.

VISUDTIBHAN, A., VISUDHIPHAN, P. and CHIEMCHANYA, S., **1999**. Stroke and seizures as the presenting signs of pediatric HIV infection. *Pediatric Neurology* **20**: 53-56.

VIZI, S.E., **2000**. Role of high-affinity receptors and membrane transporters in non-synaptic communications and drug action in the central nervous system. *Pharmacological Reviews* **52**: 63–89.

VLOK, N., MALAN, S.F., CASTAGNOLI JR., N., BERGH, J. J. and PETZER, J. P., **2006**. Inhibition of monoamine oxidase B by analogues of the adenosine A2A receptor antagonist (E)-8-(3-chlorostyryl)caffeine (CSC). *Bioorganic and Medicinal Chemistry* **14**: 3512-3521.

VOLZ, H.P., **1997**. Controlled clinical trials of hypericum extracts in depressed patients-an overview. *Pharmacopsychiatry* **30**: 72-76.

WALKER, A.R., **1953**. Usages pharmaceutique des plantes spontanees du Gabon. *Bulletin Institut d'Etudes Centrafricaines (N.S.)* (a) **5**: 19–40, (b) **6**: 275–329.

WALKER, J., **1996**. *Wild flowers of KwaZulu - Natal* . W.R. Walker family Trust, Pinetown

WALSH, R.N., **1990**. *The Spirit of Shamanism*. Los Angeles, Jeremy P. Tarcher Inc.

WANG, L.S., ZHOU, J., SHAO, X.M. and TANG, X.C., **2002**. Huperzine A attenuates cognitive deficits and brain injury in neonatal rats after hypoxia-ischemia. *Brain Research* **949**: 162–170.

WASSON, R.G. **1962**. A new Mexican psychotropic drug from the mint family. *Botanical Museum Leaflets, Harvard University* **20**: 77-84.

WASSON, R.G. **1963**. Notes on the present status of ololuiqui and the other hallucinogens of Mexico. *Botanical Museum Leaflets, Harvard University* **20**: 161-193.

WATT, J.M. and BREYER-BRANDWIJK, M.G., **1927**. Suto medicines. Part 1. *Bantu Studies* **3**: 73–100.

WATT, J.M. and BREYER-BRANDWIJK, M.G., **1962**. *The Medicinal and Poisonous Plants of Southern and Eastern Africa*. 2nd edn. London, Livingstone.

WATT, J.M., **1967**. African plants potentially useful in mental health. *Lloydia* **30**: 1–22.

WEISSBACH, H., SMITH, T.E., DALY, J.W., WITKOP, B. and UDENFRIED, S., **1960**. A rapid spectrophotometric assay of monamine oxidase based on the rate of disappearance of kynuramine. Journal of Biological Chemistry **235**, 1160-1163.

WERMUTH, C.G. and BIZIERE, K., **1986**. Pyridazinyl-GABA derivatives: a new class of synthetic $GABA_A$ antagonists. *TIPS* **7:**421–424.

WHO, **1999**. WHO Director-General unveils new global strategies for mental health. Press Release WHO/99-67. http://www.who.int/inf-pr-1999/en/pr99-67.html.

WIJEWEERA, P., ARNASON, J.T., KOSZYCKI, D. and MERALI, Z., **2006**. Evaluation of anxiolytic properties of Gotukola – (*Centella asiatica*) extracts and asiaticoside in rat behavioral models. *Phytomedicine* **13**: 668–676.

WILLIAMSON, E. and EVANS, F.J., **1988**. *Potters New Cyclopedia of Botanical Drugs and Preparations*. Saffron Walden, England, C.W Daniel.

WILLIAMSON, J., **1974**. *Useful Plants of Malawi*. Limbe, University of Malawi.

WINK, M., **2000**. *Interference of Alkaloids with Neuroreceptors and Ion Channels*. In: Atta-ur-Rahman (Ed) *Bioactive Natural Products*. Amsterdam: Elsevier Science; p. 3-122.

WINKELMAN, M. and DOBKIN DE RIOS, M., **1989**. Psychoactive properties of !Kung Bushmen medicine plants. *Journal of Psychoactive Drugs* **21**: 51–59.

WISE, R.A., **1998**. Drug-activation of brain reward pathways. *Drug and Alcohol Dependence* **51**: 13–22.

WOLLENWEBER, E. and JAY, M., **1988**. *Flavones and Flavonols*. In: Harborne, J.B., Editor, 1988. *The Flavonoids—Advances in Research since 1980*, Chapman and Hall, London, pp. 233–302.

WOOLEY, D.W. and SHAW, E., **1954**. A biochemical and pharmacological suggestion about certain mental disorders. *Proceedings of the National Academy of Sciences of the United States of America* **40**: 228–231.

WORLD HEALTH ORGANISATION, **1976.** International Classihcation of Diseases for Oncology. Geneva.

WORLD HEALTH ORGANISATION, **1978**. *Primary Health Care, Report of the International Conference on Primary Health Care*, Alma Ata, Geneva, World Health Organisation, Geneva:

WORLD HEALTH ORGANIZATION, **2000**. General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine. World Health Organization, Geneva, Switzerland.

WORLD HEALTH ORGANIZATION, **2001**. WHO, 2001, Legal Status of Traditional Medicine and Complementary/Alternative Medicine: A Worldwide Review. World Health Organization, Geneva, Switzerland.

WORLD HEALTH ORGANIZATION, 2002. WHO Policy Perspectives on Medicine No. 2 May 2002, Traditional Medicine – Growing Needs and Potential. World Health Organization, Geneva, Switzerland.

WORLD HEALTH ORGANIZATION, 2004. *Neuroscience of psychoactive substance use and dependence*. World Health Organization, Geneva, Switzerland.

WORLD HEALTH REPORT. 1998. World Health Organization, Geneva, Switzerland. *Executive Summary*. www.who.int/whr/1998/ exsum98e.htm.

WREFORD, J., **2005**. Missing Each Other: Problems and Potential for Collaborative Efforts between Biomedicine and Traditional Healers in South Africa in the Time of AIDS. *Social Dynamics* **31**: 55-89.

WU, C.-L. HUANG Y.-M. and CHEN, J.-R., **1996**. (−)-Ledol from the liverwort *Cephaloziella recurvifolia* and the clarification of its identity. *Phytochemistry* **42**: 677–679.

WURTMAN, R.J. and AXELROD, J., **1963**. A sensitive and specific assay for the estimation of monoamine oxidase. *Biochemistry and Pharmacology* **12**: 1439–1441.

YAMADA, H., ISOBE, K., TANI, Y. and HIROMI, K., **1979**. A differential determination procedure for spermine and spermidine with beef plasma oxidase. *Agricultural and Biological Chemistry* **43**: 2487-2491.

YAMADA, M. and YASUHARA, H., **2004**. Clinical pharmacology of MAO inhibitors: safety and future. *Neurotoxicology* **25**: 215–221.

YAMBA, C. B., **1997**. Cosmologies in turmoil: Witchfinding and AIDS in Chiawa, Zambia. *Africa*, **67**: 200–221.

YAN, F. and ROTH, B.L., **2004**. Salvinorin A: A novel and highly selective n-opioid receptor agonist. *Life Sciences* **75**: 2615-2619.

YOUDIM, K.A., QAISER, M.Z., BEGLEY, D.J., RICE-EVANS, C.A. and ABBOTT, N.J., **2004**. Flavonoid permeability across an in situ model of the blood–brain barrier. *Free Radical Biology and Medicine* **36**, 592–604.

ZELGER, J.L. and CARLINI, E.A., **1980**. Anorexigenic effects of two amines obtained from Catha edulis Forsk (khat) in rats. *Pharmacology Biochemistry and Behavior* **12**: 701–705.

ZELGER, J.L. and CARLINI, E.A., **1981**. Influence of cathinone (a-aminopropriophenone) and cathine (phenylpropanolamine) on circling behavior and on the uptake and release of [^{3}H]dopamine in striatal slices of rats. *Neuropharmacology* **20**: 839-843.

ZELGER, J.L., SCHORNO, H.X. and CARLINI, E.A., **1980**. Behavioural effects of cathinone, an amine obtained from *Catha edulis* Forsk: comparisons with amphetamine, norpseudoephedrine, apomorphine and nomifensine. *Bulletin of Narcotics* **32**: 67-81.

ZHANG, W., KOEHLER, K.F., ZHANG, P. and COOK, J.M., **1995**. Development of a comprehensive pharmacophore model for the benzodiazepine receptor. *Drug Design and Discovery* **12**: 193-248.

ZHOU, M. and PANCHUL-VOLOSHINA, N., **1997**. A One-Step Fluorometric Method for the Continuous Measurement of Monoamine Oxidase Activity. *Analytical Biochemistry* **253**: 169-174.