MEDICINAL PLANT USE IN THE DWARSRIVIER VALLEY, STELLENBOSCH

MELISSA LIEZEL ARENDSE

A thesis submitted in fulfilment of the requirements for the degree of Magister Scientiae in the Department of Biodiversity and Conservation Biology, University of the Western Cape.

April 2013

Supervisor: Mr F.M Weitz

(Department of Biodiversity and Conservation Biology)

Co-supervisor: Prof. W. Mabusela

(Department of Chemistry)

MEDICINAL PLANT USE IN THE DWARSRIVIER VALLEY, STELLENBOSCH

Melissa L. Arendse

KEYWORDS

Medicinal plants

Dwarsrivier valley

Ethnobotanical survey

Questionnaires

Documentation

Intellectual property rights

Elemental analysis

Biological activity

Concentration range

Phytochemical screening

Thin layer chromatography (TLC)

Gas chromatography coupled to mass spectroscopy (GC-MS)

Secondary metabolites

Geographical location

ABSTRACT

MEDICINAL PLANT USE IN THE DWARSRIVIER VALLEY, STELLENBOSCH.

M.L. ARENDSE

Magister Scientiae (MSc) Thesis, Department of Biodiversity and Conservation Biology, Faculty of Natural Sciences, University of the Western Cape.

Plants have been used as medicine since antiquity. Passed on by word of mouth through the generations, this oral tradition is at risk of becoming extinct due to westernization and lack of documentation. The community in the Dwarsrivier valley is one such community who continues to use these medicinal plant remedies on a daily basis. However, these remedies are at risk of becoming extinct due to a lack of interest from the younger generation. The objectives of the study were to identify the plants used for medicinal purposes, provide an inventory and select several plants for elemental analysis and phytochemical screening.

Individuals were selected based on their ethnobotanical knowledge and agreed to participate in the study. Over 40 individuals were interviewed from the four communities; Pniel, Lanquedoc, Meerlust and Kylemore. Of the 40 participants interviewed, 25 participants agreed to complete questionnaires. The survey yielded 53 plant species belonging to 31 families of which only 24 are indigenous. Although more exotic plants are used compared to indigenous, the indigenous plants were the more popular choice for medicinal use. Most plants belonged to the Lamiaceae family followed by Asteraceae, Alliaceae, Apiaceae and Rosaceae. The most popular plants in terms of ranking are *Agathosma crenulata* followed by *Artemisia afra* and *Helichrysum petiolare*. This is the first report of *Protea cynaroides, Cissus rhombifolia*, *Canna* spp. and *Dilatris viscosa* used for medicinal purposes.

Plants from the Dwarsrivier valley were selected for elemental analysis and phytochemical screening. These plants were selected due to them being indigenous as well as potential candidates for evaluation. The plants will be compared at phytogeographical levels and seasonal variation will be taken into account. *Cliffortia odorata*, *Asparagus capensis* and *Vernonia oligocephala* are common plants used as medicine yet limited literature is available regarding these plants.

Elemental analysis was carried out on eleven plant species from the Dwarsrivier valley. A hydrogen peroxide: sulphuric acid digestion mixture and a Unicam Solaar M Series Atomic Absorption Spectrometer (AA) were used to analyse the total element concentrations present in the plant samples. The concentration of calcium (Ca), chromium (Cr), copper (Cu), magnesium (Mg), manganese (Mn), sodium (Na), phosphorous (P), potassium (K), selenium (Se) and zinc (Zn) were evaluated.

Aloe arborescens contained the highest concentration of Ca and the second highest concentration of Mg, Na and Zn. The highest concentration of Cu was present in *Agathosma crenulata* while *Carpobrotus edulis* contained the highest concentration of both Mg and Na. *Leonotis leonurus* (narrow leaf) contained the highest concentration in both P and Na while the highest concentration of Zn was present in *Vernonia oligocephala. V. oligocephala* contained the highest concentration of chromium and the highest concentration of selenium was present in *Artemisia afra*. Although the community claimed that *Leonotis leonurus* (broad leaf) was more effective than *Leonotis leonurus* (narrow leaf), the elemental analyses revealed that *L. leonurus* (narrow leaf) had higher concentrations in all the elements except Mg and Mn. The level of the elements present in the plant samples were within the normal range except for Na which was higher and P which was lower.

Eleven species were selected for evaluation by thin layer chromatography (TLC) while the oils of *Agathosma crenulata, Salvia africana-caerulea* and *Pelargonium capitatum* were assessed by gas chromatography coupled to mass spectroscopy (GC-MS). Plants such as *Asparagus capensis*, *Cliffortia odorata* and *Vernonia oligocephala* were evaluated for the first time.

Phytochemical screening revealed the presence of tannins in all plants except *A. crenulata, A. arborescens* and *Vernonia oligocephala* and saponins were evident in all plants except *A. crenulata* and *Aloe arborescens.* Essential oils were present in all plants except *Carpobrotus edulis*, *Cliffortia odorata*, *Asparagus capensis* and *A. arborescens.* Glycosides were evident in all plants with the exception of *S. africana-caerulea*, *L. leonurus* (both narrow and broad*)*, *C. edulis*, *A. capensis* and *A. crenulata*. All plants contained flavonoids and phenols with anthraquinones present in all plants except *C. edulis*, *L. leonurus* (narrow and broad) and *P. reniforme.* Secondary metabolites evident in *Artemisia afra*, *Leonotis leonurus* and *Salvia africana-caerulea* were similar to previous studies.

The percentage of predominant compounds present in the oils of *A. crenulata*, *S. africana-caerulea* and *P. capitatum* evaluated by GC-MS was much higher compared to previous studies. This could be directly associated to the geographical locations and the season of plant collection.

DECLARATION

I declare that *Medicinal plant use in the Dwarsrivier valley, Stellenbosch* is my own work and that it has not been submitted before for any degree or examination in any other university. All sources used, or quotes, have been acknowledged and indicated by means of complete references.

Melissa L. Arendse

________________________ April 2013

This thesis is dedicated to my Lord and Saviour, Jesus Christ, and my mother Nicolette Barends. For all the prayers, love and support - I am eternally grateful.

The Lord is my light and my salvation-whom shall I fear? The Lord is the stronghold of my life- of whom shall I be afraid? - Psalm 27:1

ACKNOWLEDGEMENTS

I wish to thank the following people for their valuable help throughout the study;

My supervisor, Mr Frans Weitz and co-supervisor, Professor Wilfred Mabusela for their guidance and assistance.

In the department of Biodiversity and Conservation Biology:

Mr Lilburne Cyster for his assistance, guidance and time in the lab as well as ordering of the materials needed in the study.

In the department of Chemistry:

Mr Henry Mwangi and Mr Ndikho Nako for their help, guidance and endless assistance in the chemistry labs.

Funding:

I wish to acknowledge the NRF for financial support.

The study area:

I would like to say thank you to everyone I spoke to in the Dwarsrivier valley for their kindness, time and sharing of their medicinal knowledge. Without them, this study would not be possible. A special thank you to Mrs Pietersen for organising the meetings. I would like to convey my biggest gratitude to Mr Lotter Cyster and Mrs Julia Cyster, Aunty Georgina Cyster and Mr Terry Williams for their help, endless assistance and kindness throughout the study. You made my visits to Pniel memorable.

I wish to thank my family and the Cloete family. Without your love, support, endless patience and motivation this would not be possible. Your prayers are what gave me strength. To my friends, Francuous Müller and Riaan Cedras, thank you for all your encouragement, words of wisdom and guidance.

CONTENTS

LIST OF TABLES AND FIGURES

CHAPTER 5

CHAPTER 1

INTRODUCTION

1.1. An overview of medicinal plant use

The use of plants as therapeutic agents forms the backbone of an integral medicine system (Gurib-Fakim, 2006). These herbal remedies have been developed over many centuries and passed on through the generations by word of mouth (van Wyk and Wink, 2004). It could be claimed that our ancestors, such as the Khoi and San in South Africa, had an inborn craving for medicine (Kim, 2005). Although certain healing practices have been proven to be inaccurate, such as the Doctrine of Signatures, this does not deter from the fact that medicinal plant therapy is based on findings that date back to ancient times (Gurib-Fakim, 2006).

Plants were once the pinnacle of all medicines in the world (van Wyk et al., 1997) until the 19^{th} century which yielded the isolation of active compounds from medicinal plants (Phillipson, 2001). Natural products provide an infinite source of medicine (Schmidt et al., 2008) representing more than half of the drugs used worldwide in a clinical setting with vascular plants contributing approximately 25% of the total (van Wyk et al., 1997; van Wyk and Wink, 2004; Gurib-Fakim, 2006). Prior to World War 2, the revolutionary unearthing of quinine from *Cinchona* bark led to the inevitable curiosity in plants which steered the discovery of novel drugs from the New World (Phillipson, 2001). Almost half a century ago heralded the discovery of potent drugs from flowering plants which include pilocarpine from *Pilocarpus* spp. to treat glaucoma as well as "dry mouth", reserpine and other antihypertensives as well as diosgenin from *Dioscorea* spp. which has resulted in anovulatory contraceptives (Gurib-Fakim, 2006). Post war gave rise to few findings of novel drugs from vascular plants with reserpine from *Rauwolfia* beckoning the age of tranquilisers and vinblastine and vincristine from *Catharanthus roseus* as effective agents for chemotherapy (Phillipson, 2001). The escalation of resistant as well as multiple drug resistant strains of microorganisms is a continuing threat (McGaw et al., 2008) and as a result, natural products isolated from plants have been targeted as an alternative against infectious diseases (Green et al., 2010).

1.1.1 Medicinal plant use in Africa

The tropical and subtropical regions of Africa is home to 40-45 000 species of potential medicinal agents of which only 5 000 species are used medicinally (Gurib-Fakim and Kasilo, 2010). *Phytolacca dodecandra* is the most prominent species hailing from Africa (Hoareau and DaSilva, 1999) as extracts have been used as an efficient molluscide to contain schistosomiasis (Lemma, 1991). Additional medicinal agents rife to Africa are *Catharanthus roseus* which yields the tumour preventative agents, vincristine and vinblastine and *Ricinus communis* from which castor oil, a laxative, is produced (Hoareau and DaSilva, 1999). Although Africa is a rich source of medicinal plants, it also poses a threat to natural habitats having one of the highest deforestation rates due to anthropogenic activities (Gurib-Fakim, 2006; Muthaura et al., 2007).

This epicentre cradles African traditional medicine, the most ancient and possibly the most varied medicinal system (van Wyk and Wink, 2004; Gurib-Fakim, 2006). This system of medicine embodies a holistic approach which involves both the body and the mind (van Wyk and Wink, 2004). Approximately, 80% of the world's population, predominantly from developing countries, depends on traditional medicine for their primary healthcare needs (Kim, 2005; Springfield et al., 2005; Mander and Le Breton, 2006; Stafford et al., 2008). Although this form of medicine is still being practiced today, it continuously evolves due to interactions with other cultures (Elvin-Lewis, 2001).

1.1.2 Plants in South Africa

South Africa boasts a floristic region with well over 30 000 species of higher plants (van Wyk et al., 1997; Mander and LeBreton, 2006) with the Cape Floral Kingdom closely rivalling tropical rainforests in terms of species richness accounting for approximately 9 000 species (van Wyk et al., 1997). However, only 3000 of these higher plants are used medicinally (van Wyk et al., 1997). The species richness in the Cape Region is remarkable not only in the African context comparing favourably with areas of the same size in the wet tropics (Goldblatt and Manning, 2000). This corroborates the claim of Mander and LeBreton (2006) that South Africa is indeed one of the most diverse floristic regions in the world. Within the estimated 3000 indigenous plant species, only 38 species have reportedly been commercialised to a certain extent (van Wyk, 2008). Certain plants such as *Aspalathus linearis* is one of the most successful and important species in the indigenous industry (van

Wyk, 2011). *Hypoxis hemerocallidea* is being extensively researched after being patented into a product (van Wyk, 2011).

1.1.3 Traditional medicine versus western medicine

The rich cultural diversity of South Africa is symbolised by two medicine systems, namely the informal (African traditional medicine) and formal (Western) systems, practiced in various regions of the country (van Wyk et al., 1997; Stafford et al., 2008; van Wyk et al., 2009). African traditional medicine is an integral aspect of the culture and customs of the African people (Fennel et al., 2004). This informal medicine system is based on an apprenticeship system occurring via the traditional healer, herbalist and shaman sharing with future generations while keeping the therapeutic plants a secret (Fabricant and Farnsworth, 2001). This results in little being recorded in structured methods (Fabricant and Farnsworth, 2001). As a result of westernization, the last 400 years has born witness to the deterioration of the culture of the San and Khoi with facets of their earliest knowledge being irretrievably lost (Scott and Hewett, 2008).

The formal medicine system was presented to the country by European explorers over 300 years ago and although newer by practice, is thoroughly documented and organised and commonly known as western medicine (van Wyk et al., 1997). The World Health Organisation defined health as a state of physical and mental wellbeing and not simply the absence of disease (Hoareau, 1999). With this in mind, each medicine system has its own distinctive diagnostic and healing aspects of disease, and while opposing, denotes harmony within the culture (van Wyk et al., 1997).

1.1.4 Traditional healers

Traditional healers are also acknowledged as “isangoma” and “inyanga” (Xhosa), “nqaka” (Sotho) as well as “bossie dokter” and “kruiedokter” (Afrikaans) (van Wyk et al., 1997). There are an estimated 255 000 indigenous healers in South Africa (Water and Forestry, 2005) with approximately 27 million South Africans utilizing herbal medicines from more than 1020 plant and 150 animal species (Stafford et al., 2005). With a reported 80% of the world’s population using traditional medicine for their primary health care needs (Kim, 2005; Springfield et al., 2005; Mander and Le Breton, 2006), traditional healers play a fundamental role in the health care system as they are accessible and share similar beliefs and values as their patients (Kubukeli, 1999).

Spanning over the last decade, the work force generated by traditional healers is a potentially important resource for primary healthcare (Springfield et al., 2005). Having considerable knowledge regarding plants and preventative measures in case of acute toxicity, traditional healers have on occasion referred patients to the hospital when conditions beyond their scope of practice arise such as tuberculosis or malignancies (Savage and Hutchings, 1987).

1.1.5 Medicinal plant trade in the world

Herbal medicine has given rise to amplified global interest, igniting both the health and international trade (Stafford et al., 2005). As natural products are increasingly seen as non-narcotic, more accessible and deemed with fewer side effects, the medicinal plant trade is rapidly developing (Kurian and Asha Sankar, 2007). More than a decade ago, Hoareau (1999) estimated the global trade in medicinal plants to be worth US $800 million whereas Kurian and Asha Sankar (2007) assessed the global trade to be worth approximately US $120 billion annually.

1.1.6 Medicinal plant trade in South Africa

In South Africa, the trade in traditional medicinal plants is a large and flourishing industry (Mander et al., 1998) motivated by high population growth, rapid urbanisation and unemployment (Dold and Cocks, 2002). This entrepreneur goldmine provides 28 million South African consumers annually with 20 000 tonnes of plant material with a street value of R270 million (Water and Forestry, 2005).

Traditional healers dominate the informal trade by providing crude traditional remedies as well as herbal remedies to consumers, however, the quantification of the plant material is problematic (Mander and Le Breton, 2006). Mander (1998) estimated approximately 6 million indigenous medicine consumers in KwaZulu-Natal spend between 4% and 8% of their annual incomes on traditional medicine. In the Eastern Cape, Dold and Cocks (2002) estimated the trade of 525 tonnes of plant material to be valued at approximately R27 million.

Fuelled by the HIV pandemic as well as being an integral cultural component, medicinal plant trade is likely to be sustained for many years to come (Mander, 1998; Mander and Le Breton, 2006). There is a strong demand for traditional medicine which is evident by 54% of traditional healers interviewed claiming an increase in patients over the past 5 years with 81% expecting a further increase citing the HIV pandemic as the reason (Dold and Cocks, 2002).

1.1.7 Integration of traditional medicine into the health care system

The strong demand for the integration of traditional medicine into the health care system is closely related to its affordability, accessibility as well as it being culturally accepted in developing countries (Zhang, 2009). The last two decades has witnessed an alteration in disease patterns (Zhang, 2000) which has steered the population to develop an increasingly critical attitude towards the chemical industry however more importantly, synthetic medicine (Zschocke et al., 2000). Renewed interest in this ancient practice of medicine has triggered concern amongst researchers as the discovery of novel compounds and the determination of scientific rationale of medicinal plants used in South Africa is crucial (Fennel et al., 2004) and only a few plant species having been scientifically validated (Springfield et al., 2005; Aburjai et al., 2007; Peltzer et al., 2008).

Concerns about this ancient practice of medicine includes the notion that traditional medicine does not keep up with changes in scientific as well as technological advancement, as its methods, medicine and training are often kept a secret (Taylor et al., 2001). This creates a matter of unease as the growing population demand for herbs require the quality and consistency of the herbs be ascertained and maintained for optimal use and efficacy (Ukoha et al., 2011). In addition to these concerns, the rational use of traditional medicine is not always well defined, often relying on indescribable forces such as witchcraft and philosophies which are difficult to rationalise (Taylor et al., 2001). These concepts might be substantiated psychologically, however, they cannot be rationalised scientifically (Addae-Mensah, 1992).

1.1.8 The importance of phytochemical screening and elemental analysis

The efficacy of medicinal plants has been linked to several chemical substances, found within the plants, which produce a definite physiological reaction in the human body (Edeoga et al., 2005). Pharmacological activity appears to be the outcome of many secondary metabolites working together (Scott et al., 2004). More than a decade ago, Stewart et al. (1999) emphasized the urgency of screening plants to enable the identification of metabolites. The presence of secondary metabolites may be a useful indicator of efficacy as well as potential toxicity (Scott et al., 2004). Phytochemical screening enables the identification of secondary compounds present within the plant which may assist in identifying a potential benefit or the presence of toxic classes of secondary metabolites.

Trace elements play a curative as well as a preventative role in disease (Ajasa et al., 2004). It is imperative to know the concentration of the element present within the plants as these elements can be dangerous and toxic at excess levels (Leśniewicz et al., 2006). Elemental analyses of medicinal plants enable the identification of the element as well as the concentration of the element present within the plant. This would ascertain its beneficial or toxic effect in the plant.

A practical search evaluating the beneficial activities of medicinal plants is a rational approach in drug research (Akinmoladun et al., 2007). The distinct need to scientifically evaluate medicinal plants is obvious and for this reason, phytochemical screening as well as elemental analysis of medicinal plants is essential to determine plants with medicinal potential.

1.2. Aims of the study

Plants are still regarded as an integral aspect of the treatment regime for disease in the Dwarsrivier Valley. Therefore, the aims of the study are to:

- Identify the plants used for medicinal purposes by the communities of the Dwarsrivier Valley.
- Provide an inventory of these plants and their uses to treat various ailments.
- To determine the elemental status of selected plants and associate the predominant elements to their biological activities.
- To compare the phytochemical profiles of selected plants to those found in the literature.

1.3. Thesis outline

The thesis will take the following direction.

Chapter two: Literature review: The literature review will include previous surveys in the literature as well as elemental analyses and phytochemical screening carried out on the selected plants. This will then provide evidence of gaps in the literature as well as a comparison of the selected plants.

Chapter three: Medicinal plant use in the Dwarsrivier valley, Stellenbosch: Chapter three will focus on the survey carried out in the Dwarsrivier Valley and disclose results from interviews within the community.

Chapter four: The elemental analysis of twelve selected plant species: Elemental analysis will be carried out on twelve plant species. These results will then be related to their biological activities.

Chapter five: The phytochemical profiles of twelve selected plant species: Chapter 5 will focus on the phytochemical profiles of the selected plants and a comparative study will then be carried out.

REFERENCES

Aburjai, T., Hudaib, M., Tayyem, R., Yousef, M., Qishawi, M., 2007. Ethnopharmacological survey of medicinal herbs in Jordan, the Ajloun Heights region. Journal of Ethnopharmacology 110, 294-304.

Addae-Mensah, I., 1992. Towards a Rational Scientific Basis for Herbal Medicine - A phytochemist's two-decade contribution. Accra: Ghana Universities Press. In**:** Birdi, T.J., Brijesh, S., Daswani, P.G., 2006. Foundation for medical research, India. Approaches towards the preclinical testing and standardization of medicinal plants. www.aifo.it/english/resources/online/books/other/tradmedicine06/TradMedicine-birdi.pdf.

Ajasa, A.M.O., Bello, M.O., Ibrahim, A.O., Ogunwande, I.A., Olawore, N.O., 2004. Heavy trace metals and macronutrients status in herbal plants of Nigeria. Food Chemistry 85, 67-71.

Akinmoladun, A.C., Ibukun, E.O., Dan-Ologe, I.A., 2007. Phytochemical constituents and antioxidant properties of extracts from the leaves of *Chromolaena odorata*. Full Length Research Paper. Scientific Research and Essay 2(6), 191-194.

Dold, A.P., Cocks, M.L., 2002. The trade in medicinal plants in the Eastern Cape Province, South Africa. South African Journal of Science 98, 589-597.

Edeoga, H.O., Okwu, D.E., Mbaebie, B.O., 2005. Phytochemical constituents of some Nigerian medicinal plants. Full Length Research Paper. African Journal of Biotechnology 4(7), 685-688.

Elvin-Lewis, M., 2001. Should we be concerned about herbal remedies. Journal of Ethnopharmacology 75, 141-164.

Fabricant, D.S., Farnsworth, N.R.., 2001. The Value of Plants Used in Traditional Medicine for Drug Discovery. Environmental Health Perspectives 109(1), 69-75.

Fennel, C.W., Lindsey, K.L., McGaw, L.J., Sparg, S.G., Stafford, G.I., Elgorashi, E.E., Grace, O.M., van Staden, J., 2004. Assessing African medicinal plants for efficacy and safety: pharmacological screening and toxicology. Review. Journal of Ethnopharmacology 94, 205-217.

Goldblatt, P., Manning, J., 2000. Cape Plants: A conspectus of the Cape Flora of South Africa. Strelitzia 9. National Botanical Institute, Cape Town.

Green, E., Samie, A., Obi, C.L., Bessong, P.O., Ndip, R.N., 2010. Inhibitory properties of selected South African medicinal plants against *Mycobacterium tuberculosis*. Journal of Ethnopharmacology 130, 151-157.

Gurib-Fakim, A., 2006. Medicinal plants: Traditions of yesterday and drugs of tomorrow. Review. Molecular Aspects of Medicine 27, 1-93.

Gurib-Fakim, A., Kasilo, O.M.J., 2010. Promoting African medicinal plants through an African herbal pharmacopoeia. African traditional medicine day. Special issue 14. Afrolib.afro.who.int.documents/2010/En/AHMSI_64_67.pdf

Hoareau, L., DaSilva, E.J., 1999. Medicinal plants: a re-emerging health aid. Electronic Journal of Biotechnology 2(2), 56-70.

Kim, H.-S., 2005. Do not put too much value on conventional medicines. Perspective paper. Journal of Ethnopharmacology 100, 37-39.

Kubukeli, P.S., 1999. Traditional healing practice using medicinal herbs. The Lancet 354, 24.

Kurian, A., Asha Sankar, M., 2007. Medicinal plants. Horticulture Science Series. Volume 2. New India Publishing Agency.

Lemma, A., 1991. The Potentials and Challenges of Endod, the Ethiopian Soapberry Plant for Control of Schistosomiasis. In: Science in Africa: Achievements and Prospects, American Association for the Advancement of Sciences (AAAS), Washington, D.C., USA.

Leśniewicz, A., Jaworska, K., Żyrnicki, W., 2006. Macro- and micro-nutrients and their bioavailability in polish herbal medicaments. Food Chemistry 99, 670-679.

Mander, M., 1998. Marketing of Indigenous Medicinal Plants in Southern Africa. A Case Study in KwaZulu-Natal. Food and Agriculture Organization, Rome.

Mander, M., Ntuli, L., Diederichs, N., Mavundla, K., 1998. Economics of the Traditional Medicine Trade in South Africa – Chapter 13. www.hst.org.za/uploads/files/chap13_07.pdf

Mander, M., Le Breton, G., 2006. Overview of the Medicinal Plants Industry in Southern Africa. In: Diederichs, N. Commercialising Medicinal Plants. A Southern African Guide. Sun Press, Stellenbosch, pp. 3-9.

McGaw, L.J., Lall, N., Meyer, J.J.M., Eloff, J.N., 2008. The potential of South African plants against *Mycobacterium* infections. Journal of Ethnopharmacology 119, 482-500.

Muthaura, C.N., Rukunga, G.M., Chhabra, S.C., Mungai, G.M., Njagi, E.N.M., 2007. Traditional phytotherapy of some remedies used in treatment of malaria in Meru district of Kenya. South African Journal of Botany 73, 402-411.

Peltzer, K., Friend-du Preez, N., Ramlagan, S., Fomundam, H., 2008. Use of traditional complementary and alternative medicine for HIV patients in KwaZulu-Natal, South Africa. Research article. BMC Public Health 8, 255.

Phillipson, J.D., 2001. Phytochemistry and medicinal plants. Phytochemistry 56, 237-243.

Savage, A., Hutchings, A., 1987. Poisoned by herbs. British Medical Journal 295, 1650-1651.

Schmidt, B., Ribnicky, D.M., Poulev, A., Logendra, S., Cefalu, W.T., Raskin, I., 2008. A natural history of botanical therapeutics. Metabolism Clinical and Experimental 57(1), S3-S9.

Scott, G., Springfield, E.P., Coldrey, N., 2004. A Pharmacognostical Study of 26 South African Plant Species Used as Traditional Medicines. Pharmaceutical Biology 42(3), 186-213.

Scott, G., Hewett, M.L., 2008. Pioneers in ethnopharmacology: The Dutch East India Company (VOC) at the Cape from 1650 to 1800. Review. Journal of Ethnopharmacology 115, 339-360.

Springfield, E.P., Eagles, P.K.F., Scott, G., 2005. Quality assessment of South African herbal medicines by means of HPLC fingerprinting. Journal of Ethnopharmacology 101, 75-83.

Stafford, G.I., Jäger, A.K., van Staden, J., 2005. Effect of storage on the chemical composition and biological activity of several popular South African medicinal plants. Journal of Ethnopharmacology 97, 107-115.

Stafford, G.I., Pedersen, M.E., van Staden, J., 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.

Stewart, M.J., Moar, J.J., Steenkamp, P., Kokot, M., 1999. Findings in fatal cases of poisoning attributed to traditional remedies in South Africa. Forensic Science International 101, 177-183.

Taylor, J.L.S., Rabe, T., McGaw, L.J., Jäger, A.K., van Staden, J., 2001. Towards the scientific validation of traditional medicinal plants. Plant Growth Regulation 34, 23-37.

Ukoha, P.O., Cemaluk, E.A.C., Nnamdi, O.L., Madus, E.P., 2011. Tannins and other phytochemical of the *Samanaea saman* pods and their antimicrobial activities. African Journal of Pure and Applied Chemistry 5(8), 237-244.

Van Wyk, B.-E., van Oudtshoorn, B., Gericke, N., 1997. Medicinal plants of South Africa. Briza Publications, Pretoria.

Van Wyk, B.-E., Wink, M., 2004. Medicinal plants of the world. Briza Publications, Pretoria.

Van Wyk, B.-E., 2008. A broad review of commercially important southern African medicinal plants. Review. Journal of Ethnopharmacology 119, 342-355.

Van Wyk, B.-E., van Oudtshoorn, B., Gericke, N., 2009. Medicinal plants of South Africa. Briza Publications, Pretoria.

Van Wyk, B.-E., 2011. The potential of South African plants in the development of new medicinal products. Review. South African Journal of Botany 77, 812-829.

Water and Forestry., 2005. Medicinal plants trade. Forestry Sub-sector studies, Briefing 5. http://www2.dwaf.gov.za/dwaf/cmsdocs/Elsa/Docs/FED/SubSector%20Medicinal%20plants%202005.pdf

Zhang, X., 2000. Integration of Traditional and Complementary Medicine into National Health Care Systems. Journal of Manipulative and Physiological Therapeutics 23(2), 139-140.

Zhang, X., 2009. The WHO perspective on integration of traditional medicine into health system. European Journal of Integrative medicine 1, 167-171.

Zschocke, S., Rabe, T., Taylor, J.L.S., Jäger, A.K., van Staden, J., 2000. Plant part substitution - a way to conserve endangered medicinal plants? Journal of Ethnopharmacology 71, 281-292.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

The custom of utilising plants to cure infirmities is an ancient practice (Dyubeni and Buwa, 2012). The subsequent demands in natural products have urged scientists to investigate various plant species in the hope of discovering alternative cures (Coopoosamy and Naidoo, 2012). To enable the identification of the most frequently used species for further scientific validation, an initial ethnobotanical survey is carried out (Igoli et al., 2005). The identification of these plants often involves attaining ethnobotanical information from the community members (Sofowora, 1996). Knowledge of these plants is imperative not only to determine substitutions for the treatment of ailments but also for conservation purposes.

Overharvesting of numerous plant species has resulted in traditional healers having to travel far distances to source relevant plant material (Coopoosamy and Naidoo, 2012). As certain plants are close to extinction, plant substitutes need to be used (Coopoosamy and Naidoo, 2012). Urgency exists to examine ethnobotanical surveys to try and authenticate these plants, identify plant alternatives as well as acknowledge and act on destructive and potentially harmful treatments.

2.1.1 Ethnobotanical surveys in the Western Cape

Ethnobotanical studies in the Western Cape are limited which is ironic considering the region is rich in both floral diversity and endemism (Philander, 2011). Remarkably, an estimated 9 000 species of vascular plants are native to the area with nearly 69% endemic (Goldblatt and Manning, 2000). Surveys were carried out in the Western Cape to document plants used medicinally. Thring and Weitz (2006) and Philander (2011) identified plants used by communities in the Bredasdorp/Elim region and by Rastafarians in regions of the Western Cape.

Thring and Weitz (2006) documented 36 species belonging to 19 plant families used for medicinal purposes in the Bredasdorp/Elim region. The most prevalent species belonged to Asteraceae and Lamiaceae (six species each) followed by Solanaceae and Alliaceae (three species each). Plants commonly used in Sotho, Xhosa and Zulu traditional medicine such as *Leonotis leonurus, Artemisia afra* and *Mentha longifolia* were featured in the survey. *A. afra* and *Ruta graveolens* had the highest use-values and were the two most popular plants used by the community. The leaves were most frequently used either as an infusion or decoction with most of the participants being female between the age of 50 and 75 years and the males ranging between 45-65 years of age.

Philander (2011) reported 181 plant species from 71 plant families used by Rastafarians in the Western Cape. The largest number of species belonged to Rutaceae and Asteraceae (13 plant species each) followed by Apiaceae (9 species), Lamiaceae and Fabaceae (8 species each) and Euphorbiaceae (7 species). The leaves were predominantly utilised (49%) followed by the root/rhizome (16%), bark (12%), bulbs (7%), whole plant (5%) and tubers (4%). Oral intake was the most common administration mode (87%) used to treat urogenital ailments (11%), gastrointestinal symptoms (11%), external applications for skin ailments (9%), cardiovascular disease (8%), metabolic or modern disease such as high cholesterol, diabetes and gout (7%) as well as respiratory and spiritual ailments (6%). The herbs most prevalent include *Dodonaea viscosa*, *Pelargonium* spp., *Sutherlandia frutescens, Haemanthus albiflos, Bulbine latifolia, Chironia baccifera, Cissampelos capensis, Tulbaghia capensis, Helichrysum* spp., *Agathosma* spp., *Hypoxis hemerocallidea, Notobubon galbanum, Kedrostis nana* and *Acorus calamus.*

2.1.2 Ethnobotanical surveys in the Eastern Cape

The Eastern Cape Province, renowned for its rich plant species (Dyubeni and Buwa, 2012), has a long history of indigenous people using these plant remedies as medicine (Grierson and Afolayan, 1999). Extensive work has been carried out to document plants used in the treatment of ear, nose and throat (ENT) infections (Dyubeni and Buwa, 2012), diabetes (Erasto et al., 2005; Oyedemi et al., 2009), cardiovascular diseases (Olorunnisola et al., 2011), eye infections (Pendota et al., 2008), gastrointestinal disorders (Olajuyigbe and Afolayan, 2012; Bisi-Johnson et al., 2010; Appidi et al., 2008), cancer (Koduru et al., 2007) and wounds (Grierson and Afolayan, 1999) in the Eastern Cape Province.

Dyubeni and Buwa (2012) identified 27 plants from 21 plant families used to treat ENT infections. Members of the Asteraceae family (three species) were the most predominant, followed by Alliaceae (two species) and Rutaceae (two species). The six most frequently mentioned plants by traditional healers were *Sansevieria hyacithoides* and *Cotyledon* *orbiculata* for ear infections while nasal infections were treated using *Asclepias fruticosa*. Throat infections were treated using *Artemisia afra*, *Allium sativum* and *Lippia javanica*. The leaf (59%) was most often used followed by the bark and roots (11% each), bulb, rhizome and stem (5%) and twigs and fruits (2%). Administration of the herbal remedies varied from drinking the boiled plant material for throat infections, squeezing the warm leaf sap directly into the ear to aid ear infections and snuffing the powdered leaves for nasal infections. Females were the chief informants with 21 females being between 37-45 years old and 15 men between 28-65 years of age.

Erasto et al. (2005) documented 14 plant species belonging to six plant families used to treat diabetes in the Eastern Cape. Members of the Asteraceae family were most commonly used constituting 50% of the plants. Other families include Hypoxidaceae (two species), Asphodelaceae (two species) while Apocynaceae, Apiaceae and Buddlejaceae had one species each. Infusions were predominantly used with the leaves being the most frequently used part of the plant contributing 40% of the herbal preparation. This was followed by the roots (30%) while the corms, twigs and whole plant were rarely used for the preparation of medicines. Four plants often mentioned and highly recommended by both rural dwellers and traditional healers were *Helichrysum odoratissimum*, *Helichrysum petiolare*, *Hypoxis hemerocallidea* and *Hypoxis colchicifolia*.

In the Nkonkobe Municipality, Oyedemi et al. (2009) identified 15 plants from 13 families used by herbalists, traditional healers and community members to treat diabetes mellitus. Reportedly, 13 species (86.67%) were not mentioned before in the study area for treating this condition. The family Alliaceae and Asphodelaceae had two species each while other species had one species each. The roots (33.3%) were predominantly used followed by the whole plant (20%) as well as the bark (13.3%) and leaves (13.3%). *Strychnos henningsii* and *Leonotis leonurus* were repeatedly mentioned by both herbalists and traditional healers to treat diabetes mellitus. The members who were interviewed consisted of married herbalists and traditional healers, both men and women, age between 40-60 years with low education.

Olorunnisola et al. (2011) reported 19 plant species from 16 families used to treat heart disease and other cardiac conditions. Two plants from the Asteraceae, Hypoxidaceae and Fabaceae families were the most commonly mentioned plant species. The leaf extracts (78%) are predominantly used followed by the bulb (53%), root (21%), and the stem (16%) and corm (5%). Infusions were used in 50% of the treatments and decoctions in 15% of the treatments. The extracts are customarily taken orally, twice a day, morning and at night. The severity of the disease determined the duration of plant use. *Tulbaghia violacea* was repeatedly mentioned to treat cardiovascular disease.

Pendota et al. (2008) documented 12 plant species from 9 families identified by herbalists, traditional healers and members of community to treat eye infections. Plant species belonging to the Sapindaceae, Rosaceae and Fabaceae (two species each) families were predominantly used. Four plants were repeatedly mentioned and recommended by traditional healers. These were *Malva parvifolia*, *Helichrysum foetidum*, *Sutherlandia frutescens* and *Hippobromus pauciflorus*. The leaves (75%) were most often used followed by the bark and roots (25%). The most common method of administration involved mixing the sap with boiling water and applying directly to infected eyes after cooling or crushing the plant material and squeezing the sap directly into infected eyes. The plant preparation was commonly applied externally, usually on a daily basis, for a short period of time.

Several studies have identified plants used to treat gastrointestinal ailments including diarrhoea. Appidi et al. (2008) documented 17 plant species belonging to 14 families often used to treat diarrhoea. Members of the family Fabaceae (three species) were most commonly used followed by Anarcardiaceae (two species) while the remaining families had one specie each. The local healers used different parts of the plant. In the remedies, the roots were prevalent followed by bark, leaves and bulbs. Decoctions and infusions were the main method of preparation with the majority of the remedies taken orally. Single plant parts were used with the dosage depending on the age of the patient. The remedies were consumed until the patient was healed.

Thirty-two plant species belonging to 26 plant families were identified in the O.R district of the Eastern Cape for the treatment of diarrhoea (Bisi-Johnson et al., 2010). The most frequently mentioned families were Fabaceae (16.67%) followed by Hyacinthaceae and Hydnoraceae (8.33% each). The roots and leaves were primarily used with decoctions and infusions the preparation methods of choice. Medication was frequently administered orally or as an enema. Certain plants were used on their own, mixed with other plant(s) or consumed as food. Reportedly, most traditional healers never used cultivated plants but source their plants from the wild.

Olajuyigbe and Afolayan (2012) documented 36 plant species from 24 plant families used to treat gastrointestinal problems in the Eastern Cape. These plants aided diarrhoea, dysentery, abdominal cramps, gut disturbances, stomach disorders, upsets as well as aches. The family Fabaceae was the most represented with five plants followed by Apiaceae, Asphodelaceae, Lamiaceae and Solanaceae with three plants each. The leaves were most frequently used (30.17%) followed by the roots (25%) and bark (21.5%), whole plant (9.62%), rhizomes (5.77%), fruits (3.87%) as well as bulb and tubers (1.92%). Decoctions and infusions were the most common method of preparation.

Koduru et al. (2007) identified 17 plant species from 13 families commonly used to treat cancer by traditional healers and herbalists as well as community members in the Eastern Cape. Plant species belonging to Hypoxidaceae and Hyacinthaceae are most frequently used. The preparation method varied with decoctions and infusions most often used and certain plants having more than one preparation method. The bulbs, roots and corms were reportedly the most commonly used plant parts (54%). Leaves and bark constitute 23% and 19% respectively while seeds, fruits and latex make up approximately 4% of the herbal concoctions. *Solanum aculeastrum* (Solanaceae) was frequently mentioned to treat cancer.

Thirty-eight plants belonging to 26 plant families were recognised for treating wounds in the Eastern Cape (Grierson and Afolayan, 1999). Asteraceae (23%), Asphodelaceae (15%) and Solanaceae (12%) were most commonly represented. Seven categories were documented for the methods of preparation of the plants. These include plant parts used as a poultice (14 species), plants used as a bandage or directly applied to the wound (10 species) as well as those from which juice is extracted and applied as a dressing or used to wash wounds (5 species each). Other plants were prepared as lotions (5 species), ground into powders (4 species) or prepared as an ointment. For some plants, more than one preparation method is used. Leaves were most frequently used (68%), followed by stem bark (19%), roots, bulbs and corms (13%). Plants were applied externally to the affected areas of the body for all preparation methods.

2.1.3 Ethnobotanical surveys in KwaZulu-Natal

Coopoosamy and Naidoo (2012) carried out an ethnobotanical survey in Durban which documented 25 species from 22 families sold on the market by traditional healers. Concern regarding endangered species such as *Siphonochilus aethiopicus*, *Warburgia salutaris* and

Haworthia limifolia being sold on the market raises the issue regarding educating plant collectors on methods to sustain harvesting from the wild. Education may include sustainable harvesting from the wild, propagation at home gardens and possible implementation of wild populations within protected areas.

The four predominant complaints of the patients were wound healing, sexual enhancers and sexual diseases, blood purifiers and oral and digestive tract diseases. Wound healing includes sunburns, rashes, sores etc. In the sexual health category, the main concerns were libido functioning, prolonged erections and early ejaculations whereas sexual diseases ranged from genital sores, STDs and HIV. Blood purifiers include immune boosters, oral diseases include mouth ulcers and digestive tract disease includes diarrhoea.

Boiling the plant material to form a concoction or decoction was the common method of preparation. To treat skin infections, superficial burns or sexual infection, the plant material was boiled to make a decoction or concoction. Heating the crushed leaves, before applying, were occasionally used for aches and pains. Oral infusions were used to enhance sexual ability and a paste was made from the plant material to treat superficial sexual infection.

Corrigan et al. (2011) carried out an ethnobotanical survey in KwaNibela Peninsula, St Lucia. The vernacular names as well as the local names of 82 plant species and ten new plant species were documented. Three species, namely *Erythroxylum delagoense*, *Putterlickia verrucosa* and *Teclea natalensis* are frequently used in the KwaNibela region. The fruit of *E. delagoense* is a local medicine used for infantile respiratory and throat ailments. *P. verucosa*, the resident cleaning ritual of sangomas, was a novel finding. Reportedly, further exploration of the fruits of *T. natalensis* and its possible role in dental care could be valuable.

The majority of the remedies recorded were used for magical purposes (24 species). Additional categories with several species recorded was food/ drink (22 species), respiratory conditions (8 species), wound healing (6 species), gastrointestinal disorders (4 species), infertility (4 species), skin disorder/ treatment (3 species), parasitic infection (3 species), pain/ anxiety/ inflammation (3 species), ear/nose/ throat ailments (2 species), hunting (2 species), repellent (insect/snake) (2 species), timber (building) (2 species), firewood (2 species), general tonic (1 species) and neurological disorders (1 species). Several remedies were used as purgatives with a few requiring a combination of plant species to be used.

Five people assisted with the survey of which three gained their knowledge from their own experiences and the other two were both isangomas who received their knowledge from elderly family members.

In KwaZulu-Natal, York et al. (2011) investigated plants used to treat respiratory infections in Maputaland. Thirty plant species belonging to 18 plant families were documented. *Lippia javanica*, an indigenous aromatic shrub, was most frequently used followed by *Eucalyptus grandis* (an exotic), *Senecio serratulloides* and *Tetradenia riparia* (both indigenous species). Plants were used independently or in combination with other plants. Twenty four combinations were reported but combinations using different parts of the same plant were also noted. An interesting observation was that *E. grandis* was used in combination with *L. javanica* in 85% of the combinations.

The leaves were predominantly used but the bark as well as the underground parts was included. It was reported that the aromatic properties of certain leaves could be a possible explanation for its frequent use. On preparation, a handful of plant material is added to cold water and brought to boil. The decoction is generally taken orally with the left over decoction used for steaming. Steaming occurred until the decoction had cooled.

De Wet et al. (2010) identified 23 plant species from 15 families to treat diarrhoea by the rural community in KwaZulu-Natal. All plants that were collected were available throughout the year and grow in the homesteads or bordering areas. The most frequently used plant species were exotics namely *Psidium guajava*, *Catharanthus roseus* and *Melia azedarach,* followed by the indigenous spp. *Sclerocarya birrea* and *Strychnos madagascariensis*. For enhanced antidiarrheal efficacy, seven plants were used in five altered combinations. These include the combination of *Brachylaena transvaalensis* and *Sclerocarya birrea or Psidium guajava* as well as *Acanthospermum glabratum* with *Krauseola mosambicina* or *Psidium guajava*. *Mangifera indica* with *Sarcophyte sanguine* is also used.

The leaves or bark are predominantly used while the use of roots is seldom. A hot or cold water infusion is frequently used and consumed orally. Women were predominantly interviewed (80%) who were on average 53 years of age. The interviewees gained their herbal knowledge from their grandmothers (33%), elders (20%), mothers (19%), neighbours (10%), fathers (9%) and grandfathers (9%).

2.1.4. Ethnobotanical survey in the Karoo

In the southeastern Karoo, Van Wyk et al. (2008) documented 86 plant species, one lichen and an additional remedy, hyraceum, in an ethnobotanical survey in the Graaff-Reinet and Murraysburg districts. Of these plants, 74 were indigenous and 14 were exotic. Many of the remedies are considered health tonics. Remedies for bladder, kidneys, stomach, back as well as other minor ailments are also common. Combinations of different plants are often used.

New records of plants extensively used in the study but have apparently never been recorded in scientific literature include *Stapelia olivaea, Peliostomum* cf. *origanoides, Aloe striata, Abutilon sonneriatum, Pentzia punctata, Eberlanzia spinosa, Helichrysum pumilio, Osteospermum herbaceum, Pachypodium succulentum* and *Rhigozum obovatum.*

2.1.5 Ethnobotanical survey in Limpopo

An ethnobotanical survey was carried out in the Limpopo province to recognise plants used to treat diabetes mellitus (Semenya et al., 2012). Twenty-four plant species from 20 families were documented. Predominant species were from Asteraceae (13%), Sapotaceae and Cucurbitaceae (8%).   The plants most frequently used by the Bapedi traditional healers were *Mimusops zeyheri* (29%), *Helichrysum caespititium* (25%), *Plumeria obtusa* (21%), *Hypoxis iridifolia* (syn *Hypoxis obtusa*), *Aloe marlothii* subsp. *marlothii* and *Moringa oleifera* (17% each). Roots and leaves were preferred while the seeds, stem bark and whole plants were less frequently used. All medicinal concoctions were taken orally for one week. A decoction is preferred with warm water typically used. Medication is taken after meals as traditional healer's claim it assists in lowering the sugar content of the consumed meal.

2.2 The importance of trace elements in health care

The body requires a certain quantity of minerals to maintain good health as the consumption of herbal medicine reportedly contributes to the intake of minerals in both infants and adults (Ajasa et al., 2004). The awareness of trace elements concentration present within plants is crucial as it may influence the production of their active constituents not to mention its pharmacological action (Rajurkar and Damame, 1997). An increase in the role trace elements play in biological activities have been noted (Mohanta et al., 2003) however elemental analysis still lags behind when compared to phytochemical screening of secondary compounds found within plants.

Jimoh et al. (2010) reported that the leaves of *Leonotis leonurus* contained Sodium, Potassium, Calcium, Magnesium, Iron, Zinc, Phosphorous, Copper, Manganese and Nitrogen. Chiban et al. (2011) reported the presence of Oxygen, Carbon, Silicon, Sulphur, Sodium, Aluminium, Magnesium and Calcium in *Carpobrotus edulis* revealed by EDX spectra. These elements are reportedly the principle elements in *C. edulis*.

It is evident that elemental analysis of plants are lacking. The identification of specific elements as well as the concentration present within the plant could enable a possible association between the concentration of the element and its biological activity.

2.3 Phytochemical screening of selected plants

A medicinal plant is a plant thought, claimed or proven to have medicinal properties (Sarmani et al., 1999). This distinguishes plants whose constituents as well as therapeutic properties have been scientifically established from those plants regarded as therapeutic but have not yet been subjected to a thorough investigation (Okigbo et al., 2009). The isolation and characterisation of chemicals from different plants raise the awareness of the connection between the chemical content present within the plant and the relationship with living beings (Macaís et al., 2007). The development of phytochemical studies would not be possible without knowledge of the chemical structure of the plant (Macaís et al., 2007). Phytochemical evaluations of plants enable the identification of beneficial compounds or possible toxicities as well as scientifically validate the claims of these medicines.

Twelve plants from the Dwarsrivier valley were selected for phytochemical screening due to them being indigenous and potential candidates for phytochemical studies. *Cliffortia odorata*, *Asparagus capensis* and *Vernonia oligocephala* are common plants used as medicine yet limited literature is available regarding these plants.

2.3.1 *Agathosma crenulata* (Rutaceae)

Agathosma crenulata is a woody shrub which grows up to 2.5m in height (Moolla, 2005). This aromatic shrub has dark green leaves which are longer than it is broad with oil glands throughout (Moolla, 2005). One to three relatively large mauve or white flowers grows on the stem (Moolla, 2005). An infusion of the leaves soaked in brandy, commonly referred to as "boegoebrandewyn", is used in the Cape to treat stomach complaints and as a stimulant tonic (Viljoen and Moolla, 2007). This aromatic shrub has been used to treat urinary tract and kidney diseases, providing symptomatic relief of rheumatism and as an external application on wounds and sores (Viljoen and Moolla, 2007).

2.3.1.1 Phytochemistry

2.3.1.1.1 Volatile secondary metabolites

Previous studies have focussed on the volatile fraction (essential oil) of *Agathosma* in an attempt to determine the chemical constituents. Fluck et al. (1961) found *Agathosma betulina* to contain (ψ)-diosphenol (an isomer of diosphenol), menthone, limonene, *l*-pulegone and diosphenol and *Agathosma crenulata* to contain menthone, limonene, large amounts of *l*-pulegone and trace amounts of diosphenol. Klein and Rojahn (1968) isolated and characterised 17 compounds in "buchu" oil.

Two monoterpenes were accountable for the characteristic odour of "buchu" and 8-mercapto-*p*-menthan-3-one was isolated and is reportedly an important component in determining the flavour and aroma of the oil (Lamparsky and Schudel, 1971). Kaiser et al. (1975) identified 120 constituents in "Buchu" leaf oil of commercial origin in addition to previously described 8-mercapto-*p*-menthan-3-one. New sulfurated terpenoid ketones as well as *p*-menthan-3-one derivatives oxygenated in the 2 or 4 position was reported for the first time. Spreeth (1976) marked the differences in the leaf morphology and chemical composition of commercially important *Agathosma* species. This led to the subdivision of *A. crenulata* (L) Pillans into *A. crenulata* (L.) Pillans pro parte and *A. serratifolia* (Curt) Spreeth comb. nov. A third species, *A. betulina* (Berg.) Pillans was retained.

Collins and Graven (1996) determined the chemotaxonomy of commercial "buchu" species. In the same year, the chemical composition of *A. betulina*, *A. crenulata* and an *A. betulina* x *A. crenulata* hybrid was evaluated (Posthumus and van Beek, 1996). The aim was to recognise plants with specific chemical composition. The young plants of *A. crenulata* were stem fed with aqueous solutions of $^{2}H_2$ and $^{18}O/^{2}H_2$-labelled monoterpene precursors (Fuchs et al., 2001). Labelled pulegone precursors were converted to corresponding labelled menthofuran, isomenthone and menthone. Moolla (2005) reported that pulegone (34.9%) and menthone (16.6%) were the major compounds present in *A. crenulata*. Limonene and isomenthone constituted 13.4% and 7.3% of the total composition respectively. Sandasi et al. (2010) used vibrational spectroscopy methods as an alternative to assess *A. betulina* and *A.*

crenulata. The results revealed the potential of using MIR spectroscopy as a quick and economical alternative to forecast the major compounds of "buchu" oil.

2.3.1.1.2 Non-volatile secondary metabolites

Campbell et al. (1986) investigated 24 species from *Agathosma*, *Diosma* and *Empleurum* (tribe Diosmeae) for the presence of coumarins. All 20 species contained coumarins and 9 simple coumarins were isolated. Campbell et al. (1987) tested 42 species from *Agathosma*, *Coleonema*, *Diosma*, *Empleureum* and *Phyllosma* (tribe Diosmeae), for the presence of alkaloids. Positive results were attained for five *Agathosma* species with skimmianine and halfordamine being isolated. Normal rutaceous alkaloids do not occur in the Diosmeae, they are uncommon with evidence to date indicating they are confined to the genus *Agathosma*.

2.3.1.2 Pharmacological activity

2.3.1.2.1 Anti-inflammatory activity

Lis-Balchin et al. (2001) discovered an initial spasmogenic activity followed by spasmolysis at high concentrations of *A. betulina* and *A. crenulata* oil. The presence of rolipam caused a significant increase in spasmolytic action of *A. betulina* while *A. crenulata* also increased although not to a significant level. In addition, *A. crenulata* did not appear to block calcium channels although *A. betulina* did. Moolla (2005) reported that the essential oil of *A. crenulata* was active in the anti-inflammatory assay (IC_{50} value of 59.15$\pm$7.44µg/ml) however the extract did not display any activity at 100µg/ml.

2.3.1.2.2 Antimicrobial activity

The oil of *A. betulina* and *A. crenulata* was investigated against *Staphyllococcus aureus, Escherichia coli*, *Saccharomyces cerevisiae*, *Pseudomonas aeruginosa* and *Enterococcus hirae* (Lis-Balchin et al., 2001). Neither of the oils (10µL, undiluted) revealed antimicrobial action against *P. aeruginosa* and *E. hirae* however, very low activity was observed against *S. aureus*, *E. coli* and *S. cerevisiae.* Moolla (2005) reported that *A. crenulata* extract exhibited good activity against *Bacillus cereus*, *Candida albicans* and *Staphyllococcus aureus* with MIC values of 2mg/ml. A study carried out by Viljoen et al. (2006a) revealed that *A. crenulata* was one of the oils most active against *B. cereus*.

2.3.1.2.3 Antioxidant activity

The antioxidant potential of *A. crenulata* was investigated using the 2,2-diphenyl-1-picryhydrazyl radical (DPPH) and 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays (Moolla, 2005). The extract and the oil of *A. crenulata* was inactive at 100 μg/ml in the DPPH assay however, the extract was reportedly active in the ABTS assay.

2.3.1.2.4 Toxicity

The extract and the oil of *A. crenulata* were investigated using microculture tetrozolium (MTT) cellular viability assay (Moolla, 2005). The extract of *A. crenulata* was not toxic in the MTT assay at the concentration tested (IC_{50} value>100μg/ml) however the essential oil was toxic (IC_{50} value>0.0001μg/ml).

2.3.1.2.5 Other

Blommart and Bartel (1976) performed a study measuring the leaf form of *A. betulina* and *A. crenulata* from local plantings. This criterion was the only taxonomic basis for distinguishing the two species. This method is reliable for purposes of identification but not for hybrid buchu.

2.3.2 *Aloe arborescens* (Asphodelaceae)

Aloe arborescens is a mass of average to large, often multi-stemmed shrubs at or near ground level (Gutterman and Chauser-Volfson, 2000a) comprising of curled, soft leaves with harmless, marginal teeth (van Wyk and Smith, 1996). It could be claimed that *A. arborescens* is possibly the most cultivated species of *Aloe* worldwide (Gutterman and Volfson, 2000a). *Aloe arborescens*, also known as the Kidachi aloe in Japan, is used to treat gastrointestinal complaints, skin injuries as well as burns (Matsuda et al., 2008).

2.3.2.1 Phytochemistry

2.3.2.1.1 Volatile secondary metabolites

Umano et al. (1999) identified 123 components from the leaves of *A. arborescens* using the two methods, simultaneous purging extraction (SPE) and dichloromethane extraction (DRP). Significant differences were noted. The components present were 42 alcohols, 23 terpenoids, 21 aldehydes, 9 esters, 8 ketones, 6 acids, 5 phenols and 9 miscellaneous compounds. Large numbers of terpenoids were evident in the oils extracted by both DRP and SPE.

2.3.2.1.2 Non-volatile secondary metabolites

The leaf juice of *Aloe arborescens* contained several biologically active constituents such as aloe emodin, aloenin, magnesium lactate, barbaloin and succinic acid (Hirata and Suga, 1977). Kudritskaya et al. (1985) investigated the presence of carotenoids in the leaves of *A. arborescens* and elucidated six carotenoids of which five were identified. The main carotenoid was lutein which possessed no vitamin A activity. Saito (1993) purified Aloctin A, a constituent present in *A. arborescens* and determined its chemical, biological and pharmacological activities. Park et al. (1998) identified thirteen phenolic compounds present in *A. arborescens* analysed by high performance liquid chromatography. Aloenin, aloenin-B, 10-hydroxyaloin A, aloin A and B and aloe emodin were identified and seasonal variation was also measured.

Gutterman and Chauser-Volfson (2000a) reported that barbaloin, aloeresin and aloenin were circulated in a peripheral defence strategy in the leaves of *Aloe arborescens* with the youngest leaves containing the highest concentration. In nearly all the leaf plants, the barbaloin content was the highest and aloenin the lowest. It was reported that the orientation of the leaf may affect the overall content of these three phenols but not their relative amounts in different parts of the leaf. In the same year, Gutterman and Chauser-Volfson (2000b) reported that the more times a leaf of *A. arborescens* is cut, higher the barbaloin content of the new growth remaining at the base of the plant. Beppu et al. (2004) evaluated the monthly variations with regard to the part and position of the leaf. It was reported that the overall activity or concentration was low in the cold season and high in the warm season.

Olennikov et al. (2008) reported that the primary phenolic compounds present in all *A. arborescens* plants were aloenin, aloins and aloe-emodin. However, anthraquinone derivatives were not characteristic for the inner part of the leaves. Glucose, fructose and sucrose were present however, in the gel, only glucose was present. The highest concentration of carotenoids and chlorophyll was observed in the leaf epidermis.

2.3.2.2 Pharmacological activity

2.3.2.2.1 Anti-carcinogenic potential

The effect of whole leaf *Aloe arborescens* was examined on the formation of aberrant crypt foci induced by azoxymethane (AOM) (Shimpo et al., 2001). The results indicate that *Aloe arborescens* inhibited AOM-induced aberrant crypt foci development in rat colorectum and might have a chemopreventative effect, at least in the initial stages, of colon carcinogenesis. In another study, Shimpo et al. (2002) reported that the ethyl acetate extract of *A. arborescens* inhibited 12-0-tetradecanoylphorbol-13-acetate-induced ear oedema, as well as an increase in putrescine and tumour promotion in mice skin. Aloenin, isobarbaloin and barbaloin was reported to be beneficial as cancer chemopreventative agents against tumour promotion. Furukawa et al. (2002) reported that *A. arborescens* prevented the formation of *N*-Nitrosobis(2-oxopropyl)amine - induced pancreatic neoplasia relative to a decrease in DNA adduct formation on target tissue.

2.3.2.2.2 Analgesic potential

The administration of partially purified aloe carboxypeptidase (CPase) to ICR mice showed substantial pain alleviation and the inhibition of abdominal dropsy effusion (Obata et al., 1993). The acceleration of vascular permeability was also inhibited in the abdominal area. Reportedly, this could be due to the vasopressor activity such as angiotension I hydrolysis and angiotension II production.

2.3.2.2.3 Anti-diabetic activity

The administration of 10KDa fraction powder (prepared from *A. arborescens* leaf skin juice using ultrafiltration and processed to obtain a freeze-dried powder) administered orally using dietary supplementation displayed an inhibitory action on the absorption of jejunum glucose in rats (Beppu et al., 2006). No anti-diabetic activity was observed in the pulp of *A. arborescens*.

2.3.2.2.4 Anti-inflammatory

Substantial healing as well as preventative effects were observed when partially purified aloe carboxypeptidase was administered to rats induced with thermal oedema and mice with abdominal acetic acid inflammation (Obata et al., 1993). In the same year, Yamamoto et al. (1999) reported for the first time that barbaloin, aloe-emodin, aloenin and β-sitosterol as well as a combination of higher straight chain alcohols in *Aloe arborescens* have an inhibitory action on rat paw oedema. These compounds reportedly play a significant role in the anti-inflammatory action on *Aloe*. Teradaira et al. (2006) investigated the antigastric effects of high molecular weight compounds present in *A. arborescens* and the results suggested both suppressive and healing effects on gastric ulcers for compounds with a molecular weight of 5000-500. Microscopic and macroscopic findings confirmed these effects.

2.3.2.2.5 Antimicrobial activity

The antifungal effect of *A. arborescens* was evaluated on guinea-pig feet induced with trichophytosis and compared to lanoconazole, an antifungal agent which is commercially available (Kawai et al., 1998). A 70% growth inhibition was noted in the animals treated with 30% freeze-dried *Aloe arborescens* for 10 days compared to the untreated animals. The *in vitro* experiment revealed that the *A. arborescens* fraction with molecular weight less than 10 000 and barbaloin, inhibited the growth progression of *Trichophyton* at a minimum concentration of 75 mg/mL and 200 µg/mL.

2.3.2.2.6 Antioxidant activity

The protective effects of *A. arborescens* on soft X-irradiation induced skin injury in mice were investigated and a significant protective effect of skin injury was observed (Sato et al., 1990). Seven days after soft X-irradiation, the scavenging activity of hydroxyl radicals as well as suppression in the superoxide dismutase activity and glutathione peroxidase was noted. Beppu et al. (2003) reported that a possible mechanism which prevents the damage of islet toxins to pancreatic islets involves the inhibition of free radical scavenging of *Aloe arborescens* and thermostable low molecular component may be an associated factor.

2.3.2.2.7 Toxicity

A one-year toxicity study of *A. arborescens* revealed that the level where no observed adverse effects were noted was 0.16% in the diet. This is equivalent to 87.7 and 109.7mg/kg/day in males and females, respectively (Matsuda et al., 2008).

2.3.3 *Artemisia afra* (Asteraceae)

Artemisia afra is an aromatic, multi-stemmed perennial herb that grows up to two metres in height (van Wyk and Wink, 2004). This aromatic herb has silver, feathery leaves and small, cream-coloured flower heads (van Wyk and Wink, 2004). The leaves are primarily used however, the roots are used occasionally (van Wyk et al., 2009). *A. afra* is used for a range of ailments such as influenza, indigestion, headache, intestinal worms, cough, sore throat and asthma (van Wyk and Wink, 2004).

2.3.3.1 Phytochemistry

2.3.3.1.1 Volatile secondary metabolites

A. afra harvested during anthesis and early seed set has been shown to yield statistically higher oil content (Graven et al., 1990). The oils were rich in α-thujone, 1.8-cineole, β-thujone and camphor. The chemical composition of four generations of the same clone was very constant with chemotypes revealed on examination of wild populations. Moody et al. (1994) reported that the main constituents present in the oil of *A. afra* were cis-2,-dimethyl-4-octene-2,7-diol (19.03%), 1.8-cineole (17.55%), tricosane (13.92%) and 3.3.6-trimethyl-1.5-heptadiene-4-one (11.67%). Minor constituents identified include y-elemene (0.84%), reportedly an artefact formed during distillation, β-santalol (90.67%), camphene (0.91%) and α-thujone (1.66%). Reportedly the pattern is mostly in agreement with previous reported work on *A. afra* except that α- and β-thujone were not found as main constituents in the present study (Moody et al., 1994). Mwangi et al. (1995) elucidated fifty compounds, constituting approximately 90%, from the oil of *A. afra* leaves by GC and GC-MS. The major constituents were 1,8-cineole (67.4%), terpinen-4-0 (6.5%) and borneol.

The oils of *A. afra* of Ethiopian origin were evaluated and yomogi alcohol (21.6-26.8%) and artemisyl acetate (24.2-32.1%) were predominant in each of the oils and the composition appeared to be independent of plant maturity (Worku and Rubiolo, 1996). Viljoen et al. (2006b) evaluated the geographical variation of *A. afra* oils and reported qualitative and quantitative variation in the oil composition within as well as among natural populations. No correlation was shown between the geographical locations. Vagionas et al. (2007) investigated the aerial parts of *A. afra* and identified 37 compounds constituting 91% of the total oil content. The major components were camphor (46.2%), α-thujone (15.2%), *Artemisia* ketone (7.4%) and 1.8-cineole (4.2%).

2.3.3.1.2 Non-volatile secondary metabolites

The flower heads of *A. afra* reportedly contained scopoletin (Goodson, 1922) while Bohlman and Zdero (1972) discovered that isomeric coumarins and five acetylenes were present in the roots of *A. afra* with the aerial parts containing thujone and umbelliferone-derivatives. It was reported that no acetylenes were present in previously investigated *Artemisia* species. High field NMR techniques elucidated five glaucolides, 10 new guaianolides and 12-hydroxy-α-cyperone from *A. afra* (Jakupovic et al., 1988).

2.3.3.2 Pharmacological activity

2.3.3.2.1 Anti-diabetic activity

The aqueous extract of *A. afra* was evaluated on the pancreas of the streptozotocin-induced diabetic rats (Afolayan and Sunmonu, 2011). It was reported that *A. afra* possessed hypoglycaemic as well as antioxidant activity.

2.3.3.2.2 Antihypertensive activity

The long chain fatty acids isolated from *A. afra* induced hypotensive effects at tested dosages with the diastolic pressure more affected than the systolic pressure (Guantai and Addae-Mensah, 1999). *In vivo*, the aqueous extract exhibited a hypotensive outcome with a biphasic effect displayed *in vitro.* Lower dosages induced a cardiostimulatory effect followed by cardiodepression while higher dosages were predominantly cardiodepressant. Scopoletin, a compound isolated from *A. afra*, prompted a decrease in inotropic effect which was dose-dependent, particularly at higher doses (Guantai and Addae-Mensah, 1999).

2.3.3.2.3 Antimicrobial activity

The essential oils from the aerial parts of *A. afra* were tested against 10 antifungal species using dry weight method (Gundidza, 1993). The essential oil displayed significant activity against *Aspergillus ochraceus*, *Candida albicans*, *Alternaria alternate*, *Geotrichum candidum*, *Aspergillum niger*, *Penicillum citrium* and *Aspergillus parasitus*. Rabe and van Staden (1997) reported that *A. afra* was one of several plants displaying high antibacterial activity. The essential oils of *A. afra, Pteronia incanum* and *Rosmarinus officinalis* were evaluated against 41 microbial strains pathogens (Mangena and Muyima, 1999). The oils were selected based on their potential to prevent food spoilage and/or poisoning using common humans and plant. The results indicated that *A. afra* and *R. officinalis* exhibited similar and higher antimicrobial activities than *P. incana*. McGaw et al. (2000) reported that *A. afra* displayed moderate anthelmintic activity.

Huffman et al. (2002) evaluated the Minimum inhibitory percentages (MIP's) of the volatile oils of *A. afra, Myrothamnus flabellifolius, Lippia scaberrima, Osmitopsis asteriscoides* and *Lippia javanica* against three strains of *Staphylococcus aureus* (two which were methicillin-resistant), one strain of *Cryptococcus neoformans*, one strain of *Pseudomonas aeruginosa* and two strains of *Candida albicans*. All organisms were inhibited by <1% of the oils except *A. afra* and *O. asteriscoides* oil against *S. aureus* and methicillin-resistant *S. aureus. A. afra* oil was one of the oils that did not inhibit *P. aeruginosa* (Huffman et al., 2002). In a study evaluating the antibacterial activity of plants used to treat tuberculosis in the Eastern Cape, *A. afra* displayed the best MIC values for the tested extracts including the aqueous extracts (Buwa and Afolayan, 2009). The aqueous extract of *A. afra* had high MIC values particularly against *E. coli*, *K. pneumoniae* and *S. aureus* and it was one of two plants displaying activity against *M. aurum* A+. The ethanol and dichloromethane extracts displayed good activity against the tested organisms however the ethanol and dichloromethane extracts displayed weak activity with regard to *M. aurum* A+ (Buwa and Afolayan, 2009). Ntutela et al. (2009) investigated the potential of *A. afra* to control mycobacterial replication. The study demonstrates the *in vitro* anti-mycobacterial activity as well as modulation of pulmonary inflammation experienced in early mycobacterial infection.

A. afra (primary plant) in combination with the essential oils of *Eucalyptus globulus*, *Osmitopsis asteriscoides* and *Agathosma betulina* were evaluated on four pathogens namely *Moraxella catarrhalis, Enterococcus faecalis, Klebsiella pneumonia* and *Osmitopsis asteriscoides* (Suliman et al., 2010). Moderate activity was exhibited by individual oils with additive interactions predominantly identified. *A. afra* in combination with *O. asteriscoides* in the 8:2 ratios against *C. neoformans* displayed the most significant synergistic interaction. No antagonistic interactions were evident.

2.3.3.2.4. Antioxidant activity

Burits et al. (2001) reported that the volatile oils of *A. afra* could possibly play a role as a non-specific donor for electrons or hydrogen atoms in the DPPH assay. Additionally, *A. afra* was revealed to be an effective radical scavenging agent in the deoxyribose degradation assay while displaying antioxidant potential in the *in vitro* assay for non enzymatic lipid peroxidation in liposomes. Chamazulene, a sesquiterpene found within *A. afra*, was proposed to be accountable for the antioxidant effect (Burits et al., 2001). The aqueous extract of *A. afra* was screened for its phenolic profile as well as antioxidant activity (Sunmonu and Afolayan, 2012). A significant amount of polyphenolic compounds were revealed that significantly inhibited DPPH and ABTS radicals as well as ferric reductive ability in a concentration dependent manner.

2.3.3.2.5 Spasmolytic properties

The ethnanolic extract of *A. afra* leaves significantly reduced agonist-induced and spontaneously rhythmic contractions of isolated mouse duodenum as well as guinea pig ileum (Mulatu and Mekonnen, 2007).

2.3.3.2.6 Toxicity

The acute and chronic toxicity of *A. afra* was evaluated through *in vivo* tests in rodents (Mukinda and Syce, 2007). In the acute toxicity test, intraperitoneal administration induced a regular dose-dependent increase in mortality and the incidence of the adverse effects and general behaviour while a dose-dependent outcome was observed for the oral route. To evaluate chronic toxicity, oral doses of *A. afra* extract (0.1 or 1g/kg/day) was given to rodents for three months and no significant changes in biological or haematological parameters, body or organ weight or the general behaviour of the rats were noted.

2.3.3.2.7 Other

Artemisia afra was used as a model to design a placebo for crude materials (Dube et al., 2007). A preparation of isolated guinea-pig tracheal muscle was used to evaluate the muscle activity of the placebo. The results revealed that not only does *A. afra* possess muscle relaxant activity but no significant activity is possessed by the placebo compared to the leaves of *A. afra*.

Rapid NMR metabolomics approach combined with principle component analyses was used to test the product of a particular company in an attempt to verify whether the capsules contained artemisinin (Van der Kooy et al., 2008). The analyses confirmed the capsules were *A. afra* and *A. annua*. However, it indicated that even though *A. annua* was used in the capsules, the dosage of artemisinin was far too low to be effective. A metabolomics investigation of *A. afra* revealed there is no *in vitro* activity in the tea infusion of *A. afra* (Liu et al., 2010). With the aid of proton NMR and 2D-NMR, more than 25 polar compounds in *A. afra* were revealed including some prenylpropanoids.

2.3.4 *Asparagus capensis* (Asparagaceae)

There are several indigenous *Asparagus* species in southern Africa (van Wyk and Gericke, 2000). *A. capensis* is a rigid, branched thorny shrublet that often grows 40-80cm in height (Adamson and Salter, 1950). Long, white inflorescences and red globular berries are present on spine tipped branches (Adamson and Salter, 1950). The young shoots of *A. capensis* are harvested the same way as the real asparagus (*Asparagus officinalis)* and is known to be used as a vegetable (van Wyk and Gericke, 2000).

Neither pharmacological nor phytochemical investigations have been carried out on *A. capensis*.

2.3.5 *Carpobrotus edulis* (Aizoaceae or Mesembryanthemaceae)

Carpobrotus edulis is a fleshy succulent with smooth leaves that are erect, triangular in cross-section and often reddish-green in colour (van Wyk et al., 1997). It is highly astringent and can be applied topically to treat eczema, wounds as well as burns (Watt and Breyer-Brandwijk, 1962). It can also be used as an effective treatment against toothache, earache and oral and vaginal thrush (van Wyk et al., 1997).

2.3.5.1 Phytochemistry

2.3.5.1.1 Non-volatile secondary metabolites

Van der Watt and Pretorius (2001) reported that the leaves of *C. edulis* had high tannin content. Falleh et al. (2011a) reported that the total polyphenol content found within the leaves of *C. edulis* varied significantly between plant parts. The stems were found to be rich in polyphenols and contained the highest total flavonoid content. Quantitatively, the leaf extract showed a significantly higher concentration of phenolics compared to the stems and especially the roots.

The phytochemical analysis of four solvent extracts (hexane, ethanol, acetone and water) of *C. edulis* leaves displayed a high percentage of phenolics in the acetone extract and a considerable amount of alkaloids and proanthocyanidins in the aqueous extract (Omoruyi et al., 2012). Tannins and saponins were major constituents in the ethanol extract and flavonoids and flavonols were at a higher concentration in the hexane extract.

2.3.5.2 Pharmacological activity

2.3.5.2.1 Antimicrobial activity

The bioactive compounds isolated from *C. edulis* demonstrated noteworthy antibacterial activity against *Moraxella catharalis* (gram negative), *Staphylococcus epidermis* and *Staphylococcus aureus* (gram positive) (van der Watt and Pretorius, 2001). Hyperoside and neohesperidin were also active against *Pseudomonas aeruginosa* however, ferrulic acid was the only compound that displayed activity against *Bacillus subtilis* and gram positive *Streptococcus pneumonia*. Martins et al. (2005) revealed the methanol extract of *C. edulis*, which is inactive against the multi-drug resistant *Mycobacterium tuberculosis* or the methicillin-resistant-*Staphylococcus aureus,* is able to impede bacterial growth once phagocytosed by monocyte derived human macrophages.

The autumn and spring extracts of *Carpobrotus edulis* were evaluated against *Escherichia coli*, *Enterococcus faecalis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Chokoe et al., 2008). The spring extracts were more effective against all the test organisms having MIC values that were lower than the autumn extracts. However, the autumn extracts showed higher efficacy than the spring samples when the total activity was taken into account. Buwa and Afolayan (2009) reported that the leaves of *C. edulis* displayed activity against *M. aurum* A+. The ethanolic extract demonstrated strong activity against *B. cereus*, *S. aureus* and *M. aurum* but displayed weak activity against *E. coli* and *K. pnuemoniae*. The ethanolic extract exhibited the weakest activity compared to both the water and dichloromethane extracts.

The activities of numerous compounds isolated from *C. edulis* were evaluated against multidrug-resistant (MDR) bacteria (Martins et al., 2011). Oleanolic acid displayed good antibacterial activity against several bacterial strains with uvaol displaying the most effective modulation of efflux activity by MDR Gram-positive strains. Ibtissem et al. (2012) reported that *C. edulis* growing on the Tunisian coast displayed antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

2.3.5.2.2 Antioxidant activity

Chokoe et al (2008) evaluated the spring and autumn extracts of *Carpobrotus edulis* and reported the presence of an antioxidant compound in the ethyl acetate, acetone and methanol extract of *C. edulis* which was particuarly evident in the autumn extracts. Ibtissem et al. (2012) evaluated the antioxidant ability of *C. edulis* and *Mesembryanthemum crystallinum* growing along the Tunisian coast and reported that *C. edulis* demonstrated higher antioxidant activity in the DPPH assay. The antioxidant activity of *C. edulis* (concentration up to 2mg/ml) was higher than that of BHA, a synthetic oxidant and the high antioxidant activity can reportedly be related to the high amount of flavonoids and phenols present in *C.edulis*. Falleh et al (2011a) characterised the antioxidant properties and phenolic compounds of *C. edulis* in the leaf, stem and root. The total antioxidant activity varied greatly in the organs with the results demonstrating that the activity was very high in the aerial parts compared to the roots. The aerial antiradical activity was significantly higher than the positive control used.

In the same year, Falleh et al. (2011b) used LC/ESI-MS/MS to characterise *Carpobrotus edulis* fractions that displayed the best antioxidant activity. The results revealed that the methanol root extract (40%), the methanol leaf extract (40%) as well as the methanol stem extract demonstrated the most promising scavenging activity against DPPH and ABTS radicals. Significant differences occurred among fractions of *C. edulis* with regard to the LC/ESI-MS/MS identification of phenols. Omoruyi et al. (2012) also evaluated the antioxidant ability of *C. edulis* and reported that the aqueous and ethanol leaf extracts of *C. edulis* displayed the best antioxidant activity.

2.3.5.2.3. Antiproliferative activity

Martins et al. (2010) reported that all compounds isolated from *C. edulis* had antiproliferative activity on both cell lines. The antiproliferative effect of catechin, oleanolic acid and uvaol were more distinct on the parental cell line. The multidrug resistant cell line was sensitive to monogalactosydiacylglycerol (MDGD). The antiproliferative effect of β-amyrin and epicatechin was not significantly different in the two cell lines. Uvaol was the most effective as well as promising compound in the reversal of multidrug resistance in MDR lymphoma cell line. This is the first time the inhibitory activity of uvaol, oleanolic acid, β-amyrin and MDGD were revealed.

2.3.5.2.4 Neurological activity

Carpobrotus edulis displayed anti-cholinesterase activity against both acetylcholinesterase and butylcholinesterase (Custódio et al., 2012). It was reported that *C. edulis* can be considered a possible candidate for novel as well as alternative therapies in the treatment of neurological disorders associated with low levels of acetylcholine in the brain.

2.3.5.2.5 Other

The root profiles as well as the competition of exotic, invasive perennial *Carpobrotus edulis* and two native shrubs in the Californian coast were evaluated (D'Antonio and Mahall, 1991). *C. edulis* significantly affected the water relations as well as shoot sizes and overall morphology of both *Haplopappus ericoides* and *Haplopappus venerus* var. *sedoides.* Van

Grunsven et al. (2009) reported that success of *Carpobrotus* species found in the Mediterranean basin may be assisted by a reduced negative net impact of the soil. Morphological as well as karyological comparisons between South African *C. edulis* and *Carpobrotus acinaformis* and their invasive counterparts in Provence were determined (Verlaque et al., 2011). Both taxa emphasized unexpected chromosomal restructuring, a distinct decrease in symmetry and increase in intraspecific variability as well as an interspecific convergence of karyotypes. This suggests that a drift enabled various crosses which has amplified through introgression or hybridization.

2.3.6 *Cliffortia odorata* (Rosaceae)

Cliffortia odorata, commonly known as "Wildewingered", is a shrub with simple, hairy leaves growing 1-2m in height (Adamson and Salter, 1950).

Whitehouse and Fellingham (2007) described seven new species of *Cliffortia* L. endemic to the Cape Floristic region. The species described are *C. anthospermoides*, *C. cruciata*, *C. ferricola*, *C. gracillima*, *C. perpendicularis*, *C. sparsa* and *C. weimarckii*. A further species from Graaf Reinet is also described.

Neither pharmacological nor phytochemical investigations have been carried out on *C. odorata*.

2.3.7 *Leonotis leonurus* (Lamiaceae)

Leonotis leonurus is a shrub that grows between two and five metres high with a thick base and pale, brown branches (van Wyk et al., 1997). Bright orange, tubular flowers grow on the stem with leaves arranged opposite each other (van Wyk et al., 1997). All parts of the plant have a strong scent (van Wyk et al., 1997). Internally decoctions are used to treat bronchitis, high blood pressure and headaches (Watt and Breyer-Brandwijk, 1962).

2.3.7.1 Phytochemistry

2.3.7.1.1 Volatile secondary metabolites

Cragg and Little (1972) reported that the leaves of *L. leonurus* contained mostly labdane diterpenoids. The volatile oil from fresh, airdried, oven dried and sun dried aerial parts of *L. leonurus* were analysed and a total of 34 compounds were isolated (Asekun et al., 2005). Cis- and trans-p ocimene (14.2-27.1%), o-pinene (14.7-16.4%), germacrene D (10.4%-12.9%) and caryophyllene oxide (5.3-9.0%) were the main components present in each sample. The monoterpenoids, although fewer in number than the sesquiterpenoids, were predominant in their oil content. Limonene was also present in relevant quantities in oils from dried plant materials but absent in oils from the fresh plant material. Wu et al. (2012) established the absolute configuration of leoleorins A and D by X-ray crystallographic analyses. All isolated compounds exhibited inhibition in excess of 50% at various central nervous system (CNS) receptors with Leoleorin C displaying moderate binding affinity for Sigma 1 receptor.

2.3.7.1.2 Non-volatile secondary metabolites

HPLC and phytochemical tests revealed the presence of saponins, alkaloids and tannins in the water extracts of *L. leonurus* (Bienvenu et al., 2002). El-Ansari et al. (2009) reported that preliminary phytochemical screening of *L. leonurus* displayed the presence of sterols, terpenes, tannins, flavonoids, alkaloids and saponins as well as ten flavonoids isolated from flowering aerial parts. In the same year, Agnihotri et al. (2009) carried out another study evaluating the flowering tops of *L. leonurus* which yielded a new diterpene ester, 1,2,3-trihydroxy-3,7,11,15-tetramethylhexadecan-1-yl-palmitate including five known metabolites. Oyedemi et al. (2011) revealed the presence of flavonoids, phenolics, saponins as well as tannins in the aqueous extract of *L. leonurus* leaf. Quanititative analysis of the aqueous leaves extract revealed high phenolic (48mg/g tannic acid equivalent) and flavonoid (4.8mg/g quercetin equivalent) content.

2.3.7.2 Pharmacological activity

2.3.7.2.1 Analgesic potential

The analgesic potential of the water extract of *L. leonurus* was evaluated in rats (Maphosa et al., 2012). All doses of *L. leonurus* (50, 100 and 200mg/kg) as well as indomethacin (10mg/kg), the reference drug, caused a decrease in writhes compared to the control. The aqueous extract (50, 100 and 200mg/kg) and indomethacin (10mg/kg) displayed high inhibition of 96.2, 100, 100 and 100%, respectively.

2.3.7.2.2 Anticonvulsant activity

The water extract of *L. leonurus* at a dose of 200 and 400mg/kg protected 37.5% and 50% of the mice used while significantly delaying pentylenetetrazole-induced tonic seizures (Bienvenu et al., 2002). The same dose of *L. leonurus* extract caused a significant delay in the onset of seizures produced by N-methyl-DL-aspartic acid (400mg/kg) and picrotoxin (8mg/kg). However, neither of the doses of *L. leonurus* aqueous extract altered seizures induced by bucuculline (20mg/kg) to any significant degree.

2.3.7.2.3 Anti-diabetic activity

The antidiabetic properties of aqueous extract of *L. leonurus* in streptozotocin induced diabetic rats were investigated with the results suggesting that *L. leonurus* possessed antihyperglycaemic as well as antihyperlipidaemic potential (Ojewole, 2003). Oral administration of the plant extract at 125, 250 and 500mg/kg for 15 days lowered the blood glucose level, feed and water intake (Oyedemi et al., 2011).

2.3.7.2.4 Anti-inflammatory potential

The aqueous extract from the leaves of *L. leonurus* possessed significant anti-oedematogenic (P>0.05) effect on carrageenan-induced paw oedema (Maphosa et al., 2012). This effect was dose dependent and comparable to indomethacin, the reference drug.

2.3.7.2.5 Antimalarial activity

Agnihotri et al. (2009) evaluated the antimalarial activity of six compounds from the flowering tops of *L. leonurus*. Luteolin 7-O-β-$_{D}$-glucopyranoside displayed antimalarial activity.

2.3.7.2.6 Antimicrobial activity

L. leonurus exhibited MIC values of >4mg/ml against *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli* and *Streptococcus pyogenes* (Steenkamp et al., 2004). Kamatou et al. (2006) tested the effects of the aerial parts of *L. leonurus* in combination with *Salvia chamelaeagnea* against two Gram positive bacteria, *Bacillus cereus* and *S. aureus* as well as *E. coli* and *Klebsiella pneumoniae* (Kamatou et al., 2006). Independently, the plants displayed good antibacterial activity against the four pathogens tested. In combination, synergistic actions were observed against the Gram-positive bacteria however antagonism, synergism and/or additive actions were observed for the various ratios tested on the Gram-negative bacteria. The flowering tops of *L. leonurus* yielded a new diterpene ester with 5 known metabolites (Agnihotri et al., 2009). None of these evaluated compounds had antimicrobial activity against *Candida albicans, E. coli, P. aeruginosa, Candida neoformans, Mycobacterium intracellulare* and *Aspergillus fumigatus*.

2.3.7.2.7 Cardiovascular activity

The cardiovascular effect of *L. leonurus* was evaluated by using invasive and non-invasive techniques to determine the arterial blood pressure as well as the heart rate in normal and spontaneously hypertensive rats (Ojewole, 2003). A significant, dose-related decrease ($P>0.005$-0.001) was noted in the arterial blood pressure and heart rate in normal, spontaneously hypertensive and anaesthetized rats administered 25-800mg/kg aqueous extract intravenously. The hypotensive effect was reportedly more distinct in the hypertensive rats than in the normal ones. The aqueous leaf extract (25-800mg/kg) administered intraperitoneally similarly decreased, in a dose dependent manner, the heart rates and arterial blood pressures of normal, conscious and spontaneously hypertensive rats (Ojewole, 2003). Kenechukwa (2004) confirmed that *Leonotis leonurus* contains cardioactive compounds with specific cardiovascular activity depending on dose and preparation administered. Mnonopi et al. (2011) reported marrubiin, a diterpenoid present in *L. leonurus* as well as the organic extract of *L. leonurus*, suppressed coagulation, platelet aggregation as well as inflammatory markers. Oyedemi et al. (2011) reported that continuous administration of water extract of the leaves of *L. leonurus* and glibenclamide for 15 days reduced the level of cholesterol, HDL and triglycerides. However, a rise in LDL was detected at certain doses.

2.3.7.2.8 Toxicity

Maphosa et al. (2008) evaluated the acute, sub-acute and chronic toxicity of the aqueous extract of *L. leonurus* leaves in female rats. A high dose as well as prolonged use of *L. leonurus* was reported to be toxic to haematological parameters, white blood cells and various organs as well as fatality. Agnihotri et al. (2009) reported that none of the compounds isolated from the flowering tops of *L. leonurus* displayed cytotoxic effects towards mammalian kidney fibroblast at a concentration of 4.76μg/mL.

El-Ansari et al. (2009) reported that both 70% methanol and chloroform extract of *L. leonurus* flowering aerial parts was found to be safe for further biological studies as no lethality was observed even at 5000mg/100g body weight per ora in rats. This was the first study to show the hepatoprotective activity of *L. leonurus* using the paracetamol induced hepatotoxicity method in rats. The 70% methanol extract of *L. leonurus* displayed no cytotoxic activity against human tumour cell lines at the selected extract concentrations (0.1, 2.5, 5 and 10μg/ml) (El-Ansari et al., 2009). In another study, Oyedemi et al. (2010)

evaluated the water extract of *L. leonurus* leaves in male and female rats and reported that alterations occurred in the liver as well as kidney function indices which revealed parameter and dose-selective risk when consumed repeatedly on a daily basis at 125, 250 and 500mg/kg body weight for 21 days.

2.3.8 Pelargonium capitatum (Geraniaceae)

Pelargonium capitatum is a shrubby, hairy plant with lobed leaves and attractive pink flowers (Viljoen et al., 1995). It can be regarded as one of the important species of *Pelargonium* used in the cultivation of hybrids from which the important geranium oil is extracted (Viljoen et al., 1995).

2.3.8.1 Phytochemistry

2.3.8.1.1 Volatile secondary metabolites

The variation and morphology of *P. capitatum* was investigated and the main constituents reported were α-pinene, α-phellandrene, p-cymene, y-terpinene, β-caryophyllene, guaua-6, 9-diene, germacrene D, citronellyl formate and an unidentified sesquiterpene (Demarne et al., 1993). Viljoen et al. (1995) investigated the essential oil composition of 40 populations and popular chemotypes were identified within *P. capitatum*. High levels of essential oil variation were displayed allowing the species to be divided into eight chemotypes.

2.3.8.2 Pharmacological activity

2.3.8.2.1 Other

A transformation system using leaf discs of *Pelargonium* x *hortorum* and *P. capitatum* was developed for the first time (Hassaneine et al., 2005). This will enable evaluation of gene function or improve geraniums. Hassanein and Dorion (2005) established a plant regeneration system for zonal and scented geraniums using leaf discs. Eiasu et al. (2008) reported that conditions of high soil water availability followed by brief water stress just before harvesting would maximise the oil yield of *P. capitatum*. Eiasu et al. (2009) observed that water conservation devoid of significant reduction in essential oil yield requires growing rose scented geranium in loamy, sandy clay soil with the maximum reduction level of available soil water (40%) within the 0.8m root zone.

2.3.9 Pelargonium reniforme (Geraniaceae)

Pelargonium reniforme Curtis. is a shrublet of up to 1-m, has kidney-shaped leaves and pink flowers (Dreyer et al., 1995). This species of *Pelargonium* is indigenous to the Eastern Cape Province and occurs mainly in coastal regions (Van der Walt et al., 1988).

2.3.9.1 Phytochemistry

2.3.9.1.1 Volatile secondary metabolites

Kayser et al. (1998) detected approximately 230 components in *P. reniforme* and *Pelargonium sidoides* of which 81 (*P. reniforme*) and 102 (*P. sidoides*) could be clearly identified. Sesquiterpenes were the dominating components in both species. The oil of *P. reniforme* contained relatively high levels of sesquiterpene hydrocarbons such as α=muurolene, cyclosativene, calamenene, β-selinene and δ-selinene which were not identified in *P. sidoides*. Fairly high levels of γ-cadinene and δ-cadinene were also present in *P. reniforme*.

2.3.9.1.2 Non-volatile secondary metabolites

Wagner and Bladt (1975) isolated 7-hydroxy-5,6-dimethoxycoumarin, its monoethyl ether and its 7-0-glucoside for the first time from the roots of *P. reniforme*. Latté et al. (2002) reported for the first time the isolation as well as structural elucidation of natural occurring *0*-Galloyl-*C*-glycosyl flavones and the flavonoid patterns of the aerial as well as roots of *P. reniforme*. Latté et al. (2008) isolated and characterised an unprecedented diterpene ester, ellagitannin, n-butyl gallate and two aryltetralin lignans.

2.3.9.2 Pharmacological activity

2.3.9.2.1 Antimicrobial activity

Kayser and Kolodziej (1997) reported that the crude extract of the root of *Pelargonium reniforme* was moderately active against all the bacteria tested which included *Escherichia coli*, *Proteus mirabilis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Haemophilus influenza, Staphylococcus aureus,* beta-hemolytic *Streptococcus* and *Streptococcus pneumoniae.* The highest activity was in the aqueous phase with distinct effects against *E. coli*, *K. pnuemoniae* and β-hemolytic *streptococcus* 1451. All the compounds displayed antibacterial activity with exception of ineffective (+)-catechin (Kayser and Kolodziej, 1997).

In an initial antibacterial evaluation, the lipophilic hexane extract of the root of *P. reniforme* demonstrated the most pronounced antimycobacterial effect particularly against *Mycobacterium aurum* (MIC 64 mg/l) (Seidel and Taylor, 2004). Oleic acid, linoleic acid and palmitic acid were the predominant compounds with linoleic acid reportedly the most potent compound against *M. aurum* (MIC 2mg/l).

P. reniforme in combination with *P. sidoides* exhibited activity against *Moraxella catarrhalis, Streptococcus pneumoniae* and *Haemophilus influenza* (Seidel and Taylor, 2004). Mativandlela et al. (2006) reported that the ethanol and acetone extracts of *P. reniforme* inhibited *Aspergillus niger* and *Fusarium oxysporum* growth. The ethanol extract of *P. reniforme* inihibited *Rhizopus stolonifer* whereas the chloroform, ethanol and acetone extract of *P. reniforme* exhibited activity against *Mycobacterium tuberculosis*.

2.3.9.2.2 Antioxidant activity

The antioxidant ability of hydrolysable tannins and flavonoids isolated from *P. reniforme* was evaluated (Latté and Kolodziej, 2004). The polyphenols exhibited higher radical scavenging ability compared to the reference antioxidant, ascorbic acid, in both the DPPH and chemiluminescence assay. Tannins were observed to have more antioxidant potential compared to flavonoids (Latté and Kolodziej, 2004).

2.3.9.2.3 Hepatoprotective potential

Adewusi (2009) evaluated the hepatoprotective as well as curative potential of *P. reniforme* on alcohol-induced liver damage in Wistar rats. The results revealed the potential of *P. reniforme* extract to protect liver cells and enhance recovery from tissue damage.

2.3.9.2.4 Toxicity

The toxicity of oral administration of aqueous *P. reniforme* roots at 100, 200 and 400mg/kg was determined in male Wistar rats (Adewusi, 2009). No significant change was seen in the white blood cell count, mean corpuscular haemoglobin concentration, basophils, neutrophils or albumin, cholesterol, high density lipoprotein cholesterol, gamma glutamyl transferase or aminotransferase levels or organ body-weight ratio of the rats. However, theuric acid, chloride and alkaline phosphatase levels were significnatly reduced and red blood cell count, haemoglobin, platelets, lymphocytes, total proteins, sodium and globulin levels were significantly increased. The plant extract is not toxic at the dosages evaluated.

2.3.10 Salvia africana-caerulea (Lamiaceae)

Salvia africana-caerulea is a branched shrub which grows up to 1m high and has green, leathery leaves and blue flowers (Adamson and Salter, 1950). *S. africana-caerulea* is known as “Bloublomsalie” and infusions are often used to treat colds and flu, bronchitis as well as abdominal cramps and indigestion (Kamatou et al., 2008).

2.3.10.1 Phytochemistry

2.1.10.1.1 Volatile secondary metabolites

Kamatou (2006) revealed that the essential oil yield for *S. africana-caerulea* collected in the South Western Cape was 0.12% with major components including spathulenol (29.1%), β-carophyllene oxide (14.6%) and ledol (6.5%). *S. africana-caerulea* and *S. lanceolata* were two species that displayed a very high correlation regarding the essential oil. Reports indicated that major fluctuations were observed in *S. africana-caerulea* composition which includes limonene (2-33%) and viridifloral (2-24%).

2.3.10.2 Pharmacological activity

2.3.10.2.1 Antimicrobial activity

Kamatou (2006) reported *S. africana-caerulea* displayed moderate antibacterial activity against *E. coli*, *K. pnuemoniae*, *B. cereus* and *S. aureus*.

2.3.10.2.2 Antiplasmodial activity

S. africana-caerulea, harvested in winter, displayed the most favourable antiplasmodial activity (Kamatou et al., 2006)

2.3.10.2.3 Anticarcinogenic activity

In the cancer assay, *S. africana-caerulea* was one of two extracts that were most active against MCF-7 and SF-268 cell lines (Kamatou, 2006).

2.3.10.2.4 Antioxidant activity

The antioxidant activity was measured using the DPPH and ABTS scavenging assays (Kamatou, 2006). Moderate antioxidant activity was observed for *S. africana-caerulea.*

2.3.10.2.5 Toxicity

In a toxicity evaluation, the student t-test revealed that the solvent extracts of *S. africana-caerulea* as well as *Salvia stenophylla* were similar and the most toxic (Kamatou, 2006).

2.3.10.2.6 Other

Scanning electron microscopy revealed non-glandular trichome, stalked capitate trichomes and sessile peltate trichomes on the lower leaf surface of *S. africana-caerulea* (Kamatou, 2006). The sessile peltate trichomes found on the lower leaf surface were covered by an intact and rigid cuticle and non-glandular unbranched multicellular trichomes. Many glandular trichomes were observed on the leaves of *S. africana-caerulea* (Kamatou, 2006).

2.3.11 Vernonia oligocephala (Asteraceae)

Vernonia oligocephala is a herbaceous perennial with erect, flowering branches that develop from a woody rootstock (van Wyk et al., 1997). The leaves are elliptic in shape and not usually longer than it is broad with a sharp point and a very short stalk (van Wyk et al., 1997). Infusions are taken to treat colic, rheumatism, diabetes and dysentery (Watt and Breyer-Brandwijk, 1962; Hutchings, 1996).

2.3.11.1 Phytochemistry

2.3.11.1.2 Non-volatile secondary metabolites

Bohlmann et al. (1978) discovered sesquiterpene lactones from several *Vernonia* species. Spectroscopic methods and chemical transformations were used to interpret the structures. Three new guaianolides were reported and two new derivatives of costunolide were present in two species. The investigation of *Vernonia oligocephala*, *V. sutherlandii* and *V. adoensis* produced minute amounts of two monoepoxides, three diepoxides and five new glaucolides (Bohlmann et al., 1984).

In summary, good attempts have been made in documenting the medicinal plants used for various ailments in the different provinces. This will ensure the preservation of medicinal knowledge for many years to come. Although ethnobotanical surveys have been carried out in different provinces, surveys documenting plants used for specific ailments would also be beneficial as all the plants are likely to be mentioned. Good attempts have been made in the

Eastern Cape as well as Kwa-Zulu Natal region regarding ailment-specific ethnobotanical surveys. These ethnobotanical surveys are mandatory to assist the identification of plants with possible medicinal potential which could undergo further screening.

Elemental analysis of medicinal plants is lagging behind in contrast to phytochemical screening. An effort should be made to screen these plants as knowledge of the concentration of particular elements within the plants is essential. In doing so, a possibility exists to associate predominant elements to their biological activities. Phytochemical screening has focused predominantly on the volatile secondary metabolites. The isolation of the compounds found within the oils by steam distillation has been shown to have antibacterial and antifungal properties (Lopes-Lutz et al., 2008). However, the presence of non-volatile metabolites could indicate whether the plant will be medicinally beneficial or if toxic compounds may be present. The biological activity of certain plants such as *Artemisia afra* and *Leonotis leonurus* has been well documented however certain plants require more attention. The identification of potential medicinal plants to undergo phytochemical screening or elemental analysis will enable the verification of biological activity and in doing so, justify its use by the communities in South Africa, as well as the rest of the world.

REFERENCES

Adamson, R.S., Salter, T.M., 1950. Flora of the Cape Peninsula. Juta & Co., Ltd. Cape Town and Johannesburg.

Adewusi, E.A., 2009. Evaluation of the effect of *Pelargonium reniforme* Curtis extract on alcohol-induced liver damage in Nkonkobe Municipality, Eastern Cape Province, South Africa. M.Sc Dissertation, University of Fort Hare.

Afolayan, A.J., Sunmonu, T.O., 2011. *Artemisia afra* Jacq. Ameliorates Oxidative Stress in the Pancreas of Streptozotocin-Induced Diabetic Wistar Rats. Biosci. Biotechnol. Biochem 75(11), 2083-2086.

Agnihotri, V.K., ElSohly, H.N., Smillie, T.J., Khan, I.A., Walker, L.A., 2009. Constituents of *Leonotis leonurus* flowering tops. Phytochemistry Letters 2, 103-105.

Ajasa, A.M.O., Bello, M.O., Ibrahim, A.O., Ogunwande, I.A., Olawore N.O., 2004. Heavy trace metals and macronutrients status in herbal plants of Nigeria. Food Chemistry 85, 67-71.

Appidi, J.R., Grierson, D.S., Afolayan, A.J., 2008. Ethnobotanical Study of Plants Used for the Treatment of Diarrhoea in the Eastern Cape, South Africa. Pakistan Journal of Biological Sciences 11(15), 1961-1963.

Asekun, O.T., Grierson, D.S., Afolayan, A.J., 2005/2006. Influence of Drying Methods on the Chemical Composition and Yield of the Essential Oil of *Leonotis Leonurus*. Journal of Sci. Res. Dev., 10, 61-64.

Beppu, H., Koike, T., Shimpo, K., Chihara, T., Hoshino, M., Ida, C., Kuzuya, H., 2003. Radical-scavenging effects of *Aloe arborescens* Miller on prevention of pancreatic islet B-cell destruction in rats. Journal of Ethnopharmacology 89, 37-45.

Beppu, H., Kawai, K., Shimpo, K., Chihara, T., Tamai, I., Ida, C., Ueda, M., Kuzuya, H., 2004. Studies on the components of *Aloe arborescens* from Japan - monthly variation and differences due to part and position of the leaf. Biochemical Systematics and Ecology 32, 783-795.

Beppu, H., Shimpo, K., Chihara, T., Kaneko, T., Tamai, I., Yamaji, S., Ozaki, S., Kuzuya, H., Sonoda, S., 2006. Antidiabetic effects of dietary administration of *Aloe arborescens* Miller components on multiple low-dose streptozotocin-induced diabetes in mice: Investigation on hypoglycaemic action and systemic absorption dynamics of aloe components. Journal of Ethnopharmacology 103, 468-477.

Bienvenu, E., Amabeoku, G.J., Eagles, P.K., Scott, G., Springfield, E.P., 2002. Anticonvulsant activity of aqueous extract of *Leonotis leonurus*. Phytomedicine 9, 217-223.

Bisi-Johnson, M.A., Obi, C.L., Kambizi, L., Nkomo, M., 2010. A survey of indigenous herbal diarrhoeal remedies of O.R. Tambo district, Eastern Cape Province, South Africa. Full Length Research Paper. African Journal of Biotechnology 9(8), 1245-1254.

Blommaert, K.L.J., Bartel, E., 1976. Chemotaxonomic aspects of the buchu species *Agathosma betulina* (Pillans) and *Agathosma crenulata* (Pillans) from local plantings. Journal of South African Botany 42(2), 121-126.

Bohlmann, F., Zdero, C., 1972. Polyacetylenic compound. CCIV. Constituents of *Artemisia afra*. Phytochemistry 11, 2329-2330.

Bohlmann, F., Brindöpke, G., Rastogi, R.C., 1978. A new type of germacrolide from Vernonia species. Phytochemistry 17(3), 475-482.

Bohlmann, F., Scheidges, C., Misra, L.N., Jakupovic, J., 1984. Further glaucolides from South African *Vernonia* species. Phytochemistry 23(8), 1795-1798.

Burits, M., Asres, K., Bucar, F., 2001. The Antioxidant Activity of the Essential Oils of *Artemisia afra*, *Artemisia abyssinica* and *Juniperus procera*. Phytotherapy Research 15, 103-108.

Buwa, L.V., Afolayan, A.J., 2009. Antimicrobial activity of some medicinal plants used for the treatment of tuberculosis in the Eastern Cape Province, South Africa. African Journal of Biotechnology 8(23), 6683-6687.

Campbell, W.E., Majal, T., Bean, P.A., 1986. Coumarins of the Rutoideae: tribe Diosmeae. Phytochemistry 25(3), 655-657.

Campbell, W.E., Finch, K.P., Bean, P.A., Finkelstein, N., 1987. Alkaloids of the Rutoideae: tribe Diosmeae. Phytochemistry 26(2), 433-434.

Chiban, M., Soudani, A., Sinan, F., Persin, M., 2011. Single, binary and multi-component adsorption of some anions and heavy metals on environmentally friendly *Carpobrotus edulis* plant. Original Research Article. Colloids and Surfaces B: Biointerfaces 82, 267-276.

Chokoe, P.K., Masoko, P., Mokgotho, M.P., Howard, R.L., Mampuru, L.J., 2008. Does seasonal variation influence the phytochemical and antibacterial properties of *Carpobrotus edulis*? Full Length Research Paper. African Journal of Biotechnology 7(22), 4164-4171.

Collins, N.F., Graven, E.H., 1996. Chemotaxonomy of commercial buchu species (*Agathosma betulina* and *A.crenulata*). Journal of Essential Oil Research 8, 229-335.

Coopoosamy, R.M., Naidoo, K.K., 2012. An ethnobotanical study of medicinal plants used by traditional healers in Durban, South Africa. Full Length Research Paper. African Journal of Pharmacy and Pharmacology 6(11), 818-823.

Corrigan, B.M., Van Wyk, B.-E., Geldenhuys, C.J., Jardine, J.M., 2011. Ethnobotanical plant uses in the KwaNdibela Peninsula, St Lucia, South Africa. South African Journal of Botany 77, 346-359.

Cragg, G.M.L., Little, G.E., 1962. The extractives of *Leonotis leonurus*. Journal of the South African Chemical Institute 15, 29-30.

Custódio, L., Ferreira, A.C., Pereira, H., Silvestre, L., Vizetto-Duarte, C., Barreira, L., Rauter, A.M., Alberício, F., Varela, J., 2012. The marine halophytes *Carpobrotus edulis* L. and *Arthrocnemum macrostachyum* L. are potential sources of nutritionally important PUFAs and metabolites with antioxidant, metal chelating and anticholinesterase inhibitory activities. Botanica Marina 55, 281-288.

D'Antonio, C.M., Mahall, B.E., 1991. Root profiles and Competition between the Invasive, Exotic Perrenial, Carpobrotus edulis, and Two Native Shrub Species in California Coastal Shrub. American Journal of Botany 78(7), 885-894.

Demarne, F.-E., Viljoen, A.M., Van der Walt, J.J.A., 1993. A Study of the Variation in the Essential Oil and Morphology of *Pelargonium capitatum* (L.) L'Hérit. (Geraniaceae). Part 1. The composition of the Oil. The Journal of Essential Oil Research 5(5), 493-499.

De Wet, H., Nkwanyana, M.N., van Vuuren, S.F., 2010. Medicinal plants used for the treatment of diarrhoea in northern Maputaland, KwaZulu-Natal Province, South Africa. Journal of Ethnopharmacology 130, 284-289.

Dreyer, L.L., Marais, E.M., van der Walt, J.J.A., 1995. A subspecific division of *Pelargonium reniforme* CURT. (Geraniaceae). S. Afr. J. Bot 61, 325-330.

Dube, A., Manthata, L.N., Syce, J.A., 2007. The Design and Evaluation of Placebo Material for Crude Herbals: *Artemisia afra* Herb as a Model. Phytotherapy Research 21, 448-451.

Dyubeni, L., Buwa, L.V., 2012. An ethnobotanical study of plants used for the treatment of ear, nose and throat (ENT) infections in Nkonkobe Municipality, South Africa. Full Length Research Paper. Journal of Medicinal Plants Research 6(14), 2721-2726.

Eiasu, B.K., Soundy, P., Steyn, J.M., 2008. High Irrigation Frequency and Brief Water Stress Before Harvest Enhances Essential Oil Yield of Rose-scented Geranium (*Pelargonium capitatum* x *P. radens*). HortScience 43(2), 500-504.

Eiasu, B.K., Steyn, J.M., Soundy, P., 2009. Rose-scented geranium (*Pelargonium capitatum* x *P. radens*) growth and essential oil yield response to different soil water depletion regimes. Agricultural Water Management 96, 991-1000.

El-Ansari, M.A., Aboutabl, E.A., Farrag, A.R.H., Sharaf, M., Hawas, U.W., Soliman, G.M., El-Seed, G.S., 2009. Phytochemical and pharmacological studies on *Leonotis leonurus*. Pharmaceutical Biology 47(9), 894-902.

Erasto, P., Adebola, P.O., Grierson, D.S., Afolayan, A.J., 2005. An ethnobotanical study of plants used for the treatment of diabetes in the Eastern Cape Province, South Africa. African Journal of Biotechnology 4(12), 1458-1460.

Falleh, H., Ksouri, R., Medini, F., Guyot, S., Abdelly, C., Magné, C., 2011a. Antioxidant activity and phenolic composition of the medicinal and edible halophyte *Mesembryanthemum edule* L. Industrial Crops and Products 34, 1066-1071.

Falleh, H., Oueslati, S., Guyot, S., Dali, A.B., Magné, C., Abdelly, C., Ksouri, R., 2011b. LC/ESI-MS/MS characterisation of procyanidins and propelargonidins responsible for the strong antioxidant activity of the edible halophyte *Mesembryanthemum edule* L. Food Chemistry 127, 1732-1738.

Fluck, A.A.J., Mitchell, W.M., Perry, H.M., 1961. Comparison of buchu leaf oil. Journal of the Science and Food Agriculture 12, 290-292.

Fuchs, S., Sewenig, S., Mosandl, A., 2001. Monoterpene biosynthesis in *Agathosma crenulata* (Buchu). Journal of Flavour and Fragrance 16, 123-135.

Furukawa, F., Nishikawa, A., Chihara, T., Shimpo, K., Beppu, H., Kuzuya, H., Lee, I-S., Hirose, M., 2002. Chemopreventive effects of *Aloe arborescens* on *N*-nitrosobis(2-oxopropyl)amine-induced pancreatic carcinogenesis in hamsters. Cancer Letters 178, 117-122.

Goldblatt, P., Manning, J., 2000. Cape Plants: A conspectus of the Cape Flora of South Africa. Strelitzia 9. National Botanical Institute, Cape Town.

Goodson, J.A., 1922. Constituents of the flowering tops of *Artemisia afra*, Jacq. Biochemical Journal 16, 489-493.

Graven, E.H., Webber, L., Venter, M., Gardner, J.B., 1990. The Development of *Artemisia afra* (Jacq.) as a New Essential Oil Crop. The Journal of essential oil research 2, 215-220.

Grierson, D.S., Afolayan, A.J., 1999. An ethnobotanical study of plants used for the treatment of wounds in the Eastern Cape, South Africa. Journal of Ethnopharmacology 67, 327-332.

Guantai, A.N., Addae-Mensah, I., 1999. Cardiovascular Effect of *Artemisia Afra* and its Constituents. Pharmaceutical Biology 37(5), 351-356.

Gundidza, M., 1993. Antifungal activity of essential from *Artemisia afra* Jacq. Central African Journal of Medicine 39(7), 140-142.

Gutterman, Y., Chauser-Volfson, E., 2000a. The distribution of the phenolic metabolites barbaloin, aloeresin and aloenin as a peripheral defense strategy in the succulent leaf parts of *Aloe arborescens*. Biochemical Systematics and Ecology 28, 825-838.

Gutterman, Y., Chauser-Volfson, E., 2000b. Peripheral defence strategy: variation of barbaloin content in the succulent leaf parts of *Aloe arborescens* Miller (Liliaceae). Botanical Journal of the Linnean Society 132, 385-395.

Hassanein, A., Dorion, N., 2005. Efficient plant regeneration system from leaf discs of zonal (*Pelargonium* x *hortorum*) and two scented (*P. capitatum* and *P. graveolens*) geraniums. Plant Cell, Tissue and Organ Culture 83, 231-240.

Hassanein, A., Chevreau, E., Dorion, N., 2005. Highly efficient transformation of zonal (*Pelargonium* x *hortorum*) and scented (*P.capitatum*) geraniums via *Agrobacterium tumefaciens* using leaf discs. Plant Science 169, 532-541.

Hirata, T., Suga, T., 1977. Biologically active constituents of leaves and roots of *Aloe arborescens* var. natalensis. Z Naturforsch C 32(9-10), 731-734.

Huffman, A., Klepser, M.E., Ernst, E.J., Keele, D., Roling, E., Viljoen, A.M., 2002. Susceptibility of Opportunistic Bacteria and Yeasts to the Volatile Oils of *Artemisia afra*, *Lippia javanica*, *Lippia scaberrima*, *Myrothamnus flabellifolius* and *Osmitopsis asteriscoides*. Journal of Infectious Disease Pharmacotherapy 5(4), 43-50.

Hutchings, A., Scott, A.H., Lewis, G., Cunningham, A.B., 1996. Zulu medicinal plants. An inventory. University of Natal Press, Pietermaritzburg.

Ibtissem, B., Abdelly, C., Sfar, S., 2012. Antioxidant and Antibacterial Properties of *Mesembryanthemum crystallinum* and *Carpobrotus edulis* Extracts. Advances in Chemical Engineering and Science 2, 359-365.

Igoli, J.O., Ogaji, O.G., Tor-Anyiin, T.A., Igoli, N.P., 2005. Traditional medicine practice amongst the Igede people of Nigeria. Part II. Afr. J. Trad. CAM 2, 134-152.

Jakupovic, J., Klemeyer, H., Bohlmann, F., Graven, E.H., 1988. Glaucolides and guaianolides from *Artemisia afra*. Phytochemistry 27(4), 1129-1133.

Jimoh, F.O., Adedapo, A.A., Afolayan, A.J., 2010. Comparison of the nutritional value and biological activities of the acetone, methanol and water extracts of the leaves of *Solanum nigrum* and *Leonotis leonurus*. Food and Chemical Toxicology 48, 964-971.

Kaiser, R., Lamparsky, D., Schudel, P., 1975. Analysis of buchu leaf oil. J. Agric. Food Chem 23(5), 943-950.

Kamatou, G.P.P., 2006. Indigenous Salvia Species − An investigation of their pharmacological activities and phytochemistry. Ph.D Dissertation, University of the Witwatersrand.

Kamatou, G.P.P., Viljoen, A.M., van Vuuren, S.F., van Zyl, R.L., 2006. *In vitro* evidence of antimicrobial synergy between *Salvia chamelaeagnea* and *Leonotis leonurus*. Short communication. South African Journal of Botany 72, 634-636.

Kamatou, G.P.P., Makunga, N.P., Ramogola, W.P.N., Viljoen, A.M., 2008. South African *Salvia* species: A review of biological activities and phytochemistry. Journal of Ethnopharmacology 119, 664-672.

Kawai, K., Beppu, H., Shimpo, K., Chihara, T., Yamamoto, N., Nagatsu, T., Ueda, H., Yamada, Y., 1998. *In Vivo* Effects of *Aloe arborescens* Miller var. *natalensis* Berger (*Kidachi aloe*) on Experimental Tinea Pedis in Guinea-pig Feet. Phytotherapy Research 12, 178-182.

Kayser, O., Kolodziej, H., 1997. Antibacterial activities of Extracts and Constituents of *Pelargonium sidoides* and *Pelargonium reniforme*. Planta Medica 63, 508-510.

Kayser, O., Latté, K., Kolodziej, H., Hammerschmidt, F.-J., 1998. Composition of the essential oils of *Pelargonium sidoides* DC. and *Pelargonium reniforme* Curt. Flavour and Fragrance Journal 13(3), 209-212.

Kenechukwa, O., 2004. Cardiovascular effect of *Leonotis leonurus* extract in normotensive rats and in isolated perfused rat heart. M.Sc Dissertation, University of the Western Cape.

Klein, E., Rojahn, W., 1968. The most important constituents of buchu leaf oil. Dragoco Report 9, 183-184.

Koduru, S., Grierson, D.S., Afolayan, A.J., 2007. Ethnobotanical information of medicinal plants used for treatment of cancer in the Eastern Cape Province, South Africa. Current Science 92(7), 906-908.

Kudritskaya, S.E., Fishman, G.M., Zagorodskaya, L.M., Chikovani, D.M., 1986. Carotenoids of *Aloe arborescens*. Khimiya Prirodnykh Soedinenii 4, 573.

Lamparsky, D., Schudel, P., 1971. *p*-Menthane-8-thiol-3-one, a new component of buchu leaf oil. Tetrahedron Letters 36, 3323-3326.

Latté, K.P., Ferreira, D., Venkatraman, M.S., Kolodziej, H., 2002. *O*-Galloyl-*C*-glycosylflavones from *Pelargonium reniforme*. Phytochemistry 59, 419-424.

Latté, K.P., Kolodziej, H., 2004. Antioxidant Properties of Phenolic Compounds from *Pelargonium reniforme*. Journal of Agricultural and Food Chemistry 52, 4899-4902.

Latté, K.P., Kaloga, M., Schäfer, A., Kolodziej, H., 2008. An ellagitannin, *n*-butyl gallate, two aryltetralin lignans, and an unprecedented diterpene ester from *Pelargonium reniforme*. Phytochemistry 69, 820-826.

Lis-Balchin, M., Hart, S., Simpson, E., 2001. Buchu (*Agathosma betulina* and *A.crenulata*, Rutaceae) essential oils: their pharmacological action on guinea-pig ileum and antimicrobial activity on microorganisms. Journal of Pharmacy and Pharmacology 53, 579-582.

Liu, N.Q., Cao, M., Frédérich, M., Choi, Y.H., Verpoorte, R., van der Kooy, F., 2010. Metabolomic investigation of the ethnopharmacological use of *Artemisia afra* with NMR spectroscopy and multivariate data analysis. Journal of Ethnopharmacology 128, 230-235.

Lopes-Lutz, D., Alviano, D.S., Alviano, C.S., Kolodziejczyk, P.P., 2008. Screening of chemical composition, antimicrobial and antioxidant activities of *Artemisia* essential oils. Phytochemistry 69, 1732-1738.

Macaís, F.A., Galindo, J.L.G., Galindo, J.C.G., 2007. Evolution and current status of ecological phytochemistry. Review. Phytochemistry 68, 2917-2936.

Mangena, T., Muyima, N.Y.O., 1999. Comparative evaluation of the antimicrobial activities of essential oils of *Artemisia afra*, *Pteronia incana* and *Rosmarinus officinalis* on selected bacteria and yeast strains. Letters in Applied Microbiology 28, 291-296.

Maphosa, V., Masika, P.J., Adedapo, A.A., 2008. Safety evaluation of the aqueous extract of *Leonotis leonurus* shoots in rats. Human and Experimental Toxicology 27, 837-843.

Maphosa, V., Adedapo, A.A., Moyo, B., Masika, P.J., 2012. Anti-inflammatory and analgesic activities of the aqueous extract of *Leonotis leonurus* leaves in rats. African Journal of Biotechnology 11(26), 6878-6883.

Martins, M., Ordway, D., Kristiansen, M., Viveiros, M., Leandro, C., Molnar, J., Amaral, L., 2005. Inhibition of the *Carpobrotus edulis* methanol extract on the growth of phagocytosed multidrug-resistant *Mycobacterium tuberculosis* and methicillin-resistant *Staphylococcus aureus*. Fitoterapia 76, 96-99.

Martins, A., Vasas, A., Schelz, Z.S., Viveiros, M., Molnár, J., Hohmann, J., Amaral, L., 2010. Constituents of *Carpobrotus edulis* Inhibit P-Glycoprotein of MDR1-transfected Mouse Lymphoma Cells. Anticancer Researh 30, 829-836.

Martins, A., Vasas, A., Viveiros, M., Molnár, J., Hohmann, J., Amaral, L., 2011. Antibacterial properties of compounds isolated from *Carpobrotus edulis*. International Journal of Antimicrobial Agents 37, 438-444.

Mativandlela, S.P.N., Lall, N., Meyer, J.J.M., 2006. Antibacterial, antifungal and antitubercular activity of (the roots of) *Pelargonium reniforme* (CURT) and *Pelargonium sidoides* (DC) (Geraniaceae) root extracts. South African Journal of Botany 72, 232-237.

Matsuda, Y., Yokohira, M., Suzuki, S., Hosokawa, K., Yamakawa, K., Zeng, Y., Ninomiya, F., Saoo, K., Kuno, T., Imaida, K., 2008. One-year chronic toxicity study of *Aloe arborescens* Miller var. *natalensis* Berger in Wistar Hannover rats. A pilot study. Food and Chemical Toxicology 46, 733-739.

McGaw, L.J., Jäger, A.K., van Staden, J., 2000. Antibacterial, anthelmintic and anti-amoebic activity in South African medicinal plants. Journal of Ethnopharmacology 72, 247-263.

Mnonopi, N., Levendal, R.-A., Davies-Coleman, M.T., Frost, C.L., 2011. The cardioprotective effects of marrubiin, a diterpenoid found in *Leonotis leonurus* extracts. Journal of Ethnopharmacology 138, 67-75.

Mohanta, B., Chakraborty, A., Sudarshan, M., Dutta, R.K., Baruah, M., 2003. Elemental profile in some common medicinal plants of India. Its correlation with traditional therapeutic usage. Journal of Radioanalytical and Nuclear Chemistry 258(1), 175-179.

Moody, J.O., Adeleye, S.A., Gundidza, M.G., Wylie, G., 1994. Analysis of the essential oil of *Artemisia afra*. Pharmazie 49, 935-936.

Moolla, A., 2005. A phytochemical and pharmacological investigation of indigenous *Agathosma* species. M.Sc Dissertation, University of the Witwatersrand.

Mukinda, J.T., Syce, J.A., 2007. Acute and chronic toxicity of the aqueous extract of *Artemisia afra* in rodents. Journal of Ethnopharmacology 112, 138-144.

Mulatu, A., Mekonnen, Y., 2007. Spasmolytic effects of *Artemisia afra* and *Artemisia rehan* in tissue preparations. Ethiopian Medical Journal 45, 371-376.

Mwangi, J.W., Achola, K.J., Sinei, K.A., Lwande, W., Laurent, R., 1995. Essential Oil Constituents of *Artemisia afra* Willd. Journal of Essential Oil Research 7(1), 97-99.

Ntutela, S., Smith, P., Matika, L., Mukinda, J., Arendse, H., Allie, N., Estes, D.M., Mabusela, W., Folb, P., Steyn, L., Johnson, Q., Folk, W.R., Syce, J., Jacobs, M., 2009. Efficacy of *Artemisia afra* phytotherapy in experimental tuberculosis. Tuberculosis 89(S1), S33-S40.

Obata, M., Ito, S., Beppu, H., Fujita, K., Nagatsu, T., 1993. Mechanism of anti-inflammatory and antithermal burn action of CPase from *Aloe arborescens* Miller var. *natalensis* Berger in rats and mice. Phytotherapy Research 7(7), S30-S33.

Ojewole, J.A.O., 2003. P-2: Hypotensive effect of *Leonotis leonurus* aqueous leaf extract in rats. American Journal of Hypertension 16(5), 40A.

Okigbo, R.N., Anuagasi, C.L., Amadi, J.E., 2009. Advances in selected medicinal and aromatic plants indigenous to Africa. Full Length Research Paper. Journal of Medicinal Plants Research 3(2), 086-095.

Olajuyigbe, O.O., Afolayan, A.J., 2012. Ethnobotanical survey of medicinal plants used in the treatment of gastrointestinal disorders in the Eastern Cape Province, South Africa. Full Length Research Paper. Journal of Medicinal Plants Research 6(18), 3415-3424.

Olennikov, D.N., Zilfikarov, I.N., Ibragimov, T.A., 2008. Investigation of chemical composition of Arborescent Aloe (*Aloe arborescens* Mill.) http://www.chem.wood/volume14/2010_03/1003_007.en.html.

Olorunnisola, O.S., Bradley, G., Afolayan, A.J., 2011. Ethnobotanical information on plants used for the management of cardiovascular diseases in Nkonkobe Municipality, South Africa. Full Length Research Paper. Journal of Medicinal Plants Research 5(17), 4256-4260.

Omoruyi, B.E., Bradley, G., Afolayan, A.J., 2012. Antioxidant and phytochemical properties of *Carpobrotus edulis* (L.) Bolus leaf used for the management of common infections in HIV/AIDS patients in Eastern Cape Province. BMC Complementary and Alternative Medicine 12, 215-235.

Oyedemi, S.O., Bradley, G., Afolayan, A.J., 2009. Ethnobotanical survey of medicinal plants used for the management of diabetes mellitus in the Nkonkobe municipality of South Africa. Full Length Research Paper. Journal of Medicinal Plants Research 3(12), 1040-1044.

Oyedemi, S.O., Yakubu, M.T., Afolayan, A.J., 2010. Effect of aqueous extract of *Leonotis leonurus* (L.) R. Br. leaves in male Wistar rats. Human and Experimental Toxicology 29(5), 377-384.

Oyedemi, S.O., Yakubu, M.T., Afolayan, A.J., 2011. Antidiabetic activities of aqueous leaves extract of *Leonotis leonurus* in streptozotocin induced diabetic rats. Full Length Research Paper. Journal of Medicinal Plants Research 5(1), 119-125.

Park, M.K., Park, J.H., Kim, N.Y., Shin, Y.G., Choi, Y.S., Lee, J.G., Kim, K.H., 1998. Analysis of 13 phenolic compounds in Aloe species by high performance liquid chromatography. Phytochemistry Analysis 9(4), 186-191.

Pendota, S.C., Grierson, D.S., Afolayan, A.J., 2008. An Ethnobotanical Study of Plants Used for the Treatment of Eye Infections in the Eastern Cape Province, South Africa. Pakistan Journal of Biological Sciences 11(16), 2051-2053.

Philander, L.A., 2011. An ethnobotany of Western Cape Rasta bush medicine. Journal of Ethnopharmacology 138, 578-594.

Posthumus, M.A., Van Beek, T.A., 1996. Chemical composition of the essential oils *Agathosma betulina*, *A.crenulata* and an *A.betulina* x *A.crenulata* hybrid (Buchu). Journal of Essential Oil Research 8, 223-228.

Rabe, T., van Staden, J., 1997. Antibacterial activity of South African plants used for medicinal purposes. Journal of Ethnopharmacology 56, 81-87.

Rajurkar, N.S., Damame, M.M., 1997. Elemental analysis of some herbal plants used in the treatment of cardiovascular diseases by NAA and AAS. Journal of Radioanalytical and Nuclear Chemistry 219(1), 77-80.

Saito, H., 1993. Purification of active substances of *Aloe arborescens* Miller and their biological and pharmacological activity. Phytotherapy research 7(7), S14-S19.

Sandasi, M., Kamatou, G.P.P., Baranska, M., Viljoen, A.M., 2010. Application of vibrational spectroscopy in the quality assessment of Buchu oil obtained from two commercially important *Agathosma* species (Rutaceae). South African Journal of Botany 76, 692-700.

Sarmani, S.B., Abugassa, I., Hamzah, A., Yahya., M.D., 1999. Elemental Analysis of Herbal Preparations for Traditional Medicines by Neutron Activation Analysis with the k_0 Standardization Method. Biological Trace Element Research, 71-72, 365-376.

Sato, Y., Ohta, S., Shinoda, M., 1990. Studies on chemical protectors against radiation. xxxi. Protection effects of *Aloe arborescens* on skin injury induced by x-irradiation. Journal of the Pharmaceutical Society of Japan 110(11), 876-884.

Seidel, V., Taylor, P.W., 2004. *In vitro* activity of extracts and constituents of *Pelargonium* against rapidly growing mycobacteria. International Journal of Antimicrobial Agents 23, 613-619.

Semenya, S., Potgieter, M., Erasmus, L., 2012. Ethnobotanical survey of medicinal plants used by Bapedi healers to treat diabetes mellitus in the Limpopo province, South Africa. Journal of Ethnopharmacology 141, 440-445.

Shimpo, K., Chihara, T., Beppu, H., Ida, C., Kaneko, T., Nagatsu, T., Kuzuya, H., 2001. Inhibition of Azoxymethane-Induced Aberrant Crypt Foci Formation in Rat Colorectum by Whole Leaf *Aloe arborescens* Miller var. *natalensis* Berger. Phytotherapy Research 15, 705-711.

Shimpo, K., Ida, C., Chihara, T., Beppu, H., Kaneko, T., Kuzuya, H., 2002. *Aloe arborescens* Extract Inhibits TPA-induced Ear Oedema, Putrescine Increase and Tumour Promotion in Mouse Skin. Phytotherapy Research 16, 491-493.

Sofowora, A., 1996. Research on Medicinal Plants and Traditional Medicine in Africa. The Journal of Alternative and Complementary Medicine 2(3), 365-372.

Spreeth, A.D., 1976. A revision of the commercially important *Agathosma* species. Journal of South African Botany 42(2), 109-119.

Steenkamp, V., Mathivha, E., Gouws, M.C., van Rensburg, C.E.J., 2004. Studies on antibacterial, antioxidant and fibroblast growth stimulation of wound healing remedies from South Africa. Journal of Ethnopharmacology 95, 353-357.

Suliman, S., Van Vuuren, S.F., Viljoen, A.M., 2010. Validating the *in vitro* antimicrobial activity of *Artemisia afra* in polyherbal combinations to treat respiratory infections. South African Journal of Botany 76, 655-661.

Sunmonu, T.O., Afolayan, A.J., 2012. Evaluation of Polyphenolic Content and Antioxidant Activity of *Artemisia afra* Jacq. Ex Willd. Aqueous Extract. Pakistan Journal of Nutrition 11(7), 520-525.

Teradaira, R., Shinzato, M., Beppu, H., Fujita, K., 2006. Antigastric ulcer effects in rats of *Aloe arborescens* Miller var. natalensis *Berger* extract. Phytotherapy Research 7(7), S34-S36.

Thring, T.S.A., Weitz, F.M., 2006. Medicinal plant use in the Bredasdorp/Elim region of the Southern Overberg in the Western Cape Province of South Africa. Journal of Ethnopharmacology 103, 261-275.

Umano, K., Nakahara, K., Shoji, A., Shibamoto, T., 1999. Aroma Chemicals Isolated and Identified from Leaves of *Aloe arborescens* Mill. Var. *natalensis* Berger. J. Agric. Food Chem 47, 3702-3705.

Vagionas, K., Graikou, K., Chinou, I.B., Runyoro, D., Ngassapa, O., 2007. Chemical Analysis and Antimicrobial Activity of Essential Oils from the Aromatic Plants *Artemisia afra* Jacq. and *Leonotis ocymifolia* (Burm. F.) *Iwarsson* var. raineriana (Vision1) *Iwarsson* Growing In Tanzania. The Journal of Essential Oil Research 19, 396-400.

Van der Kooy, F., Verpoote, R., Marion Meyer, J.J., 2008. Metabolomic quality control of claimed anti-malarial *Artemisia afra* herbal remedy and *A. afra* and *A. annua* plant extracts. South African Journal of Botany 74, 186-189.

Van der Walt, J.J.A., Vorster, P.J., 1988. Pelargoniums of southern Africa, third ed. National Botanic Garden Kirstenbosch, South Africa., pp. 129-131.

Van der Watt, E., Pretorius, J.C., 2001. Purification and identification of active antibacterial components in *Carpobrotus edulis* L. Journal of Ethnopharmacology 76, 87-91.

Van Grunsven, R.H.A., Bos, F., Ripley, B.S., Suehs, C.M., Veenendaal, E.M., 2009. Release from soil pathogens plays an important role in the success of invasive *Carpobrotus* in the Mediterranean. South African Journal of Botany 75, 172-175.

Van Wyk, B.-E., Smith, G., 1996. Guide to the *Aloes* of South Africa. Briza Publications, Pretoria.

Van Wyk, B.-E., Van Oudtshoorn, B., Gericke, N., 1997. Medicinal plants of South Africa. Briza Publications, Pretoria.

Van Wyk, B.-E., Gericke, N., 2000. People’s plants: A guide to useful plants of South Africa. Briza Publications, Pretoria.

Van Wyk, B.-E., Wink, M., 2004. Medicinal plants of the world. Briza Publications, Pretoria.

Van Wyk, B.-E., de Wet, H., Van Heerden, F.R., 2008. An ethnobotanical survey of medicinal plants in the southeastern Karoo, South Africa. South African Journal of Botany 74, 696-704.

Van Wyk, B.-E., Van Oudtshoorn, B., Gericke, N., 2009. Medicinal plants of South Africa. Briza Publications, Pretoria.

Verlaque, R., Affre, L., Diadema, K., Suehs, C.M., Médail, F., 2011. Unexpected morphological and karyological changes in invasive *Carpobrotus* (Aizoaceae) in Provence (S-E France) compared to native South African species. C.R. Biologies 334, 311-319.

Viljoen, A.M., Van der Walt, J.J.A., Swart, J.P.J., Demarne, F.-E., 1995. A Study of the Variation in the Essential Oil of *Pelargonium capitatum* (L.) L'Herit. (Geraniaceae). Part II. The Chemotypes of *P. capitatum*. The Journal of Essential Oil research 7(6), 605-611.

Viljoen, A.M., Moolla, A., van Vuuren, S.F., van Zyl, R.L., Baser, K.H.C., Demirci, B., Özek, T., Trinder-Smith, T.H., 2006a. The Biological Activity and Essential Oil Composition of 17 *Agathosma* (Rutaceae) Species. Journal of Essential Oil Research 18, 2-16.

Viljoen, A.M., van Vuuren, S.F., Gwebu, T., Demirci, B., Baser, K.H.C., 2006b. The Geographical Variation and Antimicrobial Activity of African Wormwood (*Artemisia afra* Jacq.) Essential Oil. Journal of Essential Oil Research 18, 19-25.

Viljoen, A., Moolla, A., 2007. Indigenous South African Medicinal Plants. Part 5: *Agathosma betulina* ('buchu'). SA Pharmaceutical Journal, October, 39.

Wagner, H., Bladt, S., 1975. Coumarins of South African Plant species, *Pelargonium*. Phytochemistry 14, 2061-2064.

Watt, J.M., Breyer-Brandwijk, B.N., 1962. Medicinal and Poisonous plants of Southern and Eastern Africa, second ed. Livingstone, London.

Whitehouse, C.M., Fellingham, A.C., 2007. New species and notes on the genus *Cliffortia* (Rosaceae). Bothalia 37(1), 9-22.

Worku, T., Rubiolo, P., 1996. Major Constiuents of *Artemisia afra* Oil. The Journal of Essential Oil Research 7, 355-357.

Wu, H., Li, J., Fronczek, F.R., Ferreira, D., Burandt Jr, C.L., Setola, V., Roth, B.L., Zjawiony, J.K., 2012. Labdane Diterpenoids from *Leonotis leonurus*. Phytochemistry http://dx.doi.org/10.1016/j.phytochem.2012.02.021.

Yamamoto, M., Masui, T., Sugiyama, K., Yokota, M., Nakagomi, K., Nakazawa, H., 1991. Anti-inflammatory Active Constituents of *Aloe arborescens* Miller. Agric. Biol. Chem. 55(6), 1627-1629.

York, T., de Wet, H., van Vuuren, S.F., 2011. Plants used for treating respiratory infections in rural Maputaland, KwaZulu-Natal, South Africa. Journal of Ethnopharmacology 135, 696-710.

CHAPTER 3

MEDICINAL PLANT USE IN THE DWARSRIVIER VALLEY, STELLENBOSCH.

ABSTRACT

The use of plants as medicine is still used by the communities in the Dwarsrivier Valley, Stellenbosch. The aim of the study was to document the knowledge of the individuals and provide an inventory of these plants. This would aid the documentation of the plants in the Western Cape region as few ethnobotanical surveys have been carried out. Over 40 individuals were interviewed from the four communities; Pniel, Lanquedoc, Meerlust and Kylemore. The majority of the interviews occurred at the church in Pniel while others took place at the homes of the individuals.

The survey yielded 53 plant species belonging to 31 families used for medicinal purposes. Of these, only 24 plant species were indigenous. Although there were more exotic plants used by the community compared to indigenous, the indigenous plants were favoured for medicinal purposes. Most of the plants used belong to Lamiaceae, Asteraceae, Alliaceae, Apiaceae and Rosaceae. The most popular plants in terms of ranking were *Agathosma crenulata*, followed by *Artemisia afra* and *Helichrysum petiolare* in second and third place respectively. This is the first report of *Protea cynaroides*, *Cissus rhombifolia*, *Canna* spp. and *Dilatris viscosa* used for medicinal purposes. The study demonstrates the importance of conducting ethnobotanical surveys in an attempt to document the knowledge as well as identify any harmful harvesting practices for conservation purposes before these plants become extinct.

Keywords: Dwarsrivier valley, ethnobotanical survey, questionnaire, medicinal plants, documentation, intellectual property rights.

CHAPTER 3

MEDICINAL PLANT USE IN THE DWARSRIVIER VALLEY, STELLENBOSCH.

3.1 INTRODUCTION

For centuries the people of South Africa have relied on herbal medicine for all aspects of their primary health care (Grierson and Afolayan, 1999a; Kelmanson et al., 2000). This tradition of collecting, processing and applying plants as well as plant-based medications has been passed down through the generations in the African societies (Olajuyigbe and Afolayan, 2012). This emphasizes the fragility of indigenous knowledge (Light et al., 2006). Zobolo and Mkabela (2006) reported that the medicinal knowledge of members of the Zulu tribe is not sufficiently passed on through the generations as they seem to treat this form of healthcare with contempt. The knowledge of medicinal plants is rapidly dwindling due to western influence, reduction in the number of traditional healers and a lack of interest from the younger generation to carry on the tradition (Bussman et al., 2006). Therefore emphasis is placed on the documentation of this knowledge to prevent it becoming irretrievably lost to the world (Hutchings et al., 1996).

Traditional knowledge, previously regarded as "uninformed" or "unscientific", has attracted attention as researchers are relying progressively more on the knowledge of the local communities regarding potential medicinal plants (Zerbe, 2005). It can be assumed that the most efficacious species are well known to respondents (Quinlan et al., 2002). Many researchers can agree that considerable advances have been made which include characterization of plant constituents and activities as well as a better understanding between plants and pharmaceuticals (Etkin, 2001). However, countless scientists spend limited time with the community members or the environment from which these plant materials are drawn (Etkin, 2001). This is not in line with the principles of ethnobotany. Ethnobotany is regarded as the study of the relationship between plants and people (Balick and Cox, 1996) with one of the common goals being to understand how plant-people interactions influence the health of indigenous populations (Quinlan et al., 2002). As ethnopharmacology is a multidisciplinary science (Mulholland, 2005; Owen et al., 2011), successful research requires the interaction of ethnobotanists, natural product chemists, pharmacologists, taxonomists, traditional healers and/or user communities (Olajuyigbe and Afolayan, 2012). Ethnobotanical surveys are recognised as one of the major approaches for selecting plants for pharmacological screening (Adebayo and Krettli, 2011) with the key goal of ethnopharmacology being to discover novel plant-based compounds, derived from the indigenous knowledge, which could be developed into pharmaceuticals (Pillay et al., 2008). The dependence on plants as medicine further raises the need for scientific evaluation of methods used by traditional healers (Kelmanson et al., 2000), identification of novel active compounds and to validate the safety and efficacy of these medicines (Olajuyigbe and Afolayan, 2012).

3.1.1 Safety and efficacy of plant medicines

The use and prescription of traditional medicine is not regulated in South Africa which could result in possible misadministration of toxic plants (Light et al., 2006). Few medicinal plant species has been scientifically validated (Gurib-Fakim and Kasilo, 2010), with scientific literature regarding the quality, safety and efficacy available for approximately 500 species of plants currently used for medicinal purposes in South Africa (Scott et al., 2004). With the extensive use of plants over the centuries, some might expect the bioactive compounds of these plants to have low toxicity (Fabricant and Farnsworth, 2001; Elgorashi et al., 2003). Certain plants used in traditional medicine or as food have undergone screening and the results have indicated mutagenic effects in *in vitro* assays (Eldeen et al., 2005). As many of these herbal remedies are widespread and promoted as being safe and efficacious, it is not always possible for a long term user to understand that these plants might be harmful (Elvin-Lewis, 2001).

Although life threatening, circumstances of medicinal plant medicine toxicity are few when compared to the thousands reported for pharmaceutical companies (Elvin-Lewis, 2001). The safety of patients in South Africa is affected as they are increasingly dependent on sellers who are incompetent to supply the correct plant medicines (Fennel et al., 2004). However, when prepared and prescribed appropriately, the safety of traditional medicine is high (Elvin-Lewis, 2001). Several reports confirm that traditional medicine is avoided unless prescribed by a traditional healer recommended from a familiar source (Fennel et al, 2004). The therapeutic value of herbal concoctions requires evaluation to determine the efficacy and assess any potential toxic effects (Ndhlala et al., 2009).

In South African traditional health care, the forms in which plant medicines are sold pose a great risk (Fennel et al, 2004; Stafford et al, 2005). The conditions of the market in which these traditional medicines are sold are generally poor (Mander, 1998) with herbal medicines being neither registered nor controlled (Gurib-Fakim and Kasilo, 2010). Post-harvest storage and processing techniques often lead to high levels of microbial contamination (Mander and Le Breton, 2006; Katerere et al., 2008) and significant stock loss (Mander and Le Breton, 2006). Many healers as well as consumers have claimed to be concerned about the quality of products obtained from the markets (Mander, 1998). However, the first signs of modernisation in African traditional medicine have been noted in the form of labelled, packaged herbal concoctions yet they lack western quality controls and safety (Ndhlala et al., 2009). When considering the increasing economic values being placed on medicinal plants not to mention the sharp decrease in the biological species across the globe, documentation not only serves as a medium to understand the utilisation of different plant species but also provides a means to conserve these natural resources (Olajuyigbe and Afolayan, 2012).

3.1.2 Conservation

The majority of traditional medicine originates from plant sources and the careless collection of plant species could lead to extinction (Kim, 2005). With an upsurge in the commercialisation of medicinal plants in South Africa resulting in overharvesting, and the strict conservation practices which regulated the collection times as well as quantities being used a distant memory, harvesting practices has become the domain of the untrained, who often use destructive means to manage the urban demands (Williams et al., 2000). While the growing demands of herbal medicine in Europe, China and India are met by large scale cultivation, in Africa, it is common practice to collect medicinal plants from the wild (Zschocke et al., 2000). As the consumer population continues to grow and the demand for medicinal plants increases, the wild stock of medicinal plants are rapidly diminishing as there are no easily available plant substitutes (Water and forestry, 2005). This has resulted in several plant species such as *Siphonochilus aethiopicus* (wild ginger) and *Warburgia salutaris* (pepper bark tree) becoming extinct outside secure areas of KwaZulu-Natal (Mander, 1998).

A long term strategy would be to educate and inform harvesters, traditional healers as well as consumers of the implication of callous harvesting to promote the best practices to improve as well as preserve quality of these medicines (Katerere et al., 2008). The declining supply of indigenous medicinal plants could possibly cause significant economic and welfare loss

(Mander, 1998) however this could also create opportunities for commercial cultivation (Water and forestry, 2005).

In South Africa, 80% of wild plants from which material has been harvestested will die as a result of harvesting practices (Mander and Le Breton, 2005). Bulbs are considered to be highly profitable and harvesting involves uprooting the entire plant (Ncube et al., 2010). A study in the Eastern Cape reported that 93% of species traded are harvested unsustainably, due to either partially or completely being removed from the ground resulting in the death of the plant (Dold and Cocks, 2002). Williams et al. (2000) conveyed that the harvesting of almost two-thirds of the species traded on the Witwatersrand would almost always result in mortality with the most common parts traded being roots (38.4%) followed by bark (25.6%). The same occurred in the Lowveld, with the majority of plant parts traded being the roots, bark or whole plant (Botha et al., 2004). In van Wyk et al. (1997), a high proportion of trees are used for medicinal ailments with the bark reportedly used in 83% of the trees. This is a cause for concern as the intensity of acute harvesting is leading to the extinction with some forests in South Africa having lost over 80% of the trees with high medicinal value (Mander and Le Breton, 1998). The extinction of indigenous species could result before their potential as budding sources of therapeutic drugs have been evaluated and applied (Shai et al., 2008).

Ring barking is considered the most destructive harvesting practice, and if performed by unskilled labourers, there is no chance of survival for the debarked tree (Zchocke et al., 2000). Possible strategies have been suggested to combat these problems. These include the establishment of conservation regions and implementing laws against bark collection (Cunningham, 1991). However, the enforcement of these laws may be difficult. Another option involves large scale cultivation although this is not a feasible option as a short term goal due to the slow growth of the affected species (Cunningham, 1991). This is a possible option in the long term as resource users in the Lowveld region are reportedly willing to cultivate medicinal plants yet cultural constraints need to be considered (Botha et al., 2004). Plant part substitution is an option that could be implemented immediately if it is not already being used. This concept encourages the collection and use of substitute plant parts such as twigs and leaves instead of the roots and bark (Zchocke et al., 2000). Harvesting of flowers, leaves and fruits are reportedly less destructive compared to the bark and roots from trees or removing the entire plant part from geophytes (Shackleton, 2001). Researchers have already taken the step to evaluate certain parts of a plant species as an alternative such as *Hypoxis* *hemerocallidea* (Katerere and Eloff, 2008) and *Leonotis leonurus* (Agnihotri et al., 2009) as well as other bulbous plants (Ncube et al., 2010). The discovery of pharmaceuticals manufactured as a result of plant knowledge requires the acknowledgement or conpensation to indigenous groups (Philander, 2011). This concept is better referred to as intellectual property rights.

3.1.3 Intellectual property rights

Genetic resources have increased in scientific and more importantly marketable value resulting in intense disputes over access to as well as proprietorship of biodiversity (Zerbe, 2005). Growing consensus indicates that local communities involved in the identification of indigenous species with therapeutic properties should be compensated for their knowledge (Van Overwalle, 2005). However, uncertainty about the appropriate legalities concerning the right to compensation exists (Van Overwalle, 2005). The African Model Law aims to develop a regional framework which governs all aspects of intellectual property rights, biodiversity management as well as the protection of indigenous knowledge (Zerbe, 2005).

A cultural renaissance toward more natural methods of healing has emerged (Makunga et al., 2008; Rybicki et al., 2012). Traditional medicine research focusses not only to validate the medicinal plants used by communities but also confirm their pharmacological activity with intent to discover new drugs (Makunga et al., 2008). Credence is lent to the fact that South Africa houses approximately 10% of higher plant species on the earth with fewer than 5% of the species being scientifically evaluated for pharmacological activity (Bamuamba et al., 2008). With a need to integrate traditional medicine as well as preserve cultural heritage, documentation as well scientific validation of indigenous medicinal plants is necessary (Frei et al., 1998; Leonti, 2011). The depth of appropriate as well as inappropriate self-medication and the depth of the medicinal plant knowledge in a community need to be assessed (Stein et al., 1989). Therefore, selecting the most important taxa is a criterion for instigating ethnopharmacological, phytochemical and toxicological studies (Frei et al., 1998). The Dwarsrivier community still uphold the traditional of using plants to treat their medicinal complaints which would aid the identification of promising medicinal plants.

Aims and objectives

- Identify the plants used as medicine by the communities in the Dwarsrivier valley.
- Provide an inventory of these plants and their uses to treat various ailments.

3.2 MATERIALS AND METHODS

3.2.1 Interviews and data analysis

Information relating to the study was assembled by means of questionnaires and interviews amongst the members of the four communities in the Dwarsrivier Valley, Stellenbosch. These communities were Pniel, Lanquedoc, Kylemore and Meerlust (Figure 1). The objectives of the study were to document the plants used for medicinal purposes in the region and to compile an inventory for future use. The objectives were clearly explained in either English or Afrikaans to each of the interviewees and a consent form was signed before the interviews were conducted. The consent form informed the interviewee that they were under no obligation to partake in the study and were also assured that it was not mandatory to share information they did not feel comfortable sharing. The interviewee was informed that the results from the study will be published in academic journals.

Figure 1: The locations of the four communities of the Dwarsrivier Valley, Stellenbosch.

Interviews were conducted at the church in Pniel where the elders usually met or at the homes of the individuals being interviewed. Often individuals were recommended by fellow members of the community due to their ethnobotanical knowledge. All interviews were scheduled by appointment and a semi-structured questionnaire was followed to identify plants used for medicinal purposes in the area. Of the 40 participants interviewed, 25 participants agreed to complete questionnaires to generate data for figures 2-7. These 25 members were selected based on their ethnobotanical knowledge. Plant species which could be directly indicated and collected were mentioned and plants were considered valid when cited at least three times by other community members. However, novel medicinal uses mentioned by community members were also recorded. Preparation methods, dosages, duration of treatment and substitute species if species x was not available were also identified. Using the data, we were able to identify the most popular plants used in the area and determine the "preference ranking" (Cotton, 1996). The data also enables the calculation of the "use value". The use value compares the information given by the informant to the information given by all the informants (Cotton, 1996). These use values were calculated using a method by Cotton (1996).

The use-values were calculated using the following equation: $UVs = \frac{\sum UVIS}{IS}$

Where UVs = the overall use of species S

UV_{IS}= the mean number of all uses of a given plants species (S) as determined by informant I.

I_S = total number of informants interviewed for species S.

3.2.2 Plant identification

Individuals partaking in the study were required to bring along a sample of their specimen to enable the identification of the specimen. This assisted the identification process immensely due to particular plants having identical or more than one common name. In cases where the plants were no longer available, illustrations were brought along to assist the identification process. Frans Weitz, a taxonomist at the Department of Biodiversity and Conservation Biology at The University of the Western Cape, verified the samples and voucher specimens were prepared of selected plants.

3.3 RESULTS

The survey yielded 53 plant species belonging to 31 families used for medicinal purposes. Of these, only 21 were indigenous (Figure 2A and Figure 2B). The family with the largest number of species are Lamiaceae (eight species), Asteraceae (six species) and Alliaceae, Apiaceae and Rosaceae (three species each) (Figure 3). The most popular plants in terms of ranking are *Agathosma crenulata*, followed by *Artemisia afra* and *Helichrysum petiolare* in second and third place respectively (Figure 2A). Although there were more exotic plants used by the community compared to indigenous, the indigenous plants were favoured for medicinal purposes.

Table 1A and Table 1B depict the botanical name, common name, administration and preparation method and the uses of plants used by the Dwarsrivier community compared to the uses in literature.

Table 2 depicts plant combinations used by the community to treat ailments. Figure 2A shows the popularity of plants in terms of ranking whereas Figure 2B and Figure 2C show the popularity of the indigenous plants compared to the exotic plants respectively.

Figure 3 reveals the plant families used by the community. Figure 4A and Figure 4B illustrates the preferred indigenous as well as the exotic plants used to treat various ailments. The calculated use-values are represented by Figure 5A and Figure 5B. All plants are colour coded - green for indigenous plant species (Figure 5A) and red for exotic plant species (Figure 5B).

Table 1A: The current uses and preparations of the indigenous medicinal plants used in the Dwarsrivier valley, Stellenbosch as well as previously recorded uses in the literature.

Family **Plant species** **(Common name)**	**Use or condition treated**	**Method of preparation and use**	**Uses in the literature**	**Other information**
Aizoaceae (or Mesembryanthemaceae) *Carpobrotus edulis* [Sour fig (Eng); Suurvy (Afr); umgongozi (Zulu); ghaukum (Khoi)] (Voucher specimen: M.L Arendse 7)	Asthma, coughs, tuberculosis, stomach ailments, sore throat, ulcers, mouth thrush, teething and skin ailments.	Sap is applied directly to the skin to treat skin ailments. For teething and mouth thrush in children, place the sap of crushed leaves in a clean cloth and allow the child to suck the juice through the cloth or rub the exudate directly onto the gums. For the treatment of cough and asthma, place the sap of crushed leaves in a quarter cup of olive oil or milk and drink directly. The leaves are chewed to relieve sore throats. For stomach ailments, TB and ulcers, leaves are crushed and the sap is collected, added to water and then drunk as a tea three times daily.	Treatment of digestive troubles[1,2,6],burns[2,6],diarrhoea[1,3,6], dysentery [1,2,6], earache[2], eczema[2,6], eye infection[3], nappy rash[3], pregnancy difficulties[6], ringworm[6], scalds[6], skin infection[3], sore throat[1,2,6], sprue[6], thrush[1,2,6], toothache[2], tuberculosis[2] and wounds[2].	
Alliaceae *Tulbaghia violacea* [Wild garlic (Eng); wilde knoffel, wilde knofflok (Afri); Isihaqa (Zulu)] (Voucher specimen: M.L Arendse 24)	Asthma, colds and flu, cough, fever, heart ailments including hypertension, liver ailments, stomach ailments, tuberculosis, worms and as a tonic.	For the treatment of jaundice, 3 slices of wild garlic is worn around the neck under clothing. For the treatment of other ailments, a tea is prepared from the bulb and one small cup is drunk three times daily.	Acts as an anthelmintic[1]. Treatment of asthma[2,3] colds[2], colic[6],constipation[3], fever[2,3],fits[2], headaches[6],oesophagus cancer[2,3] paralysis [2], pulmonary tuberculosis[1,2], rheumatism[2] and stomach problems[2,6].	

Amaryllidaceae *Gethyllis afra* [Kukumakranka (Eng); Koekemakranka (Afri, Khoi)]	Headache, stomach ailments and as a tonic.	Usually drunk as an alcohol tincture, where *Gethyllis* leaves are stored in brandy and one cup is drunk morning and at night.	Treatment of colic[1], flatulence[1] and stomach ailments[3].	
Apiaceae *Notobubon galbanum* [Blister bush (Eng); Bergseldery (Afr)]	Bladder and kidney ailments as well as pain.	A tea is prepared by adding a few leaves to IL of boiling water and allow to cool down. Strain. Drink one cup three times daily for not more than 5 days.	Abortifacient[1], arthritis[2,3], bladder and kidney complaints[1, 2,3,5] glandular swelling[2], gout[3], hypertension[2], obesity in men[2,5] ,slimming remedy[3] and water retention[2].	Contact with *Notobubon galbanum* causes blistering[5].
Asparagaceae *Asparagus capensis* [Wild Asparagus (Eng); Katdoring (Afr)] (Voucher specimen: M.L Arendse 5)	Arthritis, heart ailments and stomach complaints.	A tea is prepared using either the leaf or the root added to one cup of boiling water and allowed to cool down. Strain. Drink one cup when necessary.	*Agathosma capensis* is eaten as a vegetable[3].	
Asphodelaceae *Aloe arborescens* [Krantz aloe (Eng); Kransaalwyn (Afr); Inkalane (Xhosa, Zulu)] (Voucher specimen: M.L Arendse 2)	Diabetes, hypertension, liver ailments, skin ailments and stomach complaints as well as a detoxification aid.	For the treatment of diabetes, hypertension, liver and stomach ailments, the leaves are crushed and the sap is spread on dry bread and then eaten. For skin ailments, the leaf is crushed and the sap applied to the affected areas of the body. Detoxification of the body requires the sap to be drunk without any water. Consume one teaspoon of the sap in the morning on an empty stomach.	Assist parturition[1], burns[2], wounds[2] and stomach ailments[6]. Acts as an anti-bacterial[2], anti-cancer [2,] anti-diabetic[2], anti-inflammatory[2] and anti-ulcer[2].	

Asphodelaceae *Bulbine frutescens* [Burn jelly plant (Eng); Balsemkopiva(Afr)] [New: Katstert] (Voucher specimen: M.L Arendse 6)	Bladder and kidney ailments, sores and wounds as well as skin ailments.	For the treatment of bladder and kidney infections, the leaf exudate is added to a quarter cup of milk and consumed. Leaf gel is applied to the affected area as often as needed to treat sores and wounds as well as skin ailments	Treatment of abrasions[3], boils[3], burns[3,6], cracked lips[3], cuts[3,6], eczema[3], fever[6], grazes[6], herpes[3], rashes[3], skin ailments[3] and wounds[3].	
Asteraceae *Artemisia afra* [Wild wormwood, African wormwood (Eng); Wildeals, Alsem(Afr); Umhlonyane (Xhosa, Zulu)] (Voucher specimen: M.L Arendse 4)	Arthritis including pain and inflammation, colds and flu, fever, cough, tuberculosis, heart ailments including hypertension, diabetes, stomach ailments and colic, headache, tonic and detoxification, bladder and kidney ailments as well as the treatment of worms.	For stomach complaints in children, place dried leaves on a cloth and sprinkle with vinegar. Place the cloth on the stomach and secure in place. For pains, sprains and inflammation (including arthritis), add leaves to a cloth and soak in vinegar. Wrap around the affected area. When dry, change as needed. To treat the remaining ailments mentioned, a tea is prepared by adding a few leaves to a cup of boiling water and allowed to cool. Strain. Drink one cup when needed.	Treatment of asthma [3,4,] coughs[1,2,3,4,6], colds[1,2,3,4,6] chills[1], colic[1,2,3,4,6], constipation [1,3,4,6,] croup [1,6,] diabetes[6], dyspepsia[1,6], earache[1,2,6] feet ailments[1], fevers[1,2,3,6] ,flatulence[3,4], gout[1,3,4,6] ,haemorrhoids[1,6], headache[1,2,3,4,6], influenza[4,6], indigestion[3,4], measles[1,6,] loss of appetite[1,2,6] , menstrual ailments[1,6], malaria[1,2,] mumps[1], pneumonia[3,6], poor appetite[3], post childbirth[1], stomachache[1], throat pain[1,3,4,6], toothache[1,6], whooping cough[1], worms[1,2,3,4,6], and as a purgative[1] and blood purifier[1,6].	
Asteraceae *Helichrysum petiolare* [Hotnotskooigoed, Kooigoed (Afri)]	Backpain, bladder and kidney ailments, colds and flu, cough, detoxification, expel placenta, feet ailments, fever, headache, heart ailments including hypertension, inflammation, pain, stomach ailments and acts as a tonic.	Tea is prepared by adding a few leaves to a cup of boiling water and allowed to cool. Drink one cup as needed to treat the ailments.	*Helichrysum petiolare* are popular as fumigants [2],[7].	

Asteraceae *Vernonia oligocephala* [Groenamara (Afri); Ihlambihloshane (Zulu); Mofolotsane (Sotho); Sefafatse (Tswana)] (Voucher specimen: M.L Arendse 25)	Diabetes, headache, hypertension, pain and stomach ailments.	A tea is prepared by adding a few leaves to one cup of boiling water and allowed to cool. This is used to treat diabetes, headache, hypertension, stomach ailments and pain.	Abdominal pain[2,3],abdominal pain during preganancy[1], colic[1,2], constipation[3], diabetes[1,2], diarrhoea[3], dysentery[1,2,3], purgative[1], rheumatism[1,2,3],stomachache[5] and ulcerative colitis[1,2].	
Boraginaceae *Lobostemon fruticosus* [Agtdaegeneesbos, douwurmbos(Afri)]	Treatment of sores and wounds and acts as a tonic.	For the treatment of sores and wounds, add a few leaves into the bath and wash the affected area. To use as a tonic, prepare as a tea using the leaves.	Bloodpurifier[2], burns[3], ringworm[1,3], skin diseases[1], sores[3], ulcers[3] and wounds[3].	
Canellaceae *Warburgia salutaris* [Pepper-bark tree (English); Peperbasboom (Afri); Isibhaha (Zulu); Mulanga, Manaka (Venda); Shibaha (Tsonga)]	Asthma, bladder and kidney ailments, colds and flu, headache and heart ailments.	The bark is broken up into smaller pieces, added to hot water and allowed to cool. Drink one cup morning and at night to treat asthma, bladder and kidney ailments, colds and flu, headache and heart ailments. *The leaves and bark are alternated for medicinal use.	Popular remedy used for colds[2,3,4,], constipation[3], coughs[2,4], coughs productive of purulent sputum[2], chest complaints[2,4], fever[3], gastric ulcers[2,3,4], headache[2,3,4] ,influenza[2,3,4], malaria[2,3,4] ,oral thrush[4], rheumatism[2,4,6],stomachache[3], toothache[2,4] and venereal diseases[2,3,4].	

Fabaceae *Sutherlandia (Lessertia) frutescens* [Cancerbush (Eng); Kankerbos, Belbos, Gansies (Afri); Unwele (Xhosa, Zulu)] (New:Wildekiertjies)	Treatment of tuberculosis and aid as a tonic.	A tea is prepared by adding a few leaves to a cup of boiling water. One cup is drunk three times daily.	Adaptogen[3,4] and bitter tonic[4]. Anxiety[3,4],asthma[3,4], backache[2],bronchitis(chronic)[3,4], cancer (internally)[1,2,3], chicken-pox[2], colds and flu[2,3,4], cough[1,3,4],diabetes[2,3,4],dysentery[3,4] fevers[2,3,4] gastric troubles[1,2,3,4],heart failure[3], inflammation[2],influenza[2,4],kidney conditions[3], liver problems[2,3,4], piles[2], poor appetite[3,4], prolapse of female genitalia[1], rheumatism[2,3,4], stress[3,4], tuberculosis[4], ulcers[3,4], urinary tract infections [3,4,] varicose veins[2], wounds[2].	If the infusion is prepared too strong, it might induce vomitting[1].
Geraniaceae *Pelargonium capitatum* [Rose-scented geranium (Eng)] (Voucher specimen: M.L Arendse 16)	Used as a carminative.	Tea is prepared from a few leaves and allowed to cool down. Drink one cup when needed to act as a carminative.	Perfume[3].	
Geraniaceae *Pelargonium reniforme* (Voucher specimen: M.L Arendse 17)	Treatment of earache and skin ailments.	For earache, the leaf is placed in the ear. To treat skin ailments, a tea is prepared by adding a few leaves to 1 cup of boiling water and allowing to cool down. Strain. Drink one cup daily.	Colic[1], delayed menses[1], diarrhoea[1], dysentery[1] and fever[1].	
Haemodoraceae *Dilatris viscosa* [Moerbossie (Afr)]	Treatment of female ailments.	Tea is prepared by adding few leaves to 1 cup of boiling water and allowing it to cool down. Strain. Drink one cup daily to treat the above-mentioned ailments.	This is the first report of the medicinal uses of *Dilatris viscosa.*	

Lamiaceae *Ballota africana* [Kattekruid, kattekruie (Afr)]	Bladder and kidney ailments, heart related ailments as well as hypertension, skin ailments, expulsion of the placenta and as a tonic.	Tea is prepared by adding a handful of leaves to approximately 1L of boiling water and allowed to cool. Drink one cup as needed to treat the ailments mentioned.	Treatment of arthritis[2],asthma[1,2,3], bronchitis[1,2], colds[1,2], colic[1], fever[3], flu[3], haemorrhoids[1,3], headaches[2,3], heart troubles[1,2], hoarseness[1, 2],hysteria[1,2], influenza[1,2], insomnia[2], liver problems[2], measles[3], sleeplessness[1], snakebite remedy[1], sores[1], stress[3], thrush[1] and typhoid fever[1,2].	
Lamiaceae *Leonotis leonurus* [(Wild dagga (Eng); Wilde dagga (Afr)] (Voucher specimen: M.L Arendse 14)	Arthritis, backpain, bladder and kidney, cold and flu, diabetes, fever, headache, heart ailments including hypertension, pain, skin ailments, sores and wounds, stomach ailments and a tonic.	Tea is prepared by adding a handful of leaves to approximately 1L of boiling water and allowed to cool. Drink one cup as needed to treat ailments.	Treatment of ammenorrheae[3],asthma[2,3], bronchitis[1,2,3,6], boils[2], colds[1,2,]3,coughs1[,2,3,6],dysentery[6] ,eczema[2], epilepsy[2,3,6], flu[3], haemarrhoids[1], headache[1,2,3,6], hypertension[2,3], itching[2], indigestion[1], influenza[2], jaundice[1,6],muscular cramps[1,2,6], skin ailments[1,2,6], snakebites[1,2,6], sores[1,6] stings[1,2,6], tonic[1], tuberculosis[1,6], viral hepatitus[2] and worms[6].	
Lamiaceae *Salvia africana-caerulea* [Bloeblomsalie (Afr)] (Voucher specimen: M.L Arendse 22)	Arthritis, bladder and kidney ailments, backpain, colds, flu and cough, fever and sore throats, colic, female ailments, heart related ailments including hypertension, stomach ailments as a detox aid and as a tonic.	To treat colds and flu, a syrup is prepared by adding the leaves to sugar. A tea is consumed three times daily for the treatment of the remaining ailments.	Remedy for abdominal cramps[3], bronchitis[3],colds[1,3] ,coughs[1], chest problems[1], colic[1], diarrhoea[1], flatulence[1], flu[3], indigestion[1,3], uterine complaints[1] and whooping cough[1].	

Melianthaceae *Melianthus comosus* [Kruidjie-roer-my-nie (Afr); Ibonya (Zulu)]	Feet ailments, inflammation, pain, sores and wounds.	Add a few leaves to a bath filled with water. Soak the affected areas in the infused water. Wash sores and wounds as well as inflamed areas and feet.	Arthritis[1,2,3,5,6] ,backache[2,3], bruises[1,2,3,6] burns[3,5], cancer[2], dyspepsia[1,6], feet ailments[1,6] ,impetigo[3], lupus[6], ringworm[2,3] ,snakebite[1,2] ,sores[1,2,3,5,6], sore throat[6], syphillis[6], tonic[1,6] and wounds[1, 2,3,5,6]	Weak infusions are consumed however, the plants are toxic. Cases have been reported on internal use leading to fatalities [3, 5, 6].
Menispermaceae *Cissampelos capensis* [Star of David (Eng); Dawidjiewortel, Dawidjies(Afr)]	Pain and stomach ailments.	A tea is prepared by adding a small piece (approximately 5cm) of root to boiling water and allowed to cool. Drink one cup three times daily when needed.	Bladder ailments[1,2], blood purifier for boils and syphillis[1,2,3], cancer[3], cholera[1,2], colic[1,2], diabetes[3],diarrhoea[1,2], dysentery[1,3], glandular swellings[3], headache[3], pain[3], urinary stones[3], sedative[3], snakebites[1,3], tuberculosis[3] and wounds[1].	
Proteacea *Protea cynaroides* [King protea (Eng); Groot suikerkan (Afr)]	Bladder and kidney ailments as well as cancer.	Prepare a tea by adding few leaves to 1 cup of boiling water and allow to cool. Strain. Drink one cup twice daily to treat bladder and kidney ailments and one cup thrice daily for the treatment of cancer.	This is the first report of the medicinal uses of *P. cynaroides*.	
Rosaceae *Cliffortia odorata* [Wildewingered (Afr)] (Voucher specimen: M.L Arendse 10)	Treatment of backpain, bladder and kidney ailments, cancer, colds and flu, cough, diabetes, feet ailments, fever, headache, hypertension, pain, stomach ailments, TB and as a detoxification aid.	Tea is prepared by adding a few leaves to 1 cup of boiling water and allowing it to cool down. Strain. Drink one cup daily to treat the ailments mentioned. For pain, add a handful of leaves to the bathwater and immerse body into the water. For the treatment of feet ailments, add a handful of leaves to a small foot bath and soak feet for 30 minutes.	Colds[1], croup[1], diphtheria[1], haemarrhoids[1], menstrual irregularities[1], miscarriages[1], mouth sprue[1], piles[1] and sore throat[1].	

Rutaceae *Agathosma crenulata* [Buchu (Eng) (Khoi), Boegoe (Afr), ibuchu (Xhosa) (Voucher specimen: M.L Arendse 1)	Arthritis including pain and inflammation, colds and flu including fever and cough, bladder and kidney ailments, headache, tuberculosis, stomach ailments, back pain, heart ailments including hypertension, detoxification aid and tonic.	For the treatment of sprains, pain and inflammation (incl. arthritis, headache and pain), place fresh leaves or dried leaves on a cloth and soak in the vinegar. Wrap the cloth around the affected area. On drying, repeat as needed. A tea is prepared by adding a few fresh or dried leaves to one cup of boiling water. Allow to cool. Drink one cup three times daily. This preparation is used to treat colds and flu as well as fever, bladder and kidney ailments including prostrate related ailments, hypertension and heart related ailments, stomach ailments, detoxification aid and as a tonic.	Tonic[8], stomach complaints[8], wounds[8], urinary tract diseases[8] and arthritis[8].	*A crenulata* is less desirable due to high levels of pulegone, a compound that is considered toxic [2,3,7] .

Table 1B: The current uses and preparations of the exotic medicinal plants used in the Dwarsrivier valley, Stellenbosch as well as previously recorded uses in the literature.

Family **Plant species** **(Common name)**	**Use or condition treated**	**Method of preparation and use**	**Uses in literature**	**Other information**
Alliaceae *Allium cepa* [Onion (Eng); Ui (Afr)]	Treatment of fever in children.	To treat fever in children, a raw onion is chopped, placed at the pulse of the foot and secured with a cloth.	Appetite loss [4], arteriosclerosis[4], asthma[4], diabetes[4], digestive disturbances[4], dysentery[4], fever[1], hypoglycaemic effects[1], influenza[1], keloids[4] and scars[4]	
Alliaceae *Allium sativum* [Garlic (Eng), Knoffel (Afri)]	Treat asthma, fever, hypertension and as a tonic.	Cloves of raw garlic are eaten to treat fever, hypertension and act as a tonic. For the treatment of asthma, seven cloves of garlic are tied around the neck and worn as a necklace for the day.	Anthelmintic[1], antiseptic[1], asthma[1], aphrodisiac[1], arteriosclerosis[4] bronchiectasis[1], carminative[1], cholera[1], colds[4], croup[1], diaphoretic[1], diuretic[1], dysenteries[1], earache[1], expectorant[1], gastrointestinal catarrh[1], headache[1], hypotensive[1], rheumatic pains[1], stimulant [1], tuberculosis[1], typhoid fever[1], ulcers[1], whooping cough[1] and wounds[1].	
Apiaceae *Foeniculum vulgare* [Fennel (Eng); Vinkel (Afr)] (Voucher specimen: M.L Arendse 12)	Backpain, bladder and kidney, heart ailments, skin ailments and as a detoxification aid.	Tea is prepared from a few leaves and allowed to cool down. Drink one cup when needed.	Appetite stimulator [2], carminative [6], catarrh[4], conjunctivitis[4], coughs (chronic)[2,4], cramps[1], diarrhoea[1], diuretic [2,3,] flatulence [2,3,4,] indigestion [2,3], influenza [4], jaundice [1,6,] menstrual irregularities [1,6], skin disorders [4] and stomach ache [1,6].	

Apiaceae *Petroselinum crispum* [Parsley (Eng)] (Voucher specimen: M.L Arendse 18)	Treatment of bladder and kidney ailments, pain including backpain, stomach ailments, sedative, heart related ailments including hypertension and as a tonic.	Tea is prepared from a few leaves and allowed to cool. Drink one small cup three times daily to treat ailments.	Diuretic[1], gastrointestinal disorders [4, 5,] emmenagogue[1], menstrual ailments[4], urinary tract ailments [4, 5] and skin ailments [4, 5].	
Apocynaceae *Catharanthus roseus* [Madagascar periwinkle (Eng)] (Voucher specimen: M.L Arendse 8)	Treatment of diabetes and hypertension.	A tea is prepared by adding seven leaves to one cup of boiling water and allow cooling down. Strain. Drink one cup when necessary.	Blood purification[6], bites[6], cancer [2,3,4,5], diabetes [2,3,4,5], liver congestion[6], menorrhagia[6], rheumatism [2,3,5], tonic [6], toothache [6] and warts [5].	
Asteraceae *Chrysanthemum parthenium* [Feverfew (Eng)]	Treatment of cough, fever, headache, hypertension and stomach ailments.	A tea is prepared by adding a few leaves to one cup of boiling water and allowed to cool down. Strain. Drink one cup when necessary.	Arthritis[1], fever[1], migraine[1], skin conditions [1] and gynaecological disorders[1].	
Asteraceae *Matricaria recutita* [Chamomile, German chamomile (Eng); Kamella (Afr)] (Voucher specimen: M.L Arendse 15)	Treatment of colic, epilepsy, fever, headache, hypertension, sedative, stomach ailments, sore eyes and worms and as a carminative.	Tea is prepared from a few flowers and leaves and allowed to cool. Drink one cup when needed.	Anxiety[4], diarrhoea[4], catarrh[4], dyspepsia [4], gastritis [4] and skin ailments [4].	
Asteraceae *Tagetes minuta* [Tall khaki bush (Eng), lang kakie bos (Afr)]	Treat colds and flu, cough, earache, feet ailments, female ailments, stomach ailments and tuberculosis.	Leaves are added to boiling water to prepare a tea. Drink one cup daily to treat the ailments mentioned.	The essential oil is used in the perfume industry and used to flavour food, beverages and tobacco[3].	
Cannabaceae *Cannabis sativa* [Marijuana, Indian hemp (Eng); Dagga (Afr); Umya (Xhosa); Matokwane (Sotho); Nsangu (Zulu); Isanga (Swati)]	Relief of asthma and cough.	Leaves are usually smoked to relieve asthma and cough.	Appetite stimulator [2,3],asthma [2,3],blood poisoning [2],bronchitis [3], chemotherapy-induced nausea [2], colds [3],depression[2], diabetes [3], epilepsy [3], glaucoma[2], headache[3], hypertension[3], insomnia [3], malaria [2], , muscle spasms[3] and snakebites[1, 2] .	

Cannaceae *Canna* spp. [New: Kanna blaar]	Treatment of arthritis, pain and inflammation.	Apply olive oil to the leaf, heat in the oven and apply to the affected area. Wait until the leaf darkens and repeat the process.	This is the first report of *Canna* spp. used for medicinal purposes.	
Euphorbiaceae *Ricinus communis* [Castor oil (Eng), Kasterolie, Olieblaar (Afr)] (Voucher specimen: M.L Arendse 19)	Treatment of asthma, arthritis including backpain and inflammation, coughs, headache, sores and wounds as well as stomach ailments.	To treat the mentioned ailments, apply camphor oil or olive oil to the leaf, heat in the oven and apply to the affected area.	Appetite stimulator[6], boils [1,2,4,6], diarrhoea [1,6], earache[3], fever[6], headache[1], mumps[3], skin disease[1], sores [1,2,4,6,] stomach ache [1,2,6], toothache [1,3,6], rheumatisms [1,6], stomach complaints[1], ulcers [6] and wounds [1,2,4,6].	
Fagaceae *Quercus laurifolia* [Swamp laurel oak, Diamond leaf oak, Laurel oak, Water oak (Eng)]	Stomach ailments.	For the treatment of stomach ailments, add a few pieces of chopped bark to boiling water and allow to cool. Strain and drink when needed.	Diarrhoea[1], dysentery[1], cholera[1], boils[1], rectal prolapse[1], boils[1], neck swelling[1],croup[1] and diphtheria[1].	
Lamiaceae *Lavandula angustifolia* [Lavender (Eng); Lavental (Afr)] (Voucher specimen: M.L Arendse 13)	Treat headaches, stomach ailments, sedative and acts as a carminative.	Tea is prepared from a few leaves and allowed to cool. Drink one cup when needed. For headaches and to aid sleeping, add a few drops of oil to the pillow before retiring to bed.	Burns [4], dyspepsia [4], headache [4], insomnia [4], migraine [4,] muscular cramps [4], tension [4] and wounds[4.]	
Lamiaceae *Mentha aquatica* [Mint (Eng); Kruisement (Afr)]	Treatment of backpain, bladder and kidney, colds and flu including cough and fever, female ailments, headache, feet ailments, stomach ailments and as a detoxification aid.	Tea is prepared by adding a few leaves to 1 cup boiling water. Allow to cool. Strain. Drink one cup when needed to treat the ailments mentioned.	Colds[6], diarrhoea[6], gynaecological complaints[1,6], menstrual irregularities[6] and respiratory ailments[6].	

Lamiaceae *Mentha longifolia* [(Wild mint (Eng); Ballerja, Kruisement (Afr)]	Bladder and kidney ailments, colds and flu including cough and fever as well as stomach ailments.	Tea is prepared by adding a few leaves to 1 cup of boiling water and allow to cool. Strain. Drink one cup when needed to treat the ailments mentioned.	Antispasmodic[1], asthma[2], carminative[1], chest inflammation[1], croup[1], colds[2,6],coughs[2], delayed pregnancy[2], diphtheria[1], epilepsy[3], fever[3], headaches[2,3,6], hysteria[2,3], fever[2], flatulence[2,6] ,flatulent colic[1],gynaecological disorders[1], headache[6], indigestion[2,3], insomnia[3], Menstrual irregularities[1,2], menstrual pain[6], oedema[1], typhoid fever, pulmonary turbuculosis[1], respiratory ailments[3,6], scarlet fever[1], stomach pain[6], swollen glands[2], swellings, urinary tract infections[2,3], whooping cough[1] and wounds[2,6]	
Lamiaceae *Rosmarinus officinalis* [Rosemary (Eng); Roosmaryn (Afr)] (Voucher specimen: M.L Arendse 20)	Treatment of colds and flu, diabetes, female ailments, liver ailments, sedative, stomach ailments, hair stimulator and to aid the body as a tonic.	A decoction is prepared and used as a rinse for hair. A tea is prepared by adding a few leaves to water and allowed to cool. Drink one cup daily to treat the ailments mentioned.	Appetite stimulator[4], carminative[4], flatulence [4], hair stimulator[1], headache[4], heart disease[1] and stomach cramps[4].	
Lamiaceae *Thymus vulgaris* [Thyme, garden thyme (Eng)] (Voucher specimen: M.L Arendse 23)	Treatment of colic and female ailments.	For the treatment of colic, a weak decoction is made and added to the baby bottle or a few leaves are chopped up and added to the milk in the baby bottle. For the treatment of female ailments, a tea is prepared by chopping up a few leaves and adding to a cup of boiling water. Drink one cup daily.	Antiparasiticide[1], antiseptic[1], antispasmodic[1], bronchitis[4] carminative[1], digestive[1], emmenagogue[1], sedative[1] and upper respiratory tract inflammation[4] .	

Lauraceae *Cinnamomum verum* [Cinnamom bark (Eng); Kaneel (Afri)]	Treatment of diabetes, female ailments and hypertension	Tea is prepared by adding a few sticks of cinnamon or half teaspoon of powder to one cup of boiling water and allowing it to cool down. Strain. Drink one cup twice daily to treat the mentioned ailments.	Arthritis[4], appetite loss [4], colds[4], diarrhoea[4,6], flatulence[4], gastrointestinal spasms [4], hypertension [6], inflammation [4], menstrual disorders [4,6] , nausea and vomiting [4,6].	
Moraceae *Ficus carica* [Fig leaf (Eng); Vyeblaar (Afr)]	Arthritis, skin ailments, sores and wounds and stomach ailments.	Tea is prepared from a few leaves and allowed to cool. Drink one cup when needed.	Diphtheria[1], diuretic[1], emollient[1], thrush[1].	
Myrtaceae *Eucalyptus globulus* [Eucalyptus, Bluegum (Eng); Bloekom(Afr)]	Colds and flu, cough, detoxification, female ailments, feet ailments, fever, headache, pain, sore throat and as a tonic.	For the treatment of feet ailments, add a few leaves or few drops of oil to warm water and soak feet for 30 minutes. A tea is prepared by adding a few leaves to 1 cup of boiling water and allow to cool. Strain. Drink one cup when needed to treat the mentioned ailments.	Colds [1,2,3,4], congestion [3,4], croup[1], cough[1], diabetes[1], diphtheria[1] and rheumatism[4].	
Myrtaceae *Psidium guajava* [Guava leaf (Eng); Koejawel blaar (Afr); Ugwava (Zulu)]	Bladder and kidney ailments, diabetes, hypertension, female ailments and pain.	Tea is prepared by adding a handful of crushed leaves to boiling water. Allow to cool. Strain. Drink one cup three times daily to treat the mentioned ailments.	Cough[1], cuts [1], diarrhoea[1], haemorrhages[1], pulmonary disorders[1] and sprains [1].	

Polygonaceae *Polygonum aviculare* [Knotweed, knotgrass (Eng)] (New: Litjiesgras)	Treatment of back pain, colic, mouth thrush and teething, stomach ailments and as a tonic.	For mouth thrush and teething, bruise leaves and rub onto the gums. For stomach ailments and colic in babies, chop up the leaves and add to boiling water to make a tea. Administer through the baby bottle. As an alternative, add chopped leaves to olive oil or into the babies' milk. For backpain and to aid as a tonic, prepare a tea made from the dried leaves added to boiling water and allow to cool. Strain and drink one cup daily.	Coughs[4], catarrh[4], expectorant[1], diarrhoea[1], diuretic [1, 4], dysentery[1], haemarrhoids[1], hypertension[1], infertility[4], liver ailments [4], nervous conditions [4], rheumatism[1], skin ailments[4] and tonic [1].	
Rutaceae *Ruta graveolens* [Herb of grace, Rue (Eng); Wynruit (Afri)] (Voucher specimen: M.L Arendse 21)	Treatment of arthritis including inflammation and pain, bladder and kidney ailments, headache, heart ailments including hypertension, sedative and acts as a tonic.	A tea is prepared by adding a few leaves to 1L boiling water and 1 cup is drunk daily. Used to treat the mentioned ailments. For headaches, the leaves are bruised, applied to the forehead and covered with a rag.	Appetite loss[4], asthma[1], childbirth[1], circulatory disorders[4], colic[1], convulsions[1], earache[1], fevers [1,4], fits (infantile)[1], heart ailments[1,2,4,5], hypertension[4], hysteria[2,4], injuries [4,5,] menstrual irregularities[1,4,5], respiratory ailments[1,2], rheumatism[2,4,5], skin diseases[4,5], spasms[4], sprains[4,5], stomachic[1] and toothache [1,4.]	

Rosaceae *Prunus persica* [Peach leaves (Eng), Perske blare (Afri)]	Treatment of stomach ailments, fever as well as worms.	For the treatment of stomach ailments and fever in infants and children, place fresh or dried leaves on a cloth and sprinkle with vinegar. Attach to the stomach area of infants and children to ease stomach pains and reduce or lower fever. For the treatment of worms, a tea is prepared using the leaves and one cup is drunk three times daily.	Anaemia[1], delayed menstruation [1] and whooping cough[1].	
Rosaceae *Rubus pinnatus* [Blackberry,Bramble, Cape Bramble(Eng); Braambos (Afr); Munambala (Venda)]	Treatment of mouth thrush, female complaints as well as sore eyes.	For the treatment of mouth thrush, the root is chopped or finely crushed and rubbed onto the inflamed gums until it induces a light bleed. Female complaints are treated by adding the roots to boiling water to prepare a tea. Drink one cup daily. For sore eyes, a decoction is prepared and used to wash the eyes.	Abdominal cramps[1], chest conditions [1,6], convulsions, diarrhoea [1,6] and toothache[6].	
Solanaceae *Datura stramonium* [Common thorn apple, Jimson weed (Eng); Gewone stinkblaar, Malpitte(Afr)] (Voucher specimen: M.L Arendse 11)	Arthritis including pain and inflammation as well as sores and wounds.	Apply olive oil to the leaf, heat in the oven and apply to the affected area. Wait until the leaf darkens and repeat the process. Useful in treating the mentioned ailments.	Boils[1], abscess[1], gout[1], rheumatism [1,3,7], insomnia[3],hypnotic [7], aphrodisiacs[3,7], headache[3], asthma [1,3], bronchitis[1], toothache [1], sore throat[1], earache[3], pain[3], inflammation[3], fractures[3], sprains[3] and wounds[3], septic sores[3]	

Urticaceae *Urtica dioica* [Stinging nettle (Eng), Brandtnetel (Afri)]	Used for the treatment of arthritis, bladder and kidney ailments, cough including fever and sore throat, diabetes, heart ailments including hypertension, measles, prostrate ailments, skin ailments, sores and wounds, tuberculosis and as a detoxification aid.	Leaves are added to boiling water to prepare a tea. Drink one cup daily to treat the ailments mentioned.	Blood purifier[1],bronchial catarrh[1], diuretic [1], dysentery[1], eczema[1], expectorant[1], gout[1], haemorrhoids[1], haemoptysis[1], haemorrhages[1], jaundice[1], oedema[1], prostate enlargement[4,5], purgative[1], rheumatism[1,4,5], snakebite remedy[1], urinary tract inflammation[4,5] and treatment of kidney gravel[4].	Skin contact with nettles causes painful itching with welt formation at the areas pricked by stinging hairs. In sensitive persons, inflammation can last for 36 hours[5].
Verbenaceae *Aloysia triphylla* [Lemon verbena, vervain (Eng)] (Voucher specimen: M.L Arendse 3)	Acts as a tonic and carminative.	Few leaves, fresh or dried, are added to a cup of boiling water as a tea and left to cool down. Drink as needed.	Antipyretic[4], antispasmodic [1,4], aromatic[1], asthma[4], colds [4], colic[4], diarrhoea[4], fever[4], flatulence[4], indigestion[4], sedative[4], stimulant[1], stomachic [1,4].	
Vitaceae *Cissus rhombifolia* [New: Plakkie] (Voucher specimen: M.L Arendse 9)	Treatment of inflammation and sores and wounds.	For inflammation and sores and wounds, leaves are cut open and applied to the affected area.	This is the first report of *Cissus rhombifolia* used for medicinal purposes.	

References: 1) Watt and Breyer-Brandwijk (1962); 2) van Wyk et al (1997); 3) van Wyk and Gericke (2000); 4) van Wyk and Wink (2004); 5) Van Wyk et al (2002); 6) Hutchings et al (1996); 7) Van Wyk et al (2009); 8) Viljoen and Moolla (2007)

Table 2: Combinations of plants used in the Dwarsrivier valley compared to other parts of South Africa.

AILMENT	COMBINATION	LITERATURE
Arthritis	-*Artemisia afra* and *Ruta graveolens*	This is the first report of plant combinations used to treat arthritis.
Back pain	-*Agathosma crenulata*, *Leonotis leonurus* and *Mentha longifolia* -*Helichrysum petiolare* and *Salvia africana-caerulea* -*Mentha acquatica* and *Petroselinum crispum*	This is the first report of plant combinations used to treat back pain
Bladder and Kidney	-*Ballota africana* and *Urtica dioica* (weak bladder) -*A. afra* and *H. petiolare* -*H. petiolare* and *S. africana-caerulea* -*A. crenulata*, *L. leonurus* and *M. longifolia.* -*Protea cynaroides* and *Warburgia salutaris* -*M. acquatica* and *Petroselinum crispum.* -*M. acquatica* and *Ruta graveolens* -*M. longifolia* and *P. crispum*	This is the first report of plant combinations used to treat bladder and kidney complaints.
Cancer	*Cliffortia odorata* and *Helichrysum petiolare* *C.odorata* and *Protea cynaroides*	The is the first report of plant combinations used to treat cancer.
Colds and flu	*A. crenulata* and *L. leonurus* *A. crenulata*, *C. odorata*, *E. globulus* and *H. petiolare* *A. crenulata* and *M. acquatica* *A. crenulata*, *M. longifolia* and *L. leonurus* *A. crenulata* and *H. petiolare* *E. globulus* and *S. africana-caerulea*	-*Aloe marlothii*, *Hypoxis* sp., *Scadoxis puniceus* and *Erythrina caffra* for the treatment of chest pain, fever and a blocked nose (York et al., 2011) -*Eucalyptus grandis* and *Lippia javanica* for the treatment of chills, coughs, runny nose, headache, chest pain, tonsillitis, sore throat, fever or earache (York et al., 2011). -*Eucalyptus grandis*, *Clausena anisata* and *Lippia javanica* for fever, cough, runny or blocked nose (York et al., 2011). -*Eucalyptus grandis*, *Combretum molle* and *Lippia javanica* to treat cough and fever (York et al., 2011). -*Eucalyptus grandis*, *Brachylaena* spp. and *Lippia javanica* to treat cough, chest pain, runny nose and fever (York et al., 2011). *Eucalyptus grandis* and *Ozoroa obovata* to treat cough, chest pain, fever, blocked or runny nose (York et al., 2011).

Diabetes	-*A. afra* and *Psidium guajava*	This is the first report of plant combinations used for diabetes.
Female ailments	-*E. globulus*, *Rosmarinus officinalis* and *P. guajava* -*S. africana-caerulea* and *Tagetes minuta* -*M. aquatica* and *R. graveolens* -*S. africana-caerulea*, *E. globulus* and *P. guajava* -*R. officinalis* and *T. minuta*	This is the first report of plant combinations for female ailments.
Fever	-*A. afra* and *Prunus persica* -*A .afra*, *R. graveolens* and *P. persica* -In children: *A. afra*, *Ruta graveolens* and *P. persica.*	-*Aloe marlothii*, *Hypoxis* sp., *Scadoxis puniceus* and *Erythrina caffra* for the treatment of chest pain, fever and a blocked nose (York et al., 2011) -*Eucalyptus grandis* and *Lippia javanica* for the treatment of chills, coughs, runny nose, headache, chest pain, tonsillitis, sore throat, fever or even earache (York et al., 2011). -*E. grandis*, *Clausena anisata* and *L. javanica* for fever, cough, runny or blocked nose (York et al., 2011). -*E. grandis*, *Combretum molle* and *L. javanica* to treat cough and fever. -*E. grandis*, *Brachylaena* spp. and *L. javanica* to treat cough, chest pain, runny nose and fever (York et al., 2011). *E. grandis* and *Ozoroa obovata* to treat cough, chest pain, fever, blocked or runny nose (York et al., 2011).
Hypertension	*A .afra* and *P. guajava.*	This is the first report of plant combinations used for treating hypertension.
Inflammation of the womb	*E. globulus*, *P. guajava*, *Rosmarinus officinalis*, *S. africana-caerulea* and *T. minuta*	This is the first report of plant combinations used for treating inflammation of the womb.
Sedative	*Lavandula angustifolia*, *Matricaria recutita*, *R. officinalis* and *R. graveolens.*	This is the first report of plant combinations used as a sedative.

Stomach ailments	-*A. crenulata*, *L. leonurus* and *M. longifolia* -*A. afra* and *P. persica* -*P. persica* and *R. graveolens*	*Brachylaena transvaalensis* and *Psidium guajava* to treat diarrhoea (de Wet et al., 2010). -*Acanthospermum glabratum* and *P. guajava* (de Wet et al., 2010).
Tonic	-*A. crenulata*, *A. afra*, *S. africana-caerulea*, *Lobostemon fruticosus* and *Ballota africana* -*A. afra* and *H. petiolare.* -*A. afra*, *A. crenulata*, *B. africana*, *L. fruticosus* and *S. africana-caerulea.* -*Lobostemon fruticosus* and *E. globulus*	This is the first report of plant combinations used as a tonic.

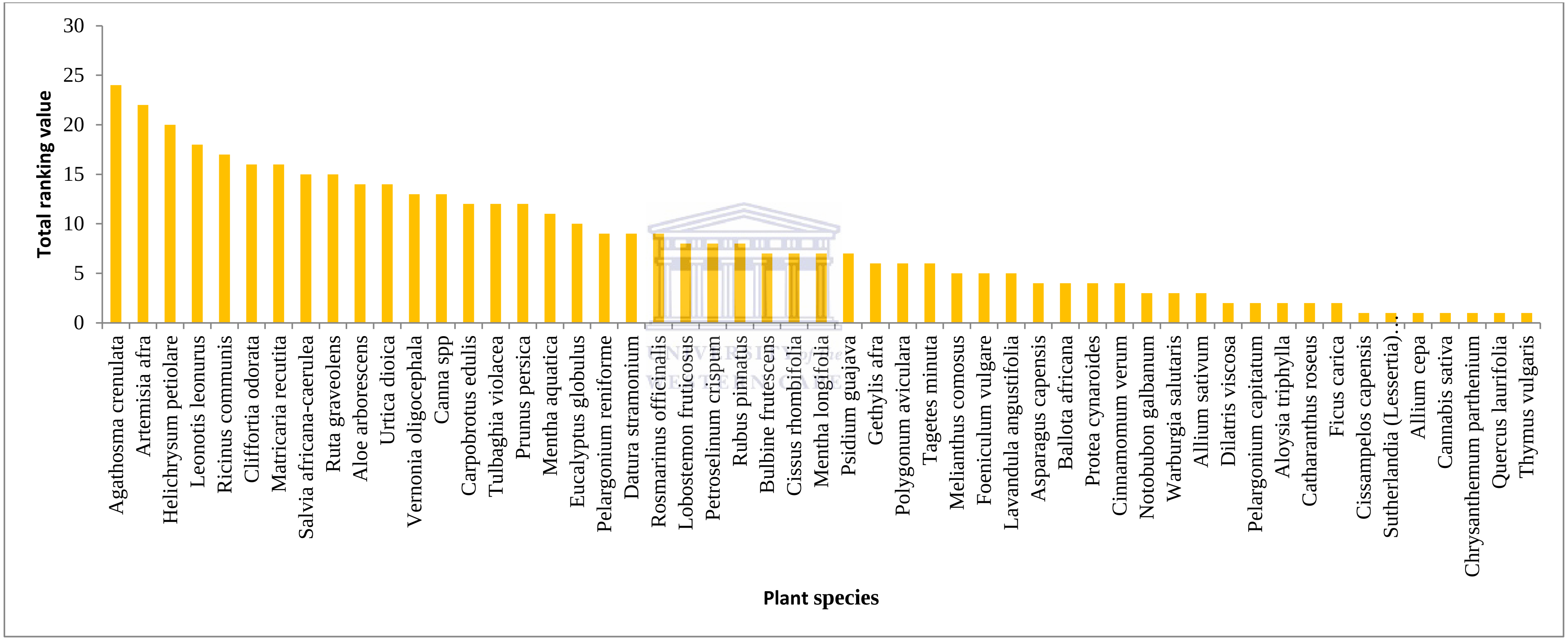

Figure 2A: Ranking value of plants in the Dwarsrivier community.

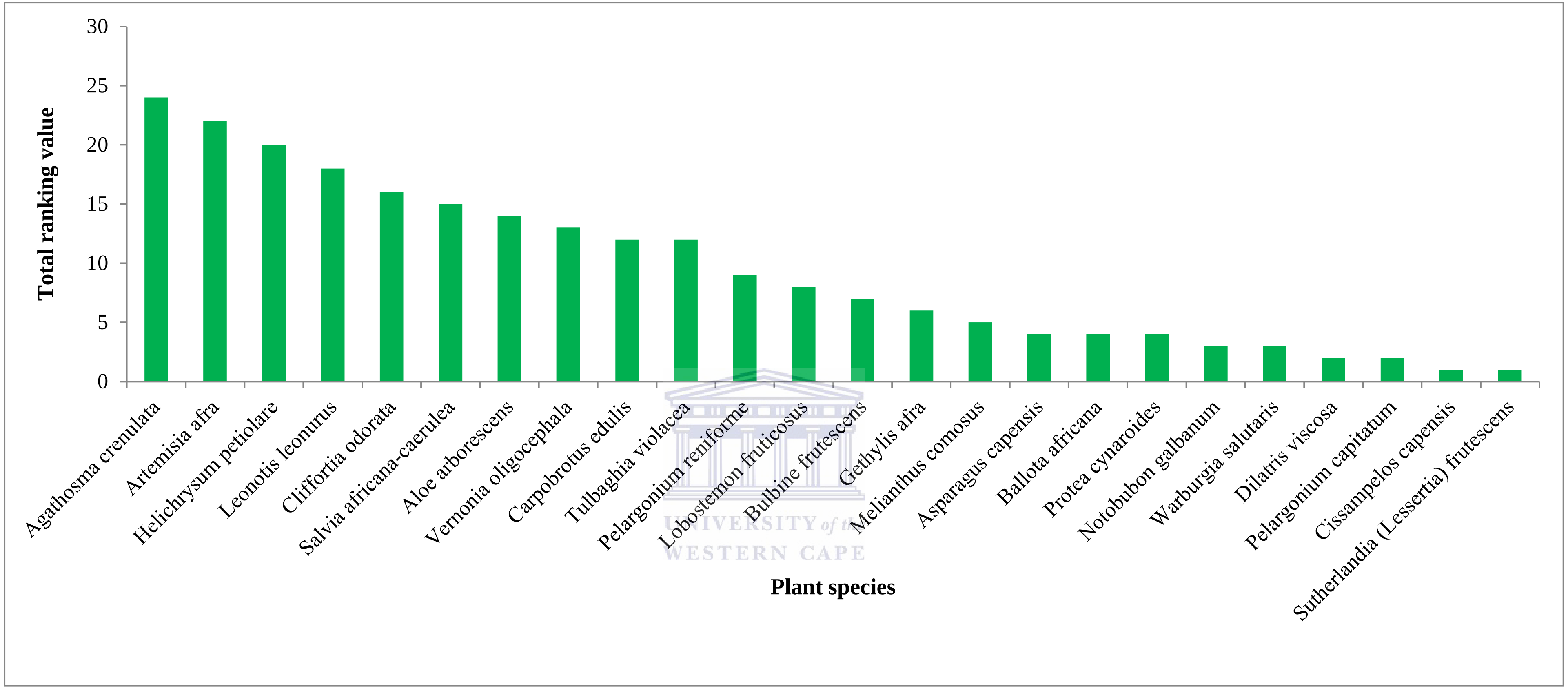

Figure 2B: Ranking value of the indigenous plants in the Dwarsrivier valley.

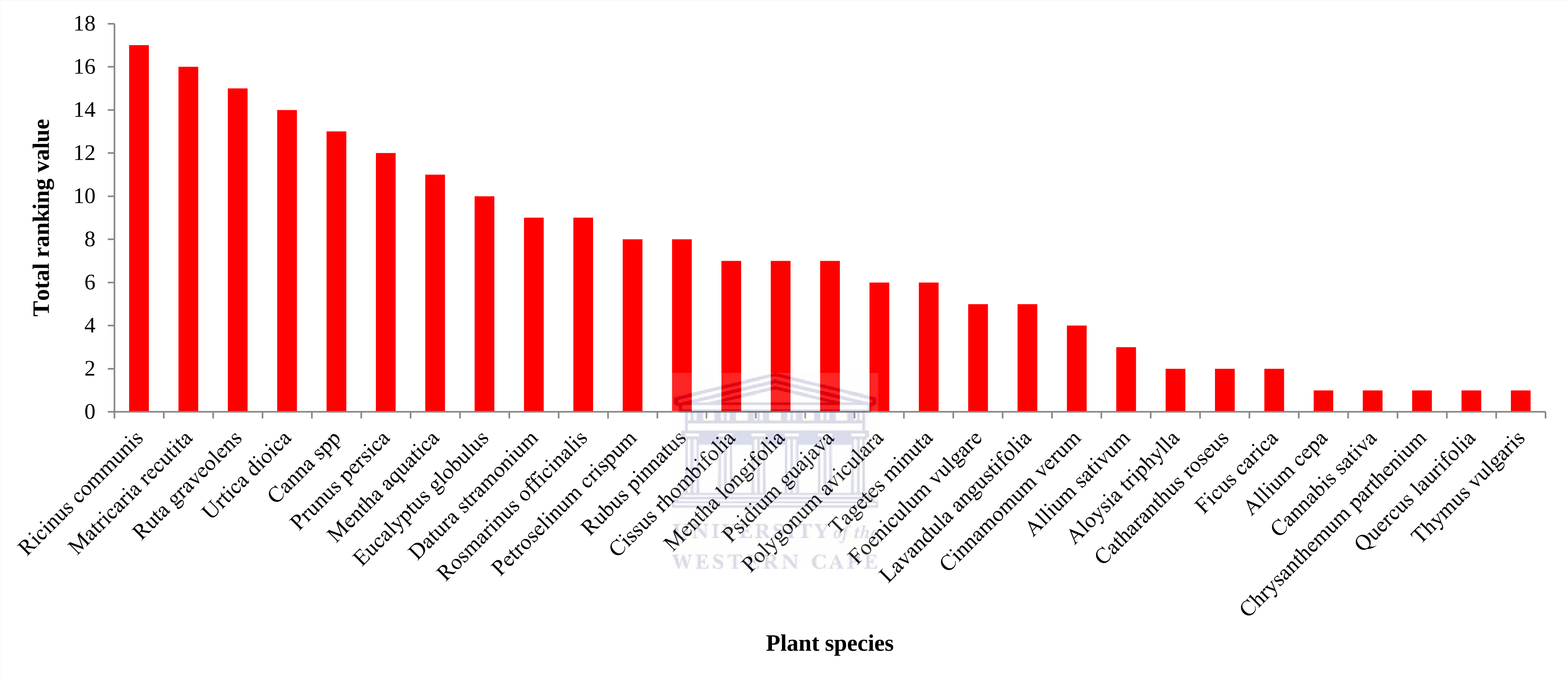

Figure 2C: Ranking value of exotic plants in the Dwarsrivier valley

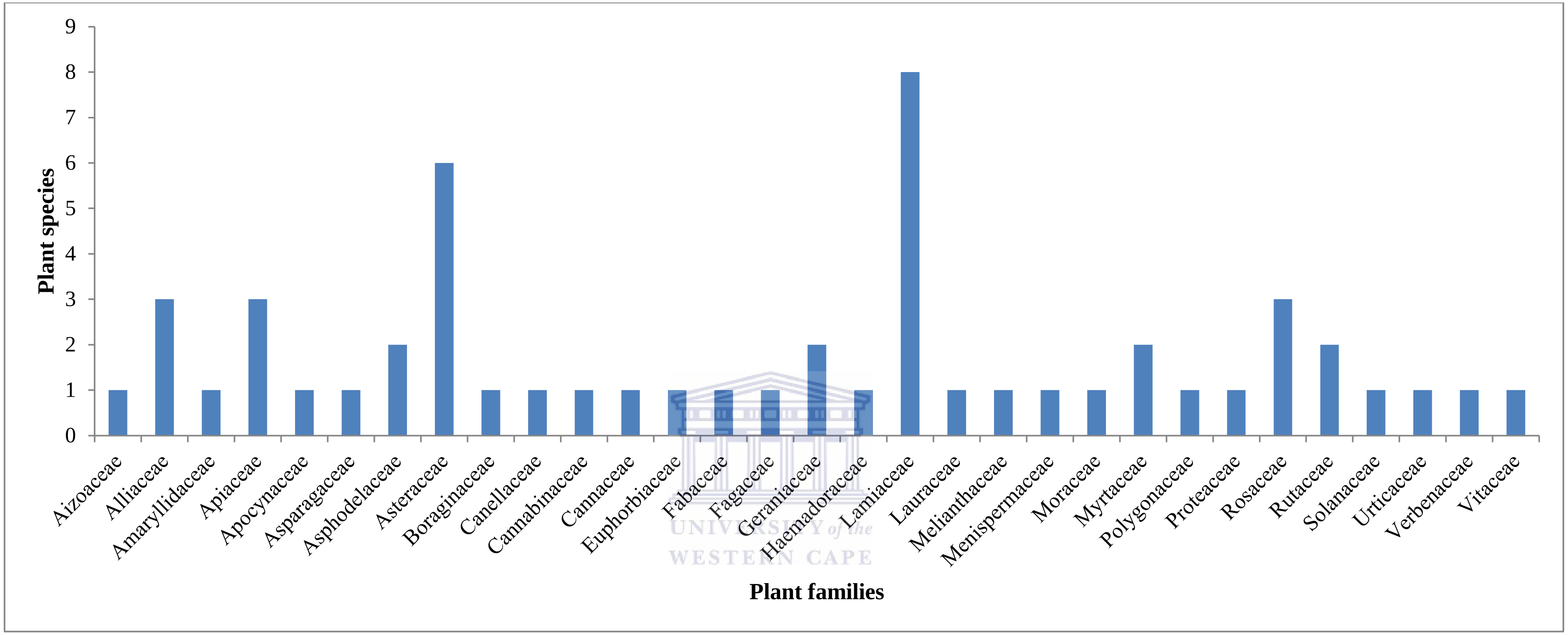

Figure 3: The most common plant families

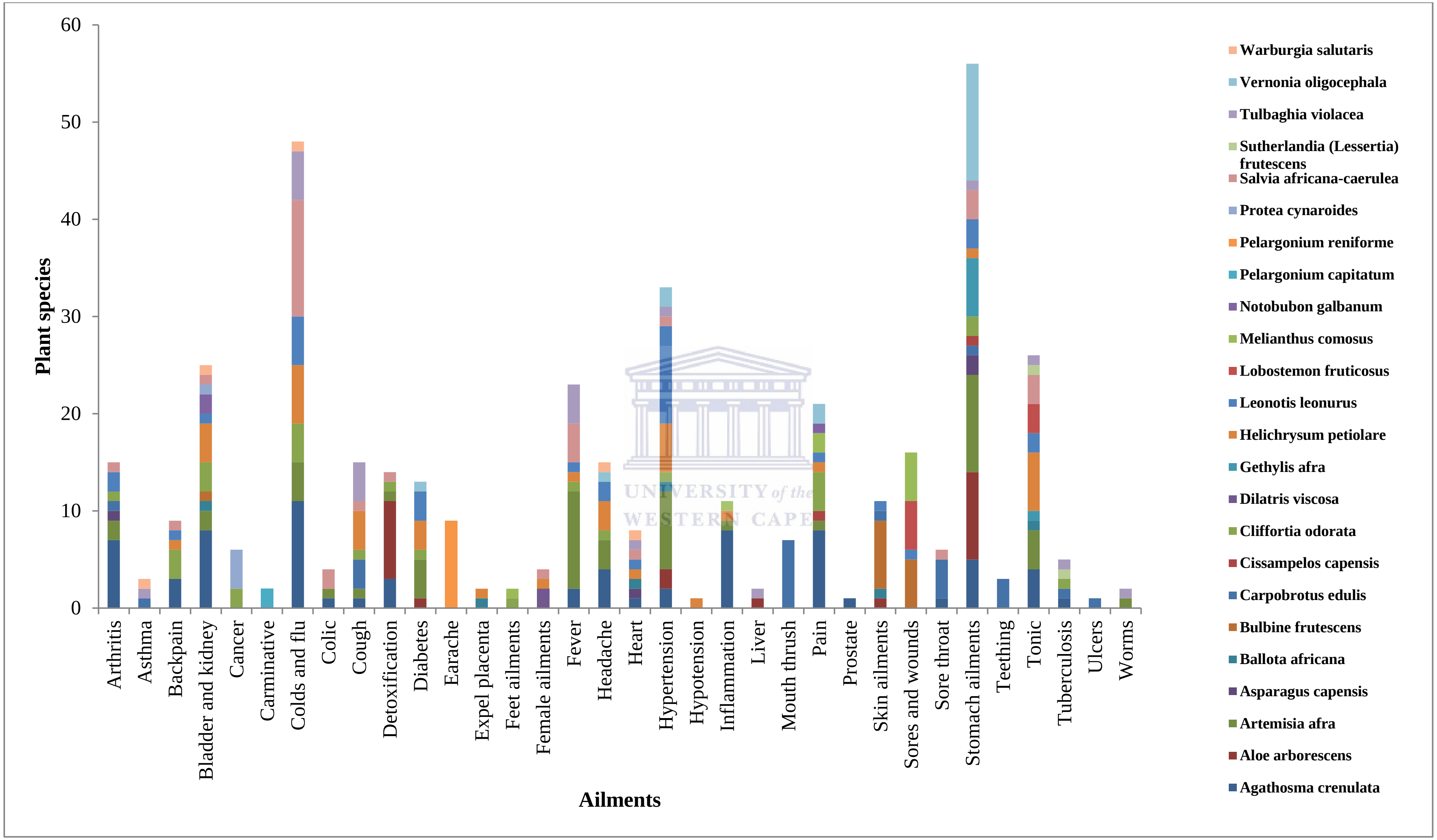

Figure 4A: Recorded uses of the indigenous plants to treat ailments in the Dwarsrivier valley.

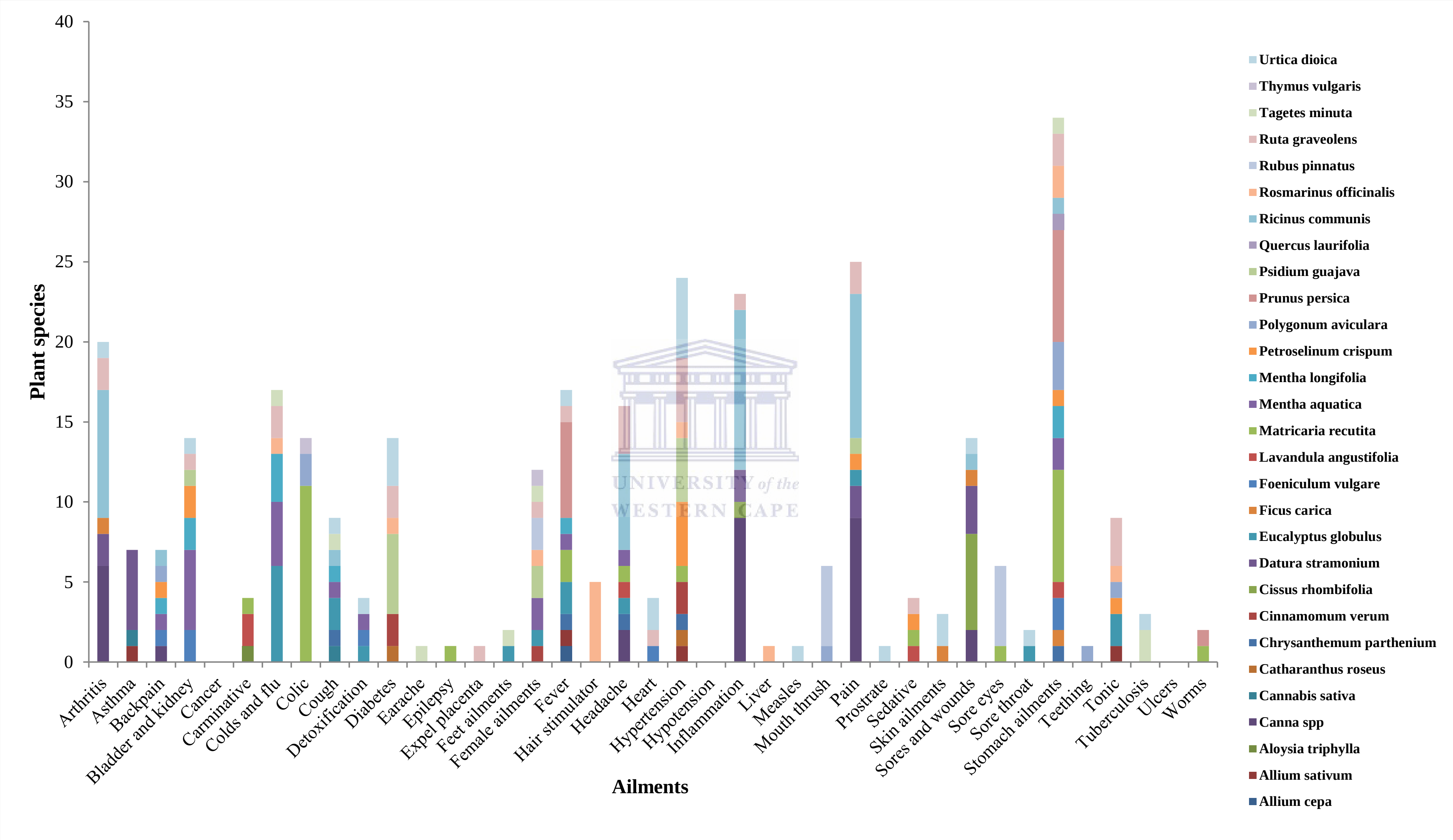

Figure 4B: Recorded uses of exotic plants to treat ailments in the Dwarsrivier valley.

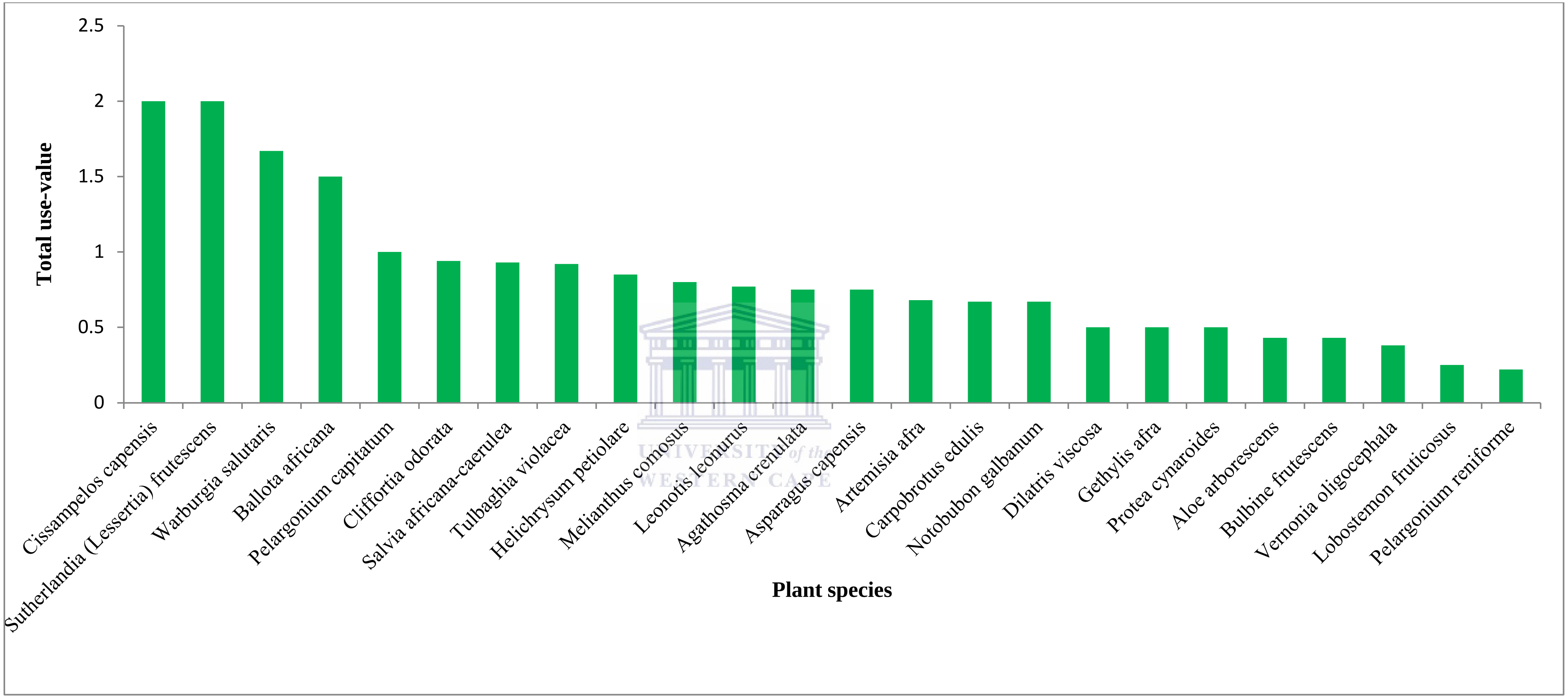

Figure 5A: Graph showing the calculated use-values for indigenous plant species.

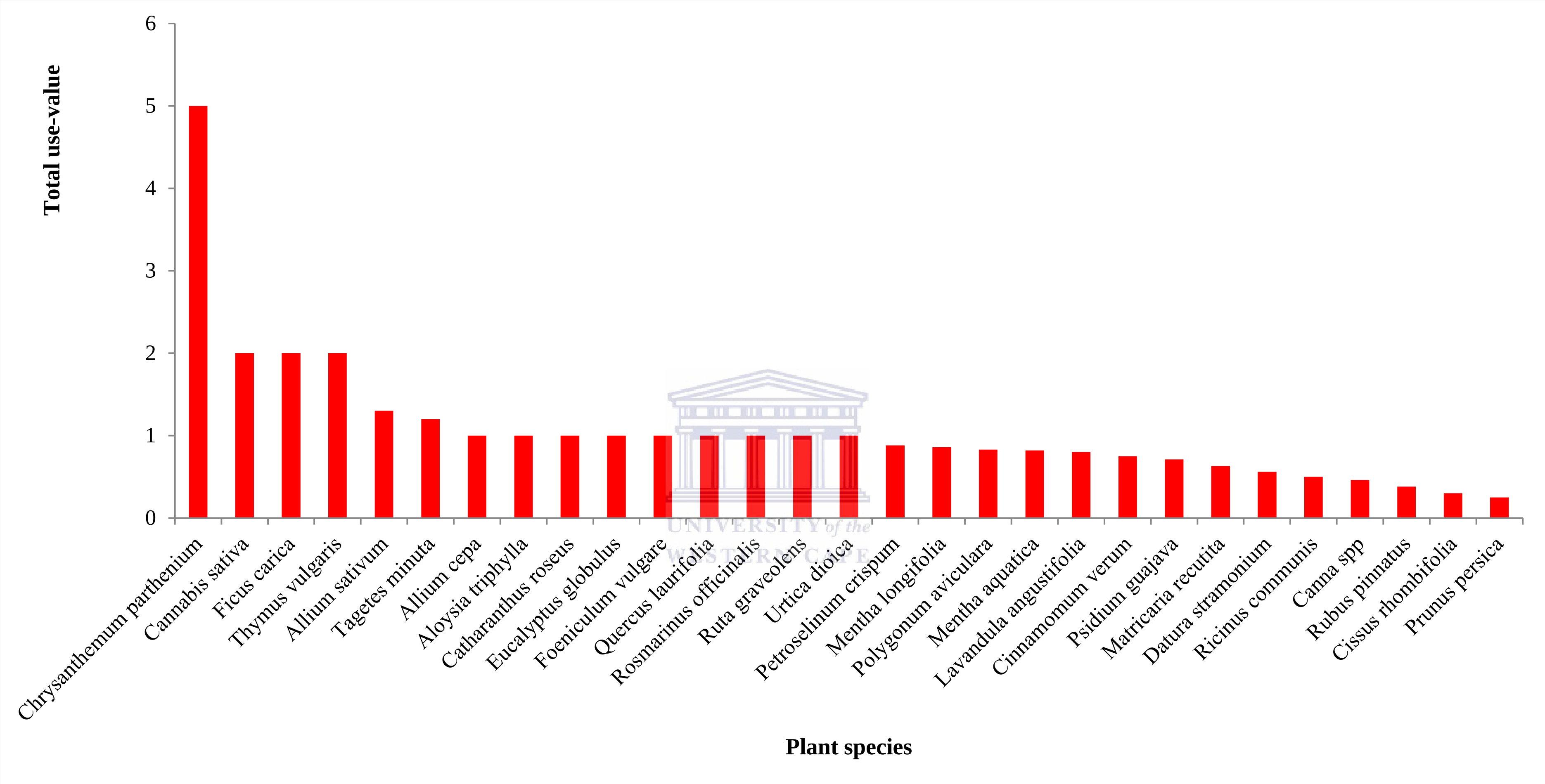

Figure 5B: Graph showing the calculated use-values for exotic plant species.

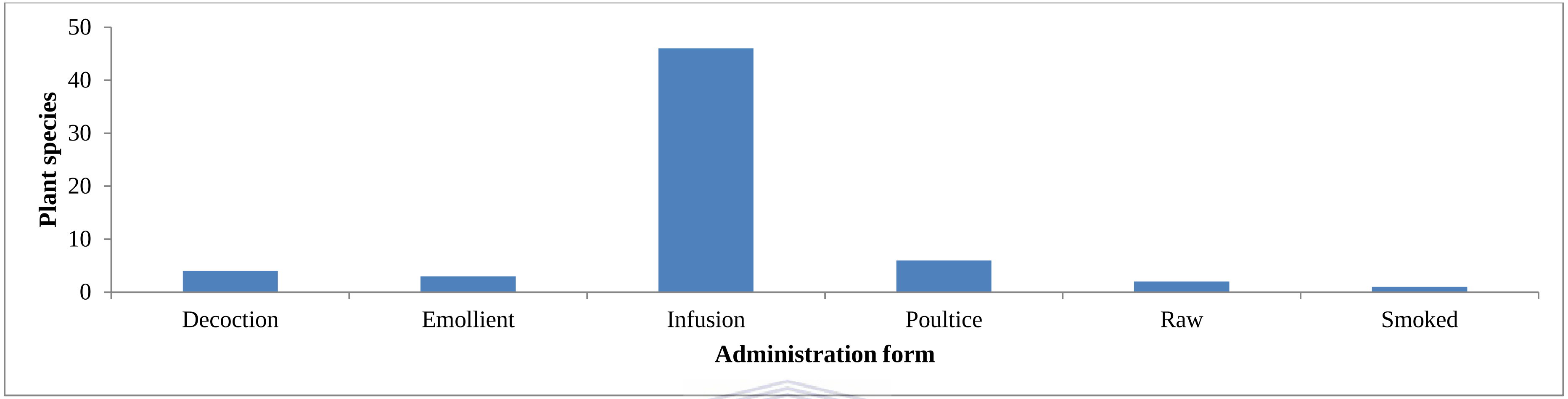

Figure 6: The predominant administration form for different plant species.

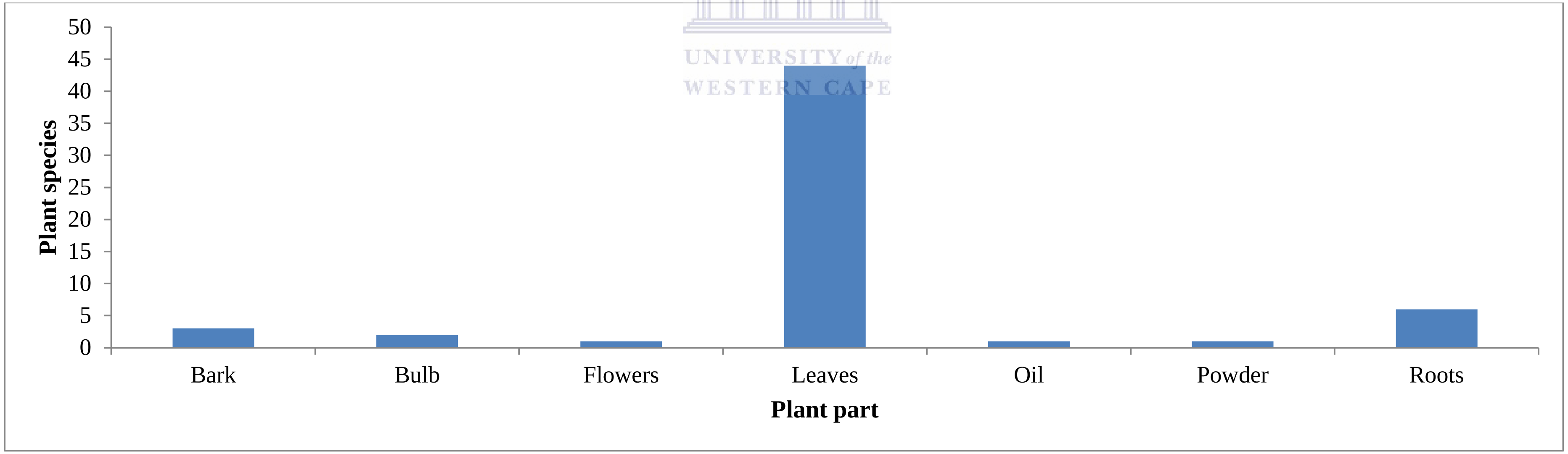

Figure 7: The predominant plant part used for different species.

3.3.1 Outcomes from the survey

Common health concerns experienced by the community in the Dwarsrivier valley were hypertension, arthritis and stomach ailments in adults and colic and fever in children. *Leonotis leonurus*, *Ricinus communis* and *Vernonia oligocephala* were most commonly used to treat hypertension, arthritis and stomach ailments respectively (Table 1A and Table 1B). *Matricaria recutita* is used to treat colic in children and *Artemisia afra* is used to assist with fever (Table 1A and Table 1B).

To treat hypertension, infusions or decoctions are administered. Arthritis is treated by spreading camphor or olive oil on a leaf before heating in the oven and applying to the affected area. An infusion of *V. oligocephala* is used to treat stomach ailments. In children, colic is treated with *M. recutita* and *A. afra* is used to treat fever. A decoction of *M. recutita* is administered. Small doses, usually a quarter cup sweetened with sugar is given to children two years and older. Very dilute herbal decoctions are given to infants in the bottle but these exceptions are rare. *M. recutita* is one of the few remedies administered as a decoction to children as other herbs are either too strong or bitter. For the treatment of stomach ailments, *A. afra* is applied to the stomach of the infant and lightly sprinkled with vinegar before securing with a cloth.

Many of the plants utilised by the Dwarsrivier community are plants frequently used in South Africa and in Zulu traditional medicine such as *Aloe arborescens*, *Artemisia afra*, *Leonotis leonurus* and *Tulbaghia violacea*. It was found that many of the plants utilised as medicine has been validated by the literature. This is important as it justifies the need for further research to be carried out to scientifically validate its use. Certain plants such as *Asparagus capensis* and *Cliffortia odorata* have very limited literature. This indicates an opportunity for new studies to be done which could possibly give rise to the developments of new treatments.

Figure 2 A depicts the ranking of the 53 plants in terms of popularity. The top four plants are all indigenous (Figure 2B). *Agathosma crenulata*, *Artemisia afra*, *Helichrysum petiolare* and *Leonotis leonurus* and *Ricinus communis* in fifth place respectively. *R. communis* is the only exotic in the top five plants (Figure 2C). The community were very knowledgeable about plants that were indigenous compared to those that were exotic. They would often favour the indigenous plants as the first method of treatment.

Figure 3 ranks the plant families in terms of the family with the most species used by the community in the Dwarsrivier valley. The family with the largest number of species belong to Lamiaceae (eight species), Asteraceae (six species) followed by Alliaceae, Apiaceae and Rosaceae with three species each.

Figure 4A and Figure 4B illustrate the different plants being utilised as medicine for different symptoms. The community felt that hypertension, arthritis and stomach ailments were the complaints requiring the most assistance. However, according to the graphs these ailments were not the most frequently cited in terms of plant remedies. The indigenous graph (Figure 4A) depicted that stomach ailments, colds and flu and hypertension were the ailments which had the most options for treatment. *Vernonia oligocephala* was featured as the herb of choice regarding stomach ailments (Figure 4A). Colds and flu is most often treated with *Salvia africana-caerulea* followed closely by *Agathosma crenulata* as the alternative (Figure 4A). Hypertension is treated using *Leonotis leonurus* (Figure 4 A). In the exotic graph, stomach ailments were also ranked first followed by pain and hypertension in second and third place. Arthritis was ranked fifth (Figure 4B). Interestingly, certain ailments such as epileptic fits, stimulators for hair growth, measles, sedation and sore eyes were only treated using exotic plants (Figure 4B).

Figure 5A and figure 5B depict the use value of the plants. It is important to note that the use value often does not necessarily correlate with the ranking value. This is illustrated by *Cissampelos capensis* which had a ranking value of 24 but a use value of two (Figure 5A). In the exotic graph *Chrysanthemum parthenium* was ranked 27^{th} but had a use value of 5 (Figure 5B). The most popular method of preparing the herbal mixture is by infusion (86%) (Figure 6) with the leaves (83%) being most commonly used (Figure7). Leaves are considered the most effective as the community believe that the therapeutic potential of the plant is found within the leaf. *Gethylis afra* is extremely rare and extremely difficult to find. *Vernonia oligocephala* or *Artemisia afra* are often used as alternatives.

A remedy that is not plant based, Hyraceum or “dassiepis”, is often used by the community. It is the dried concentration of hyrax urine which is often used in infusions. It is used to treat arthritis, colds and flu, as a tonic and to facilitate the expulsion of the placenta. Another popular remedy used for sores and wounds as well as skin ailments is “duiwelpoeier”, a mushroom that is left to burst and the ash is often blown onto the sores and wounds as a topical relief.

According to the literature, this is the first report of the medicinal use of *Protea cynaroides*, *Cissus rhombifolia*, *Canna* spp and *Dilatris viscosa*.

3.4 DISCUSSION

Scientists are investigating the possibility of various plant species as potential cures for various ailments (Coopoosamy and Naidoo, 2012). The comparison of various surveys enables the identification of plants which could be subjected to further scientific evaluation.

A common complaint of the community in the Dwarsrivier valley is arthritis which is treated by plants such as *Agathosma crenulata*, *Artemisia afra*, *Cliffortia odorata*, *Datura stramonium*, *Leonotis leonurus* and *Ricinus communis*. Similar plants were used in the Elim/Bredasdorp region as well as other parts of the Western Cape. Thring and Weitz (2006) reported that *L. leonurus* and *R. communis* are used by communities to aid arthritis. Philander (2011) reported that a topical application of *C. odorata* is used to treat arthritis in some areas of the Western Cape.

Surveys have been conducted throughout South Africa to document plants used to treat colds and flu as well as the symptoms associated with it (York et al., 2011; Dyubeni and Buwa, 2012). Colds and flu are treated using plants such as *A. crenulata*, *A. afra*, *C. odorata*, *Eucalyptus globulus*, *Helichrysum petiolare*, *Mentha aquatica*, *Mentha longifolia*, *L. leonurus*, *Salvia africana-caerulea* and *Warburgia salutaris* to name a few. Thring and Weitz (2006) reported that *A. afra*, *Helichrysum crispum*, *L. leonurus* and *M. longifolia* are used to treat colds and flu. Similar plants such as *A. afra*, *M. longifolia* and *S. africana-caerulea* are used in other areas of the Western Cape (Philander, 2011). In KwaZulu-Natal, *A. afra* is used to treat colds and flu whereas *W. salutaris* is used to aid sinusitis (Coopoosamy and Naidoo, 2012).

The community in the Dwarsrivier valley use plant combinations to treat colds and flu, claiming that different plants have different therapeutic effects. i.e. one plant might boost immunity while another might assist in clearing the nasal passages. Popular combinations include *Eucalyptus globulus* together with *Agathosma crenulata*, *Cliffortia odorata* and *Helichrysum petiolare*. Another combination includes *E. globulus* and *S. africana-caerulea*. In KwaZulu-Natal, *Aloe marlothii*, *Hypoxis* sp., *Scadoxus puniceus* and *Erythrina caffra* is a popular combination used to treat a blocked nose, fever and chest pain (York et al., 2011). In the Dwarsrivier valley, *Aloe arborescens* is not used to treat respiratory ailments. The Dwarsrivier community treat sore throats using *Eucalyptus globulus*, *Carpobrotus edulis* and *Urtica dioica*. *Carpobrotus* spp. is used in the Elim/Bredasdorp area to treat sore throats (Thring and Weitz, 2006) while *Allium sativum*, *Leonotis leonurus*, *Tulbaghia violacea* and *Salvia microphylla* are used in the Eastern Cape (Dyubeni and Buwa, 2012).

Detoxification is an important routine in the Dwarsrivier valley as it is regarded as cleansing the body. *Agathosma crenulata*, *Aloe arborescens*, *Cliffortia odorata*, *Eucalyptus globulus*, *Helichrysum petiolare*, *Mentha aquatica*, *Mentha longifolia* and *Urtica dioica* are used in the Dwarsrivier valley. *A. arborescens*, *A. capensis* and *Leonotis leonurus* are used as a blood purifier in the Western Cape region (Philander, 2011) and *Aloe ferox* is used in the Durban area (Coopoosamy and Naidoo, 2012). However, in Jordan, *Aloe vera* is used as an expectorant (Al-Qura'n, 2009).

In South Africa, the number of people suffering from diabetes has risen steadily over the last two decades (Erasto et al., 2005). In the Dwarsrivier valley, *Aloe arborescens*, *Artemisia afra*, *Catharanthus roseus*, *Cliffortia odorata*, *Psidium guajava*, *Ruta graveolens* and *Vernonia oligocephala* are a few of the plants used to treat diabetes in the Dwarsrivier valley. *L. leonurus*, *R. graveolens* and *V. oligocephala* are used in the Elim/Bredasdorp region (Thring and Weitz, 2006) and *A. afra* and *C. roseus* are used to treat diabetes in the Eastern Cape (Erasto et al., 2005).

Hypertension was the primary health concern experienced by the community in the Dwarsrivier valley. *Agathosma crenulata*, *Artemisia afra*, *Allium sativum*, *Asparagus capensis*, *Catharanthus roseus*, *Cliffortia odorata*, *Helichrysum petiolare*, *Leonotis leonurus*, *Tulbaghia violacea* and *Vernonia oligocephala* are a few of the plants used by the community to treat hypertension. *Leonotis leonurus* is primarily used to treat hypertension in the Dwarsrivier valley. In the Elim/Bredasdorp area, *L. leonurus* and *T. violacea* are used by the community to treat hypertension (Thring and Weitz, 2006) while *Asparagus capensis* is reportedly used by the Rastafarians to treat hypertension in other areas of the Western Cape (Philander, 2011). In the Eastern Cape, *Leonotis leonurus* and *Tulbaghia violacea* are also used to treat hypertension (Olorunnisola et al., 2011). This is in accordance with the community in Burkina Faso, who used *A. sativum* (Nadembega et al., 2011), and the communities in India who used *C. roseus* (Rajakumar and Shivanna, 2009) to treat hypertension. This is in agreement with its use in the Dwarsrivier valley.

Skin ailments such as eczema, psoriasis etc are treated using *Aloe arborescens*, *Ballota africana, Bulbine frutescens*, *Leonotis leonurus*, *Carpobrotus edulis, Pelargonium reniforme* and *Urtica dioica* either internally or as an emollient. Thring and Weitz (2006) reported similar plants such as *Aloe ferox*, *Bulbine logopus* and *Carpobrotus* spp. used in the treatment of wounds in Bredasdorp region while Philander (2011) reported that *A. arborescens* as well as *C. edulis* is used in areas in the Western Cape. In Durban, *Aloe ferox* and *Bulbine frutescens* are used (Coopoosamy and Naidoo, 2012). *Aloe* spp. is also used throughout the world to treat skin conditions or wounds. In Nepal, *Aloe vera* gel is applied to burns (Shresthna and Dhillion, 2003) whereas the community in Burkina Faso use *Aloe buettneri* as an anti-inflammatory (Nadembega et al., 2011).

Sores and wounds are treated using *Cliffortia odorata*, *Datura stramonium*, *Ficus carica*, *Leonotis leonurus*, *Lobostemon fruticosus*, *Melianthus comosus* and *Ricinus communis* in the Dwarsrivier valley. *Bulbine frutescens* and *M. comosus* are used in the Bredasdorp/Elim region (Thring and Weitz, 2006). In the Eastern Cape region, *Leonotis leonurus* was also one of many plants used to treat wounds (Grierson and Afolayan, 1999b). In Durban, sexual complaints were the main concern with *Bulbine frutescens* applied to the genital areas (Coopoosamy and Naidoo, 2012). This is in contrast to the complaints by the Dwarsrivier community which were primarily chronic complaints such as hypertension. This could be due to the age difference of medicinal plant users in the Dwarsrivier community which was between 50 to 65 years compared to the younger population in Durban.

Stomach complaints were one of the major concerns reported by the community in the Dwarsrivier valley including constipation, diarrhoea and cramps associated with diarrhoea. *Vernonia oligocephala* was the most common remedy used for stomach ailments in the Dwarsrivier valley with other plants such as *Aloe arborescens*, *Agathosma crenulata*, *Artemisia afra*, *Carpobrotus edulis*, *Cissampelos capensis*, *Cliffortia odorata*, *Gethyllis afra*, *Helichrysum petiolare*, *Leonotis leonurus*, *Mentha aquatica*, *Mentha longifolia*, *Prunus persica* and *Ricinus communis* also being used.

Numerous studies have been conducted in South Africa that specifically document plants used to treat diarrhoea (Appidi et al., 2008; de Wet et al., 2010; Bis-Johnson., 2010) as well as gastrointestinal complaints (Olajuyigbe and Afolayan, 2012). Appidi et al (2008) reported that *Prunus persica* is used to treat diarrhoea in the Eastern Cape. *C. edulis* was one of many plants used to treat diarrhoea in the O.R Tambo district of the Eastern Cape (Bisi-Johnson et al., 2010). *Cissampelos capensis* is used in the KwaZulu-Natal region to treat diarrhoea (de Wet et al., 2010) while Olajuyigbe and Afolayan (2012) reported that *C. capensis*, *Mentha aquatica* and *M. longifolia* are used to treat stomach aches in the Eastern Cape. Similar plants are utilised in other parts of the world to treat stomach complaints. In Jordon, *Matricaria chamomilla* is used to treat intestinal colic and *Mentha longifolia* is used to relieve spasm as well as flatulence (Al-Qura'n, 2009). This is in accordance with the uses in the Dwarsrivier valley as *Matricaria recutita* is used to treat infantile colic and both *M. aquatica* and *M. longifolia* is used to treat stomach ailments.

Plant combinations are often used by the community in the Dwarsrivier valley to treat ailments. This is a common occurence in South Africa as traditional healers reportedly rarely use single plants in their plant extracts (Eldeen et al., 2005). Although research has been done on popular plants used in South Africa to treat gynaecological complaints (Steenkamp, 2003), anti-inflammatory and anticholinesterase properties of plants (Fawole et al., 2010) and cancer (Steenkamp and Gouws et al., 2006), very few surveys have documented the different plant combinations used to treat various ailments. At present, plant combinations to treat diarrhoea (de Wet et al., 2010) and respiratory infections (York et al., 2011) have been documented. However, a study carried out in Kwa-Zulu Natal has documented commercial herbal mixtures commonly sold to the public (Ndhlala et al., 2011).

The most common method of preparation used by the Dwarsrvier community is a tea. When needed, a few leaves are picked and immediately brewed. This is in accordance with the traditional medicine method of preparation of "herbal drinks" for oral intake as the herbal plants are boiled for 30-60 minutes (Kolachi et al., 2010). In the Dwarsrivier valley, the leaf is considered the most effective part of the plant as many community members believe it has the most therapeutic potential. In many surveys conducted in South Africa, the leaves were predominantly used. However, the surveys using medicinal plants to treat diabetes (Oyedemi et al., 2009; Semenya et al., 2012) and diarrhoea (Appidi et al., 2008; Bisi-Johnson et al., 2010) utilised the roots of the plant. This is detrimental to the plant. Van Vuuren (2008) reported that researchers are possibly avoiding root studies due to the destructive nature of this harvesting procedure.

The community members often use the plants as soon as the complaint arises as they feel it tends to be more effective and immediately deals with the issue at hand. Medication is usually taken twice a day after meals. Philander (2011) reported that the bush doctors in the Western Cape sometimes advised the plants to steep overnight and was drunk the next day. This is in agreement with the Bapedi healers in the Limpopo province who consume medication after a meal (Semenya et al., 2012). Infusions and decoctions are often stored in the refrigerator overnight but never longer than three days as it is not considered effective if stored for a long period of time.

Plant medications are never consumed for longer than seven days by the community members. Philander (2011) reported that Rastafarians advised the maximum period of plant use to be two weeks. This is in contrast with Oyedemi et al (2009) who reported that traditional healers and herbalists advised extracts to be taken orally for six to twelve months depending on the severity of the ailment whereas Olorunnisola et al (2011) reported that the extracts were usually taken for a long time depending on the severity of the disease. It is interesting to note that in the Dwarsrivier community rectal administration of herbal preparations are not utilised compared with other areas in South Africa (Olajuyigbe and Afolayan, 2012).

Many of the informants (52%) obtained their plant material from the mountain where they grow naturally. They are very aware of the importance of plant conservation as well as the extinction of plants. For this reason, only what is needed is taken and often replanted in their gardens. Certain plants such as *Melianthus comosus* and *Notobubon galbanum* are not planted in the gardens. These plants are often bought from street vendors instead of obtaining it from the mountain. The community in the Dwarsrivier valley also practice plant-part substitution alternating between the leaves and the bark of *Warburgia salutaris*. The community members (70%) in the Bredasdorp/ Elim region obtain the plant from their gardens or along the main road. When the plants are not available they purchase what is needed from the supermarket or from the farmers (Thring and Weitz, 2006).

However, this is not the case in the O.R. Tambo district in the Eastern Cape Province where traditional healers never use cultivated plants only those sourced from the wild (Bisi-Johnson et al., 2010). This is a concern as the predominant part used to treat diarrhoea in this area was the root with unsustainable harvesting method such as root excavation and bark stripping reported (Bisi-Johnson et al., 2010).

Standardization of the medicine is a major concern as many of the dosage forms used is measured using a cup. This is also evident in the Bredasdorp/ Elim region (Thring and Weitz, 2006). A study conducted by Olajuyigbe and Afolayan (2012) where the traditional healers were interviewed revealed that oral dosages were administered using lids, spoons, cups, pinches and handfuls.

Many of the community members do visit the local clinic for their monthly chronic medication but many still prefer to use medicinal plants. Often the chronic medication is used in addition to the plant medicine which could lead to certain drug interactions. With trial and error, the community isolated the toxic plants and may only use them in miniscule doses. Pregnant women are not recommended to use any of the herbal medicines as many of these herbal formulations have not been tested and may be harmful to the foetus. This reiterates the importance of scientifically verifying these medicinal plants. Similar findings occurred in the Bredasdorp/ Elim region as community members use western medicine as they feel it works faster even though plant medicine does provide relief (Thring and Weitz, 2006).

In the Dwarsrivier valley, the majority of the users were females between the age of 50 and 65 years. This was evident in the Bredasdorp / Elim region with most of the users being elderly women (Thring and Weitz, 2006) and the rural homesteads in KwaZulu-Natal (De Wet et al., 2010). It has become evident that the knowledge possessed by the community has been passed down through the generations. Many members acknowledge the importance of documentation as they feared the knowledge may become extinct especially as the younger generation show no interest in continuing the plant tradition. The youngsters felt that medicinal plants are too slow in providing relief compared to conventional medicine often claiming the bitter taste was too strong and overwhelming.

3.5 CONCLUSION

The survey yielded 53 plant species from 31 families of which only 21 were indigenous. The families with the largest number of species used in the Dwarsrivier valley are Lamiaceae (eight species), Asteraceae (six species) and Alliaceae, Apiaceae and Rosaceae (three species each). The most popular plants in terms of ranking are *Agathosma crenulata*, followed by *Artemisia afra* and *Helichrysum petiolare* in second and third place respectively. Although there were more exotic plants used by the community compared to indigenous, the indigenous plants were favoured for medicinal purposes. The plants used in the survey were justified by the literature with many of the plants having the same uses. This advocates that ethnobotanical surveys can be a reliable source to discover as well as separate plants needing further investigation. An important observation is that not much literature is available regarding plant combinations except De Wet et al. (2010) and York et al. (2011). This is alarming as many communities do use different plant combinations as well as different plant parts to achieve the most effective remedy.

A major concern is the lack of standardization. The integration of traditional medicine into mainstream medicine is dependent on validation, standardisation and characterisation of these medicinal plants (Ginsburg and Deharo, 2011). In order for traditional medicine to be viewed in the same light as western medicine, steps need to be taken to ensure the safety as well as efficacy of these medicines.

REFERENCES

Adebayo, J.O., Krettli, A.U., 2011. Potential antimalarials from Nigerian plants: A review. Journal of Ethnopharmacology 133, 289-302.

Agnihotri, V.K., ElSohly, H.N., Smillie, T.J., Khan, I.A., Walker, L.A., 2009. Constituents of *Leonotis leonurus* flowering tops. Phytochemistry Letters 2, 103-105.

Al-Qura'n, S., 2009. Ethnopharmacological survey of wild medicinal plants in Showbak, Jordan. Journal of Ethnopharmacology 123, 45-50.

Appidi, J.R., Grierson, D.S., Afolayan, A.J., 2008. Ethnobotanical Study of Plants Used for the Treatment of Diarrhoea in the Eastern Cape, South Africa. Pakistan Journal of Biological Sciences 11(15), 1961-1963.

Balick, M.J., Cox, P.A., 1996. Plants, People, and Culture. The Science of Ethnobotany. Scientific American Library.

Bamuamba, K., Gammon, D.W., Meyers, P., Dijoux-Franca, M-G., Scott, G., 2008. Anti-mycobacterial activity of five plant species used as traditional medicines in the Western Cape Province (South Africa). Journal of Ethnopharmacology 117, 385-390.

Bisi-Johnson, M.A., Obi, C.L., Kambizi, L., Nkomo, M., 2010. A survey of indigenous herbal diarrhoeal remedies of O.R. Tambo district, Eastern Cape Province, South Africa. Full Length Research Paper. African Journal of Biotechnology 9(8), 1245-1254.

Botha, J., Witkowski, E.T.F., Shackleton, C.M., 2004. Market profiles and trade in medicinal plants in the Lowveld, South Africa. Environmental Conservation 31(1), 38-46.

Bussman, R.W., Genevieve, G.G., Solio, J., Lutura, M., Lutuluo, R., Kunguru, K., Wood, N., Mathenge, S., 2006. Plant use of the Maasai of Sekenani Valley, Maasai Mara, Kenya. J. Ethnobio, Ethnomed 2, 22.

Coopoosamy, R.M., Naidoo, K.K., 2012. An ethnobotanical study of medicinal plants used by traditional healers in Durban, South Africa. Full Length Research Paper. African Journal of Pharmacy and Pharmacology 6(11), 818-823.

Cotton, C.M., 1996. Ethnobotany: Principles and Applications. John Wiley and Sons LTD, Chichester.

Cunningham, A.B., 1991. Development of a conservation Policy on commercially exploited medicinal plants : a case study from South Africa. In: Akarele, O., Heywood, V., Synge, H. (Eds.), Conservation of Medicinal Plants. Cambridge University Press, p. 337.

De Wet, H., Nkwanyana, M.N., van Vuuren, S.F., 2010. Medicinal plants used for the treatment of diarrhoea in northern Maputaland, KwaZulu-Natal Province, South Africa. Journal of Ethnopharmacology 130, 284-289.

Dold, A.P., Cocks, M.L., 2002. The trade in medicinal plants in the Eastern Cape Province, South Africa. South African Journal of Science 98, 589-597.

Dyubeni, L., Buwa, L.V., 2012. An ethnobotanical study of plants used for the treatment of ear, nose and throat (ENT) infections in Nkonkobe Municipality, South Africa. Full Length Research Paper. Journal of Medicinal Plants Research 6(14), 2721-2726.

Eldeen, I.M.S., Elgorashi, E.E., van Staden, J., 2005. Antibacterial, anti-inflammatory, anti-cholinesterase and mutagenic effects of extracts obtained from some trees used in South African traditional medicine. Journal of Ethnopharmacology 102, 457-464.

Elgorashi, E.E., Taylor, J.L.S., Maes, A., van Staden, J., De Kimpe, N., Verschaeve, L., 2003. Screening of medicinal plants used in South African traditional medicine for genotoxic effects. Toxicology Letters 143, 195-207.

Elvin-Lewis, M., 2001. Should we be concerned about herbal remedies? Journal of Ethnopharmacology 75, 141-164.

Erasto, P., Adebola, P.O., Grierson, D.S., Afolayan, A.J., 2005. An ethnobotanical study of plants used for the treatment of diabetes in the Eastern Cape Province, South Africa. African Journal of Biotechnology 4(12), 1458-1460.

Etkin, N.L., 2001. Perspectives in ethnopharmacology: forging a closer link between bioscience and traditional empirical knowledge. Journal of Ethnopharmacology 76, 177-182.

Fabricant, D.S., Farnsworth, N.R., 2001. The Value of Plants Used in Traditional Medicine for Drug Discovery. Environmental Health Perspectives 109(1), 69-75.

Fawole, O.A., Amoo, S.O., Ndhlala, A.R., Light, M.E., Finnie, J.F., Van Staden, J., 2010. Anti-inflammatory, anticholinesterase, antioxidant and phytochemical properties of medicinal plants used for pain-related ailments in South Africa. Journal of Ethnopharmacology 127, 235-241.

Fennel, C.W., Lindsey, K.L., McGaw, L.J., Sparg, S.G., Stafford, G.I., Elgorashi, E.E., Grace, O.M., van Staden, J., 2004. Assessing African medicinal plants for efficacy and safety: pharmacological screening and toxicology. Review. Journal of Ethnopharmacology 94, 205-217.

Frei, B., Baltisberger, M., Sticher, O., Heinrich, M., 1998. Medical ethnobotany of the Zapotecs of the Isthmus-Sierra (Oaxaca, Mexico): Documentation and assessment of indigenous uses. Journal of Ethnopharmacology 62, 149-165.

Ginsburg, H., Deharo, E., 2011. A call for using natural compounds in the development of new antimalarial treatments - an introduction. Malaria Journal 10(1), S1. http://www.malariajournal.com/content/10/S1/S1.

Grierson, D.S., Afolayan, A.J., 1999a. Antibacterial activity of some indigenous plants used for the treatment of wounds in the Eastern Cape, South Africa. Short communication. Journal of Ethnopharmacology 66, 103-106.

Grierson, D.S., Afolayan, A.J., 1999b. An ethnobotanical study of plants used for the treatment of wounds in the Eastern Cape, South Africa. Journal of Ethnopharmacology 66, 327-332.

Gurib-Fakim, A., Kasilo, O.M.J., 2010. Promoting African medicinal plants through an African herbal pharmacopoeia. African traditional medicine day. Special issue 14. Afrolib.afro.who.int.documents/2010/En/AHMSI_64_67.pdf

Hutchings, A., Scott, A.H., Lewis, G., Cunningham, A.B., 1996. Zulu medicinal plants. An inventory. Unitversity of Natal Press, Pietermaritzburg.

Katerere, D.R., Eloff, J.N., 2008. Anti-bacterial and anti-oxidant activity of *Hypoxis hemerocallidea* (Hypoxidaceae): Can leaves be substituted for corms as a conservation strategy? South African Journal of Botany 74, 613-616.

Katerere, D.R., Stockenström, S., Thembo, K.M., Rheeder, J.P., Shephard, G.S., Vismer, H.F., 2008. A preliminary survey of mycological and fumonisin and aflatoxin contamination of African traditional herbal medicines sold in South Africa. Human & Experimental Toxicology 27, 793-798.

Kelmanson, J.E., Jäger, A.K., van Staden, J., 2000. Zulu medicinal plants with antibacterial activity. Journal of Ethnopharmacology 69, 241-246.

Kim, H.-S., 2005. Do not put too much value on conventional medicines. Perspective paper. Journal of Ethnopharmacology 100, 37-39.

Kolachi, N.F., Kazi, T.G., Afridi, H.I., Khan, S., Wadhwa, S.K., Shah, A.Q., Shah, F., Baig, J.A., Sirajuddin., 2007. Determination of selenium content in aqueous extract of medicinal plants used as herbal supplement for cancer patients. Food and Chemical Toxicology 48, 3327-3332.

Leonti, M., 2011. The future is written: Impact of scripts on the cognition, selection, knowledge and transmission of medicinal plant use and its implications for ethnobotany and ethnopharmacology. Journal of Ethnopharmacology 134, 542-555.

Light, M.E., Sparg, S.G., Stafford, G.I., van Staden, J., 2005. Riding the wave: South Africa's contribution to ethnopharmacological research over the last 25 years. Journal of Ethnopharmacology 100, 127-130.

Makunga, N.P., Philander, L.E., Smith, M., 2008. Current perspectives on an emerging formal natural products sector in South Africa. Journal of Ethnopharmacology 119, 365-375.

Mander, M., 1998. Marketing of Indigenous Medicinal Plants in Southern Africa. A Case Study in KwaZulu-Natal. Food and Agriculture Organization, Rome.

Mander, M., Le Breton, G., 2006. Overview of the Medicinal Plants Industry in Southern Africa. In: Diederichs, N. Commercialising Medicinal Plants. A Southern African Guide. Sun Press, Stellenbosch., pp. 3-9.

Mulholland, D.A., 2005. The future of ethnopharmacology A southern African perspective. Perspective paper. Journal of Ethnopharmacology 100, 124-126.

Nadembega, P., Boussim, J.I., Nikiema, J.B., Poli, F., Antognoni, F., 2011. Medicinal plants in Baskoure, Kourittenga Province, Burkina Faso: An ethnobotanical study. Journal of Ethnopharmacology 133, 378-395.

Ncube, B., Finnie, J.F., Van Staden, J., 2010. Seasonal variation in antimicrobial and phytochemical properties of frequently used medicinal bulbous plants from South Africa. South African Journal of Botany doi: 10.1016/j.sajb.2010.10.004

Ndhlala, A.R., Stafford, G.I., Finnie, J.F., Van Staden, J., 2009. *In vitro* pharmacological effects of manufactured herbal concoctions used in KwaZulu-Natal South Africa. Journal of Ethnopharmacology 122, 117-122.

Ndhlala, A.R., Stafford, G.I., Finnie, J.F., Van Staden, J., 2011. Commercial herbal preparations in KwaZulu-Natal, South Africa: The urban face of traditional medicine. Review. South African Journal of Botany 77, 830-843.

Olajuyigbe, O.O., Afolayan, A.J., 2012. Ethnobotanical survey of medicinal plants used in the treatment of gastrointestinal disorders in the Eastern Cape Province, South Africa. Full Length Research Paper. Journal of Medicinal Plants Research 6(18), 3415-3424.

Olorunnisola, O.S., Bradley, G., Afolayan, A.J., 2011. Ethnobotanical information on plants used for the management of cardiovascular disease in Nkonkobe Municipality, South Africa. Full Length Research Paper. Journal of Medicinal Plants Research 5(17), 4256-4260.

Owen, P.L., Johns, T., Etkin, N.L., 2011. Bridging the "two cultures" in ethnopharmacology: Barriers against interdisciplinarity in postgraduate education. Ethnopharmacological communication. Journal of Ethnopharmacology 134, 999-1005.

Oyedemi, S.O., Bradley, G., Afolayan, A.J., 2009. Ethnobotanical survey of medicinal plants used for the management of diabetes mellitus in the Nkonkobe municipality of South Africa. Full Length Research Paper. Journal of Medicinal Plants Research 3(12), 1040-1044.

Philander, L.A., 2011. An ethnobotany of Western Cape Rasta bush medicine. Journal of Ethnopharmacology 138, 578-594.

Pillay, P., Maharaj, V.J., Smith, P.J., 2008. Investigating South African plants as a source of new antimalarial drugs. Review. Journal of Ethnopharmacology 119, 438-454.

Quinlan, M.B., Quinlan, R.J., Nolan, J.M., 2002. Ethnophysiology and herbal treatments of intestinal worms in Dominica, West Indies. Journal of Ethnopharmacology 80, 75-83.

Rajakumar, N., Shivanna, M.B., 2009. Ethno-medicinal application of plants in the eastern region of Shimoga district, Karnataka, India. Journal of Ethnopharmacology 126, 64-73.

Rybicki, E.P., Chikwamba, R., Koch, M., Rhodes, J.I., Groenewald, J.-H., 2012. Plant-made therapeutics: An emerging platform in South Africa. Research review paper. Biotechnology Advances 30, 449-459.

Scott, G., Springfield, E.P., Coldrey, N., 2004. A Pharmacognostical Study of 26 South African Plant Species Used as Traditional Medicines. Pharmaceutical Biology 42(3), 186-213.

Semenya, S., Potgieter, M., Erasmus, L., 2012. Ethnobotanical survey of medicinal plants used by Bapedi healers to treat diabetes mellitus in the Limpopo Province, South Africa. Journal of Ethnopharmacology 141, 440-445.

Shackleton, C.M., 2001. Re-examining local and market orientated use of wild species for the conservation of biodiversity. Environmental Conservation 28, 270-278.

Shai, L.J., McGaw, L.J., Masoko, P., Eloff, J.N., 2008. Antifungal and antibacterial activity of seven traditionally used South African species active against *Candida albicans*. South African Journal of Botany 74, 677-684.

Shrestha, P.M., Dhillion, S.S., 2003. Medicinal plant diversity and use in the highlands of Dolakha district, Nepal. Journal of Ethnopharmacology 86, 81-96.

Stafford, G.I., Jäger, A.K., van Staden, J., 2005. Effect of storage on the chemical composition and biological activity of several popular South African medicinal plants. Journal of Ethnopharmacology 97, 107-115.

Steenkamp, V., 2003. Traditional herbal remedies used by South African women for gynaecological complaints. Journal of Ethnopharmacology 86, 97-108.

Steenkamp, V., Gouws, M.C., 2006. Cytotoxicity of six South African medicinal plant extracts used in the treatment of cancer. Short communication. South African Journal of Botany 72, 630-633.

Stein, C.M., Gora, N.P., Macheka, B.M., 1989. Self-medication in urban and rural Zimbabwean communities. Br. J. Clin. Pharmac. 27, 741-747.

Thring, T.S.A., Weitz, F.M., 2006. Medicinal plant use in the Bredasdorp/Elim region of the Southern Overberg in the Western Cape Province of South Africa. Journal of Ethnopharmacology 103, 261-275.

Van Overwalle, G., 2005. Protecting and sharing biodiversity and traditional knowledge: Holder and user tools. Ecological Economics 53, 585-607.

Van Vuuren, S.F., 2008. Antimicrobial activity of South African medicinal plants. Review. Journal of Ethnopharmacology 119, 462-472.

Van Wyk, B.E., Van Oudtshoorn, B., Gericke, N., 1997. Medicinal plants of South Africa. Briza Publications, Pretoria.

Van Wyk, B.-E., Gericke, N., 2000. People's plants: A guide to useful plants of South Africa. Briza Publications, Pretoria.

Van Wyk, B.-E., Van Heerden, F.R., Van Oudtshoorn, B., 2002. Poisonous Plants of South Africa. Briza Publications, Pretoria.

Van Wyk, B.-E., Wink, M., 2004. Medicinal plants of the world. Briza Publications, Pretoria.

Van Wyk, B.-E., Van Oudtshoorn, B., Gericke, N., 2009. Medicinal plants of South Africa. Briza Publications, Pretoria.

Viljoen, A., Moolla, A., 2007. Indigenous South African Medicinal Plants. Part 5: *Agathosma betulina* ('buchu'). SA Pharmaceutical Journal, October, 39.

Water and Forestry, 2005. Medicinal plants trade. Forestry Sub-sector studies, Briefing 5. http://www2.dwaf.gov.za/dwaf/cmsdocs/Elsa/Docs/FED/SubSector%20Medicinal%20plants%202005.pdf

Watt, J.M., Breyer-Brandwijk, B.N., 1962. Medicinal and poisonous plants of Southern and Eastern Africa, second ed. Edinburgh, Churchill Livingstone.

Williams, V.L., Balkwill, K., Witkowski, E.T.F., 2000. Unraveling the commercial market for medicinal plants and plant parts on the Witwatersrand, South Africa. Economic Botany 54(3), 310-327.

York, T., de Wet, H., van Vuuren, S.F., 2011. Plants used for treating respiratory infections in rural Maputaland, KwaZulu-Natal, South Africa. Journal of Ethnopharmacology 135, 696-710.

Zerbe, N., 2005. Biodiversity, ownership, and indigenous knowledge: Exploring legal frameworks for community, farmers and intellectual property rights in Africa. Ecological Economics 53, 493-506.

Zobolo, A.M., Mkabela, Q.N., 2006. Traditional knowledge transfer of activities practiced by Zulu women to manage medicinal and food plant gardens. African Journal of Range and Forage Science 23(1), 77-80.

Zschocke, S., Rabe, T., Taylor, J.L.S., Jäger, A.K., van Staden, J., 2000. Plant part substitution- a way to conserve endangered medicinal plants? Journal of Ethnopharmacology 71, 281-292.

CHAPTER 4

ELEMENTAL ANALYSIS FOR TWELVE SELECTED PLANTS FROM THE DWARSRIVIER VALLEY, STELLENBOSCH.

ABSTRACT

Elemental analyses were carried out on eleven plant species from the Dwarsrivier Valley, Stellenbosch. The content of Calcium (Ca), Chromium (Cr), Copper (Cu), Magnesium (Mg), Manganese (Mn), Sodium (Na), Phosphorous (P), Potassium (K), Selenium (Se) and Zinc (Zn) were evaluated within these plants. A hydrogen peroxide: sulphuric acid digestion mixture and a Unicam Solaar M Series Atomic Absorption Spectrometer (AA) were used to analyse the total element concentrations present within the plant samples.

Aloe arborescens contained the highest concentration of Calcium (Ca), had the second highest concentration of Magnesium (Mg), Sodium (Na) and Zinc (Zn) and featured third in the Manganese (Mn) concentration. *Agathosma crenulata* contained the highest concentration of Copper (Cu) and had the second highest concentration of Mn. *Artemisia afra* had the highest concentration of Mn and Selenium (Se). The concentration of Na and Mg was the highest in *Carpobrotus edulis*. *C. edulis* also had the second highest concentration of Ca, Phosphorous (P) and Potassium (K) and had the third highest concentration of Zn. *Leonotis leonurus* (narrow leaf) had high concentrations of P and K and *Vernonia oligocephala* was the highest in Chromium (Cr) and Zn. Although the community claimed that *Leonotis leonurus* (broad leaf) was more effective than *Leonotis leonurus* (narrow leaf), the elemental analyses revealed that *L. leonurus* (narrow leaf) had higher concentrations in all the elements except Mg and Mn. The concentration of the elements were within the normal range in the plant leaves for all except P, which was lower, and Na which was higher.

Keywords: Medicinal plants, elemental analysis, Unicam Solaar M Series Atomic Absorption Spectrometer, biological activity, concentration range

CHAPTER 4

ELEMENTAL ANALYSIS FOR TWELVE SELECTED PLANTS FROM THE DWARSRIVIER VALLEY, STELLENBOSCH.

4.1 INTRODUCTION

A delicate balance exists between antioxidant defence and free radical generation at cellular level in healthy conditions (Zuo et al., 2006). Aerobic cells have developed their own defence system, Cu-Zn SOD (superoxide dismutase), to control free radicals (Zuo et al., 2006). The inability to scavenge these oxygen derived free radicals may result in oxidative damage to cells (Shikanga et al., 2010) which include lipid, protein and DNA damage (Temple et al., 2000; Zuo et al., 2006).

The consumption of antioxidant vitamins and minerals have been associated with reduced cardiovascular risk as the upsurge in oxidative stress has been linked to many disease factors such as rheumatism, numerous autoimmune diseases (Kaur and Kapoor, 2001), cancer and cardiovascular disease (Chehade et al., 2009). The implementation of an antioxidant rich diet or treatment regimen would be beneficial as antioxidants are associated with the prevention of disease (Balch, 2006). For this reason, we will discuss how trace elements can benefit chronic diseases.

4.1.1 Elements

Minerals are inorganic constituents which exist in all tissues of the body and play an essential role in the maintenance of certain physicochemical processes imperative for living (Soetan et al., 2010). Minerals can generally be classified as macro (major) and micro (trace) elements (Soetan et al., 2010). Criteria exist to determine whether an element is essential or not. This includes the existence of the element within strong, healthy tissue, its existence in the foetus and new-borns and whether the body exerts homeostatic control over its uptake in the tissue or the blood stream as well as its excretion (Leśniewicz et al., 2006). Trace elements are an essential feature regarding optimum human health due to their diverse metabolic characteristics and functions (Zargar et al., 1998) with recent interest in the environmental concentrations further validating its importance for proper functioning (Narendhirakannan et al., 2005).

Trace elements partake in five roles in living organisms (Nielsen, 1991);

1. Some elements play an integral part in the catalytic centres where biological chemical reactions occur.
2. Certain elements accept or donate electrons in reduction or oxidation reactions as redox reactions are important in generating and utilizing metabolic energy.
3. Some elements, predominantly iron, transport, release and bind oxygen in the body.
4. Certain elements have structural roles, conveying stability as well as three dimensional structures to important biological molecules.
5. Some elements are necessary for proteins to form "finger loops" capable of site-specific DNA binding to double-stranded DNA. These "finger loops" control genetic transcription or gene expression by molecular means.

The elements that will be focussed on here are Calcium, Chromium, Copper, Magnesium, Manganese, Phosphorous, Potassium, Selenium, Sodium and Zinc.

4.1.1.1 Calcium (Ca)

Calcium is a vital electrolyte and the most abundant mineral in the body (Vaskonen, 2003). This nutrient plays a role in cardiovascular health preventing cardiac disease by lowering cholesterol levels as well as maintaining consistent heartbeat and conduction of nerve impulses (Balch, 2006). It is also a component of bones and teeth (Soetan et al., 2010).

Ca is a crucial plant nutrient (White and Broadley, 2003) playing a structural role in the cell wall and membranes, as an intracellular messenger in the cytosol and a counter cation for organic and inorganic ions in the vacuole (Marschner, 1995). Ca deficiency in nature is a rare occurrence although excessive Ca restricts plant species on calcareous soils which result in tiny yellow "specks" in the cell walls (White and Broadley, 2003).

Ca can be found in dark green leafy vegetables, dairy foods, salmon (with bones), sardines and seafood (Gennari, 2001; Balch, 2006). Ca deficiency can lead to muscle cramps, insomnia, eczema, aching joints, cardiac conditions such as elevated blood cholesterol, hypertension and heart palpitations (Balch, 2006).

4.1.1.2 Chromium (Cr)

Chromium (Cr) generally occurs in trivalent Cr (III) and hexavalent Cr (VI) forms in the environment (Shrivastava et al., 2002). This heavy metal occurs in numerous oxidation states which ranges from Cr^{2-} to Cr^{6+} with the oxidation state and concentration determining the beneficial as well as toxic effect in both humans and animals (Zayed et al., 1998). The trivalent and hexavalent forms are the only forms necessary for human health (Dayan and Paine, 2001). Cr maintains stable blood sugar levels by assisting insulin utilisation which could be beneficial for individuals suffering from diabetes and hypoglycaemia (Balch, 2006).

Cr has never been recognised as an essential element for plant growth (Samantaray et al., 1998; Shanker et al., 2005) although studies on its stimulatory effects have been reported (Samantaray et al., 1998). In certain cases, Cr stimulated the growth of plants at lower concentrations whereas higher concentrations had a retarding effect (Samantaray et al., 1998).

Sources of Cr include organ meats, mushrooms, prunes, cereals, nuts, wholegrain bread, asparagus, tea, cocao and coffee as well as alcoholic drinks which include wine and beer (Mutuma et al., 1999). The quantity of Cr consumed daily is important although specific foods may have a detrimental effect on Cr status such as foods laden with simple sugars (Anderson, 1998). Increased risk of arteriosclerosis, inadequate amino acid metabolism, fatigue, anxiety and glucose intolerance (particularly in people with diabetes) are a result of Cr deficiency (Balch, 2006).

4.1.1.3 Copper (Cu)

Copper (Cu) is a transition metal with three oxidation states (Uauy et al., 1998). The cupric state is most frequently found within biological systems (Uauy et al., 1998). Cu is involved in oxidative metabolism which can produce superoxide radicals (Nielsen, 1991). Being part of the Cu-Zn superoxide dismutase, it is also involved in the disproportionation of superoxide free radical ions (Nielsen, 1991). Cu is an important element playing a role in bone and connective tissue integrity, blood vessels and neurotransmitter production (Zima et al., 2001). Together with zinc and vitamin C, Cu assists in the formation of elastin, an important skin protein (Balch, 2006). It is involved in the healing process, is necessary for healthy joints and plays a role in glucose stabilization (Balch, 2006).

Copper is necessary for plant nutrition playing a role in the enzymes vital for cell metabolism (Fernandes and Henriques, 1991). Copper plays an important role in superoxide dismutase which is mostly involved with chloroplasts in vascular plants (Asada et al., 1977). Lack of this element results in deficiency symptoms which affect the young leaves and reproductive organs of the plant (Yruela, 2005).

Deficiency of copper include anemia (Soetan et al., 2010). Most food sources of Cu provided to mammals are from food rather than water (Gaetke and Chow, 2003). Food sources include liver, molasses, legumes, nuts, shellfish as well as other seafood (Soetan et al., 2010). The absorption of Cu in the human body is affected by factors such as chemical form and dietary factors (Gaetke and Chow, 2003).

4.1.1.4 Magnesium (Mg)

Magnesium (Mg) is the fourth most plentiful cation found within the body with roughly half found within bones and the remainder in soft tissues (Bilbey and Prabhakaran, 1996). The functions of Mg are either structural (in the bone) or biochemical (Bilbey and Prabhakaran, 1996). Mg is a facilitator in enzyme activity particularly enzymes involved in energy production (Balch, 2006). This cation prevents soft tissue calcification and protects the arterial lining from stress caused by sudden changes in blood pressure (Balch, 2006).

In plants, Mg plays a role in photosynthesis and is the most common enzyme activator related to energy transport (Mayland, 1983; Wilkinson et al., 1990). This ion is required for ribosomal particle stability and functional RNA protein particles require Mg to carry out sequential reactions needed for photosynthesis (Wilkinson et al., 1990).

Mg is present in most food especially dairy products, fish, meat and seafood (Balch, 2006). Due to the kidneys ability to excrete Mg load, severe hypermagnesaemia is uncommon however, Mg homeostasis can be disrupted and may result in Mg deficiency (Bilbey and Prabhakaran, 1996). Deficiency may be the result of malnutrition, malabsorption, renal tubular damage, diuretic therapy and chronic alcoholism (Bilbey and Prabhakaran, 1996).

4.1.1.5 Manganese

Manganese (Mn) is a crucial trace metal present in all tissues and plays a role in regulating blood sugar and cellular energy, normal immune function and aids the functioning of numerous organ systems within the body (Aschner and Aschner, 2005; Balch, 2006). It is considered indispensable to life as a result of its function in mammalian tissue (Finley and Davis, 1999) as higher concentrations of Mn is reportedly found in mitochondria-rich tissues

(Burch et al., 1975). Mn is required for normal metabolism of amino acids, lipids and carbohydrates (Aschner and Aschner, 2005; Balch, 2006) in addition to being used in cartilage and synovial fluid formation (Balch, 2006).

A small amount of Mn within the plant stimulates plant growth (Kelley, 1914). Mn assists the synthesis of chlorophyll, plays a role in enzyme activation of fat biosynthesis and nitrate assimilation (Dučić and Polle, 2005). Mn is a prerequisite for the water-splitting step of photosynthesis (Williams, 1992).

The Mn concentrations found in foods vary (Burch et al., 1975). The predominant Mn source is found within the diet with major sources including grain, rice and nuts (Aschner and Aschner, 2005) as well as avocado's, seeds and seaweed (Balch, 2006). Fruits and vegetables are excellent sources of Mn (Finley and Davis, 1999). Deficiency of Mn (which is rare) can lead to cardiac problems such as atherosclerosis, high cholesterol and hypertension, confusion, convulsion, eye and hearing difficulties, memory loss, tremors and teeth grinding (Balch, 2006).

4.1.1.6 Phosphorous

Phosphorous (P) is needed for normal heart rhythm, heart muscle contraction, kidney function and blood clotting (Balch, 2006) as well as bone and tooth formation (Balch, 2006; Soetan et al., 2010). It assists cell growth by facilitating the utilization of vitamins and the conversion of food to energy (Balch, 2006).

P is a constituent of fundamental molecules such as phospholipids, nucleic acids and ATP and as a result, plants cannot grow without this crucial nutrient (Schachtman et al., 1998). Phosphorous is the second most frequent macronutrient able to limit plant growth (Schachtman et al., 1998).

Significant amounts of phosphorous can be found in foods such as asparagus, wholegrains, poultry, salmon and meat, seeds (sesame, pumpkin and sunflower seeds), eggs, legumes, nuts and brewer's yeast and corn (Balch, 2006). Phosphorous deficiency is uncommon but can lead to symptoms such as skin sensitivity, irregular breathing, fatigue, bone pain and anxiety (Balch, 2006).

4.1.1.7 Potassium (K)

Potassium (K) is a principle intracellular cation involved predominantly in membrane potential and electrical excitation of nerve and muscle cells (Vaskonen, 2003). This cation plays a role in acid-base balance, conducting nerve impulses and the regulation of osmotic pressure (Soetan et al., 2010). K is crucial for maintaining stable blood pressure and the transmission of electrochemical impulses (Balch, 2006).

K functions in protein synthesis as well as the activation of enzymes within plants (Williams, 1992). This intracellular cation is required for the function of water balance which affects osmosis as well as the operation of the stomata (Williams, 1992).

Food sources of K include wholegrains, vegetables, poultry, meat, fish, fruit and dairy foods (Balch, 2006). K deficiency results in high cholesterol levels and low blood pressure, cognitive impairment, acne, insatiable thirst, abnormally dry skin, constipation and depression (Balch, 2006).

4.1.1.8 Selenium

Selenium (Se) is a crucial nutrient required by animals, humans as well as microorganisms (Ellis and Salt, 2003). It is regarded as an essential trace element and is a co-factor for glutathione peroxidase (Devasagayam et al., 2004). This essential nutrient is often found in substantial amounts in tissues such as the liver, spleen and lymph nodes (Rayman, 2000). The valence state of Se affects the toxicity and bioavailability (Fan and Kizer, 1990). Due to it occurring naturally, this antioxidant appears to preserve tissue elasticity by delaying polyunsaturated fatty acid oxidation (Zima et al., 2001). Selenium plays an essential role aiding antioxidant function through glutathione peroxidases which eradicate potentially harmful radicals due to oxidative stress (Maggini et al., 2007).

Selenium in combination with vitamin E is a vital antioxidant, with the potential to impact immune processes by acting in the extracellular space, together with cell membranes and specifically the gastrointestinal tract as well as the cell cytosol (Arthur et al., 2003). Se represents a nutritional conundrum as it is both toxic and beneficial (Brown and Arthur, 2001).

The essentiality of Se as a micronutrient in land plants has not been conclusively demonstrated (Ellis and Salt, 2003; Sors et al., 2005).

The major dietary sources of Se intake are meat, poultry, grain and grain products as well as seafood (Fan and Kizer, 1990). Se deficiency might occur in geographical areas as a result of the soil that lacks Se (Chehade et al., 2009). Selenium deficiency has been associated with high cholesterol levels, exhaustion, growth impairment, pancreatic insuffiency, cancer and heart disease as well as sterility (Balch, 2006).

4.1.1.9 Sodium

Sodium (Na) is the principle cation of extracellular fluid and a major intravascular fluid volume determinant (Logan, 2006; Doyle, 2008). This essential nutrient plays a crucial role in the regulation of acid-base balance and plasma volume (Soetan et al., 2010) with excessive sodium intake possibly resulting in high blood pressure, oedema as well as liver and kidney disease (Balch, 2006).

Certain plants, particularly halophytes, require Na ions for growth (Blumwald et al., 2000). However, Na ions are detrimental to most plants as elevated salt levels represent a water deficit or osmotic stress due to a decrease in osmotic potential in the soil solution (Zhu, 2007).

Virtually all foods contain Na (Balch, 2006). Deficiency of Na can include anorexia, weight loss, recurrent infections, low blood pressure, dizziness and headaches, depression, confusion and abdominal cramps (Balch, 2006).

4.1.1.10 Zinc

Zinc is an essential trace element (Devasagayam et al., 2004). This essential element is abundant in plants, micro-organisms as well as animals (Burch et al., 1975; Soetan et al., 2010) and has three distinct physiological roles – regulatory, catalytic and structural (Zima et al., 2001). It assists as a catalyst for enzymes gene transcription, DNA replication and RNA and protein synthesis (Rostan et al., 2002; Salgueiro et al., 2002). Zn plays a fundamental role in the enzyme Cu Zn-SOD as the Zn status within the body influences the activity of this enzyme and therefore oxidative stress (Gaetke and Chow, 2003; Ho, 2004). The antioxidant role of Zn is mainly the result of Cu-Zn SOD (Zuo et al., 2006). Its antioxidant role as well as its ability to stabilize membranes suggests a preventative role in free radical induced injury during inflammatory processes (Prasad, 2008a). The levels of Zn may influence conditions facilitated by oxidative damage such as cardiovascular disease, alcoholism and liver cirrhosis, cutaneous and rheumatologic disease (Rostan et al., 2002). Zinc is essential for a healthy immune system influencing both acquired and innate immune functions (Maggini et al., 2007; Prasad, 2008b).

Foods rich in Zn include red meat and seafood (Ho, 2004; Balch, 2006), with legumes and whole grains a much less bioavailable source of zinc (Ho, 2004). Diet induced Zn deficiency is prevalent amongst poor populations that consume predominantly cereals and foods of vegetable origin (Sandstead, 1984). Infertility, loss of taste and smell, increased susceptibility to infection and slow wound healing, acne, fatigue, delayed sexual maturity, growth impairment and high cholesterol levels are a result of Zn deficiency (Balch, 2006).

Table 3: Concentration ranges of the focus elements in plants.

ELEMENT	PLANT CONCENTRATION (mg/kg)
Calcium (Ca)	400 - 13 000 mg/kg [1]
Chromium (Cr)	Not detectable - 18mg/kg [2,3]
Copper (Cu)	4 - 20 mg/kg [1]
Magnesium (Mg)	700 - 9000 mg/kg [1]
Manganese (Mn)	3 - 1000mg/kg [1]
Phosphorous (P)	100 - 10 000 mg/kg [1]
Potassium (K)	1000 – 68 000mg/kg [1]
Selenium (Se)	0.01 – 100 mg/kg [4,5,6,7,8]
Sodium (Na)	20 – 1500mg/kg [1]
Zinc (Zn)	1 – 400mg/kg [1]

References: 1) Larchner (2003); 2) Pawlisz (1997); 3) Cary and Kubota (1999); 4) White and Broadley (2005); 5) Sager (2006); 6) Fordyce (2005); 7) Marschner (1995); 8) Wilber (1980)

Research has shown that many edible plants are rich in phytochemicals which may have health promoting effects. Investigating the biological significance of trace element composition in plants is crucial to develop new strategies of drug design based on natural resources (Mohanta et al., 2003).

The community in the Dwarsrivier valley utilise many plants as medicine and by investigating the predominant elements it might be possible to associate certain elements found within the plant to certain ailments.

Aims and objectives

- To determine the elemental status of selected plants.
- Associate the predominant elements found in selected plants to the ailments of the community in the Dwarsrivier valley.

4.2 MATERIALS AND METHODS

4.2.1 Plant collection

Twelve plant species (Table 4) were collected from the wild and from the gardens of individuals who participated in the study in the Dwarsrivier community near Stellenbosch in July 2011. One sample of each species at three different localities were collected and transported to the Department of Biodiversity and Conservation Biology at the University of the Western Cape to be identified by Mr Frans Weitz, a taxonomist in the department. Voucher specimens were prepared for plants and deposited in the herbarium (M.L Arendse 1-25).

Healthy, mature leaves were selected free of insect predation and harsh weathering of the climate. Plant material was rinsed with deionised water to eradicate any soil or insects that may be present. The leaves were immediately placed in brown paper bags to be oven dried at 70°C for at least 72 hours. Dried samples were finely ground using a mortar and pestle.

4.2.1.1 Sample preparation

The plant material was digested using a hydrogen peroxide: sulphuric acid digestion mixture (Allen et al., 1986). The digestion mixture was prepared as follows: 175 ml of 100 volume H_2O_2 (hydrogen peroxide) was added to 7g $Li_2SO_4.H_2O$. and mixed well. The flask containing the mixture was kept on ice while carefully adding 210 ml of concentrated H_2SO_4. The plant material weighing between 0.4-0.5g were individually wrapped in cigarette paper (Rizzler) and placed in Kjeldahl flasks independently where 5 ml of the digestion mixture was added to each flask and covered with a glass funnel. The flasks were placed in a digestion block at 150°C until the initial reaction subsided. The temperature of the digestion block was increased to 250^0C and left for 30 minutes after which it was increased to 380^0C. The digestion procedure continued until a clear, almost colourless solution was obtained. The solution was left to cool, then diluted with deionised water and filtered through Whatman 42 filter paper and further diluted to 100ml in a volumetric flask. The sample solution was then transferred and stored in 100 ml polyethylene bottles until needed. Three replicates for each site was used for the elemental analysis.

4.2.2 Plant analyses

4.2.2.1 Cations

The samples were analysed for Calcium (Ca), Chromium (Cr), Copper (Cu), Magnesium (Mg), Manganese (Mn), Phosphorous (P), Potassium (K), Selenium (Se), Sodium (Na) and Zinc (Zn) using a Unicam Solaar M Series Atomic Absorption Spectrophotometer (AAS). Ca and Mg were analysed with the flame system using a nitrous oxide/acetylene flame. Cu, Na, K and Zn were analysed with the flame system using an air/acetylene flame. Cr and Mn were analysed using the GF95 graphite furnace.

4.2.2.2 Selenium (Se)

The total Se concentration was determined using the VP 100 vapour system with the EC90 furnace.

4.2.2.3 Phosphorous (P)

The total P concentration was determined using the Murphy and Riley (1962) method. A pale yellow solution was obtained by combining the following reagents: sulphuric acid, ascorbic acid, potassium antimonyl tartrate and ammonium molybdate. Of the sample solution, 1ml was added to 50 ml volumetric flasks with eight ml of Murphy and Riley solution, and diluted to volume with deionised water. The solutions were left for 60 minutes until the colour developed. The absorbance was measured at a wavelength of 882nm using a Shimadzu 160-A UV/visible spectrophotometer. The P concentration was calculated using the following formula:

$$P\ (mg/kg) = \frac{\text{Concentration in } \mu g \times \text{solution volume (100ml)}}{\text{Aliquot (3ml)} \times \text{sample mass (g)}}$$

(Allen et al., 1986)

Table 4: List of selected plant species

Agathosma crenulata	***Leonotis leonurus* (narrow leaf leaf)**
Aloe arborescens	***Leonotis leonurus* (broad leaf)**
Artemisia afra	***Pelargonium capitatum***
Asparagus capensis	***Pelargonium reniforme***
Carpobrotus edulis	***Salvia africana-caerulea***
Cliffortia odorata	***Vernonia oligocephala***

4.3 RESULTS

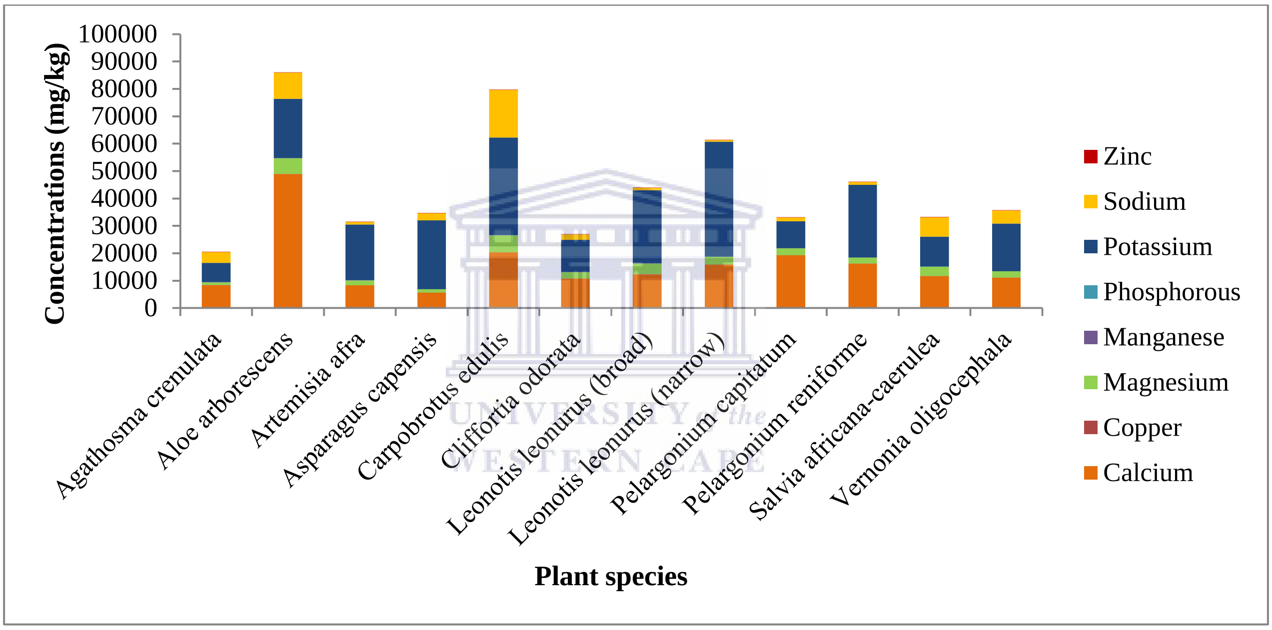

Figure 8: The concentration of elements in selected plant species

Aloe arborescens contained the highest concentration of Ca and the second highest concentration of Mg, Na and Zn (Figure 8). The highest concentration of Cu was present in *Agathosma crenulata* while *Carpobrotus edulis* contained the highest concentration of both Mg and Na (Figure 8). *Leonotis leonurus* (narrow leaf) had the highest concentration in both P and K while the highest concentration of Zn was present in *Vernonia oligocephala (*Figure 8). The level of the elements present in the plant samples were within the normal range (Table 3) except for Na which was higher and P which was lower (Figure 8) .

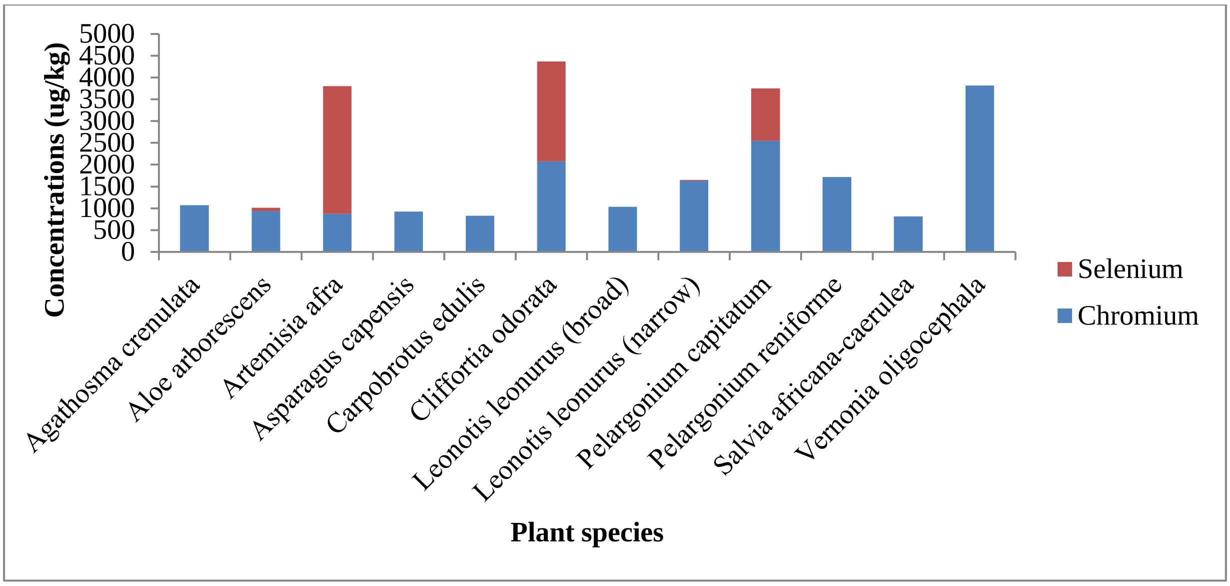

Figure 9: The concentrations of elements in selected plant species

Vernonia oligocephala contained the highest concentration of Cr and the highest concentration of Se was present in *Artemisia afra*.

4.4 DISCUSSION

4.4.1 Calcium (Ca)

The concentrations of Ca present within the plants were within the recommended range (Table 3). Jimoh et al. (2010) reported that the leaves of *L. leonurus* in the Eastern Cape contained Ca, however, the concentration was lower than *L. leonurus* in the Dwarsrivier valley. This could be due to locality, collection season and drying methods. The Dwarsrivier sample was collected in July whereas Jimoh et al. (2010) carried out plant collection in November.

A. arborescens and *L. leonurus* (narrow leaf) are used by the community in the Dwarsrivier valley to treat diabetes, hypertension and cardiac related ailments such as cholesterol. The antidiabetic potential of *A. arborescens* has been evaluated. Beppu et al. (2006) reported that a 10KDa fraction powder of *A. arborescens* inhibited the absorption of glucose in the jejunum of rats. This validates the use of *A. arborescens* by the community in the Dwarsrivier valley to treat diabetes mellitus. Studies evaluating other species of *Aloe* are encouraging and justify further studies to confirm these biological activities of *A. arborescens*.

The cardiovascular potential (Ojewole, 2003) and hypoglycaemic ability (Oyedemi et al., 2011) of *Leonotis leonurus* has been confirmed. This lends credence to the community in the Dwarsrivier valley utilizing *L. leonurus* (narrow leaf) to treat diabetes, hypertension as well as other cardiac related conditions.

4.4.2 Chromium (Cr)

This is the first elemental evaluation of *Vernonia oligocephala*, *Pelargonium capitatum* as well as *Cliffortia odorata.* These plants contained the highest concentration of Cr. The concentration of Cr present in the sample was within the expected range for plants (Table 3).

V. oligocephala, *C. odorata* and *L. leonurus* (broad leaf) are used by the Dwarsrivier community to treat diabetes. Oyedemi et al. (2011) confirmed the hypoglycaemic activity of *L. leonurus*. At present, no studies are available evaluating the biological activity of *C. odorata* or *V. oligocephala*. The relationship between Cr supplementation and diabetes has been well covered by studies (Riales and Albrink, 1981; Felcman and Bragance, 1988; Striffler et al., 1995; Anderson et al., 2001). This validates the utilisation of *L. leonurus*, *V. oligocephala* and *C. odorata* as an anti-diabetic aid in the Dwarsrivier valley.

V. oligocephala and *C. odorata* are used to treat hypertension in the Dwarsrivier valley. *L. leonurus* (broad leaf) is used to treat hypertension as well as cardiac related ailments. The cardiac potential of *L. leonurus* has been confirmed. Studies by Schroeder (1967), Riales and Albrink (1981) and Cefalu et al. (2002) were consistent with the beneficial influence on lipid metabolism. This lends credence to the use of *L. leonurus* as an aid to combat high cholesterol by the community in the Dwarsrivier valley.

4.4.3 Copper (Cu)

This is the first elemental evaluation of the presence of Cu within *Agathosma crenulata*, *Salvia africana-caerulea* and *Artemisia afra* which were the three plants containing the highest concentration. The concentration of Cu was found to be within the range expected in plants (Table 3). Jimoh et al. (2010) reported that the leaves of *L. leonurus* in the Eastern Cape contained Cu however it was higher than the sample found in the Dwarsrivier. This could be a result of the geographical location, seasonal variation and drying methods.

A. afra and *L. leonurus* (broad and narrow leaf) are used by the community to treat diabetes. The hypoglycaemic activities of *A. afra* (Afolayan and Sunmonu, 2011) and *L. leonurus*

(Oyedemi et al., 2011) have been confirmed. Zargar et al. (1998) reported that plasma Cu levels were significantly elevated in diabetic patients. This validates the use of *A. afra* and *L. leonurus* to treat diabetes mellitus in the Dwarsrivier valley.

L. leonurus (broad and narrow leaf) is a popular remedy used to treat sores and wounds. Rabe and van Staden (1997) and Steenkamp et al. (2004) confirmed the antibacterial activity of *L. leonurus*. The efficacy of Cr in healing wounds has been extensively evaluated (Maquart et al., 1993; Canapp et al., 2003; Cangul et al., 2006) which further lends credence to the use of *L. leonurus* as an aid to treat sores and wounds.

4.4.4 Magnesium (Mg)

The levels of Mg present in the leaves were within the normal range expected in plants (Table 3). The Mg concentration found within the leaves of *L. leonurus* in the Dwarsrivier valley was high compared to the concentrations of *L. leonurus* found by Jimoh et al. (2010) in the Eastern Cape. This can be related to the geographical location, differences in the seasons when collection took place as well as different drying methods.

Aloe arborescens and *Leonotis leonurus* are popular remedies used by the community in the Dwarsrivier valley to treat hypertension as well as other cardiac related disorders. The cardiac potential of *L. leonurus* was confirmed by Ojewole (2003) and Oyedemi et al. (2011). The cardiac potential of *A. arborescens* has not yet been determined. Several studies suggest Mg may assist in lowering blood pressure validating the use of *L. leonurus* and *A. arborescens* to treat cardiac related ailments (Witteman et al., 1994; Itoh et al., 1997; Kawano et al., 1998; Al-Delaimy et al., 2004).

Carpobrotus edulis, *Aloe arborescens* and *Leonotis leonurus* are all used to assist stomach ailments which include diarrhoea and stomach cramps. Maphosa et al. (2012) confirmed the ability of aqueous *L. leonurus* to reduce pain and inflammation in rats. Intravenous administration of Mg resolved tetany and muscle pains in a military recruit (Bilbey and Prabhakaran, 1996). This justifies its use in treating diarrhoea and cramps by the community in the Dwarsrivier valley.

4.4.5 Manganese (Mn)

This is the first elemental evaluation of Mn in *A. afra*, *A. crenulata* and *A. arborescens.* These three plants contained the highest concentration of Mn. The level of Mn was within the normal range expected in plants (Table 3). The concentration of Mn in *L. leonurus* found in the Dwarsrivier valley was low compared to that of Jimoh et al. (2010) in the Eastern Cape. This could be due to geographical location, seasonal variation as well as the drying methods employed for the plants. All three plants are used to treat diabetes in the Dwarsrivier valley. The hypoglycaemic activity was confirmed for *A. arborescens* (Beppu et al., 2006) and *A. afra* (Afolayan and Sunmonu, 2011). The hypoglycaemic activity of *A. crenulata* is yet to be investigated. Few studies have been carried out evaluating the preventative role of Mn in diabetes and varied outcomes have been reported (Walter et al., 1991; Kazi et al., 2008).

4.4.6 Phosphorous (P)

This is the first elemental analysis evaluating P within *C. edulis*. The levels of P found in the plants were lower than the normal range expected (Table 3). However, the P concentration within *L. leonurus* found in the Dwarsrivier valley was high compared to that of Jimoh et al. (2012) in the Eastern Cape. This could be associated with geographical location, seasonal variation of collection as well as drying methods.

Leonotis leonurus (narrow leaf) is used to treat hypertension as well as cardiac related ailments. The cardiac potential of *L. leonurus* has been confirmed (Ojewole; 2003; Oyedemi et al., 2011). Although the P concentration was low within the plants this was not a concern as high serum P levels even within the range are associated with cardiovascular disease (Ix et al., 2009; Foley et al., 2009; Alonso et al., 2010). Therefore, the use of *L. leonurus* as an aid to treat hypertension and other cardiac related ailments by the community in the Dwarsrivier valley is validated.

4.4.7 Potassium (K)

The level of K present within the plants were within the normal range expected (Table 3). *Leonotis leonurus* found within the Dwarsrivier valley had a high concentration of K compared to that found by Jimoh et al. (2010) in the Eastern Cape. This could be related to the difference in collection seasons, geographical variation as well as differences in drying methods. *L. leonurus* (both narrow and broad leaf) are used to treat hypertension as well as cardiac related ailments and its cardiac potential has been confirmed (Ojewole, 2003; Oyedemi et al, 2011). The cardiovascular benefit of K has been investigated (Linas and Marzec-Calvert, 1986; He et al., 2009; Oberleithner et al., 2009).

4.4.8 Selenium (Se)

The level of Se present in the plants was within the normal range (Table 3). This is the first elemental analysis evaluating the level of Se in *Artemisia afra*, *Cliffortia odorata*, *Pelargonium capitatum* and *Leonotis leonurus*.

Artemisia afra, *Cliffortia odorata* as well as *Leonotis leonurus* aid the treatment of arthritis in the Dwarsrivier valley. The antioxidant ability of *A. afra* (Afolayan and Sunmonu, 2011; Sunmonu and Afolayan, 2012) and pain and inflammation reducing ability of *L. leonurus* (Maphosa et al., 2012) has been confirmed. Several studies evaluated the role of low Se in the development of arthritis (O'Dell et al., 1991, Heliövaara et al., 1994; Yazar et al., 2005). This justifies the folkoric use of *A. afra* and *L. leonurus* in the Dwarsrivier valley to treat arthritis.

Cliffortia odorata was the only plant used for the treatment of cancer by the community in the Dwarsrivier valley. However, biological tests evaluating the anti-carcinogenic ability of *C. odorata* are yet to be established. Interestingly *Artemisia afra* contained the highest concentration of Se yet it is not used as an aid for treating cancer. Compounds within *A. afra* have been reported to induce apoptosis in cancer cells (Singh and Lai, 2004; Mu et al., 2008). The protective effect of Se as well as the effect of low Se in the development of cancer has been investigated (Brooks et al., 2001; Duffield-Lillico et al., 2003 ;van den Brandt., 2003; Li et al., 2004; Zuo et al., 2006). This finding validates the potential of using *A. afra* as an anti-carcinogenic agent.

4.4.9 Sodium (Na)

The level of Na found within plants was higher than the normal range expected within plants (Table 3). This is the first elemental analysis evaluating the presence of Na in *A. arborescens* and *Salvia africana-caerulea. A. arborescens* and *Salvia africana-caerulea* contained the highest concentrations of Na. The level of Na found in the Dwarsrivier valley was high compared to the values reported by Jimoh et al. (2010) in the Eastern Cape. This could be related to geographical location, variations in the season of collection as well as drying methods.

The negative effects of excess Na have been documented (Doyle, 2008; Strazzulio et al., 2009). Interestingly, all the plants containing high amounts of Na such as *A. arborescens, V. oligocephala* and *A. crenulata* with the exception of *C. edulis* and *S. africana-caerulea* are used to treat hypertension in the Dwarsrivier valley. This is a concern as one of the major complaints of the community was hypertension.

At present, the cardiac potential of *C. edulis*, *A. arborescens* and *V. oligocephala* has not been evaluated. Several studies have shown that moderate restriction of dietary sodium may lower blood pressure (Midgley et al., 1996; Graudal et al., 1998; He and MacGregor, 2002; Cook et al., 2007; He et al., 2009) and reduce long term cardiovascular implications (He et al., 1999; Cook et al., 2007). However, certain surveys contradicted these findings (Aiderman et al., 1998; Harsha et al., 2004; Bibbins-Domingo et al., 2010).

4.4.10 Zinc (Zn)

This is the first elemental evaluation of Zn in *Vernonia oligocaphala*, *Aloe arborescens* and *Carpobrotus edulis*. The level of Zn found within the sample was within the normal range (Table 3). Elemental analysis revealed the presence of Zn in *L. leonurus* leaves in the Eastern Cape (Jimoh et al., 2010) however the concentration was higher than that of *L. leonurus* collected in the Dwarsrivier valley. This can be related to the geographical location as well as differences in the seasons.

Leonotis leonurus (broad leaf) is a popular remedy used in the Dwarsrivier valley to boost the immune system. Jansen et al. (2009) reported the importance of Zn in aiding the immune system. This validates the use of *L. leonurus* as a tonic by the people in the Dwarsrivier valley.

Vernonia oligocephala, *Aloe arborescens* as well as *Leonotis leonurus* are used to treat diabetes mellitus. The hypoglycaemic effect has been confirmed for *A. arborescens* (Beppu et al., 2006) as well as *L. leonurus* (Oyedemi et al., 2011). At present the hypoglycaemic potential of *V. oligocephala* has not been evaluated. The role of Zn in diabetes mellitus has been extensively investigated (Chausmer, 1998; Zargar et al., 1998; Singh et al., 1998; Jansen et al., 2012) further validating the use of *V. oligocephala*, *A. arborescens* and *L. leonurus* to treat diabetes mellitus.

Aloe arborescens and *Carpobrotus edulis* are used to treat skin ailments whereas *Leonotis leonurus* is used to aid the healing of sores and wounds in the Dwarsrivier valley. The antibacterial activity of *Leonotis leonurus* has been confirmed by Rabe and van Staden (1997) and Steenkamp et al. (2004). McGaw et al. (2000) reported that *Aloe arborescens* has no antibacterial activity. However, the protective effects of *A. arborescens* on soft X-irradiation induced skin injury has been confirmed (Sato et al., 1990). This validates the community using *A. arborescens* to soothe irritated skin in conditions such as eczema and psoriasis. The use of Zn in treating wounds as well as a soothing emollient has been investigated (Pories et al., 1967; Baldwin et al., 2001). This further lends justification to the use of *A. arborescens, C. edulis* and *L. leonurus* by the Dwarsrivier community to treat sores and wounds as well as skin conditions.

4.5 CONCLUSION

Currently not many studies are available evaluating the concentration of elements within plants. Compared to phytochemical screening, elemental analysis lags behind. This is detrimental to phytomedicine as it would be beneficial to scientifically evaluate whether a relationship exists between the elements found in plants and their biological activity.

The level of the elements present in the plant samples were within the normal range except for Na which was higher and P which was lower. Hypertension is one of the major concerns by the community in the Dwarsrivier valley yet several of the plants used such as *A. arborescens*, *V. oligocephala* and *A. crenulata* all contained high levels of Na. The level of P was low compared to the normal ranges expected in plants. However, this is not a concern as studies have reported that high serum P levels, even within the normal range, may be a risk factor for coronary artery arteriosclerosis in healthy young adults. *L. leonurus* contained the highest level of P and it is also the predominant treatment for hypertension. Many of the community members have claimed that *L. leonurus* (broad leaf) has a stronger effect than *L. leonurus* (narrow leaf). However, the elemental analysis revealed that *L. leonurus* (narrow leaf) had higher concentrations than *L. leonurus* (broad leaf) in all the elements except Mg and Mn. A great challenge faces natural medicine researchers to scientifically justify to modern man what the San and Khoi already knew thousands of years ago.

REFERENCES

Afolayan, A.J., Sunmonu, T.O., 2011. *Artemisia afra* Jacq. Ameliorates Oxidative Stress in the Pancreas of Streptozotocin-Induced Diabetic Wistar Rats. Biosci. Biotechnol. Biochem 75, 2083-2086.

Aiderman, M.H., Cohen, H., Madhovan, S., 1998. Dietary sodium intake and mortality: the National Health and Nutritional Examination Survey (NHANES I). The Lancet 351, 781-785.

Al-Delaimy, W.K., Rimm, E.B., Willet, W.C., Stampfer, M.J., Hu, F.B., 2004. Magnesium Intake and Risk of Coronary Heart Disease among Men. Original Research. Journal of the American College of Nutrition 23(1), 63-70.

Allen, S.E., Grimshaw, H.M., Rowland, A.P., 1986. Chemistry Analysis. In: Moore, P.D., Chapman, S.B. (Eds.), Methods in Plant Ecology, second ed. Blackwell Scientific Publications, London, pp. 302-303, 331-332.

Alonso, A., Nettleton, J.A., Ix, J.H., de Boer, I.H., Folsom, A.R., Bidulescu, A., Kestenbaum, B.R., Chambless, L.E., Jacobs, D.R Jr., 2010. Dietary Phosphorous, Blood Pressure, and Incidence of Hypertension in the Atherosclerosis Risk in Communities Study and the Multi-Ethnic study of Atherosclerosis. Hypertension 55, 776-784.

Anderson, R.A., 1998. Chromium, Glucose Intolerance and Diabetes. Review Article. Journal of the American College of Nutrition 17(6), 548-555.

Anderson, R.A., Roussel, A.-M., Zouari, N., Mahjoub, S., Matheau, J.-M., Kerkeni, A., 2001. Potential Antioxidant Effects of Zinc and Chromium Supplementation in People with Type 2 Diabetes Mellitus. Original Research. Journal of the American College of Nutrition 20(3), 212-218.

Arthur, J.R., McKenzie, R.C., Beckett, G.J., 2003. Selenium in the Immune system. The Journal of Nutrition 133, 1457S-1459S.

Asada, K., Kanematsu, S., Uchida, K., 1977. Superoxide dismutases in photosynthetic organisms. Arch. Biochem. Biophys. 179, 243-256.

Aschner, J.L., Aschner, M., 2005. Nutritional aspects of manganese homeostasis. Review. Molecular Aspects of Medicine 26, 353-362.

Balch, P.A., 2006. Prescription for Nutritional Healing. Fourth Edition. Penguin Group.

Baldwin, S., Odio, M.R., Haines, S.L., O'Connor, R.J., Englehart, J.S., Lane, A.T., 2001. Skin benefits from continuous topical administration of a zinc oxide/petrolatum formulation by a novel disposable diaper. European Academy of Dermatology and Venereology 15(1), 5-11.

Beppu, H., Shimpo, K., Chihara, T., Kaneko, T., Tamai, I., Yamaji, S., Ozaki, S., Kuzuya, H., Sonoda, S., 2006. Antidiabetic effects of dietary administration of *Aloe arborescens* Miller components on multiple low-dose streptozotocin-induced diabetes in mice: Investigation on hypoglycemic action and systemic absorption dynamics of aloe components. Journal of Ethnopharmacology 103, 468-477.

Bibbons-Domingo, K., Chertow, G.M., Coxson, P.G., Moran, A., Lightwood, J.M., Pletcher, M.J., Goldman, L., 2010. Projected Effect of Dietary Salt Reductions on Future Cardiovascular Disease. The New England Journal of Medicine 362(7), 590-599.

Bilbey, D.I.J., Prabhakaran, V.M., 1996. Muscle cramps and magnesium deficiency: case reports. Canadian Family Physician 42, 1348-1351.

Blumwald, E., Aharon, G.S., Apse, M.P., 2000. Sodium transport in plant cells. Review. Biochimica et Biophysica Acta 1465, 140-151.

Brooks, J.D., Metter, E.J, Chan, D.W., Sokoll, L.J., Landis, P., Nelson, W.G., Muller, D., Andres, R., Carter, H.B., 2001. Plasma selenium level before diagnosis and the risk of prostate cancer development. The Journal of Urology 166, 2034-2038.

Brown, K.M., Arthur, J.R., 2001. Selenium, selenoproteins and human health: a review. Public Health Nutrition 4(2B), 593-599.

Burch, R.E., Hahn, H.K.J., Sullivan, J.F., 1975. Newer Aspects of the Roles of Zinc, Manganese, and Copper in Human Nutrition. Review. Clin. Chem 21(4), 501-520.

Canapp, S.O Jr., Farese, J.P., Schultz, G.S., Gowda, S., Ishak, A.M., Swaim, S.F., Vangilder, J., Lee-Ambrose, L., Martin, F.G., 2003. The Effect of Topical Tripeptide-Copper Complex on Healing of Ischemic Open Wounds. Veterinary Surgery 32, 515-523.

Cangul, I.T., Gul, N.Y., Topal, A., Yilmaz, R., 2006. Evaluation of the effects of topical tripeptide-copper complex and zinc oxide on open-wound healing in rabbits. European Society of Veterinary Dermatology 17, 417-423.

Cary, E.E., Kubota, J., 1990. Chromium concentration in Plants: Effects of Soil Chromium Concentration and Tissue Contamination by Soil. J. Agric. Food Chem. 38(1), 108-114.

Cefalu, W.T., Wang, Z.Q., Zhang, X.H., Baldor, L.C., Russel J.C., 2002. Oral Chromium Picolinate Improves Carbohydrate and Lipid Metabolism and Enhances Skeletal Muscle Glut-4 Translocation in Obese, Hyperinsulinemix (JCR-LA Corpulent) Rats. J. Nutri132, 1107-1114.

Chausmer, A.B., 1998. Zinc, Insulin and Diabetes. Review Article. Journal of the American College of Nutrition 17(2), 109-115.

Chehade, J.M., Sheikh-Ali, M., Mooradian, A.D., 2009. The Role of Micronutrients in Managing Diabetes. Diabetes Spectrum 22(4), 214-218.

Cook, N.R., Cutler, J.A., Obarzanek, E., Buring, J.E., Rexrode, K.M., Kumanyika, S.K., Appel, L.J., Whelton, P.K., 2007. Long term effects of dietary sodium reduction on cardiovascular disease outcomes: observational follow-up of the trials of hypertension prevention (TOHP). BMJ, doi:10.1136/bmj.39147.604896.55 (published 20 April 2007)

Dayan, A.D., Paine, A.J., 2001. Mechanisms of chromium toxicity, carcinogenicity and allergenicity: Review of the literature from 1985 to 2000. Human & Experimental Toxicology 20, 439-451.

Devasagayam, T.P.A., Tilak, J.C., Boloor, K.K., Sane, K.S., Ghaskadbi, S.S., Lele, R.D., 2004. Free Radicals and Antioxidants in Human Health: Current Status and Future Prospects. Review Article. JAPI 52, 794-804.

Doyle, M.E., 2008. Sodium Reduction and Its Effects on Food Safety, Food Quality, and Human Health. FRI Briefings 1-10.

Dučić, T., Polle, A., 2005. Transport and detoxification of manganese and copper in plants. Braz. J. Plant Physiol 17(1), 103-112.

Duffield-Lillico, A.J., Dalkin, B.L., Reid, M.E., Turnbull, B.W., Slate, E.H., Jacobs, E.T., Marshall, J.R., Clark, L.C., 2003. Selenium supplementation, baseline plasma selenium status and incidence of prostate cancer: an analysis of the complete treatment period of the Nutritional Prevention of Cancer Trial. BJU International 91, 608-612.

Ellis, D.R., Salt, D.E., 2003. Plants, selenium and human health. Current Opinion in Plant Biology 6, 273-279.

Fan, A.M., Kizer, K.W., 1990. Selenium Nutritional, Toxicologic, and Clinical Aspects. The Western Journal of Medicine 153, 160-167.

Felcman, J., Braganca, M.L.T., 1988. Chromium in plants. Comparison Between the Concentration of Chromium in Brazilian Nonhypo and Hypoglycaemic Plants. Biological Trace Element Research 17, 11-16.

Fernandes, J.C., Henriques, F.S., 1991. Biochemical, Physiological, and Structural Effects of Excess Copper in Plants. The Botanical Review 57(3), 246-273.

Finley, J.W., Davis, C.D., 1999. Manganese deficiency and toxicity: Are high or low dietary amounts of manganese cause for concern? Biofactors 10, 15-24.

Foley, R.N., Collins, A.J., Herzog, C.A., Ishani, A., Kalra, P.A., 2009. Serum Phosphorous Levels Associate with Coronary Atherosclerosis in Young Adults. Journal of the American Society of Nephrology 20, 397-404.

Fordyce, F.M., 2005. Selenium deficiency and toxicity in the environment. In: Selinus, O., Allowat, J.A., Finkelman, J.B., Fuge, R., Lindh, U., Smedley, P. (Eds.), Essentials of Medical Geology. Elsevier, pp.373-415.

Gaetke, L.M., Chow, C.K., 2003. Copper toxicity, oxidative stress, and antioxidant nutrients. Toxicology 189, 147-163.

Gennari, C., 2001. Calcium and vitamin D nutrition and bone disease of the elderly. Public Health Nutrition 4(2B), 547-559.

Graudal, N.A., Galløe, A.M., Garred, P., 1998. Effects of Sodium Restriction on Blood Pressure, Renin, Aldosterone, Catecholamines, Cholesterols, and Triglyceride. A Meta-analysis. Review. JAMA 279(17), 1383-1391.

Harsha, D.W., Sacks, F.M., Obarzanek, E., Svetkey, L.P., Lin, P.-H., Bray, G.A., Aickin, M., Conlin, P.R., Miller III, E.R., Appel, L.J., 2004. Effect of Dietary Sodium Intake on Blood Lipids: Results from the DASH-Sodium Trial. Hypertension 43, 393-398.

He, J., Ogden, L.G., Vupputuri, S., Bazzano, L.A., Loria, C., Whelton, P.K., 1999. Dietary Sodium Intake and Subsequent Risk of Cardiovascular Disease in Overweight Adults. JAMA 282(21), 2027-2034.

He, F.J., MacGregor, G.A., 2002. Effect of modest salt reduction on blood pressure: a meta-analysis of randomised trials. Implications for public health. Original Article. Journal of Human Hypertension 16, 761-770.

He, J., Gu, D., Chen, J., Jaquish, C.E., Rao, D.C., Hixson, J.E., Chen ,J.-c., Duan, X., Huang, J.-f., Chen, C.-S., Kelly, T.N., Bazzano, L.A., Whelton, P.K., 2009. Gender Difference in Blood Pressure Responses to Dietary Sodium Intervention in the GenSalt Study. J Hypert. 27(1), 48-54.

Heliövaara, M., Knekt, P., Aho, K., Aaran, R.-K., Alfthan, G., Aromaa, A., 1994. Serum antioxidants and risk of rheumatoid arthritis. Annals of the Rheumatic Diseases 53, 51-53.

Ho, E., 2004. Zinc deficiency, DNA damage and cancer risk. Reviews: Current Topics. Journal of Nutritional Biochemistry 15, 572-578.

Itoh, K., Kawasaki, T., Nakamura, M., 1997. The effects of high oral magnesium supplementation on blood pressure, serum lipids and related variables in apparently healthy Japanese subjects. British Journal of Nutrition 78(5), 737-750.

Ix, J.H., De Boer, I.H., Peralta, C.A., Adeney, K.L., Duprez, D.A., Jenny, N.S., Siscovick, D.S., Kestenbaum, B.R., 2009. Serum Phosphorous Concentrations and Arterial Stiffness among Individuals with Normal Kidney Function to Moderate Kidney Disease in MESA. Clinical Journal of the American Society of Nephrology 4, 609-615.

Jansen, J., Karges, W., Rink, L., 2009. Zinc and diabetes - clinical links and molecular mechanisms. Reviews: Current Topics. Journal of Nutritional Biochemistry 20, 399-417.

Jansen, J., Rosenkrantz, E., Overbeck, S., Warmuth, S., Mocchegiani, E., Giacconi, R., Weiskirchen, R., Karges, W., Rink, L., 2012. Disturbed zinc homeostasis in diabetic patients by *in vitro* and *in vivo* analysis of insulinomimetic activity of zinc. www.sciencedirect.com.

Jimoh, F.O., Adedapo, A.A., Afolayan, A.J., 2010. Comparison of the nutritional value and biological activities of the acetone, methanol and water extracts of the leaves of *Solanum nigrum* and *Leonotis leonurus*. Food and Chemical Toxicology 48, 964-971.

Kaur, C., Kapoor, H.C., 2001. Antioxidants in fruits and vegetables - the millennium's health. Review. International Journal of Food Science and Technology 36, 703-725.

Kawano, Y., Matsuoka, H., Takishita, S., Omae, T., 1998. Effects of Magnesium Supplementation in Hypertensive Patients: Assessment by Office, Home, and Ambulatory Blood Pressures. Hypertension 32, 260-265.

Kazi, T.G., Afridi, H.I., Kazi, N., Jamali, M.K., Arain, M.B., Jalbani, N., Kandhro, G.A., 2008. Copper, Chromium, Manganese, Iron, Nickel, and Zinc Levels in Biological Samples of Diabetes Mellitus Patients. Biol Trace Elem Res 122, 1-18.

Kelley, W.P., 1914. The Function of Manganese in Plants. Botanical Gazette, 57(3), 213-227.

Larcher, W., 2003. Physiological Plant Ecology, Fourth ed. Springer-Verlag Berlin Heidelberg New York, p. 513.

Leśniewicz, A., Jaworska, K., Zyrnicki, W., 2006. Macro- and micro-nutrients and their bioavailability in Polish herbal medicaments. Food Chemistry 99, 670-679.

Li, H., Stampfer, M.J., Giovannucci, E.L., Morris, J.S., Willett, W.C., Gaziano, J.M., Ma, J., 2004. A Prospective Study of Plasma Selenium Levels and Prostate Cancer Risk. Journal of the National Cancer Institute 96(9), 696-703.

Linas, S.L., Marzec-Calvert, R., 1986. Potassium depletion ameliorates hypertension in spontaneously hypertensive rats. Hypertension 8, 990-996.

Logan, A.C., 2006. Dietary Sodium Intake and Its Relation to Human Health: A Summary of the Evidence. Introduction to Supplement. Journal of the American College of Nutrition 25(3), 165-169.

Maggini, S., Wintergerst, E.S., Beveridge, S., Hornig, D.H., 2007. Selected vitamins and trace elements support immune function by strengthening epithelial barriers and cellular and humoral immune responses. British Journal of Nutrition 98, S29-S35.

Maphosa, V., Adedapo, A.A., Moyo, B., Masika, P.J., 2012. Anti-inflammatory and analgesic activities of the aqueous extract of *Leonotis leonurus* leaves in rats. Full Length Research Paper. African Journal of Biotechnology 11(26), 6878-6883.

Maquart, F.-X., Bellon, G., Chaqour, B., Wegrowski, J., Patt, L.M., Trachy, R.E., Monboisse, J.-C., Chastang, F., Birembaut, P., Gillery, P., Borei, J.-P., 1993. *In Vivo* Stimulation of Connective Tissue Accumulation by the Tripeptide-Copper Complex Glycyl-L-histidyl-L-lysine-Cu^{2+} in Rat Experimental Wounds. J.Clin.Invest 92, 2368-2376.

Marschner, H., 1995. Mineral Nutrition of Higher Plants. Academic Press, London.

Mayland, H.F., 1983. Role of Magnesium in Plants. Proceedings of John Lee Pratt International Symposium on the Role of Magnesium in Animal Nutrition. Virginia Polytechnic Institute and State University Blacksburg, Virginia.

McGaw, L.J., Jäger, A.K., van Staden, J., 2000. Antibacterial, anthelmintic and anti-amoebic activity in South African medicinal plants. Journal of Ethnopharmacology 72, 247-263.

Midgley, J.P., Matthew, A.G., Greenwood, C.M.T., Logan, A.G., 1996. Effect of Reduced Dietary Sodium on Blood Pressure. A Meta-analysis of Randomised Controlled Trials. Review. JAMA 275, 1590-1597.

Mohanta, B., Chakraborty, A., Sudarshan, M., Dutta, R.K., Baruah, M., 2003. Elemental profile in some common medicinal plants of India. Its correlation with traditional therapeutic usage. Journal of Radioanalytical and Nuclear Chemistry 258(1), 175-179.

Mu, D., Zhang, W., Chu, D., Liu, T., Xie, Y., Fu, E., Jin, F., 2008. The role of calcium, P38 MAPK in dihydroartemisinin-induced apoptosis of lung cancer PC-14 cells. Original Article. Cancer Chemother Pharmacol 61, 639-645.

Murphy, J., Riley, J.P., 1962. A modified single solution method for the determination of phosphate in natural waters. Analytica Chimica Acta 27, 31-36.

Mutuma, S., Amuna, P., Shukla, H., Sumar, S., 1999. Chromium in food, nutrition and health - an introduction. Nutrition and Food Science 2, 81-88.

Narendhirakannan, R.T., Subramanian, S., Kandaswamy, M., 2005. Mineral Content of Some Medicinal Plants Used in the Treatment of Diabetes Mellitus. Biological Trace Element Research 103, 109-115.

Nielsen, F.H., 1991. Trace and ultratrace elements in health and disease. Comprehensive therapy 17(3), 20-26.

Oberleithner, H., Callies, C., Kusche-Vihrog, K., Schillers, H., Shahin, V., Riethműller, C., MacGregor, G.A., de Wardener, H.E., 2009. Potassium softens vascular endothelium and increases nitric oxide release. PNAS 106(8), 2829-2834.

O'Dell, J.R., Lemley-Gillespie, S., Palmer, W.R., Weaver, A.L., Moore, G.F., Klaasen, L.W., 1991. Serum selenium concentrations in rheumatoid arthritis. Annuals of the Rheumatic Diseases 50, 376-378.

Ojewole, J.A.O., 2003. P-2: Hypotensive effect of *Leonotis leonurus* aqueous leaf extract in rats. American Journal of Hypertension 16(5), 40A.

Oyedemi, S.O., Yakubu, M.T., Afolayan, A.J., 2011. Antidiabetic activities of aqueous leaves extract of *Leonotis leonurus* in streptozotocin induced diabetic rats. Full Length Research Paper. Journal of Medicinal Plants Research 5(1), 119-125.

Pawlisz, A. V., 1997. Canadian water quality guidelines for Cr. Environ. Toxicol. Water Qual. 12(2), 123–161.

Pories, W.J., Hentzel, J.H., Rob, C.G., Strain, W.H., 1967. Acceleration of Healing with Zinc Sulphate. Annals of Surgery 165(3), 432-436.

Prasad, A.S., 2008a. Clinical, immunological, anti-inflammatory and antioxidant roles of zinc. Mini Review. Experimental Gerontology 43, 370-377.

Prasad, A.S., 2008b. Zinc in Human Health: Effect of Zinc on Immune Cells. MOL MED 14(5-6), 353-357.

Rabe, T., van Staden, J., 1997. Antibacterial activity of South African plants used for medicinal purposes. Journal of Ethnopharmacology 56, 81-87.

Rayman, M.P., 2000. The importance of selenium to human health. Review. The Lancet 356, 233-241.

Riales, R., Albrink, M.J., 1981. Effect of chromium chloride supplementation on glucose tolerance and serum lipids including high-density lipoprotein of adult men. The American Journal of Clinical Nutrition 34, 2670-2678.

Rostan, E.F., DeBuys, H.V., Madey, D.L., Pinnell, S.R., 2002. Evidence supporting zinc as an important antioxidant for skin. International Journal of Dermatology 41, 606-611.

Sager, M., 2006. Selenium in agriculture, food and nutrition. Pure Appl. Chem. 78(1), 111 – 133.

Salgueiro, M.J., Zubillaga, M.B., Lysionek, A.E., Caro, R.A., Weill, R., Boccio, J.R., 2002. The Role of Zinc in the Growth and Development of Children. Review Article. Nutrition 18, 510-519.

Samantaray, S., Rout, G.R., Das, P., 1998. Role of chromium on plant growth and metabolism. Review. Acta Physiologiae Plantarum. 20(2), 201-212.

Sandstead, H.H., 1984. Trace Metals in Human Nutrition. Nutrition in the 20th century. John Wiley and Sons Inc.

Sato, Y., Ohta, S., Shinoda, M., 1990. Studies on chemical protectors against radiation. xxxi. Protection effects of *Aloe arborescens* on skin injury induced by x-irradiation. Journal of the Pharmaceutical Society of Japan 110(11), 876-884.

Schachtman, D.P., Reid, R.J., Ayling, S.M., 1998. Phosphorous Uptake by Plants: From Soil to Cell. Plant Physiol 116, 447-453.

Schroeder, H.A., 1967. Cadmium, Chromium, and Cardiovascular Disease. Circulation 35, 570-582.

Shanker, A.K., Cervantes, C., Loza-Tavera, H., Avudainayagam, S., 2005. Chromium toxicity in plants. Review article. Environmental International 31, 739-753.

Shikanga, E.A., Combrinck, S., Regnier, T., 2010. South African *Lippia* herbal infusions: Total phenolic content, antioxidant and antibacterial activities. South African Journal of Botany 76, 567-571.

Shrivastava, R., Upreti, R.K., Seth, P.C., Chaturvedi, U.C., 2002. Effects of chromium on the immune system. Mini Review. FEMS Immunology and Medical Microbiology 34, 1-7.

Singh, R.B., Niaz, M.A., Rastogi, S.S., Bajaj, S., Gaoli, Z., Shoumin, Z., 1998. Current Zinc Intake and Risk of Diabetes and Coronary Artery Disease and Factors Associated with Insulin Resistance in Rural and Urban Populations of North India. Original Paper. Journal of the American College of Nutrition 17(6), 564-570.

Singh, N.P., Lai, H.C., 2004. Artemisinin Induces Apoptosis in Human Cancer Cells. Anticancer Research 24, 2277-2280.

Soetan, K.O., Olaiya, C.O., Oyewole, O.E., 2010. The importance of mineral elements for humans, domestic animals and plants: A review. African Journal of Food Science 4(5), 200-222.

Sors, T.G., Ellis, D.R., Salt, D.E., 2005. Selenium uptake, translocation, assimilation and metabolic fate in plants. Review. Photosynthesis Research 86, 373-389.

Steenkamp, V., Mathivha, E., Gouws, M.C., van Rensburg, C.E.J., 2004. Studies on antibacterial, antioxidant and fibroblast growth stimulation of wound healing remedies from South Africa. Journal of Ethnopharmacology 95, 353-357.

Strazzulio, P., D'Elia, L., Kandala, N.-B., Cappuccio, F.P., 2009. Salt intake, stroke, and cardiovascular disease: meta-analysis of prospective studies. BMJ 339, 1-9.

Striffler, J.S., Law, J.S., Polansky, M.M., Bhathena, S.J., Anderson, R.A., 1995. Chromium Improves Insulin Response to Glucose in Rats. Metabolism 44(10), 1314-1320.

Sunmonu, T.O., Afolayan, A.J., 2012. Evaluation of Polyphenolic Content and Antioxidant Activity of *Artemisia afra* Jacq. Ex Willd. Aqueous Extract. Pakistan Journal of Nutrition 11(7), 520-525.

Temple, N.J., 2000. Antioxidants and disease: More questions than answers. Nutrition Research 20(3), 449-459.

Uauy, R., Olivares, M., Gonzales, M., 1998. Essentiality of copper in humans. American Journal of Clinical Nutrition 67, 952S-959S.

Van den Brandt, P.A., Zeegers, M.P.A., Bode, P., Goldbohm, R.A., 2003. Toenail Selenium Levels and the Subsequent Risk of Prostrate cancer: A Prospective Cohort Study. Cancer Epidemiology, Biomarkers & Prevention 12, 866-871.

Vaskonen, T., 2003. Dietary minerals and modification of cardiovascular risk factors. Journal of Nutritional Biochemistry 14, 492-506.

Walter, R.M Jr., Uriu-Hare, J.Y., Olin, K.L., Oster, M.H., Anawalt, B.D., Critchfield, J.W., Keen, C.L., 1991. Copper, Zinc, Manganese, and Magnesium Status and Complications of Diabetes Mellitus. Diabetes Care 14(11), 1050-1056.

White, P.J., Broadley, M.R., 2003. Calcium in Plants. Review Article. Annals of Botany 92, 487-511.

White, P.J., Broadley, M.R., 2005. Biofortifying crops with essential mineral elements. TRENDS in Plant Science 10(12), 586-593.

Wilber, C.G., 1980. Toxicology of selenium: a review. Clin. Toxicol. 17, 171–230.

Wilkinson, S.R., 1990. Magnesium in Plants Uptake, Distribution, Function, and Utilization by Man and Animals. In: Sigel and Sigel, A (Eds). Metal ions in biological systems. Volume 26, Marcel Dekker Inc, New York and Basel.

Williams , U.S., 1992. A Textbook of Biology, third ed. In: Soetan, K.O., Olaiya, C.O., Oyewole, O.E., 2010. The importance of mineral elements for humans, domestic animals and plants: A review. Review Paper. African Journal of Food Science 4(5), 200-222.

Witteman, J.C.M., Grobbee, D.E., Derkx, F.H.M., Bouillon, R., de Bruijn, A.M., Hofman, A., 1994. Reduction of blood pressure with oral magnesium supplementation in women with mild to moderate hypertension. American Journal of Clinical Nutrition 60, 129-135.

Yazar, M., Sarban, S., Kocyigit, A., Isikan, U.E., 2005. Synovial Fluid and Plasma Selenium, Copper, Zinc, and Iron Concentrations in Patients with Rheumatoid Arthritis and Osteoarthritis. Biological Trace Element Research 106, 123-132.

Yruela, I., 2005. Copper in plants. Braz. J. Plant Physiol. 17(1), 145-156.

Zargar, A.H., Shah, N.A., Masoodi, S.R., Laway, B.A., Dar, F.A., Khan, A.R., Sofi, F.A., Wani, A.I., 1998. Copper, zinc and magnesium levels in non-insulin dependent diabetes mellitus. Postgrad Med J 74, 665-668.

Zayed, A., Lytle, C.M., Qian, J.-H., Terry, N., 1998. Chromium accumulation, translocation and chemical speciation in vegetable crops. Planta 206, 293-299.

Zhu, J.-K., 2007. Plant Salt Stress. Encyclopedia of lifesciences.

Zima, T., Fialová, L., Mestek, O., Janebová, M., Crkovská, J., Malbohan, I, Stípek, S., Mikulíková, L., Popov, P., 2001. Oxidative Stress, Metabolism of Ethanol and Alcohol-Related Diseases. Journal of Biomedical Science 8, 59-70.

Zuo, X.L., Chen, J.M., Zhou, X., Li, X.Z., Mei, G.Y., 2006. Levels of Selenium, Zinc, Copper, and Antioxidant Enzyme Activity in Patients with Leukemia. Biological Trace Element Research 114, 41-53.

CHAPTER 5

PHYTOCHEMICAL SCREENING FOR TWELVE SELECTED PLANTS FROM THE DWARSRIVIER VALLEY, STELLENBOSCH.

ABSTRACT

Twelve plants were selected for evaluation by thin layer chromatography (TLC) while the oils of *Agathosma crenulata, Salvia africana-caerulea* and *Pelargonium capitatum* were assessed by gas chromatography coupled to mass spectroscopy (GC-MS). Plants such as *Asparagus capensis*, *Cliffortia odorata* and *Vernonia oligocephala* were evaluated for the first time.

Phytochemical screening revealed the presence of tannins in all plants except *A. crenulata*, *A. arborescens* and *Vernonia oligocephala* and saponins were evident in all plants except *A. crenulata* and *Aloe arborescens.* Essential oils were present in all plants except *Carpobrotus edulis*, *Cliffortia odorata*, *Asparagus capensis* and *A. arborescens.* Glycosides were evident in all plants with the exception of *S. africana-caerulea*, *L. leonurus* (both narrow and broad*), C. edulis*, *A. capensis* and *A. crenulata*. All plants contained flavonoids and phenols with anthraquinones present in all plants except *C. edulis*, *L. leonurus* (narrow and broad) and *P. reniforme.* Secondary metabolites evident in *Artemisia afra*, *Leonotis leonurus* and *Salvia africana-caerulea* were similar to previous studies.

The percentage of predominant compounds present in the oils of *A. crenulata*, *S. africana-caerulea* and *P. capitatum* evaluated by GC-MS was much higher compared to previous studies. This could be directly associated to the geographical locations and the season of plant collection.

Key words: Medicinal plants; thin layer chromatography (TLC); gas chromatography coupled to mass spectroscopy (GC-MS); secondary metabolites; variables

CHAPTER 5

PHYTOCHEMICAL SCREENING FOR TWELVE SELECTED PLANTS FROM THE DWARSRIVIER VALLEY, STELLENBOSCH

5.1 INTRODUCTION

5.1.1 An overview

The world is an emporium of medicinal plants which provide society with endless health options (Krishnaiah et al., 2009) with many infectious diseases having been treated with medicinal plant remedies (Parekh and Chanda, 2007). The medicinal properties of plants are linked to the secondary compounds found within the plant and are not associated with a single entity but an amalgamation of these metabolites (Scott et al., 2004; Parekh et al., 2005; Joshi et al., 2011). The presence of secondary metabolites may be useful indicators of both efficacy and potential toxicity (Scott et al., 2004).

5.1.2 The role of secondary metabolites

Phytochemicals are present in an array of plants, including seeds, herbs, fruits and vegetables, and are considered an important component of the human diet (Okwu, 2005). These bioactive compounds work together with nutrients and fibres to act as defense against disease (Krishnaiah et al., 2007). They provide plants with their characteristic colour, texture, flavour and scent (Ukoha et al., 2011). The medicinal action of these plants is distinctive to a plant species or group (Joshi et al., 2011). This is sustained by the notion that the arrangement of secondary products found in a specific plant is taxonomically distinct (Parekh et al., 2005). These compounds can be categorised as primary or secondary constituents established by their metabolic role (Krishnaiah et al., 2007). Primary constituents include purines and pyrimidines of nucleic acids, proteins and amino acids whereas secondary components are the residual plant compounds such as phenolics, terpenes, alkaloids etc. (Krishnaiah et al., 2007). Secondary metabolites from vascular plants act as a defense mechanism against invading micro-organisms (Kähkönen et al., 1999).

As reported before, the beneficial characteristics of medicinal plants are linked to secondary compounds working in combination (Joshi et al., 2009). The secondary metabolites that will be focussed on include alkaloids, essential oils, flavonoids, saponins and tannins.

5.1.2.1 Alkaloids

The alkaloids encompass the largest single class of secondary plant substances (Barbosa-Filho et al., 2006). These secondary metabolites are derived mostly from amino acids (Zieglar and Facchini, 2008). Alkaloids within the plant are a defense strategy against pathogens and herbivores (Zieglar and Facchini, 2008). The effective biological activity of several alkaloids has led to abuse and resulted in the development of stimulants, narcotics, pharmaceuticals and poisons (Facchini, 2001).

Alkaloids have an extraordinary range of pharmacological activity although certain alkaloids have toxic effects (Barbosa-Filho et al., 2006). Plant-derived alkaloids used in clinical settings include morphine and codeine (analgesics), the anticancer agent vinblastine, scopolamine (sedative), the gout suppressant better known as colchicine, ajmaline (antiarrythmic) and the antibiotic known as sanguinarine (Facchini, 2001).

5.1.2.2 Essential oils

Essential oils are characterised by a strong aroma and are produced as secondary metabolites in aromatic plants (Bakkali et al., 2008). These volatile oils are complex mixtures containing between 20-60 constituents at various concentrations and are characterised by two or three major components at relatively high concentrations (Bakkali et al., 2008). Depending on the plant and the extraction procedure used, various classes of molecules such as terpenoids, phenolics, aromatics, sulphur-and nitrogen containing compounds can be isolated (Nakatsu et al., 2000). Terpenoids encompass the largest organic chemical group, not only in essential oils but also in natural products (Nakatsu et al., 2000).

These oils have been used as medicine since antiquity (Nakatsu et al., 2000) as an antiseptic, food preservative, antimicrobial, spasmolytic, analgesic, sedative, anti-inflammatory and as local anaesthetic remedies (Bakkali et al., 2008). Their lipophilic ability allow them to move through the cell wall and membrane and in the process, disrupt the arrangement of phospholipids, polysaccharides and fatty acids resulting in permealisation (Bakkali et al., 2008).

5.1.2.3 Flavonoids

Flavonoids belong to a group of polyphenol compounds which are present in all foods of plant origin (Ross and Kasum, 2002) and are frequent components of the human diet (Cao et al., 1997). These widely distributed compounds are characterised by a benzo-y-pyrone structure (Ross and Kasum, 2002). It is this structure together with relative moieties within the molecule that determine the biochemical activities of flavonoids and their metabolites (Yao et al., 2004). Due to their chemical nature, flavonoids are viewed as antioxidants (Hollman and Katan, 1999).

The ease with which a hydrogen atom can be donated to a free radical, as well as the ability of an aromatic compound to support an unpaired electron due to delocalisation, determines the antioxidant capacity of phenolic compounds (Ross and Kasum, 2002). The biological as well as pharmacological effects of a flavonoid compound may depend upon its behaviour as either an antioxidant or a prooxidant (Cao et al., 1997).

5.1.2.4 Saponins

Saponins are naturally occurring surface-active glycosides (Francis et al., 2002) distributed in plants as well as marine and animal kingdoms (Lacaille-Dubois and Wagner, 1996). They are classified into two groups, steroidal and triterpenoid saponins, based on their aglycone skeleton (Sparg et al., 2004). Saponins consist of a sugar moiety that typically contains glucose, galactose, glucoronic acid, xylose, rhamnose or methylpentose glycosidically linked to a hydrophobic aglycone (sapogenin) which may be triterpenoid or steroid (Francis et al., 2002; Vincken et al., 2007). These surface-active glycosides are characterised by surfactant properties which give rise to soap-like, stable foam in aqueous solutions (Osbourne, 1996; Francis et al., 2002) which distinguish these compounds from other glycosides (Sparg et al., 2004). This soap-like behaviour is due to the arrangement of polar and non-polar structural elements in their molecules (Francis et al., 2002).

Normally, immature plants of a species were found to have greater saponin content compared to mature plants of the same species (Francis et al., 2002). The mechanism of action of saponins is somewhat nonspecific, and can be assumed to affect all eukaryotic organisms with sterols in their membranes (Osbourne, 1996). The interaction of saponins with cholesterol in the cell membrane may create pore-like structures which may cause the membrane to burst (Lacaille-Dubois and Wagner, 1996). This may cause hypersecretion which would explain its expectorant activity (Lacaille-Dubois and Wagner, 1996).

5.1.2.5 Tannins

Tannins are polyphenol compounds of plant origin and are amongst the most widely distributed plant secondary products (Mc Sweeney et al., 2001). These secondary metabolites are found in an array of vascular plant species of both herbaceous and woody types (Scalbert, 1991).

Tannins can be categorised into two groups based on their properties and structures (Bhat, 1998). Hydrolysable tannins are composed of esters of ellagic acid (ellagitannins) or gallic acid (gallotannins) with a sugar core which is readily hydrolysed by acids or enzymes (Bhat, 1998). Condensed tannins, composed of flavonoid units, are also known as polymeric proanthocyanidins and are more abundant in tree barks and woods compared to hydrolysable tannins (Bhat, 1998). Additionally, condensed tannins are more resistant to microbial attacks than hydrolysable tannins and are more toxic to microorganisms (Bhat, 1998).

The foreseen interaction of tannins with biological systems stems primarily from its characteristic capability to form complexes with metal ions and macromolecules as well as its anti-oxidative and radical scavenging properties (De Bruyn et al., 1999).

Medicinal plants are rich in constituents which could be potential candidates for natural drugs serving as cheap, safe and alternative antimicrobial agents (Kuete et al., 2010). They are generating interest as alternative antimicrobial agents as they are regarded as being safer than synthetic medication (Ali et al., 2012). Despite the extensive application of these plants in traditional medicine, information on their phytochemical constituents is limited (Njoku and Obi, 2009). The establishment of a scientific foundation is essential to enable more rational awareness of African medicinal plants as candidates for new medicines (Kuete et al., 2010). Many isolated compounds lack comprehensive biological and clinical evaluation with many plants with pharmacological properties devoid of proper chemical identification (Graz et al., 2007). Phytochemical screening is the first step to scientifically justify the remedies used by cultures for medicinal purposes.

Aims and objectives

- To compare the phytochemical profiles of selected plants to those found in the literature.

5.2 MATERIALS AND METHODS

5.2.1 Plant material collection

5.2.1.1 Plant collection for thin layer chromatography

Eleven plant species were collected from the wild as well as the gardens of individuals who participated in the study in the Dwarsrivier community near Stellenbosch in August 2011. One sample of each species at three different localities were collected and transported to the Department of Biodiversity and Conservation Biology at the University of the Western Cape to be identified by Mr Frans Weitz, a taxonomist in the department. Voucher specimens have been deposited in the Herbarium at the University of the Western Cape.

Healthy, mature leaves were selected free of insect predation and harsh weathering of the climate. The plant material was rinsed with deionised water to eradicate any soil or insects which may be present. The leaves were then immediately placed in brown paper bags to be oven dried at 70°C for at least 72 hours. Dried samples were finely ground using a mortar and pestle.

5.2.1.2 Plant collection for gas chromatography

Three plant species, namely *Agathosma crenulata*, *Pelargonium capitatum* and *Salvia africana-caerulea* were collected from the gardens of individuals who participated in the study in the Dwarsrivier community in October 2011. Plants were identified by Frans Weitz, a taxonomist at the University of the Western Cape and voucher specimens were deposited in the Herbarium at the University of the Western Cape. Plants were then stored in the freezer until needed.

A known quantity of fresh plant material was subjected to hydrodistillation in a Clevenger apparatus for eight hours one day after collection. The technique is based on the principle of evaporating volatile compounds via steam (Moolla, 2005). The essential oils were collected in vials, sealed and stored in the refrigerator until analysis.

5.2.2 Extraction of plant materials

5.2.2.1 Phytochemical screening

For the purpose of phytochemical screening, rations of the ground samples were soaked separately in five solvents namely: hexane (Hex), dichloromethane (DCM), ethyl acetate

(EtOAc), methanol (MeOH) and water (H_2O) at room temp (25^oC) for 24 hours and the derived extracts were evaporated on a rotary evaporator at $\leq 45\ ^oC$.

5.2.2.2 Alkaloid screening

Plant material dried at 40^0C was finely ground with a mortar and pestle, mixed with 15 ml 0.05M H_2SO_4 and left to stand at room temperature for 20 minutes. After filtering, the remaining plant solid was extracted with 5ml 0.05M $H_2SO_{4.}$ The aqueous phases were combined, applied to glass columns with 24g course grade celite, alkalinized with ammonia (4ml), and extracted once with 100 ml CH_2Cl_2. The CH_2Cl_2 extracts were dried over anhydrous Na_2SO_4. The solvent was then evaporated under reduced pressure.

5.2.3 Thin layer chromatography

Analytical TLC was conducted on normal-phase Merck Silica gel 60 PF_{254} precoated aluminium plates. Separated compounds were visualized under UV light at 254 and 366 nm.

For the detection of alkaloids, brown or orange-brown zones appear immediately on spraying with quinine used as the reference standard for alkaloids.

For the detection of anthraquinones, the plate is sprayed with potassium hydroxide reagent which shows red fluorescence in UV-366nm. 1.4-Naphthoquinone was used as the standard reference for anthraquinones.

For the detection of essential oils, the plate is sprayed with vanillin-sulphuric acid reagent and heated for 1-2 minutes on a heating plate. The plates were then evaluated. The essential oils were depicted as dark blue, green or red zones and camphor was used as the standard reference for essential oils.

For the detection of flavonoids and phenols, the plate was sprayed with Sodium reagent and then visualised under UV light. Apigenin was used as a reference standard.

5.2.4 Alkaloid test

Merck 60 F254 silica gel plates (0.25MM layer thickness) were developed in $CHCl_3$: cyclohexane: Et2NH (4:5:1). The plates were dried at 100^oC for 3 minutes, studied under UV_{254} and UV_{365} and then sprayed with Dragendorff reagent.

5.2.5 General phytochemical screening test

5.2.5.1 Saponins

The presence of saponins was determined using the frothing test. 1g of dried plant material was added to 5 ml of deionised water in the test tube and then heated. The test tube was vigorously shaken for a few minutes. The presence of froth suggests the existence of saponins.

5.2.5.2 Tannins

A small quantity (1g) of plant material was boiled in 5 ml of deionised water. The decoction was allowed to cool and then filtered. A few drops of ferric chloride solution was added to the filtrate. The presence of tannins is indicated by a blue black precipitate.

5.2.6 Gas chromatography

The analysis of essential oils by gas chromatography coupled to mass spectroscopy (GC-MS) was performed using an Agilent 6890N GC with a CTC CombiPAL Autosampler and Agilent 5975B MS. The system was operated under the following conditions; column: DB-FFAP (60m, 0.25mm ID, 0.5µm film thickness) with model number, J&W 122-3263, temperatures: injector temperature 240^{o}C, column initially 70 ^{o}C for 10 min, ramp 1 for 3 minutes at 142 ^{o}C and ramp 2 for 5 minutes at 225 ^{o}C for 3 minutes.

Table 5.1: The twelve selected plants

Agathosma crenulata	***Leonotis leonurus*** **(narrow leaf)**
Aloe arborescens	***Leonotis leonurus*** **(broad leaf)**
Artemisia afra	***Pelargonium capitatum***
Asparagus capensis	***Pelargonium reniforme***
Carpobrotus edulis	***Salvia africana-caerulea***
Cliffortia odorata	***Vernonia oligocephala***

5.3 RESULTS

5.3.1 Thin layer chromatography

Table 5.2: Alkaloids

A. capensis	-
A. crenulata	+
A. afra	-
A. arborescens	-
C. edulis	-
C. odorata	-
L. leonurus (narrow)	+
L. leonotis (broad)	+
P. capitatum	-
P. reniforme	-
S. africana-caerulea	-
V. oligocephala	-

Table 5.3: Saponins and Tannins

Plant species	**SAPONINS**	**TANNINS**
A. crenulata	+++	-
A. arborescens	-	-
A. afra	++	+
A. capenses	+++	+
C. edulis	+++	+
C. odorata	+++	+
L. leonurus (narrow)	+	+
L. leonurus (broad)	+	+
P. capitatum	++	+
P. reniforme	++	+
S. africana-caerulea	++	+
V. oligocephala	-	-

Table 5.4: TLC results for *A. crenulata*

	Essential oils	Anthraquinones	Flavonoids and Phenols	Glycosides
Hex	+	-	+	
DCM	+	-	+	
EtOAc	+	-	-	-
MeOH	+	+	+	-
H_20	+	+	+	-

Table 5.5: TLC results for *A. arborescens*

	Essential oils	Anthraquinones	Flavonoids and Phenols	Glycosides
Hex	-	+	+	
DCM	-	+++	+	
EtOAc	-	++	+	-
MeOH	-	+	+	++
H_20	-	-	+	-

Table 5.6: TLC results for *A. afra*

	Essential oils	Anthraquinones	Flavonoids and Phenols	Glycosides
Hex	+	+++	+	
DCM	+	+	+	
EtOAc	+	-	-	+++
MeOH	+	+	+	+
H_20	+	+	+	+++

Table 5.7: TLC results for *A. capensis*

	Essential oils	Anthraquinones	Flavonoids and Phenols	Glycosides
Hex	-	+	+	
DCM	-	-	+	
EtOAc	-	+	+	-
MeOH	-	+	+	-
H_20	-	+	+	-

Table 5.8: TLC results for *C. edulis*

	Essential oils	Anthraquinones	Flavonoids and Phenols	Glycosides
Hex	-	-	+	
DCM	-	-	+	
EtOAc	-	-	+	-
MeOH	-	-	+	-
H_20	-	-	-	-

Table 5.9: TLC results for *C. odorata*

	Essential oils	Anthraquinones	Flavonoids and Phenols	Glycosides
Hexane	-	-	-	
DCM	-	+	+	
EtOAc	-	-	+	++
MeOH	-	-	+	-
H_20	-	-	+	-

Table 5.10: TLC results for *L. leonurus* (narrow leaf)

	Essential oils	Anthraquinones	Flavonoids and Phenols	Glycosides
Hex	+	-	-	
DCM	+	-	-	
EtOAc	+	-	-	-
MeOH	+	-	+	-
H_20	+	-	+	-

Table 5.11: TLC results for *L. leonurus* (broad leaf)

	Essential oils	Anthraquinones	Flavonoids and Phenols	Glycosides
Hex	+	-	-	
DCM	+	-	-	
EtOAc	+	-	-	-
MeOH	+	-	+	-
H_20	+	-	+	-

Table 5.12 TLC results for *P. capitatum*

	Essential oils	Anthraquinones	Flavonoids and Phenols	Glycosides
Hex	+	-	+	
DCM	+	-	+	
EtOAc	+	-	-	+
MeOH	+	-	+	++
H_20	+	-	+	-

Table 5.13: TLC results for *P. reniforme*

	Essential oils	Anthraquinones	Flavonoids and Phenols	Glycosides
Hexane	+		-	
DCM	+		-	
EtOAc	+	+	-	++
MeOH	+	-	+	++
H_20	+	-	-	+

Table 5.14: TLC results for *S. africana-caerulea*

	Essential oils	Anthraquinones	Flavonoids and Phenols	Glycosides
Hexane	+	-	-	
DCM	+	-	-	
EtOAc	-	-	-	-
MeOH	+	+	+	-
H_20	+	++	+	-

Table 5.15: TLC results for *V. oligocephala*

	Essential oils	Anthraquinones	Flavonoids and Phenols	Glycosides
Hex	-	++	+	
DCM	-	+	+	
EtOAc	-	-	-	-
MeOH	+	-	+	+
H_20	+	-	+	-

5.3.2 Gas chromatography

Table 5.16: Gas chromatography results for *A. crenulata*

α-pinene	Moolla, 2005
Camphene	Moolla,2005
β-Pinene	Moolla,2005
β-Phellandrene	
Cyclohexane,1-methylene-4-(1-methylethenyl)	
Bicyclo[4.1.0]hept-2-ene,3,7,7-trimethyl	
D-limonene	
Limonene	
Bicyclo[3.1.0]hex-2-ene,4-methyl-1-1-(1-methylethyl)	
1,3,8-p-Menthatriene	
(R) -(+)-3-Methylcyclopentanone	
1,3,6-Octratriene,3,7-dimethyl-,E)	
Benzene,1-methyl-2-(1-methylethyl)	
Cyclohexanone,3-methyl	
Benzene,1-methyl-4-(1-methylethenyl)	
Limonene oxide,cis	
Limonene oxide, trans	
Cyclohexanone-5-methyl-2-(1-methylethyl)-trans	
Benzofuran,4,5,6,7-tetrahydro-3,6-dimethyl	
Bicyclo[2.2.1]heptan-2-one,1,7,7-trimethyl-,(IS)	
1,6-Octadien-3-ol,3,7-dimethyl	
Cyclohexanol,5-methyl-2-(1-methylethenyl)-,[1R-(1.α.,2.β.,5.α.)]	
Cyclohexanol,5-methyl-2-(1-methylethenyl)	
Isopulegol	
Cyclohexanone,5-methyl-2-(1-methylethenyl)-,trans	
6-Methyl-3,5-heptadiene-2-one,	
3-Cyclohexen-1-ol,4-methyl-1-(1-methylethyl)	
Cyclohexanone,2-methyl-5-(1-methylethenyl)-,trans	
Cyclohexanone,2-methyl-5-(1-methylethenyl)	
Cyclohexanone,5-methyl-2-(1-methylethylidene)	
p-menth-1-en-8-ol	
Bicyclo[3.1.1]hept-3-en-2-one,4,6,6-trimethyl-,(1S)	
2-Cyclohexen-1-ol,2-methyl-5-(1-methylethylethenyl)-acetate,cis	
2-Cyclohexen-1-one,3-methyl-6-(1-methylethyl)	
2-Cyclohexen-1-one,2-methyl-5-(1-methylethenyl-,(S)	
6-Octen-1-ol,3,7-dimethyl-,(R)	
2-Caren-10-al	
2-Cyclohexen-1-ol,2-methyl-5-(1-methylethenyl)-,cis	

1-Cyclohexene-1-methanol,4-(1-methylethenyl)	
Benzenemethanol,4-(1-methylethyl)	
1H-Cycloprop[e]azulen-7-ol,decahydro-1,1,7-trimethylene-,[1ar-(1a.α.,4a.α.,7β.,7a.β.,7b.α.)]	
Acetophenone,4-methoxy-	
1H-Cycloprop[e]azulen-7-ol,decahydro-1,1,7-trimethyl-4-methylene-,[1ar-(1a.α.,4a.α.,7.β.,7a.beta.,7b.α.)]	
Mint furanone	

Table 5.17: Gas chromatography results for *S. africana-caerulea*

α-Pinene	Kamatou,2007
Camphene	Kamatou, 2007
β-Pinene	Kamatou, 2007
3-Carene	Kamatou, 2007
α-Phellandrene	Kamatou, 2007
Cyclohexane, 1-methylene-4-(1-methylethenyl)	
(+)-4-Carene	
2,6-Dimethyl-1,3,5,7-octatetraene	
D-limonene	
1,3,6-Octatriene,3,7-dimethyl-,E)	
Bicyclo[3.1.0]hex-2-ene,2-methyl-5-(1-methylethyl)	
Benezene,1-methyl-2-(1-methylethyl)	
Bicyclo[4.1.0]hept-2-ene,3,7,7-trimethyl	
Cyclohexene,1-methyl-4-(1-methylethylidene)	
6-Hepten-1-ol, 2-methyl	
5-Hepten-2-ol,6-methyl	
α-Cubene	
Epizonarene	
Isoledene	
Naphthalene,1,2,3,5,6,8a-hexahydr	
Ylangene	
Copaene	
Cyclohexanone,5-methyl-2-(1-methylethyl)-,(2S-trans)	
Bicyclo[2.2.1]heptan-2-one,1,7,7-trimethyl-,(1R)	
1H-Cycloprop[e]azulene, 1a.2,3,4,4a,5,6,7b-octahydro-1,1,4,7-tetramethyl-,[1a.α.,4.α.,4a.β.,7b.α.)]	
Delta-Semilene	
Aristolene	
1-H-Cyclopropa[a]naphthalene,1a,2,3,5,6,7,7a,7b-octahydro-1,1,4,7-tetramethyl-,[1aR-(-(1a-α.,7a.α.,7b.α.)]	
Caryophyllene	Kamatou, 2007
3-Cyclohexen-1-ol,4-methyl-1-(1-methylethyl)-,(R)	
Naphthlene,1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethylidene)-,(4aR-trans)	
1H-Cycloprop[e]azulene,decahydro1,1,7-methyl-4-methylene-,[1aR-(1a.α.,4a.α.,7.α.,7a.β.,7b.α.)]	
1,1,4a-Trimethyl-5,6-dimethylenedecahydronaphthalene	
Aromadene	
Pulegone	
α-Carophylene	
Naphthalene,1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-,1.α.,4a,α.,8a.α.)	
Naphthalene,2,3,4,4a,5,6-hexahydro-1,4a-dimethyl-7-(1-methylethyl)	

1H-Cycloprop[e]azulene,1a,2,3,5,6,7,7a,7b-actahydro-1,1,4,7-tetramethyl-,[1aR-(1a.α.,7α.,7a.β.,7b.α.)]	
Cyclohexene,1-methyl-4-(5-methyl-1-methylene-4-hexenyl)-,(S)	
α-Farnesene	
Naphthalene,1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methyl-,(1S-cis)	
Ledol	Kamatou, 2007
(-)-Globulol	

Table 5.18: Gas chromatography results for *Pelargonium capitatum*

1,4-Cyclohexadiene, 1-methyl-4-1-methylethyl)	
Benzene, 1-methyl-4-(1-methylethyl)	
Benzene, 1-methyl-2-(1-methylethyl)	
2-Furanmethanol, 5-ethenyltetrahydro-.alpha.,.alpha.,5-trimethyl-,cis	
2H-Pyran, 3,6-dihydro-4-methyl-2-2-methyl-1-propenyl)	
1H-Cyclopenta[1,3]cyclopropa[1,2]b enzene, octahydro-7-methyl-3-methylene-4-(1-methylethyl)-, [3aS-(3a.alpha.,3b.beta.,4.beta.,7.alpha.,7 aS*)]	
α- Cubebene	
Benzaldehyde	
Spiro[5.5]undeca-1,8-diene, 1,5,5, 9-tetramethyl-, (R)	
1H-3a,7-Methanoazulene, 2,3,4,7,8,8a-hexahydro-3,6,8,8-tetramethyl-,[3R (3.alpha.,3a.beta.,7.beta.,8a .alpha.)]	
3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)	
1H-3a,7-Methanoazulene, octahydro-3,8,8-trimethyl-6-methylene-, [3R-(3.alpha.,3a.beta.,7.beta.,8a.alpha.)]	
1H-Cycloprop[e]azulene, decahydro- 1,1,7-trimethyl-4-methylene	
10s,11s-Himachala-3(12),4-diene	
1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene-, [1aR -(1a.alpha.,4a.beta.,7.alpha.,7a.beta.,7b.alpha.)]	
1H-Cycloprop[e]azulene, decahydro- 1,1,7-trimethyl-4-methylene-, [1aR -(1a.alpha.,4a.beta.,7.alpha.,7a.beta.,7b.alpha.)]	
Cyclohexanone, 5-methyl-2-(1-methylethenyl)	
2,6-Octadienal, 3,7-dimethyl-, (Z)	
1,6-Cyclodecadiene, 1-methyl-5-met hylene-8-(1-methylethyl)-, [s-(E,E)]	
1H-Cyclopenta[1,3]cyclopropa[1,2]benzene, octahydro-7-methyl-3-methylene-4-(1-methylethyl)-, [3aS-(3a.alpha.,3b.beta.,4.beta.,7.alpha.,7aS*)]	
Naphthalene, 1,2,3,4,4a,5,6,8a-oct ahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1.alpha.,4a.alpha.,8a.alpha.)	
Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-,(1S-cis)	
Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1.alpha.,4a.beta.,8a.alpha.)	
1H-Cyclopropa[a]naphthalene, 1a,2, 3,5,6,7,7a,7b-octahydro-1,1,7,7a-tetramethyl-, [1aR-(1a.alpha.,7.alpha.,7a.alpha.,7b.alpha.)]	
Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl	
Benzoic acid, 2-hydroxy-, methyl ester	
Benzenemethanol, .alpha.,.alpha.,4-trimethyl-	
Butanoic acid, 3,7-dimethyl-2,6-octadienyl ester, (E)	
Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-	

methylethenyl)-, [2R-(2.alpha.,4a.alpha.,8a.beta.)]	
Benzene, 1,2-dimethoxy-4-(2-propenyl)	
Nerolidol 2	
1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, [S-(Z)]-	
1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)	
Ledol	(Demarne et al., 1993)
Globulol	
Azulene, 1,2,3,3a,4,5,6,7-octahydr o-1,4-dimethyl-7-(1-methylethenyl) -, [1R-(1.alpha.,3a.beta.,4.alpha.,7.beta.)]	
1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1a.alpha.,4a.alpha.,7.beta., 7a.beta.,7b.alpha.)]	
(-)-Spathulenol	
Thymol	
Phenol, 2-methyl-5-(1-methylethyl)	
Naphthalene, 1,2,3,4,4a,5,6,8a-oct ahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1.alpha.,4a.beta.,8a.alpha.)	
Naphthalene, 1,2,3,4,4a,5,6,8a-oct ahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1.alpha.,4a.alpha.,8a.alpha.)	
Copaene	(Demarne et al., 1993)
4-Hydroxy-3-methylacetophenone	
.alpha.-Cadinol	

5.4 DISCUSSION

5.4.1 Agathosma crenulata

Agathosma crenulata is a remedy used in the Dwarsrivier valley to treat many ailments ranging from sores and wounds to aiding the body as a tonic. Phytochemical screening has revealed the presence of flavonoids in the dichloromethane and methanol extracts of *A. crenulata* from the Dwarsrivier valley. The presence of phenolic compounds indicates the ability of the plant to act as an antimicrobial agent (Okwu and Okwu, 2004). Moolla et al. (2007) extracted fresh plant material using methanol and dichloromethane (1:1) and reported antimicrobial activity exhibited against the four pathogens tested namely *Bacillus cereus*, *Staphylococcus aureus*, *Klebsiellae pneumoniae* and *Candida albicans.* This justifies its use as an antimicrobial agent and validates it being used as a remedy to treat sores and wounds by the community in the Dwarsrivier valley.

A. crenulata is used to treat arthritis is the Dwarsrivier valley. GC-MS indicated the presence of α-pinene in the oils. The essential oil of *A. crenulata* was reportedly active in the anti-inflammatory assay (Moolla, 2005) and α-pinene has been shown to possess nociceptive ability (Him et al., 2008). This validates the use of *A. crenulata* to treat arthritis in the Dwarsrivier valley.

Stomach ailments are one of the main complaints in the Dwarsrivier valley with *A. crenulata* often used as a treatment. The presence of α-pinene and β-pinene were detected in the oils of *A. crenulata*. The anti-inflammatory activity of *A. crenulata* has been confirmed (Moolla, 2005). The compounds α-pinene and β-pinene have been shown to display antibacterial activity (Dorman and Deans, 2000) with β-pinene exhibiting antispasmodic activity (Sadraei et al., 2001) which justifies its use as an aid to treat diarrhoea by the community in the Dwarsrivier valley.

Flavonoids are effective radical scavengers (Hollman et al., 1996) and have received considerable attention due to its beneficial effect in the prevention of disease such as cardiovascular disease and cancer as well as gastric and duodenal ulcers, allergies and viral and bacterial infections (Yao et al., 2004). Moolla (2005) reported the extract and oil of *A. crenulata* to be inactive in the DPPH assay however the extract was active in the ABTS assay. Phenolics are generally known for their antioxidant ability (Brookes and Dutton, 2007). Several compounds present in the oil of *A. renulata* reportedly have antioxidant potential. These include α-pinene (Wei and Shihamoto, 2007; Wang et al., 2008), β-pinene (Wang et al., 2008), limonene (Wei and Shihamoto, 2007) and isopulegol (Singh et al., 2012). This authenticates the use of *A. crenulata* to treat arthritis, cardiovascular ailments and colds and flu.

The percentage of α-pinene, β-pinene and camphene present in the essential oils of *A. crenulata* in the Dwarsrivier valley were higher than that which was found by Moolla (2005). The major compounds found in the sample from the Dwarsrivier valley varied greatly to that found by Moolla (2005). This could be related to geographical location as well as the collection season.

5.4.2 Artemisia afra

Phytochemical screening of the dichloromethane extract of *Artemisia afra* revealed the presence of flavonoids, phenols as well as essential oils and the water extract showed positive results for flavonoids, phenols and essential oils. The dichloromethane extract of *A. afra* produced compounds with significant antimicrobial activity capable of inhibiting the replication of *Mycobacterium tuberculosis* and *Mycobacterium aurum* (Ntutela et al., 2009) while the water extract showed activity against *M. aurum* A+ strain (Buwa and Afolayan, 2009). The presence of phenolic compounds indicates the potential of a plant as an antimicrobial agent (Rauha et al., 2000). Although *A. afra* is not used by the community in the Dwarsrivier valley to treat tuberculosis it could be used as a possible treatment method.

5.4.3 Carpobrotus edulis

Carpobrotus edulis is one of two plants used to treat tuberculosis by the community in the Dwarsrivier valley. Phytochemical screening revealed the presence of flavonoids in the methanol extract of *C. edulis*. Van der Watt and Pretorius (2001) reported antibacterial activity for the crude methanol extract of *C. edulis* against *Mycobacterium catharrhalis.* Martins et al. (2005) revealed that although the methanol extract of *C. edulis* is inactive against *Staphylococcus aureus* (Methicillin-resistant) and *Mycobacterium tuberculosis* (multi-drug resistant), once phagocytosed by human macrophages derived from monocytes it inhibits the growth of these bacteria. This validates the use of *C. edulis* by the community in the Dwarsrivier valley as a treatment for tuberculosis.

C. edulis extract from the Dwarsrivier valley displayed positive results for the presence of tannins and the methanol extract of *C. edulis* revealed the presence of flavonoids and phenols. In a study by Van der Watt and Pretorius (2001), six flavonoids isolated from the methanol extract of *C. edulis* showed significant antibacterial activity against *Mycobacterium catharralis*, *Staphylococcus aureus* and *Staphylococcus epidermis*. These include rutin, neohesperidin, hyperoside, cactichin, ferulic acid and an unknown flavonoid. Plants containing tannins are astringent, have the ability to draw tissues together and are effective in stopping the flow of blood or other secretions (Olajuyigbe and Afolayan, 2012). The presence of flavonoids and tannins in *C. edulis* found in the Dwarsrivier valley justifies its use as an antimicrobial agent used to soothe irritated skin as well as sores.

5.4.4 Leonotis leonurus

L. leonurus tested positive for the presence of saponins and tannins and the water extract revealed the presence of flavonoids. *L. leonurus* is used to treat hypertension as well as other cardiac related ailments in the Dwarsrivier valley. Jimoh et al. (2010) reported that *L. leonurus* could potentially act as an antioxidant while Ojewole (2003) confirmed the hypotensive activity of *L. leonurus*. Other cardiovascular benefits of *L. leonurus* were confirmed by Mnonpi et al. (2011). Increased oxidative stress has been associated with the development of cardiovascular disease and cancer (Chehade, 2002) and flavonoids have been associated with antioxidant ability (Hollman and Katan, 1999). The beneficial effect of saponins in the prevention of cholesterol has been confirmed (Sidhu and Oakenfull, 1986; Krishnaiah et al., 2009) with proanthocyanidins (condensed tannins) reportedly playing a role in cardiovascular diseases (Wang et al., 2011). This lends credence of the use of *L. leonurus* as an aid to treat hypertension and cholesterol in the Dwarsrivier valley.

The methanol extract of *L. leonurus* revealed the presence of flavonoids and phenols with essential oils being present in both the methanol and water extract. The saponins and tannins test confirmed positive results for *L. leonurus*. Okwu and Okwu (2004) reported plants possessing phenolic compounds could serve as potential antimicrobial agents. Jimoh et al. (2010) confirmed the antibacterial activity of the methanol extract of *L. leonurus* against *Bacillus cereus*, *Serratia marcescens*, *Staphylococcus epidermis*, *Staphylococcus aureus*, *Micrococcus kristinae*, *Streptococcus kristinae*, *Steptococcus pyrogens*, *Escherichia coli*, *Salmonella pooni*, , *Pseudomonas aeruginosa* but not *Klebsiella pneumoniae* justifying its use as an antimicrobial agent.

Steenkamp et al. (2004) reported that the methanol as well as aqueous extract of *L. leonurus* presented MIC values of >4 mg/ml against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli* and *Pseudomonas aeruginosa*. Maphosa et al. (2012) reported the potential of *L. leonurus* to reduce pain and inflammation. Pandey et al. (2012) reported that clinical studies have revealed the ability of terpenoids to strengthen the skin, increase the concentration of antioxidants in wounds and increase the blood supply to inflamed tissues thus restoring it. Saponins are used to stop bleeding and treat wounds as it helps to induce blood coagulation (Okwu and Josiah, 2006) while tannins are astringent and hasten the healing of wounds as well as inflamed mucous membranes. Krishnaiah et al. (2009) reported that flavonoids are known for their analgesic properties. This justifies the use of *L. leonurus* (broad and narrow leaf) to treat sores and wounds by the community in the Dwarsrivier valley.

5.4.5 Pelargonium capitatum

The percentage of α-cubebene, copaene and ledol found in the *Pelargonium capitatum* sample found in the Dwarsrivier valley was higher than that of Demarne et al. (1993). This could be attributed to the geographical location as well as the collection season. Demarne et al. (1993) observed variation amongst the diverse populations with only 20 compounds regarded as significantly important which could contribute to more that 5% of the total oil.

5.4.6 Pelargonium reniforme

The methanol and the water extract of *Pelargonium reniforme* revealed the presence of flavonoids. Plants containing flavonoids are potential antimicrobial agents (Okwu and Okwu, 2004). Kayser and Kolodziej (1997) reported the water extract of *P. reniforme* displayed better antibacterial activity than the ethyl acetate extract against *E. coli*, *K. pneumoniae* and β-hemolytic *Streptococcus* 1451. The hexane extract of *P. reniforme* displayed the most distinct antimycobacterial effect particularly against *Mycobacterium aurum* (Seidel and Taylor, 2004). A methanol sample of *P. reniforme* displayed higher radical scavenging activities compared to the reference antioxidant in both the DPPH and chemiluminescence assay (Latté and Kolodziej, 2004). Flavonoids have been reported to possess antioxidant potential. Although *P. reniforme* is not used as an aid to treat tuberculosis in the Dwarsrivier valley it could be used by the community to assist the treatment.

4.4.7 Salvia africana-caerulea

Salvia africana-caerulea is a popular remedy to treat colds and flu, inflammation, cardiovascular conditions, stomach ailments and arthritis by the Dwarsrivier commuity. The methanol and water extracts of *S. africana-caerulea* revealed positive results for essential oils, flavonoids and phenols. Kamatou et al. (2008) conducted a study and found that the antibacterial activity of the spring sample of *S. africana-caerulea* had the best activity against *Escherichia coli, Staphylococcus aureus* and *Bacillus cereus* with the exception of *Klebsiella pneumoniae*. Other studies have validated the use of essential oils as antimicrobial agents (Cox et al., 2000; Chorianopoulos et al., 2004; Iscan et al., 2006; Gachkar et al., 2007). This justifies the use of *S. africana-caerulea* in the treatment of stomach ailments such as diarrhoea.

Salvia africana-caerulea contained the following compounds which had antibacterial activity: α-pinene (Dorman and Deans, 2000), β-pinene (Dorman and Deans, 2000) and limonene (Dorman and Deans, 2000), whereas α-pinene (Sadraei *et al*., 2001) and β-pinene (Sadraei et al., 2001) also have anti-spasmodic activity. This justifies the use of *S. africana-caerulea* in the treatment of colds and flu. According to Kamatou et al. (2008), *Salvia africana-caerulea* exhibited poor anti-inflammatory activity, however, it exhibited antioxidant activity. Even though Kamatou et al. (2008) reported S. *africana-caerulea* exhibited poor anti-inflammatory activity, the compounds camphene (Mulyaningsih et al, 2010) as well as β-carophyllene (Tambe et al., 1996) found in the oil of S. *africana-caerulea* in the present study possess anti-inflammatory potential. Further research indicates essential oils from other plant species possess anti-inflammatory and analgesic activity (Iscan et al., 2006; Iwalewa et al., 2007). This would justify the use of *S. africana-caerulea* as an aid in arthritis as well as anti-inflammatory conditions by the community in the Dwarsrivier valley.

The percentage of α-pinene, β-pinene and camphene found in the oil of *Salvia africana-caerulea* in the Dwarsrivier was higher compared to that of Kamatou (2006). The major compounds found within the oil of *S. africana-caerulea* in the Dwarsrivier valley such as carophyllene and ledol were lower than that found by Kamatou (2006). The major compounds found by Kamatou (2006) were spathulenol, β-carophyllene oxide, ledol and α-pinene. These oils were found in low concentrations or not present at all in the oil of *S. africana-caeruleae* in the Dwarsriver valley. This can be attributed to the geographical location as well as collection season.

5.5 CONCLUSION

Phytochemical screening is a step towards scientifically validating the folkloric uses of traditional medicine. Plants such as *Asparagus capensis*, *Cliffortia odorata* and *Vernonia oligocephala* have been evaluated for the first time. These plants are popular remedies used by the communities in the Dwarsrivier valley. Phytochemical evaluation of *Artemisia afra*, *Leonotis leonurus* and *Salvia africana-caerulea* revealed similar findings to previous studies (Scott et al., 2004). The predominant compounds detected in the oils of *Agathosma crenulata*, *Salvia africana-caerulea* and *Pelargonium capitatum* analysed by gas chromatography coupled to mass spectroscopy (GC-MS) were higher compared to other studies previously carried out (Moolla, 2005; Kamatou, 2006; Demarne et al., 1993). This could be directly associated with geographical location as well as seasonal variation.

REFERENCES

Ali, A., Akhtar, N., Khan, B.A., Khan, M.S., Rasul, A., Uz-Zaman, S., Khalid, N., Waseem, K., Mahmood, T., Ali, L., 2012. *Acacia nilotica*: A plant of multipurpose medicinal uses. Review. Journal of Medicinal Plants Research 6(9), 1492-1496.

Bakkali, F., Averbeck, S., Averbeck, D., Idaomar, M., 2008. Biological effects of essential oils - A review. Food and Chemical Toxicology 46, 446-475.

Barbosa-Filho, J.M., Pitivesam, M.R., Moura, M.D., Silva, M.S., Lima, K.V.P., da-Cunha, E.V.L., Fechine, I.M., Takemura, O.S., 2006. Anti-inflammatory activity of alkaloids: A twenty-century review. Brazilian Journal of Pharmacognosy 16(1), 103-139.

Bhat, T.K., Singh, B., Sharma, O.P., 1998. Microbial degradation of tannins - A current perspective. Biodegradation 9, 343-357.

Brookes, K.B., Dutton, M.F., 2007. Bioactive components of the uteroactive medicinal plant, *Gunnera perpensa* (or ugobo). South African Journal of Science 103, 187-189.

Buwa, L.V., Afolayan, A.J., 2009. Antimicrobial activity of some medicinal plants used for the treatment of tuberculosis in the Eastern Cape Province, South Africa. Full Length Research Paper. African Journal of Biotechnology 8(23), 6683-6687.

Cao, G., Sofic, E., Prior, R.L., 1997. Antioxidant and prooxidant behaviour of flavonoids: Structure-activity relationships. Free Radical Biology & Medicine 22(5), 749-760.

Chehade, J.M., Sheikh-Ali, M., Mooradian, A.D., 2009. The Role of Micronutrients in Managing Diabetes. Diabetes Spectrum 22(4), 214-218.

Chorianopoulos, N., Kalpoutzakis, E., Aligiannis, N., Mitaku, S., Nychas, G.-J., Haroutounian, S.A., 2004. Essential oils of Satureja, Origanum, and Thymus Species: Chemical Composition and Antibacterial Activities Against Foodborne Pathogens. Journal of Agriculture and Food Chemistry 52, 8261-8267.

Cox, S.D., Mann, C.M., Markham, J.L., Bell, H.C., Gustafson, J.E., Warmington, J.R., Wyllie, S.G., 2000. The mode of antimicrobial action of the essential oil of *Melaleuca alternifolia* (tea tree oil). Journal of Applied Microbiology 88, 170-175.

De Bruyn, T., Pieters, L., Deelstra, H., Vlietinck, A., 1999. Condensed vegetable tannins: Biodiversity in structure and biological activities. Biochemical systematics and Ecology 27, 445-459.

Demarne, F.-E., Viljoen, A.M., Van der Walt, J.J.M., 1993. A Study of the Variation in the Essential Oil and Morphology of *Pelargonium capitatum* (L.) L'Hérit. (Geraniaceae). Part 1. The composition of the Oil. The Journal of Essential Oil Research 5(5), 493-499.

Dorman, H.J.D., Deans, S.G., 2000. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. Journal of Applied Microbiology 88, 308-316.

Facchini, P.J., 2001. ALKALOID BIOSYNTHESIS IN PLANTS: Biochemistry, Cell Biology, Molecular Regulation, Metabolic Engineering Applications. Annu. Rev. Plant. Physiol. Plant. Mol. Biol. 52, 29-66.

Francis, G., Kerem, Z., Makkar, H.P.S., Becker, K., 2002. The biological action of saponins in animal systems: a review. British Journal of Nutrition 88, 587-605.

Gachkar, L., Yadegari, D., Rezaei, M.B., Taghizadeh, M., Astaneh, S.A., Rasooli, I., 2007. Chemical and biological characteristics of *Cuminum cyminum* and *Rosmarinus officinalis* essential oils. Food Chemistry 102, 898-904.

Graz, B., Elisabetsky, E., Falquet, J., 2007. Beyond the myth of expensive clinical study: Assessment of traditional medicines. Commentary. Journal of Ethnopharmacology 113, 382-386.

Him, A., Ozbek, H., Turel, I., Oner, A.C., 2008. Antinoceptive activity of alpha-pinene and fenchone. Pharmacologyonline 3, 363-369.

Hollman, P.C.H., Hertog, M.G.L., Katan, M.B., 1996. Analysis and health effects of flavonoids. Food Chemistry 57(1), 43-46.

Hollman, P.C.H., Katan, M.B., 1999. Dietary Flavonoids: Intake, Health Effects and Bioavailability. Food and Chemical Toxicology 37, 937-942.

Iscan, G., Kirimer, N., Kűrkcűoglu, M., Arabaci, T., Kűpeli, E., Hűsnű Can Baser, K., 2006. Biological Activity and Composition of the Essential Oils of *Achillea schischkinii* Sosn. And *Archillea aleppica* DC. subsp. *aleppica*. Journal of Agricultural and Food Chemistry 54, 170-173.

Iwalewa, E.O., McGaw, L.J., Naidoo, V., Eloff, J.N., 2007. Inflammation: the foundation of diseases and disorders. A review of phytomedicines of South African origin used to treat pain and inflammatory conditions. African Journal of Biotechnology 6(25), 2868-2885.

Jimoh, F.O., Adedapo, A.A., Afolayan, A.J., 2010. Comparison of the nutritional value and biological activities of the acetone, methanol and water extracts of the leaves of *Solanum nigrum* and *Leonotis leonurus*. Food and Chemical Toxicology 48, 964-971.

Joshi, B., Sah, G.P., Basnet, B.B., Bhatt, M.R., Sharma, D., Subedi, K., Pandey, J., Malla, R., 2011. Phytochemical extraction and antimicrobial properties of different medicinal plants: *Ocimum sanctum* (Tulsi), *Eugenia caryophyllata* (Clove), *Achyranthes bidentata* (Datiwan) and *Azadirachta indica* (Neem). Full Length Research Paper. Journal of Microbiology and Antimicrobials 3(1), 1-7.

Kähkönen, M.P., Hopia, A.T., Vuorela, H.J., Rauha, J.P., Pihlaja, K., Kujala, T.S., Heinonen, M., 1999. Antioxidant Activity of Plant Extracts Containing Phenolic Compounds. J. Agric. Food Chem. 47(10), 3954-3962.

Kamatou, G.P.P., 2006. Indigenous *Salvia* Species − An investigation of their pharmacological activities and phytochemistry. Ph.D dissertation, University of the Witwatersrand.

Kamatou, G.P.P., Van Zyl, R.L., Van Vuuren, S.F., Figueiredo, A.C., Barroso, J.G., Pedro, L.G., Viljoen, A.M., 2008. Seasonal variation in essential oil composition, oil toxicity and the biological activity of solvent extracts of three South African *Salvia* species. South African Journal of Botany 74, 230-237.

Kayser, O., Kolodziej, H., 1997. Antibacterial activities of Extracts and Constituents of *Pelargonium sidoides* and *Pelargonium reniforme*. Original Paper. Planta Medica 63, 508-510.

Krishnaiah, D., Sarbatly, R., Bono, A., 2007. Phytochemical antioxidants for health and medicine - A move towards nature. Standard Review. Biotechnology and Molecular Biology Review 1(4), 97-104.

Krishnaiah, D., Devi, T., Bono, A., Sarbatly, R., 2009. Studies on phytochemical constituents of six Malaysian medicinal plants. Full Length Research Paper. Journal of Medicinal Plants Research 3(2), 067-072.

Kuete, V., Tabopda, T.K., Ngameni, B., Nana, F., Tshikalange, T.E., Ngadjui, B.T., 2010. Antimycobacterial, antibacterial and antifungal activities of *Terminalia superba* (Combretaceae). South African Journal of Botany 76, 125-131.

Lacaille-Dubois, M.A., Wagner, H., 1996. A review of the biological and pharmacological activities of saponins. Phytomedicine 2(4), 363-386.

Latté, K.P., Kolodziej, H., 2004. Antioxidant Properties of Phenolic Compounds from *Pelargonium reniforme*. Journal of Agricultural and Food Chemistry 52, 4899-4902.

Maphosa, V., Adedapo, A.A., Moyo, B., Masika, P.J., 2012. Anti-inflammatory and analgesic activities of the aqueous extract of *Leonotis leonurus* leaves in rats. Full Length Research Paper. African Journal of Biotechnology 11(26), 6878-6883.

Martins, M., Ordway, D., Kristiansen, M., Viveiros, M., Leandro, C., Molnar, J., Amaral, L., 2005. Inhibition of the *Carpobrotus edulis* methanol extract on the growth of phagocytosed multidrug-resistant *Mycobacterium tuberculosis* and methicillin-resistant *Staphyllococcus aureus.* Short report. Fitoterapia 76, 96-99.

McSweeney, C.S., Palmer, B., McNeill, D.M., Krause, D.O., 2001. Microbial interactions with tannins: nutritional consequences for ruminants. Animal Feed Science and Technology 91, 83-93.

Mnonopi, N., Levendal, R.-A., Davies-Coleman, M.T., Frost, C.L., 2011. The cardioprotective effects of marrubiin, a diterpenoid found in *Leonotis leonurus* extracts. Journal of Ethnopharmacology 138, 67-75.

Moolla, A., 2005. A phytochemical and pharmacological investigation of indigenous Agathosma species. MSc dissertation, University of the Witwatersrand.

Moolla, A., Van Vuuren, S.F., Van Zyl, R.L., Viljoen, A.M., 2007. Biological activity and toxicity profile of 17 *Agathosma* (Rutaceae) species. South African Journal of Botany 73, 588-592.

Mulyaningsih, S., Youns, M., El-Readi, M.Z., Ashour, M.L., Nibret, E., Sporer, F., Herrmann, F., Reichling, J., Wink, M., 2010. Biological activity of the essential oil of *Kadsura longipedunculata* (Schisandraceae) and its major components. Journal of Pharmacy and Pharmacology 62, 1037-1044.

Nakatsu, T., Lupo, A.T., Chinn, J.W., Kang, R.K.L., 2000. Biological activity of essential oils and their constituents. Studies in Natural Products Chemistry 21, 571-631.

Njoku, O.V., Obi, C., 2009. Phytochemical constituents of some selected medicinal plants. Full Length Research Paper. African Journal of Pure and Applied Chemistry 3(11), 228-233.

Ntutela, S., Smith, P., Matika, L., Mukinda, J., Arendse, H., Allie, N., Estes, D.M., Mabusela, W., Folb, P., Steyn, L., Johnson, Q., Folk, W.R., Syce, J., Jacobs, M., 2009. Efficacy of *Artemisia afra* phytotherapy in experimental tuberculosis. Tuberculosis 89(S1), S33-S40.

Ojewole, J.A.O., 2003. P-2: Hypotensive effect of *Leonotis leonurus* aqueous leaf extract in rats. American Journal of Hypertension 14, 40A.

Okwu, D.E., Okwu, M.E., 2004. Chemical composition of *spondias mombin linn* plant parts. J. Sustain. Agric. Environ., 62(2), 140-147.

Okwu, D.E., 2005. Phytochemicals, Vitamins and Mineral Contents of Two Nigerian Medicinal Plants. International Journal of Molecular Medicine and Advance Sciences 1(4), 375-381.

Okwu, D.E., Josiah, C., 2006. Evaluation of the Chemical Composition of two Nigerian medicinal plants. African Journal of Biotechnology 5(4), 357-361.

Olajuyigbe, O.O., Afolayan, A.J., 2012. Ethnobotanical survey of medicinal plants used in the treatment of gastrointestinal disorders in the Eastern Cape Province, South Africa. Full Length Research Paper. Journal of Medicinal Plants Research 6(18), 3415-3424.

Osbourne, A., 1996. Saponins and plant defence - a soap story. Trends in plant science 1(1), 4-9.

Pandey, I.P., Ahmed, S.F., Chhimwal, S., Pandey, S., 2012. Chemical Composition and Wound healing activity of Volatile oil of Leaves of *Azardirachta indica* A.juss. Advances in Pure and Applied Chemistry 1(3), 62-66.

Parekh, J., Jadeja, D., Chanda, S., 2005. Efficacy of Aqueous and Methanol Extracts of Some Medicinal Plants for Potential Antibacterial Activity. Turk J Biol 29, 203-210.

Parekh, J., Chanda, S.V., 2007. *In vitro* Antimicrobial Activity and Phytochemical Analysis of Some Indian Medicinal Plants. Turk J Biol. 31, 53-56.

Rauha, J.-P., Remes, S., Heinonen, M., Hopia, A., Kähkönen, M., Kujala, T., Pihlaja, K., Vuorela, H., Vuorela, P., 2000. Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. International Journal of Food Microbiology 56, 3-12.

Ross, J.A., Kasum, C.M., 2002. Dietary flavonoids: Bioavailability, Metabolic Effects, and Safety. Annu. Rev. Nutr 22, 19-34.

Sadraei, H., Asghari, G.R., Hajhashemi, V., Kolagar and Ebrahim M., 2001. Spasmolytic activity of essential oil and various extracts of *Ferula gummosa Boiss*. on ileum contractions. Phytomedicine 8(5), 370-376.

Scalbert, A., 1991. Antimicrobial properties of tannins. Review Article Number 63. Phytochemistry 30(12), 3875-3883.

Scott, G., Springfield, E.P., Coldrey, N., 2004. A Pharmacognostical Study of 26 South African Plant Species Used as Traditional Medicines. Pharmaceutical Biology 42(3), 186-213.

Seidel, V., Taylor, P.W., 2004. *In vitro* activity of extracts and constituents of *Pelargonium* against rapidly growing mycobacteria. International Journal of Antimicrobial Agents 23, 613-619.

Sidhu, G.S., Oakenfull, D.G., 1986. A mechanism for the hypocholesterolaemic activity of saponins. British Journal of Nutrition 55, 643-649.

Singh, H.P., Kaur, S., Megi, K., Kumari, S., Saini, V., Batish, D.R., Kohli, R.K., 2012. Assessment of *in vitro* antioxidant activity of essential oil of *Eucalyptus citriodora* (lemon-scented Eucalypt; Myrtaceae) and its major constituents. LWT-Food Science and Technology 48(2), 237-241.

Sparg, S.G., Light, M.E., van Staden, J., 2004. Biological activities and distribution of plant saponins. Review. Journal of Ethnopharmacology 94, 219-243.

Steenkamp, V., Mathivha, E., Gouws, M.C., van Rensburg, C.E.J., 2004. Studies on antibacterial, antioxidant and fibroblast growth stimulation of wound healing remedies from South Africa. Journal of Ethnopharmacology 95, 353-357.

Tambe, Y., Hitomi, T., Gisho, H., Yasumasa, I., Shigeo, T., 1996. Gastric Cytoprotection of the Non-Steroidal Anti-Inflammatory Sesquiterpene, β-Caryophyllene. Planta Medica 62, 469-470.

Ukoha, P.O., Cemaluk, E.A.C., Nnamdi, O.L., Madus, E.P., 2011. Tannins and other phytochemical of the *Samanaea saman* pods and their antimicrobial activities. Full Length Research Paper. African Journal of Pure and Applied Chemistry 5(8), 237-244.

Van der Watt, E., Pretorius, J.C., 2001. Purification and identification of active antibacterial components in *Carpobrotus edulis* L. Short communication. Journal of Ethnopharmacology 76, 87-91.

Vincken, J-.P., Heng, L., de Groot, A., Gruppen, H., 2007. Saponins, classification and occurrence in the plant kingdom. Review. Phytochemistry 68, 275-297.

Wang, W., Wu, N., Zu, Y.G., Fu, Y.J., 2008. Antioxidative activity of *Rosmarinus officianalis* L.essential oil compared to its main components. Food Chemistry 108(3), 1019-1022.

Wang, S., Melnyk, J.P., Tsao, R., Marcone, M,F., 2011. How natural dietary antioxidants in fruits, vegetables and legumes promote vascular health. Review. Food Research International 44, 14-22.

Wei, A., Shihamoto, T., 2007. Antioxidant Activities and Volatile Constituents of Various Essential Oils. J. Agric. Food Chem 55(5), 1737-1742.

Yao, L.H., Jiang, Y.M., Shi, J., Tomás-Barberán, F.A., Datta, N., Singanusong, R., Chen, S.S., 2004. Flavonoids in Food and Their Health Benefits. Plant Foods for Human Nutrition 59, 113-122.

Ziegler, J., Facchini, P.J., 2008. Alkaloids biosynthesis: metabolism and trafficking. Annu.Rev.Plant.Biol. 59, 735-769.

CHAPTER 6

SUMMARY AND RECOMMENDATIONS

Plants have been used since time immemorial to provide humans with food and medicine. The various ways that humans utilise plants are complex and dynamic (Heinrich et al., 2006). Phytochemical and pharmacological evaluation of plants has led to the elucidation of novel structures to manufacture new drugs (Geyid et al., 2005). Ethnobotanical surveys provide a platform to identify potential plants for further investigation as well as plants which could possibly be at risk of becoming extinct. As a result, documentation of medicinal plants is imperative.

Phytochemicals or secondary metabolites are present in plants and are formed during its normal metabolic processes (Okigbo et al., 2009). Plants possess a wide range of natural antioxidants due to the structural diversity of the secondary metabolites (Teffo et al., 2010). Phytochemical screening enables the identification of secondary metabolites present within the plants which could be beneficial or toxic to the body. Trace elements are also present within the plants. Awareness of the concentration of these trace elements is essential as it might influence pharmacological action or may be toxic to the body. Elemental analysis enables the identification of these elements and if possible, the association to the beneficial or toxic effects of plants.

Chapter 3 revealed that the survey identified 53 plant species belonging to 31 plant families used for medicinal purposes. Of these, only 21 were indigenous. Although more exotic plants are used by the community members compared to indigenous, the indigenous plant species are favoured for medicinal purposes. The plants used for medicinal purposes were justified by the literature with many of the plants having similar uses (Hutchings, 1996; van Wyk et al., 1997; van Wyk and Gericke, 2000; van Wyk and Wink, 2004; van Wyk et al., 2009). This lends credence to the folkloric use of these remedies. Most of the plants used belong to Lamiaceae, Asteraceae, Alliaceae, Apiaceae and Rosaceae. The most popular plants in terms of ranking belong to *Agathosma crenulata*, followed by *Artemisia afra* and *Helichrysum petiolare.* This is the first report of *Protea cyanaroides*, *Cissus rhombifolia*, *Canna* spp and *Dilatris viscosa* used for medicinal purposes.

Twelve plants were selected for elemental analysis (Chapter 4). A hydrogen peroxide: sulphuric acid digestion mixture and Unicam Solaar M Series Atomic Absorption Spectrometer (AA) were used to analyse the total element concentration present within the samples. Calcium (Ca), Chromium (Cr), Copper (Cu), Magnesium (Mg), Manganese (Mn), Sodium (Na), Phosphorous (P), Potassium (K), Selenium (Se) and Zinc (Zn) concentrations were evaluated within the plants.

Aloe arborescens contained the highest concentration of Ca, had the second highest concentration in Mg, Na and Zn and featured third in the Mn concentration. *Agathosma crenulata* contained the highest concentration of Cu and the second highest Mn concentration. *Artemisia afra* contained the highest concentration of Mn and Se. The concentration of Na and Mg was highest in *Carpobrotus edulis*. *C. edulis* also had the second highest concentration of Ca, P and K and the highest concentration of Zn. *Leonotis leonurus* (narrow leaf) had high concentrations of P and K and *Vernonia oligocephala* was high in Cr and Zn. Although the community reported *L. leonurus* (broad leaf) to be more effective than *L. leonurus* (narrow leaf), the elemental analysis revealed *L. leonurus* (narrow leaf) had higher concentrations in all the elements except P, which was lower, and Na which was higher.

Thin layer chromatography was carried out on twelve selected plants while the oils of *Agathosma crenulata*, *Salvia africana-caerulea* and *Pelargonium capitatum* were assessed by gas chromatography coupled to mass spectroscopy. *Asparagus capensis*, *Cliffortia odorata* and *Vernonia oligocephala* were evaluated for the first time.

Flavonoids and phenols were detected in all the plants with anthraquinones present in all the plants except *Carpobrotus edulis*, *Leonotis leonurus* (narrow and thin leaf) and *Pelargonium reniforme*. Saponins were present in all plants except *A. crenulata*, *A. arborescens* and *Vernonia oligocephala*. The presence of essential oils was evident in all plants except *C. edulis*, *C. odorata*, *A. capensis* and *A. arborescens*. Glycosides were evident in all plants except *S. africana-caerulea*, *L. leonurus* (both narrow and broad), *C. edulis*, *A. capensis* and *A. crenulata*. Secondary metabolites present in *Artemisia afra*, *L. leonurus* and *S. africana-caerulea* were the same as a previous study carried out (Scott et al., 2004).

Interesting observations were noted for plant species evaluated by GC-MS. The percentage of predominant compounds present in the oils of *A. crenulata*, *S. africana-caerulea* and *P.capitatum* were much higher compared to previous studies (Moolla, 2005; Kamatou, 2006;

Demarne et al., 1993). This could be directly associated to geographical locations and the collection season.

6.1 Recommendations

Plant combinations as well as different plant parts are frequently used by communities to achieve the most effective remedy. However, concerns eminent in the survey were a lack of documentation of plant combinations. At present, not many studies are available evaluating the presence of elements within the plants. Compared to phytochemical screening, elemental analysis lags behind. This is detrimental to phytomedicine as it would be beneficial to scientifically evaluate whether a relationship exists between elements present in plants and its biological activities. Therefore an effort should be made to investigate the elemental analysis of plants as well as the secondary metabolites present within the plants.

REFERENCES

Demarne, F.-E., Viljoen, A.M., Van der Walt, J.J.M., 1993. A Study of the Variation in the Essential Oil and Morphology of *Pelargonium capitatum* (L.) L'Hérit. (Geraniaceae). Part 1. The composition of the Oil. The Journal of Essential Oil Research 5(5), 493-499.

Geyid, A., Abebe, D., Debella, A., Makonnen, Z., Aberra, F., Teka, F., Kebede, T., Urga, K., Yersaw, K., Biza, T., Haile Mariam, B., Guta, M., 2005. Screening of some medicinal plants of Ethiopia for their anti-microbial properties and chemical profiles. Journal of Ethnopharmacology 97, 421-427.

Heinrich, M., Kufer, J., Leonti, M., Pardo-de-Santayana, M., 2006. Ethnobotany and ethnopharmacology-Interdisciplinary links with the historical sciences. Commentary. Journal of Ethnopharmacology 107, 157-160.

Hutchings, A., Scott, A.H., Lewis, G., Cunningham, A.B., 1996. Zulu medicinal plants. An inventory. University of Natal Press, Pietermaritzburg.

Kamatou G.P.P., 2006. Indigenous Salvia Species − An investigation of their pharmacological activities and phytochemistry. Ph.D Dissertation, University of the Witwatersrand.

Moolla, A., 2005. A phytochemical and pharmacological investigation of indigenous *Agathosma* species. M.Sc Dissertation, University of the Witwatersrand.

Okigbo, R.N., Anuagasi, C.L., Amadi, J.E., 2009. Advances in selected medicinal and aromatic plants indigenous to Africa. Full Length Research Paper. Journal of Medicinal Plants Research 3(2), 086-095.

Teffo, L.S., Aderogba, M.A., Eloff, J.N., 2010. Antibacterial and antioxidant activities of four kaempferol methyl ethers isolated from *Dodonaea viscosa* Jacq. Var. *angustifolia* leaf exracts. South African Journal of Botany 76, 25-29.

Van Wyk, B.-E., Van Oudtshoorn, B., Gericke, N., 1997. Medicinal plants of South Africa. Briza Publications, Pretoria.

Van Wyk, B.E., Gericke, N., 2000. People's plants: A guide to useful plants of South Africa. Briza Publications, Pretoria.

Van Wyk, B.-E., Wink, M., 2004. Medicinal plants of the world. Briza Publications, Pretoria.

Van Wyk, B.E., Van Oudtshoorn, B., Gericke, N., 2009. Medicinal plants of South Africa. Briza Publications, Pretoria.

APPENDIX A

DWARSRIVIER QUESTIONNAIRE

NAME:

CONTACT NUMBER:

1. When someone is ill, what action is taken to deal with the illness/disease?

a) Attend a modern health centre, private clinic etc.

b) Self-medication with western medicine

c) Self-medication with herbal medication

d) Consult traditional healers

e) Others (Please specify)

f) No action taken against illness

2. Where did you obtain your knowledge regarding herbal medicine?

3. What role in the family or community did this individual have (mother, father etc)?

4. Which are the most widely used herbs?

5. What symptoms are they often used for?

6. What are the common symptoms/ailments in your community?

____
____
____

7. Where do you obtain the herbs (garden, shop etc)?

____
____
____

8. If these herbs are not available, which alternatives or options do you use?

____
____
____
____

9. Are there any limitations of the herbs you may take from certain areas?

____
____
____

10. How long have you been using herbal medications in your family?

________________Months

________________Years

11. Who in your family utilises the herbs? Children?

____
____
____

12 What part of the plant do you use? Why?

____
____
____
____

13. How do you prepare the plant for medicinal purposes (tea, alcohol, poultice etc)?

____
____
____

14. How much of the plant mixture do you consume?

15. How should the plant preparations be taken? What is the dosage?

16. At what stage of illness should the medicinal preparations be taken? How long should it be taken for?

17. Have you experienced any side effects? (Vomiting, headaches, nausea?)

18. Has any toxicity been reported?

19. Can any of the herbs be combined? If so, please name the combinations.

20. Are these herbs safe to use during pregnancy?

21 Can people on high blood pressure medication and on blood thinning medication use these herbal preparations?

22. Can the herbs be stored for later use? If so, how long can it be stored for and are there any specific storage conditions that need to be adhered to? (dried, extracted in alcohol) If not, why?

APPENDIX B

List of the informants who contributed to the survey in Chapter 3.

Mr Lotter Cyster

Mrs Julia Cyster

Aunty Georgina Cyster

Mr Terry Williams

Aunty Sally

Mrs Mabel Isaacs

Mrs Sarah Stubbs

Mr Bezuidenhout

Mrs Caroline Williams

Aunty Rita

Mrs Solomon

Mr Goshai

Mrs Joanna Patience

Mrs Anne Mentoor

Aunty Steyn

Aunty Evelyn

Mr Charls Natus

Aunty Maudie

Mrs Adams

Mr Authur Williams

Mr Brand

Aunty Rosie

Mr Valte Arnoldes

Mrs Mina Arnoldes

Aunty Dorothy

Aunt Rosie

Mrs Linda Fortuin

Mr Bernard Mentoor

Mrs Hazel Hendricks

APPENDIX C

Referencing instructions to authors wanting to publish in the Journal of Ethnopharmacology

Downloaded from:

www.journals.elsevier.com/journal-of-ethnopharmacology

Click on the “Guides for authors” link.

Reference style

Text: All citations in the text should refer to:

1. Single author - the author's name (without initials, unless there is ambiguity) and the year of publication.

2. Two authors - both authors' names and the year of publication.

3. Three or more authors - first author's name followed by "et al." and the year of publication.

Citations may be made directly (or parenthetically). Groups of references should be listed first alphabetically, then chronologically.

Examples: "as demonstrated (Allan, 1996a, 1996b, 1999; Allan and Jones, 1995). Kramer et al. (2000) have recently shown ...."

List: References should be arranged first alphabetically and then further sorted chronologically if necessary. More than one reference from the same author(s) in the same year must be identified by the letters “a”, “b”, “c”, etc., placed after the year of publication. Please use full journal names.

Examples:

Reference to a journal publication

Van der Geer, J., Hanraads, J.A.J., Lupton, R.A., 2000. The art of writing a scientific article. Journal of Scientific Communication. 163, 51-59.

Reference to a book

Strunk Jr., W., White, E.B., 1979. The Elements of Style, third ed. Macmillan, New York.

Reference to a chapter in an edited book

Mettam, G.R., Adams, L.B., 1999. How to prepare an electronic version of your article, In: Jones, B.S., Smith , R.Z. (Eds.), Introduction to the Electronic Age. E-Publishing Inc., New York, pp. 281-304.