AN INVESTIGATION OF THE PLANTS USED MEDICINALLY IN SELF-CARE IN THE BREDASDORP / ELIM REGION

TAMSYN SHARON ALYSON THRING

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

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Supervisor: Mr. F.M. Weitz
(Department of Biodiversity and Conservation Biology)

Co-Supervisor: Prof. Q. Johnson
(Department of Medical Biosciences)

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Tamsyn S. A. Thring

KEYWORDS

South Africa

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Bulbine lagopus

Chironia baccifera

Conyza scabrida

Dodonaea angustifolia

Disc-diffusion bioassay

Minimum Inhibitory Concentration

Thin Layer Chromatography

Bioautography

ABSTRACT

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T.S.A. Thring

Magister Scientiae [MSc] Thesis, Department of Biodiversity and Conservation Biology, Faculty of Natural Sciences, University of the Western Cape

Much of the traditional medicinal plant knowledge is to a large extent known by the older generations. This knowledge is at risk of disappearing due to not being passed down to younger members of the respective families and communities. The Bredasdorp / Elim area in the Southern Overberg has many individuals who possess such knowledge. The aims of this study were to identify what plants were in use in the area, to document this knowledge and to choose certain plants to test in antimicrobial bioassays.

Individuals who had knowledge of using plants in medicines were identified and approached and were found to be willing to participate. Over 40 individuals were interviewed to find out the uses, preparations and dosages of the plants mentioned. The information was gathered using semi-structured and structured questionnaires to yield 36 plant species belonging to 19 families being used. Among these plants were

many plants which are commonly used around South Africa. The families with the largest number of species were found to be the Asteraceae, Lamiaceae, Alliaceae and the Solanaceae. Using a structured questionnaire where only 15 knowledgeable people participated, it was found that the most popular plants were found to be *Artemisia afra* and *Ruta graveolens*. Here the results were determined using preference ranking methods and calculating the use-values of each plant. The knowledge was collected from elderly people and mostly from women.

Plant material was then collected for four species which are in use in the area for testing in antimicrobial bioassays. These species were chosen because not much appears to be known about the plants in the literature regarding their antimicrobial activity. The plants chosen were: *Bulbine lagopus*, *Chironia baccifera*, *Conyza scabrida* and *Dodonaea angustifolia*. These plants were extracted in ethanol, ethyl-acetate, methanol and water and tested against the following microorganisms: *Candida albicans* (yeast), *Mycobacterium smegmatis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The disc-diffusion bioassay and the liquid dilution assay were used to test all four plant species for antimicrobial activity. The liquid dilution assay was found to be more sensitive and thus showed better antimicrobial results. The best activity was found in the methanol and ethyl acetate extracts overall. *C. scabrida* and *D. angustifolia* both showed good activity against *M. smegmatis*. Due to both of these plants being used to treat symptoms of tuberculosis such as coughs and fevers it was decided to investigate the ethanol, methanol and ethyl-acetate extracts of these plants further against this bacterium. This was done using thin layer chromatography (TLC) to achieve separation of the extracts followed by inoculating the TLC plates with agar containing the bacterium. After incubation for 48 hours the plates were removed and the overlay sprayed with 0.2mg/ml 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). The zones of inhibition could then be observed. All three extracts for each plant showed at least two zones of inhibition against *M. smegmatis*. From this it appears that these plants are potential candidates for further testing against *M. tuberculosis*. This study shows that ethnobotanical surveys can be useful in finding plants which have antimicrobial activity as well as being able to justify the uses of certain plants in their treatment of various conditions.

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DECLARATION

I declare that *An investigation of the plants used medicinally in self-care in the Bredasdorp / Elim region* 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 quoted, have been acknowledged and indicated by means of complete references.

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CONTENTS

LIST OF TABLES AND FIGURES

CHAPTER 1

INTRODUCTION

1.1. Background

Southern Africa has over 30 000 higher plant species out of which approximately 3000 species are used in traditional medicine. The Cape alone has nearly 9000 species and is said to have the most diverse temperate flora on earth (van Wyk et al., 1997). With such a rich biodiversity as well as a rich cultural heritage it is not surprising that there are many users of traditional medicine (Mander, 1998). It is estimated that there are 27 million indigenous medicine consumers in South Africa and with respect to the demand for popular plant species, the demand exceeds supply. With so many consumers and a decline in the availability of certain plants there will be a large effect on consumers and the supporting industries of traditional medicine in the future (Mander, 1998).

It is estimated that between 70% and 80% of black people in South Africa use traditional medicine and consult with traditional doctors (Donaldson and Scott, 1994). This applies mainly to rural areas or areas which are far way from modern health care facilities (Veale et al., 1992). Western allopathic medicine, Western herbalism, homeopathy as well as Ayurvedic medicine from India and Chinese medicine are also practiced in South Africa (van Wyk and Gericke, 2000).

Indigenous plants provide the basis for traditional medicine and are generally obtained from wild stocks around South Africa. Due to the high demand for these plants and a lack of notable management of these resources, there is a decline of many species of indigenous medicinal plants (Mander, 1998). In Kwa-Zulu Natal alone, over 4000 tonnes of plant material are traded within a year. This amounts to an estimated value of US$ 13 million. In South Africa as a whole, approximately 20 000 tonnes of plant material may be sold in a year amounting to about US$ 60 million (Mander, 1998). With such a demand, the incessant harvesting of wild plants poses a threat to biodiversity, particularly to the 700 plant species that are traded actively in South Africa (Mander, 1998). A suggestion was made to cultivate indigenous medicinal plants for marketing due to the decreasing supply of medicinal plants and localized extinctions which have occurred (Mander, 1998). There was little response to this suggestion due to lack of knowledge of the economics of producing indigenous plants and the associated markets (Mander, 1998).

There are said to be approximately 200 000 indigenous traditional healers in South Africa (van Wyk et al., 1997). These are known as "inyanga" and "isangoma" (Zulu, plural: "izinyanga" and "izangoma"), "ixwele" and "amaquira" (Xhosa), "nqaka" (Sotho) and in the Western and Northern Cape, "bossiedokter" and "kruiedoktor" (van Wyk et al., 1997). Herbalists and diviners are often used to describe the terms "izinyanga" and "izangoma" which are said to be "spiritually empowered" along with other spirit mediums, intuitives and spiritual healers (van Wyk et al., 1997). Many of the older generation have an extensive herbal knowledge and many of their own herbal remedies (van Wyk et al., 1997).

Plants were once the main source of all medicines and still many drugs are made from natural products. Some of the most important drugs used in medicine today are derived from plants, for example; taxol (*Taxus brevifolius*) and vincristine (*Catharanthus roseus*) which are used in chemotherapeutic drugs (van Wyk et al., 1997). *Aloe ferox* (Cape aloe) and *Harpagophytum procumbens* (Devil's claw) are examples of South African plants which are used worldwide in medicines (van Wyk et al., 1997). Discovering useful compounds from plants involves many disciplines; ethnobotany, the study of plant use by various cultures; ethnomedicine, how these herbs are used therapeutically; and ethnopharmacology where the active ingredients are studied with regard to their chemistry and toxicity (Huxtable, 1992). The ethnobotanical approach to drug discovery is therefore a viable start for pharmaceutical research due to many drugs being discovered through the study of indigenous knowledge systems (Huxtable, 1992; van Wyk et al., 1997). It is therefore important that plant biodiversity is conserved in order to discover what active compounds may be present in unexamined plants. It has been said that extinctions of species may result in the loss of a potentially significant compound. *Ginkgo biloba* has been cited as an example because it is an ancient plant that apparently was saved from extinction by human interference. This tree contains gingkolides which have the potential to treat cerebral ischemia (Huxtable, 1992). It is suggested that more investigations should be launched in the above mentioned fields as this is an efficient way for discovering new drug activities (Huxtable, 1992).

In recent years traditional medicine has been declining all over the world and this may lead to much valuable information about healing properties of many plants being lost (Harsha et al., 2002). However, there is an increasing interest in looking at traditional medicine systems and trying to evaluate certain plants and to justify their uses. Due to western drugs being expensive or not readily available as well as new resistant strains of harmful microbes emerging, there is a need to discover new treatments and compounds with novel mechanisms of fighting diseases (Rojas et al., 2003). Higher plants produce a wide range (hundreds and thousands) of compounds which have varying biological activity. These compounds are thought to play an important role in attracting pollinators and in defending the plant with chemicals against insect, animal and even microbial attack (Rojas et al,. 2003). Antimicrobial activity has been found in plant species and many drugs have been derived from plant species.

In South Africa there is a lack of detailed information regarding the uses of plants in traditional medicine (van Wyk et al., 1997). Due to what is described as rapid urbanisation and the degeneration of cultural heritage, knowledge that may traditionally be passed on from generation to generation could be lost (van Wyk et al., 1997). It is therefore important to learn as much as possible so as to try and document knowledge so that it may provide missing links both ethnobotanically as well as culturally. In doing a survey such as this, the status of the availability of certain plant species must be determined in order to see if these species are being exploited. If a plant is found to be under threat then measures can be taken in order to begin to conserve it. There may be different uses for plants nationwide. For example, one group may use this plant as an anti-bacterial agent while another group may have found it more effective in the treatment of intestinal parasites. This could lead to the isolation of new active compounds which could one day be present in a life saving drug. Another reason for carrying out this study is to try and find a way of benefiting the people of that region in return for their input. An example of this could be helping the local traditional medicine practitioners to cultivate a certain plant species which may be extremely hard to find or too expensive to buy.

1.2. The study area

The Bredasdorp / Elim area is situated in the Southern Overberg, in the Cape Floristic Region. The Southern Overberg has a rich flora of about 2500 species. Approximately 300 species are endemic (restricted to the area) and 32 species appear in the Red Data Book (Mustart et al., 1997). The Bredasdorp Formation limestones are associated with an endemic rich vegetation. The soils are alkaline and organic rich. The plants growing on this terrain are known as limestone proteoid fynbos. Bredasdorp and surrounding areas are made up of three vegetation types: limestone proteoid fynbos , restioid fynbos and neutral sand proteoid fynbos. In seasonally waterlogged sands restioid plants such as *Chondropetalum tectorum* are found. This is known as wet restioid fynbos where woody plants cannot live due to annoxic and wet conditions. Dry restioid fynbos is found in well-drained sands where these shallow rooted plants thrive because they are able to absorb most of the moisture in the sands leaving little for deeper rooted plants. Neutral sand proteoid fynbos is found in the bases of limestone outcrops, where calcium has been leached out of the limestone-derived soils. A typical example of a plant found growing in this type of environment is *Protea susannae*. Geophytes such as *Lachenalia bulbifera* and *Bobartia longicyma* are found here (Mustart et al., 1997). With such a wide range of plants available in the area, particularly endemic species, it would seem that there is the potential to uncover some new plants being used. Also, Smith (1966), documents some plant species which have been utilized in medicines in this area and these plants, and others, may still be in use and these uses are worth documenting.

When studying the uses of traditional medicines within a community it is necessary to ask permission to carry out the study from the relevant authorities. Establishing trust from the local people who are willing to partake in such a study is also vital. It was suggested that in the initial interviews that no written notes be taken so as not to arouse suspicion or resentment (Lipp, 1989). Selecting informants is also an important step, often it is the older generation, as well as the traditional healers, who have first hand experience of using plants for healing (Lipp, 1989; Malamas and Marselos, 1992). Mission hospitals have also yielded information regarding the use of indigenous plants in medicines (Fourie et al., 1992). The next step is to compile an inventory followed by botanical identification of the plants used (Hedberg, 1993). In a survey of medicinal plants performed in Greece, information was gathered from 35 interviews with local individuals, particularly elderly people. In the interviews, recipes, local names, descriptions and samples of herbs as well as personal experiences of the users were all discovered (Malamas and Marselos, 1992). Voucher specimens were collected and identified and then an inventory could be compiled (Malamas and Marselos, 1992). It is then possible to consult previous studies and literature so that comparisons may be made to see which plants are still used in remedies (Hedberg, 1993). Following this, several other steps may follow: the screening of plant extracts, the isolation and identification of any active compounds discovered, pharmacological and toxicological studies, clinical testing and finally, the production of a drug (Hedberg, 1993).

All of the above was taken into account when surveying the traditional plant use in the Bredasdorp and Elim regions. Informants were carefully selected and approached and ultimately treated with due respect. Sitting and talking to the people at first helped to establish a rapport before specific questions were asked. Then questionnaires were devised to document the relevant information of the plants mentioned. As large a sample as possible was used to ensure the data is representative and all information acquired has been compared with the available literature. This data is presented in Chapter 3 of this thesis along with analysis to find out which plants are most popular and which are best to treat specific ailments using methods of data representation by Phillips and Gentry (1993a,b).

From the results of the survey, four plant species were chosen; *Bulbine lagopus, Chironia baccifera*, *Conyza scabrida* and *Dodonaea angustifolia*. These plants were chosen because little is known about their antimicrobial activity and these plants are easily accessed in this area. The plants were collected from the study area and extracted in water, ethanol, ethyl acetate and methanol and then tested against four available microbial species: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Mycobacterium smegmatis* and *Candida albicans*. The extracts were tested *in vitro* in the disc diffusion (Salie et al., 1996) and liquid dilution assays (Eloff, 1998). *C. scabrida* and *D. angustifolia* were used in a thin layer chromatography bioassay where *M. smegmatis* was overlaid in agar to establish if there were any antimicrobial compounds present. This part of the study comprises Chapter 4 of this thesis.

1.3. Aims of this study

Due to plants still being used medicinally in self-care in the Bredasdorp / Elim region, the aims of this study were:

- discover which plants are used in this area for medicinal purposes
- provide an inventory of these plants and how they are utilized
- test certain plant extracts in antimicrobial bioassays in order to possibly discover new antimicrobial compounds

Achieving these aims would help to answer the research question which asks: Are traditional knowledge systems declining in the Bredasdorp area, and could this impede the discovery of important links in the quest for new antimicrobial drugs from plant sources? By answering this question the hypothesis, that traditional knowledge is declining due to not being passed down from generation to generation in the Bredasdorp region, and the many plant species that are used medicinally contain potential compounds that may be effective against harmful microbes, can be investigated.

1.4. Thesis outline

This thesis will take the following direction: The literature review, (Chapter 2), will encompass examples of previous surveys from the literature. Evidence to support the use of medicinal plants as potential antimicrobial candidates will then be presented as well as looking at intellectual property rights. The next chapter (Chapter 3) will then focus on the survey in the Bredasdorp / Elim area and divulge results from the interviews with the community. Chapter 4 consists of the antimicrobial experiments as discussed previously. Finally, the discussion (Chapter 5), will focus on the results of the survey and the bioassays and confirm the hypothesis. The referencing format for this thesis is based on the Journal of Ethnopharmacology (please refer to Appendix D for referencing guidelines from this journal).

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CHAPTER 2

LITERATURE REVIEW

Plants are still widely utilized as medicines around the world in traditional cultures to treat a variety of illnesses and symptoms. Hence traditional medicine is interlinked with the availability of many plant species as well as the knowledge of how to use them. Both plant species and the knowledge are threatened. Habitat changes or habitat destruction affects plant populations as can the over-harvesting or exploitation of these species. The knowledge of these plants is then affected due to the resulting loss of diversity along with modern-day issues such as modernization, economics and urbanization (Tabuti et al., 2003). However, plant based medicines and medicinal plants are also becoming increasingly accepted as alternatives to synthetic drugs and natural remedies are becoming more commercialized (van Wyk and Wink, 2004). With medicinal plants being more widely researched, there is good reason to conserve plant biodiversity which in turn will aid in the conservation of traditional knowledge. Also, as mentioned in the introduction, microbial species are becoming more resistant to antibiotics and many people die from nosocomial infections each year (Gnanamani et al., 2003).

This literature review aims to look at some well-known plant derived drugs and how they were discovered. Then to look at surveys which have been performed around the world followed by instances where plants chosen from their uses in traditional medicine have been found to have antimicrobial activity. Thus showing that the ethnobotanical approach is a good starting point to discovering new anti-infectives. The review will also demonstrate the importance of gathering information about medicinal plants before this information is lost. This evidence will support the basis for the research question and the hypothesis.

2.1. The "ethnobotanical approach to drug discovery"

Compounds based on, or derived from plant natural products are present in at least 25% of prescription drugs in the USA and Canada (Huxtable, 1992; Cox, 1994) and 30% including prescription drugs in western medical systems in the world (Scott, 1993). However, it is estimated that out of 250 000 higher plant species in the world only 5-15% have been investigated for medicinal value (Rojas et al., 2003). Many of the drugs in current use were discovered from ethnobotanical leads (Cox, 1994). These leads have resulted in three types of drug discovery according to Cox (1994); the first being unmodified natural plant drugs such as digoxin where ethnomedical use suggests clinical effectiveness. Secondly, unmodified natural products discovered from traditional plant use information which only vaguely suggested any therapeutic value (e.g. vincristine). And finally, instances where natural products from plants used in traditional medicine have been changed or made synthetically for the required use (e.g. aspirin).

Two very important drugs, digoxin and proscillaridin, which are widely used today have been derived from ethnobotanical leads. Digoxin was discovered in 1775 by William Withering an English physician. He was told by a folk healer about the leaves of the foxglove, *Digitalis purpurea*, being useful in the treatment of dropsy.

This condition caused by inadequate pumping of the heart results in swelling of the body. When Withering was treating some of his dropsy patients with the leaves, he found out that this plant had a powerful cardiotonic effect. Since Withering's discovery, more than 30 cardiac glycosides have been isolated from this plant (Cox, 1994). Similarly, Gerard in 1597, mentioned that the sea squill, *Drimia maritima*, had possible pharmacological activity due to being used in folk medicine to treat dropsy. *D. maritima* has since been found to contain a drug called proscillaridin which is a cardiotonic drug (Gerard, 1597 cited by Cox, 1994). Some uses of plant medicines have been recorded even earlier than the two previously mentioned plants. One such plant (a species of which is used in the survey in Chapter 3) is *Artemisia absinthium*. The use of this plant has been recorded in Chinese pharmaceutical books dating to roughly A.D. 340 as being a principal ingredient of a malaria remedy. Extracts from this plant were tested in the 1960s and early 1970s and this plant was shown to have good antimalarial activity (Swerdlow, 2000).

Therefore, it does appear that there is a good chance of finding potential candidates for drugs to treat many diseases from plants used in traditional or folk medicine. This has been seen in the search for anticancer activity where in one survey, 10% of the randomly screened plants showed positive responses. When plants found to be used medicinally were screened, it was found that 20% of the plants used to treat cancers, 29% of plants used to treat worms, 39% and 52% of plants used as fish poisons and arrow poisons respectively showed positive responses (Spjut and Perdue, 1976 cited by Huxtable, 1992). There are over 50 drugs used today in medicine which were derived from ethnobotanical leads. These include atropine, used as a pupil dilator, derived from *Atropa belladonna*; codeine, an analgesic and antitussive and morphine, an analgesic, from *Papaver somniferum*; and vinblastine (Hodgkin's disease) and vincristine (paediatric leukemia), isolated from *Catharanthus roseus* (Cox, 1994).

2.2. Ethnobotanical surveys around the world

Surveys have been performed in many different countries of the world including Greece, India, México, Ethiopia, Uganda, Swaziland and South Africa. These surveys looked at which plant species were used to treat many different ailments. Some of these surveys have also led to the preliminary pharmacological testing of certain plants resulting in bioactivity being found thus justifying the uses of certain plants in the treatment of the various complaints they are used to treat.

In one ethnobotanical study performed in Greece, local people were interviewed to discover what plants were still in use. Over 1100 species of plants are found in this region in north western Greece. This study was performed because many of the local people still use plants in self-care and these people were interviewed to find out more about the reported plant use. Mostly elderly people were interviewed because they form the majority of the people who still use plants. Thirty-five interviews were performed in total to yield 36 species of plants in use in the area and nine of the plant species in use were found to be species that had not previously been mentioned in literature from that region (Malamas and Marselos, 1992). One of the interesting outcomes is the use of *Hypericum perforatum* (St. Johns's Wort) in a preparation which is used to treat the viral infection H*erpes zoster* to relieve the pain. This plant has also been thought to have antiviral properties that may affect the human immunodeficiency virus as well as the herpes virus. The preparation, where the flowers are placed in olive oil in the sun for a week until the flowers disintegrate, is applied to the affected area (Malamas and Marselos, 1992). This preparation could be useful in finding antiviral activity and, if not for this survey, could have been lost if the "recipe" was not recorded or passed on by the person or people who use it.

In India the traditional knowledge system is declining due to tribal exploitation by modern society and often being forbidden to use the forest or natural resources they have been using for years. There is therefore a need to record information regarding plant use among the many communities before this information is lost (Katewa et al., 2004). The decline of practicing herbal medicine may also lead to valuable information never being discovered (Harsha et al., 2002). Folk herbal medicines have been recorded from the largest state of India, Rajastan (Katewa et al., 2004). The vegetation in this area has a wide biodiversity including subtropical forest which has contributed to the culture of the local tribal people and supplies them with a source of medicinal plants. Most of this knowledge about plant use is passed down orally through folk lore and is often kept secret. It was found to be known by people over the age of 60 years old and was evenly spread between men and women. From these interviews, interesting uses of plants not mentioned in the literature were found among the 61 plants belonging to 38 families in use in the area. Thirty-eight of these plants were used to treat skin diseases and related conditions, 14 were used for digestive disorders and the rest for other conditions including respiratory ailments and diabetes (Katewa et al., 2004).

Harsha et al. (2002) examined the ethnomedical knowledge of the Kunabi tribe of a district in India called Karnataka. This district also has a large biodiversity and it was found that 45 plant species belonging to 26 families are currently in use in herbal medicines by the Kunabi people. The data was collected via many interviews and discussions and 24 types of conditions were found to be treated. Due to these plants being largely accepted as effective remedies by the people in the tribe, these plants deserve further testing and investigation to see if their uses are justified in traditional remedies (Harsha et al., 2002). All the plants were listed in both the above surveys with their mode of preparation and dosage thus preserving some of this knowledge for future generations.

In one district of México, 119 people were interviewed to find out about plants specifically used in the treatment of gastrointestinal infections. Out of the 119 interviewed, 88 people stated that they relied on plant based medicines to treat these stomach conditions. It was found here that the majority of the information was known by older people between the ages of 60 and 90 years of age and by wives and mothers. Informal interviews, followed by semi structured interviews were used to gather the information and a wide range of people were interviewed such as housewives, farmers and healers. 44 plant species from 25 families were found to be in use here (Hernández et al., 2003).

Conducting ethnobotanical surveys can also be of benefit when examining cultural heritage. In a study in Mexico, two Macro-Mayan ethnic groups (Lowland Mixe and Zoque-Popoluca communities) were considered in two independent ethnobotanical studies (Heinrich et al, 1992; Leonti et al, 2001; Leonti, et al., 2003). Direct contact between the people in each community is very rare even with the advances in road infrastructure and telecommunications and so the data from each study was compared to see if there were any plants and their uses in common. It was found that out of 123 shared plant species 62 species had at least one common use which could exclude pure coincidence (Leonti et al., 2003). These results indicate a possible common cultural heritage as well as suggest common selection criteria. This particular study identified a small group of medicinal plants thought to belong to a "proto-Mixe/Zoque" culture. These types of studies combined with linguistic evidence can help to verify the antiquity of medicinal plant usage in particular regions (Leonti et al, 2003).

2.3. Ethnobotanical surveys in Africa

In Africa many people still rely on traditional medicine and traditional healers, however, self-care using plants is often found. Mothers were found in an Ethiopian study to be the healers of the family, treating injuries and ailments with available plants (Gedif and Hahn, 2003). In this instance, mothers were used as informants to attempt to document the plants used in self-care in a rural community. Here, 25 plant species belonging to 21 families were used. It was found that 1/3 of the plants used in the study were plants which are often used in food and/or spices as well as being available in Ethiopian markets. The most plants in use belong to the families Lamiaceae and Solanaceae. Herbal medicine is favored here because of its perceived efficacy, accessibility as well as being a cheaper alternative to modern medicine. There is good reason to investigate frequently used plants in pharmacological tests (Gedif and Hahn, 2003) because these plants must provide some degree of relief for the condition they are used to treat otherwise the plant would not be used so often.

In Uganda, where traditional medicine is still relied upon, it was found by Tabuti et al. (2003) that it was necessary to perform pilot studies to identify key informants. From this, 23 key informants were interviewed using both open- and closed-ended questionnaires. It was found that informants were more likely to share their knowledge in the presence of someone they knew or trusted and so research assistants were hired that had grown up in the area. This combined with establishing rapport with the people helped to record a total of 229 medicinal plants belonging to 68 families with the largest number of plants belonging to the families Fabaceae, Euphorbiaceae, Asteraceae and Solanaceae. The families Rubiaceae, Asteraceae and Euphorbiaceae were also found to contain many plants which are used medicinally by traditional medicine practitioners in a study in Swaziland (Amusan et al., 2002). Here, 41 herbal remedies were found to be used for 25 illnesses. The remedies comprised of 47 plant species from 32 families and some of these have not been described before in the Swaziland flora. It was found that scientific activities of some of the plants and plant families did not explain the activities reported by the people and so more research is needed to discover whether these plants can be warranted in their uses (Amusan et al., 2002).

2.3.1 Ethnobotany in South Africa

In South Africa, there is still a need to perform surveys regarding the details of plants used medicinally due to the "fragility of oral-tradition knowledge" along with urbanization and acculturation (van Wyk et al., 1997). Recording of African medicinal plants began early in the Cape. In 1850 the book "Florae Capensis Medicae Prodromus" by Pappe (Liengme, 1983) was published which comprised a collection of the notes of the uses of medicinal plants recorded in the early 19th century such as Burchell (1822), Kay (1833) and other travelers. Liengme (1983) also reported that in 1888, Smith wrote a book on the medicinal plants of the Eastern Cape. In the 20th century, Watt and Breyer-Brandwijk (1962) contributed to the knowledge on medicinal plants in Southern and Eastern Africa in their book which describes, in detail, many plants, their uses in traditional medicine and the chemical compositions of some of these plants. Traditional medicinal plants used by the Zulu, Sotho and Xhosa people have been recorded (Hutchings, 1989). In this survey, 794 plant species with specific uses were recorded. Hutchings et al. (1996) later investigated the plants utilized by the Zulu people and a survey was performed on 1032 species and an inventory compiled listing the uses and preparations of these plants. These plants were listed using both literature sources as well as interviews with traditional healers and individuals using plants (Hutchings et al., 1996).

In South Africa, market surveys have also been performed due to the large numbers of plants which are sold in the medicine trade. Mander (1998) conducted a large study in KwaZulu-Natal looking at the marketing of medicinal plants. Here, key traders, gatherers, indigenous healers and patients were among the people interviewed. This survey listed the plants most frequently used and it was estimated that the value of the trade in raw products was approximately US$13 million a year. This amount equates to almost one third of the value of the annual maize harvest in KwaZulu-Natal. On average, 4% to 8% of household's annual incomes were spent on indigenous medicine (Mander, 1998). Similar market studies have been done in the Witwatersrand region of South Africa to determine which plants were being sold and where these plants had come from (Williams, 1996).

Another reason for studying plants used in traditional medicine is that some of these plants are toxic and can account for the deaths of individuals particularly children (Bye and Dutton, 1991). In KwaZulu-Natal, herbalists are supposed to register with the Nyanga National Association under Zulu Law, Act 6 of 1981 (Bye and Dutton, 1991). However the registration seems to be unsuccessful (Cunningham, 1988). So there is the risk of being treated by an unqualified person which can lead to deaths related to inappropriate plant use. Suspected herbal-related deaths (often infants) have affected the communications between doctors, patients and traditional healers and caused friction between western doctors and healers (Bye and Dutton, 1991). One of the dominant themes in the culture of black South Africans is that of fertility and thus there are many plants which are used in pregnancy and childbirth. Many of these plants that are used are, in fact, toxic and studies that look into these plants and their effects on pregnant women and the foetus should be encouraged (Veale et al., 1992). Veale et al. (1992) revealed in a study which focused on plants used during pregnancy and childbirth that 16 plants out of 57 used were reported to be toxic.

2.4. Medicinal plants as sources of antimicrobial compounds

Tuberculosis (TB) is becoming a serious threat worldwide. TB is an ancient disease and evidence of spinal TB has been found in fossil bones dating from around 8 000BC (Newton et al., 2002). TB remains to be the largest cause in deaths around the world particularly in developing countries and currently one third of the world's population is infected with this contagious disease (Newton et al., 2002). In developing countries, it is estimated that 30%-60% of adults are infected with *Mycobacterium tuberculosis* and roughly 8-10 million people die each year from this disease (Lall and Meyer, 1999). Individuals who have the human immunodeficiency virus (HIV) are susceptible to TB and often become infected before the onset of AIDS (Lall and Meyer, 1999; Newton et al., 2002). The World Health Organization (WHO) predicted that between the years 2000 and 2020, approximately one billion people would be newly infected and that 200 million would develop TB and 35 million would die from it (WHO 2000 cited by Newton et al., 2002). Drugs such as Ethambutol, Isoniazid, Rifampim and Streptomycin to name but a few, are drugs that have helped immensely in the treatment and control of TB but new multi-drug resistant strains of *M. tuberculosis* have been on the increase (Lall and Meyer, 1999; Newton et al., 2002). This means that more treatment is required for longer periods of time and different medications need to be used and sometimes, even surgery is necessary to remove affected parts of lung tissue (Lall and Meyer, 1999). By increasing the amount of treatment, there is also the problem of patients not taking the medication to its completion and thus getting re-infected and passing on the resistant strains (Newton et al., 2002). In South Africa, TB is a big problem with over three in every thousand people dying from the disease making it the highest rate in the world. One out of every 200 people suffers from TB (Lall and Meyer, 1999). The Western Cape Province has one of the highest records of infected people in the world (Salie et al., 1996).

Studies are being done looking at plants which are used in traditional medicine and in the literature for TB related symptoms such as coughs and fevers. In a study in the Western Cape, four species of the Asteraceae indigenous to South Africa were screened against *M. smegmatis* (Salie et al., 1996). Out of the four species, two were found to have antimycobacterial activity and one of these plants in particular, *Helichrysum crispum*, is reported to be used to treat coughs, bronchitis and tuberculosis and so is justified in its use in traditional medicine (Salie et al., 1996). *M. smegmatis* is often used as a test organism because it is non-pathogenic, faster growing and behaves similarly to *M. tuberculosis* and thus if activity is found, then *M. tuberculosis* can be used (Newton et al., 2002).

Lall and Meyer (1999) chose 20 plant species reported to be used by South African people to treat TB symptoms and tested them against drug-resistant and drug-sensitive strains of *M. tuberculosis*. Six plant species were found to have active extracts against the resistant strain of TB thus backing up these plants being used in traditional medicine for the treatment of TB. These plant species were *Chenopodium ambrosioides*, *Ekebergia capensis*, *Euclea natalensis*, *Helichrysum melanacme*, *Nidorella anomala* and *Polygala myrtifolia*.

In another study (Newton et al., 2002), 43 plant species were chosen from around the globe to be tested for antimycobacterial activity on the basis of being used in traditional medicine to treat TB and leprosy or the symptoms of these diseases (Newton et al., 2002). Here, two *Mycobacterium* strains were used; *M. smegmatis* and *M. aurum* (also used as a non-pathogenic alternative to *M. tuberculosis*) and activity was found in five plant species against *M. aurum* and in two species against *M. smegmatis* in the initial screening. This study also isolated some important compounds and highlighted certain plant species which suggest further investigation using *M. tuberculosis*. Three plant species in particular were highlighted for future investigation: *Commiphora mukul* (Rutaceae) used in India and Pakistan as an expectorant and antiseptic and to treat bronchitis, whooping cough and pulmonary

TB; *Psoralea corylifolia* (Leguminosae) also from India and Pakistan and is used as an anthelmintic and to treat leprosy; *Sanguinaria canadensis* (Papaveraceae) from Canada and North America which is used in cough preparations as well as for fevers among other uses (Newton et al., 2002). The reported traditional uses in the literature for these plants appear to be justified in treating TB.

Gastrointestinal diseases are responsible for the deaths of many people in developing countries (Heinrich et al., 1992). In many rural communities, western drugs may not be available or may be too expensive and so people visit local healers and take plant based decoctions to alleviate the problem (Heinrich et al., 1992; Hernández et al., 2003). An ethnobotanical study was performed in a village in México to determine which plants were in use to treat these gastrointestinal illnesses (Hernández et al, 2003). Here it was revealed that 73.95% of the informants used plant medicines to treat gastrointestinal disorders. Out of the 44 plants said to treat these disorders, eight of these plants were extracted and tested against 14 bacterial strains and all the hexane extracts were shown to have activity against both Gram-positive and Gram-negative bacteria. Two plants, *Lippia graveolens* and *Lantana achyranthifolia*, were mentioned most frequently as treatments and were found to have the best antibacterial activities with Minimum Inhibitory Concentrations (MIC) less than 0.30mg/ml. These findings show that these plants are justified in their use in traditional medicine (Hernández et al, 2003).

Crossopetalum gaumeri, a plant used in the medicines of the Yucatec Maya in México to treat diarrhea was found to contain terpenoids which possess antimicrobial activity which may support the plants use in the treatment of this condition (Ankli et al., 2002). This is yet another ethnobotanical survey which has found biological activity in plants which helps to provide evidence that their use is valid in traditional remedies.

In a similar study, Heinrich et al. (1992) used a different method to verify plants used in the treatment of gastrointestinal disorders. Here, instead of testing against microorganisms, each plant's characteristic smell and taste were examined. It was found that plants with astringent, bitter or aromatic properties are used most often to treat stomach disorders. These plants were then tested for the presence of essential oils and bitter compounds and a large number of the plants contained one of these compounds. This means these plants have the potential to act in the same ways as the tannin-containing drugs used to treat the same conditions (Heinrich et al., 1992).

Essential oils have been investigated as potential antimicrobial agents. One of the most popular plants used in traditional medicine in South Africa is *Artemisia afra* Jacq. (Asteraceae) and this oil was been found to inhibit the growth of 23 out of 25 bacteria in one study. The oil was found to have an effect on the bacterium *Klebsiella pneumoniae* which is interesting because this plant is regarded as a good treatment for coughs and colds and conditions related to this bacterium, suggesting that the plant does in fact help to treat these conditions. The oil was found to be more successful as an antimycotic (Graven et al., 1992). However, when *A. afra* was extracted in methanol and water extracts, neither extract showed any activity against *K. pneumoniae* (Rabe and Van Staden, 1997) suggesting that the volatile oil component is responsible for the activity seen by Graven et al., in 1992.

The oil of *Senecio graveolens* (Asteraceae) was found to have activity against *Candida albicans* which indicates potential as an antifungal agent. Even though this plant is not used to treat fungal related problems, it is used in other ways as an emmenagogue, a digestive and cough suppressant as well as to aid with mountain sickness (Pérez et al., 1999). Another example is that of *Osmitopsis asteriscoides* (Asteraceae), a South African plant used to treat chest complaints and inflammation, swelling and cuts and is a Cape Dutch remedy. This plant contains essential oils which have been shown to have good antifungal activity against *C. albicans* (Viljoen et al., 2003).

Microbes such as *S. aureus* and *Pseudomonas aeruginosa* are often responsible for wound and burn infections. In India, burns are a public health issue and often people die in hospital as a result of their wounds becoming infected with new resistant microbial strains (Gnanamani et al., 2003). Plants may provide a source of compounds which may help in wound management. In Peru, Rojas et al., (2003) screened plants chosen on the basis of being used in traditional medicine for the treatment of infectious or inflammatory diseases. Antimicrobial activity was found in 25 out of 36 ethanol extracts. The extracts were tested against eight pathogens including *S. aureus* (inhibited by 15 extracts), *P. aeruginosa* (inhibited by 13 extracts) and *C. albicans* (inhibited by 13 extracts). *Datura alba* and *Celosia argentea* are both plants used in India to treat skin diseases and as antiseptics and the alcoholic extracts of these plants were shown to have good activity against eight pathogens which infect wounds and burns (Gnanamani et al., 2003).

In the Eastern Cape province of South Africa, Grierson and Afolayan (1999) found that some plants used by people in the treatment of wounds were justified by testing these plants against microbes. The information was gathered using questionnaires and approaching local people, healers and *Sangomas* (spiritual healers) and four plants were found to be recommended for the treatment of wounds. The plants chosen were *Grewia occidentalis* (Tiliaceae), *Malva parviflora* (Malvaceae), *Polystichum pungens* (Aspidiaceae) and *Cheilanthes viridis* (Adiantaceae). These were extracted in water, acetone and methanol. They were then tested against 10 microbes including *S. aureus* and *P. aeruginosa*. The results showed that some of these plants are justified in their use in traditional medicine (Grierson and Afolayan, 1999). These are just a few examples of plants which may have the potential to be used in wound management. It seems that plants used by people can be a good source of new treatments especially when it comes to conditions such as wounds and burns.

Not all plants used in medicines are justifiable for example, soft soaps are often used by traditional healers to help treat boils, ring worm and wounds in Nigeria (Moody et al., 2004). These soaps are locally made but the healers often incorporate plants such as *Aloe vera* and *Ageratum conyzoides* into these soaps to treat these conditions. Consequently, these two plants were investigated for antibacterial and antifungal activity but in the preliminary tests, no activity was found that would substantiate the healer's claims. Some of the test soap samples did show a degree of activity and so perhaps more understanding of interactions between secondary metabolites in the extracts is perhaps needed as well as looking at pH and the age of the plants collected before adequate conclusions can be drawn (Moody et al., 2004).

2.5. Intellectual property rights and indigenous knowledge

In any study which incorporates the knowledge of an indigenous people or a community there is always the question of intellectual property rights and remuneration to the people who contributed. It is felt that the resources of a country belong to the people of that country. If a group of people have been using a plant for a purpose after much trial and error for a long period of time, it is only fair that these people should see the benefits if their knowledge is the basis of a new or even revolutionary drug (Soejarto, 1993; Barton, 1994). It must be remembered that a plant itself is not novel, a plant cannot be patented purely on the basis that it has medicinal value (Barton, 1994), but if a compound from a plant has been isolated then it may be patented. If the finding of this chemical was achieved through ethnobotanical means then the people who divulged the knowledge should be duly compensated (Barton, 1994). However, implementing standards for intellectual property protection could also cause a problem in that pharmaceutical prices could increase thus reducing people's access to these medicines particularly in developing countries (Timmermans, 2003).

South Africa has been said to have a "well-established intellectual property framework in place" (Wolson, 2001). South Africa has an obligation under Article 8(j) of the Convention on Biological Diversity to:

> Subject to its national legislation, respect, preserve, and maintain knowledge, innovations and practices of indigenous and local communities...and promote their wider application...and encourage the equitable sharing of benefits

arising from the utilization of such knowledge, innovations and practices (Wolson, 2001).

Although there is still much to be done in South Africa in this regard, indigenous knowledge is becoming an important topic in the research field and much is being done to protect knowledge and to develop more research capacity in this field (NRF, 2003). With regards to this study, it has been decided that all the information gathered from the interviews is the property of the people who divulged it. This information will be distributed to the people in such a way that they can make further use of it should they wish to.

This literature review has shown that it is possible to use ethnobotany as a tool to verify some uses of plants in traditional medicine and use this information to discover new drugs. It has been seen that many important drugs contain compounds derived from plants and can benefit the lives of many individuals suffering from diseases such as leukemia and heart disease. By performing surveys important information can be gathered regarding the uses of plants in medicines. From the surveys mentioned in this review, elderly people are frequently found to be the most knowledgeable individuals (Malamas and Marselos, 1992; Katewa et al., 2004). Many of these people have not been able to pass on their knowledge and if it is not documented, and these people die, this information is lost. So ethnobotanical surveys can provide a means of preserving or documenting this information. These studies have also been used to study cultural heritage and to verify aspects such as the antiquity of medicinal plant usage (Leonti et al., 2003).

Ethnobotanical surveys can also be of benefit when choosing plants for pharmacological testing. Plants which have a high frequency of use for the treatment of a specific condition may certainly be worth testing to see whether or not these plants contain compounds which can help in the treatment of the condition. Plants which have been used in traditional medicine to treat coughs and fevers (symptoms of tuberculosis) have been found to be active against the bacteria causing this disease, *Mycobacterium tuberculosis* (Lall and Meyer, 1999). This activity aids in the justification of certain plants in the treatment of certain conditions. Gastrointestinal infections are also problematic in many developing countries and surveys have been performed in these countries to record which plants are used to treat these disorders. Following testing against microbes responsible for gastrointestinal infections, many of these plants were found to be active against these microbes thus supporting their use in the treatment of these infections (Ankli et al., 2002; Hernández et al, 2003).

It is important to recognize and acknowledge the input from traditional medicine systems as being valuable sources of information which can provide many answers to our medical questions. However, it is also vital that scientists do not exploit the knowledge obtained and that this knowledge is preserved for future generations.

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CHAPTER 3

MEDICINAL PLANT USE IN THE BREDASDORP / ELIM REGION

ABSTRACT

There are many individuals in the Bredasdorp / Elim area who still use plants as medicines to treat many conditions. This study aimed to document some of this knowledge and present an inventory of all the plants in use in the area. Over 40 individuals were interviewed from old age homes, community centres for the elderly as well as people who were known for their knowledge in this matter. The information was gathered by means of questionnaires after establishing a rapport with the people. In total, 36 plant species from 19 families were found to be in general use in the area. Only 58% of these plants are indigenous to South Africa, 33% are introduced species and 9% are naturalized species. The dominant families were Asteraceae, Lamiaceae, Alliaceae and the Solanaceae. Many of the plants in use are plants which are in common use in traditional medicine around South Africa and share many of the same uses. Some uses which have not been seen in the consulted literature have also been documented. Following the initial questionnaire, a more structured questionnaire was used to try and determine which plants were preferred by the individuals and which plant was best for particular ailments. Only 15 individuals were found who were willing to partake in this part of the study. However, these individuals were highly regarded for their knowledge in self-care using plants. From this part, it was found that *Artemisia afra* and *Ruta graveolens* were the most popular plants out of the 16 plants which were found to be used more frequently. It was found that most of the knowledge was held by elderly women and that much of this knowledge has already been lost. This study shows that ethnobotanical surveys can be useful in preserving information for future generations as well as discovering new uses for plants.

Keywords: South Africa; Southern Overberg; ethnobotanical survey; medicinal plants; questionnaires

1. INTRODUCTION

In South Africa, many people still use plants as medicines as an alternative or supplement to visiting a western health care practitioner (van Wyk et al., 1997). This is not surprising due to South Africa's cultural diversity as well as large floral biodiversity. South Africa is home to over 30 000 species of higher plants and 3000 of these species have been found to be used in traditional medicine across the country (van Wyk et al., 1997). There are over 27 million users of indigenous medicine (Mander, 1998) and an estimated 200 000 indigenous traditional healers which up to 60% of the population consult with (van Wyk et al., 1997). Knowledge of these plants is very important because not only is there the potential to discover new alternatives for the treatments of illnesses but also from a conservation point of view. If certain plant species are found to be under threat due to a high demand for plant medicines then measures can be implemented to try and ensure sustainability of the plant species. It is also important from a cultural point of view because much of the knowledge is being lost due to not being passed on from one generation to the next. So it is important to document this knowledge for future generations who may one day need the information.

Many important sources of information in the literature are available from previous surveys performed in South Africa: Hutchings et al., (1996) performed a survey looking at around 1032 plant species (near 25% of the flora of KwaZulu-Natal) used in Zulu traditional medicine. This survey was compiled from existing literature as well as from interviews with traditional healers, hospital patients and people who use plants themselves. This shows that performing surveys on indigenous plant use and looking at plants used either by healers or in self-medication can provide information which may help in the conservation of certain plant species as well as contribute to the South African Pharmacopoeia. Important literature sources detailing medicinal plant use in South Africa include Watt and Breyer-Brandwijk (1962); Watt (1967); Cunningham (1988); Hutchings (1989); Hutchings et al., (1996); Mander (1998); van Wyk et al. (1997); and van Wyk and Gericke (2000). This study, in the Bredasdorp / Elim region, aims to capture knowledge from individuals who use plants in self-care in the hope that it may add to our current knowledge and maybe help to substantiate certain plant uses for the illnesses they are reported to treat. Studies similar to this have been performed with success in other parts of the world.

1.1. Medicinal plant use in the world

Plants are still in use in many parts of the world for treating illnesses and other ailments. A study was performed in Greece where local people were interviewed to discover what plants were still in use. This is an area where over 1100 species of plants are found many of which are still used by the local people in self-mediciation. Here mainly people who personally use plants as medicines (mostly elderly people) were interviewed and a total of 35 interviews were performed to yield 36 species of plants in use in the area. Nine of the plant species in use were found to be species that had not previously been mentioned in literature from that region (Malamas and Marselos, 1992). This means that there is the potential to discover new plants to investigate in the fields of Ethnobotany and Ethnopharmacology.

In a study performed by Gedif and Hahn (2003) in rural central Ethiopia, plants are still widely used in traditional medicine but much of this knowledge has not been recorded. In this instance mothers were interviewed due to them being the healers of the family as is common in many developing countries (Gedif and Hahn, 2003). The interviews aimed at documenting how plants are used and what conditions they are used to treat. 25 species out of 21 plant families were found to be used in self-care in the Butajira community and a third of these species are available in Ethiopian markets and already have recorded uses in cooking (Gedif and Hahn, 2003). In one survey performed in Mexico, 119 people were interviewed to find out about plants specifically used in the treatment of gastrointestinal infections. Out of the 119 interviewed 88 people stated that they relied on plant based medicines to treat these stomach conditions. This information was gathered using informal interviews and then semi structured interviews and a wide range of people were interviewed such as housewives, farmers and healers (Hernández et al., 2003).

If specific plants are found to be used frequently, a platform is provided to investigate the phytochemical and pharmacological actions of plants and so studies such as this can prove very useful in the discovery of new medicinal compounds. Surveys where traditional plant use is recorded can also be of benefit in the justification of herbal drugs currently on the market if the same plant is used in the study area for the same condition as the one in the store. Conversely, the market product can also justify the use of the plant by a person unaware of the product thereby showing that traditional medicine is an important source of information when looking for plant based remedies. However, this is also an area which is open to exploitation.

1.2. Intellectual property rights

There is a political and very important consideration when utilizing indigenous knowledge for drug discovery; the question of intellectual property rights and remuneration to the local people. Concerned scientists feel that the plant and animal resources of a country belong to the people of that country. The knowledge obtained has been discovered through many years of trial and error by the people of that region, therefore a share of any money obtained from the natural product should be given back to the people who divulged that knowledge (Barton, 1994; Soejarto, 1993). Professional bodies such as the International Society of Ethnobiology, Society of Economic Botany, and American Anthropology Associations strongly encourage researchers conducting ethnobotanical studies to impart appropriate benefits to the local or indigenous people who divulged their knowledge (King et al., 1996). In South Africa, much is being done to encourage researchers to protect indigenous property rights. The National Research Foundation (NRF) has made this topic one of its key focus areas (NRF, 2003). South Africa is also said to have a well-established intellectual property framework in place and is bound under Article 8(j) of the Convention on Biological Diversity to protect, respect and preserve indigenous knowledge (Wolson, 2001). In this study in the Southern Overberg, it was decided that the information gathered would be compiled and given back to the communities via the people who participated. This information would be compiled in such a way that it could be used by the community to create a booklet should they wish to do so.

1.3. The Study Area

The Bredasdorp / Elim region is situated in the Southern Overberg in the Western Cape Province of South Africa. The Overberg region has been inhabited by humans for at least half a million years and these early hunter-gatherer inhabitants would have been reliant on the indigenous flora and fauna of the region (Mustart et al., 1997). The Southern Overberg has a rich flora of about 2500 species out of which approximately 300 are endemic (restricted to the area) and 32 Red Data Book species (Mustart et al., 1997). Since the 1940s a shift has occurred from predominately veld based grazing to cereal crops and introduced pastures. This, along with increasing alien invasive species, has led to the demise of much of the natural renosterveld and poses a threat to existing endemic flora (Mustart et al., 1997). The Bredasdorp Formation limestones are associated with an endemic rich vegetation (Mustart et al., 1997; Cowling, 1992). The soils are alkaline and organic rich. The plants growing on this terrain are known as limestone proteoid fynbos. Bredasdorp and surrounding areas are made up of three vegetation types: limestone proteiod fynbos , restioid fynbos and neutral sand proteoid fynbos (Mustart et al., 1997).The study area has many individuals who still rely on plants as a source of treatment for various conditions. Therefore the focus of this study was to determine which plants are in use in the area and to see how these plants are prepared for application in treating various complaints.

2. METHODOLOGY

2.1. Interviews and data analysis

Interviews were conducted in places where elderly people generally meet, places such as old age homes and community centres for the elderly. In some cases, individuals were recommended by other members in the community for their knowledge and so these people were interviewed as well. Preliminary interviews took place by appointment and a basic questionnaire was followed where possible to see which plants are in use and how and what they are used for. In follow-up interviews, more specific questionnaires were designed using the information already gathered. These were aimed at finding out more about what had already been mentioned and finding out the preparations and dosages of the various medicines and if there were any alternate species if species *x* was not available. An example of the questionnaires can be seen in Appendix A. In all interviews either a tape recorder was used or notes were written. A new more specific questionnaire or rather worksheet was designed (Appendix B) which listed all the plants mentioned thus far in the study and asked informants to rank the plants that they used in order of personal importance. This worksheet also listed the most common ailments mentioned in the study and asked each individual to specify which plant(s) they thought was most useful to treat that ailment. In the case where there is more than one plant suggested the person was asked to list the plants in order of importance. From this data, a list could be calculated to determine what the most popular plants are in use in the area, termed "preference ranking" (Cotton, 1996; Phillips and Gentry, 1993a,b). For example; if a person used five plants he or she was asked to rank them in order of importance. Then the plant termed the most important would be given a ranking of 5 (in the case of five plants) and the fifth most important assigned a value of 1. These values from each informants list were summed for each plant to generate a list of the plants in decreasing order of popularity. Also this data permitted the calculation of each plants "use-value" to determine how useful a particular plant is. These values were calculated using a method by Cotton (1996) and Phillips and Gentry (1993a,b).

The use-values were calculated using the following equation:

$$UV_S = \frac{\sum UV_{IS}}{I_S}$$

Where UV_S = the overall use of species S

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

I_S= total number of informants interviewed for species S

Over 40 individuals were interviewed in total and the data can be seen in Table 1. Out of these 40 individuals, 15 participants agreed to complete questionnaires to generate the data in Figures 1,2 and 3.

2.2. Plant identification

Due to some plants having the same or more than one common name there was an initial problem involving the identification of several plants. This problem was overcome by taking illustrated books to the interviews so that the interviewees could page through the books and look at the photographs and recognize certain plants. This helped tremendously in coming to an agreement for each plant in use and also helped in triggering memories of plants that were still used and that were used in the past. Many of the informants also helped in the identification of plants by bringing along samples of plant material to the meetings. This also promoted much discussion and debate amongst the people interviewed. Where possible the samples were kept and taken to other meetings to use in discussions with different informants which also helped to verify the names of the plants. The plant specimens were also verified by taxonomist Mr. Frans Weitz of the Herbarium in the Department of Biodiversity and Conservation Biology at the University of the Western Cape. Herbarium specimens of the lesser known species were deposited in this herbarium.

3. RESULTS

Thirty-six species belonging to 19 families were found to be in use in the study area (Table 1). The families with the largest number of species are the Asteraceae (six species), Lamiaceae (six species) and the Alliaceae and Solanaceae (three species each). Twelve out of the 36 species are introduced species, 3 species are naturalized and 21 species are indigenous to South Africa. Table 1 shows the botanical name, common name, condition treated, preparation and uses recorded in the literature of each plant. Figure 1 shows a list of the most widely used plants as determined by the worksheet in Appendix B where 15 willing informants were asked to rank the plants that they use in order of importance. Figure 2 shows the results from the worksheet where the 15 participants were asked to list which plants were best to treat specific conditions. To determine these figures, a plant was assigned a value of one if it was used to treat a condition and a zero if it was not used. The "ones" were summed for each plant and for each condition to give the results in Figure 2. This graph shows which plants are preferred to treat particular ailments. Figure 3 shows the calculated use-values. Each species is colour-coded in order to be able to differentiate between the different graphs and plants.

Table 1. The current uses and preparations of medicinal plants used in the Bredasdorp / Elim region and previously recorded uses in the literature

Family **Plant species** **(Common name)**	**Use/Condition treated**	**Method of preparation and use**	**Uses in the literature**	**Other information**
Alliaceae *Allium cepa* L. (Ui, Onion) (I)	Liver problems	Administered in cooking or eaten raw	Internally for bronchial and gastric infections and externally for acne and boils[d]	
Allium sativum L. (knoffel, garlic) (I)	Arthritis, backache, fever, rheumatism and worms	Cloves are eaten raw. Taken on a daily basis for arthritis and pains	Febrifuge, tuberculosis (TB), stimulant, carminative, antiseptic, anthelmintic, diaphoretic, expectorant, diuretic, hypotensive, whooping cough[a]	It has been suggested that on the first day take one clove and on the second two and on the third three, then two then one on the following days and repeat for arthritis
Tulbaghia violacea Harv. (wilde knoffel, wild garlic) (Ind.)	Earache, fever, high and blood pressure	Clove pieces are placed in castor oil to make eardrops. For fever and high blood pressure a tea is made from bulbs and a small cup taken three times daily	fever, colds, asthma, Stomach complaints[e,f], TB and anthelmintic[a,f]	

Amaryllidaceae *Gethyllis spp.* (koekmakranka) (Ind. Possibly endemic)	Convulsions, heart and stomach problems, sleeplessness	Seed pods are soaked in brandy and a small amount (±25 ml) taken at night for sleeplessness and heart condition or symptomatically to relieve stomach pain or treat convulsions	Colic, indigestion[a,b,f], flatulence, teething trouble, boils, bruises and insect bites[a]	
Apiaceae *Foeniculum vulgare* Mill. (vinkel, fennel) (I)	Arthritis, fever, milk stimulant in pregnant women, diuretic for weight loss	An infusion made from the leaves is drunk, to treat arthritis and fever and to stimulate milk production, green fruit is chewed to reduce fat	Flatulence, cough, diuretic, digestive problems[f], diarrhea, stomachache and cramps[a]	The infusion can also be used as a wash in which arthritic joints can be soaked in
Petroselenium crispum (Mill.) A.W. Hill (pietersielie, parsley) (I)	Arthritis, bladder and kidneys, liver problems, cough, diabetes, rheumatism	Taken as a tea made from a handful of leaves taken when needed	Diuretic and emmenagogue[a]	Also used in cooking to help with the listed ailments
Asphodelaceae *Aloe ferox* Mill. (alwyn, aloe) (I)	Skin problems	Leaf juice is applied to affected area	Laxative[a,f,g], arthritis, eczema, conjunctivitis, hypertension and stress, other species leaf sap used to treat irritations, burns, bruises[f], opthalmia, purgative, syphilis and venereal sores[a,e]	

Bulbine lagopus (Thunb.) N.E.Br. (geel katstert) Herbarium specimen no. (TSAT 011) (E)	Wounds, sores, skin conditions	leaf is broken and exudate applied to affected area	Leaf sap of *Bulbine* species used to treat burns, wounds, skin conditions[a,f] (such as acne, eczema and rashes[e]), cracked lips, herpes,[f] and stomach upsets[e]	
Asteraceae *Artemisia afra* Jacq. Ex Willd. (Wilde als, wormwood) (I)	Bladder and kidney disorders, coughs, colds, influenza, convulsions, diabetes, fever, headache, heart, inflammation, rheumatism, stomach disorders and worms	A tea made from a handful of the leaves can be taken daily to treat bladder and kidney trouble, coughs, colds, influenza, heart, headache. A syrup can also be made by boiling the leaves with sugar for coughs. For inflammation and fever leaves are made into a poultice with brandy or vinegar and wrapped around affected area (inflammation) or around the stomach (fever). For diabetes a small amount of the tea is taken twice daily continuously.	This popular plant has many uses in the literature including uses as a tonic, anthelmintic[b,e], eyewash[b], coughs, colds and influenza, fever, loss of appetite, colic, headache, earache, malaria, worms[a,e,f], constipation, diabetes and as blood purifiers for acne and boils[e]	

Elytropappus rhinocerotis (L.f.) Less. (renosterbos) (Ind.)	Bladder and kidney disorders, convulsions, diabetes, fever, headache, stomach disorders, worms and wounds/ sores (tea has also been mentioned to be good for heart and cancer)	place leaves in brandy or vinegar for stomach ailments and take small amount; tea made from leaves drunk for bladder and kidneys, convulsions, diabetes, fever, headache, and worms; tea used to wash wounds and sores	Stomach problems, dyspepsia, indigestion, ulcers, cancer, stomachic and appetite stimulant[a,b,f,g]	Was used as a remedy during an influenza epidemic in 1918[b]
Conyza scabrida DC. (paddabossie) Previously named *C. ivaefolia* Less also known as "bakbos" or "oondbos" because plant was used as a brush to sweep out ovens[b,a] Herbarium specimen no. (TSAT 013) (N)	Chest, heart, fever, diabetes, rheumatism, colds and flu, inflammation	leaf infusions drunk as a tea, take 25ml morning and evening for fever, rheumatism, heart, stomach ailments coughs, colds and flu, leaves (fresh or dried) are placed on a cloth with vinegar/brandy and wrapped around sore area (headaches, stomach ache) as a poultice to relieve pain. Can also be used as an eyewash	Influenza, chest, stomach, heart afflictions[b], convulsions in children [a,c], fever, to hasten the birth of the placenta and pleuritic pain in children[a], sprains and fractures[d,e]	Showed evidence as a remedy for influenza during the 1918 influenza epidemic. Dried leaves keep a long time and no reported difference between the dried and fresh herb has been mentioned. "groenamara" has been said to be an alternative if *Conyza* not available.
Helichrysum crispum (L.) D. Don (kooigoed) (Ind.)	arthritis, bladder and kidneys, colds and flu, cough, fever, headache, heart, sleeplessness and rheumatism	Dried leaf infusions drunk as tea, approx 25ml taken 2-3 times per day until symptoms disappear. For sleeplessness place small amount of leaves under pillow at bedtime.	Colds, headache[b,e,f], Coughs, fever, infections, menstrual pain[b,f], heart, backache, kidneys[a], stomachache and used as a circumcision wound dressing, and as a blood purifier for boils[e]	

Eriocephalus paniculatus (Cass.) (wilde roosmaryn, wild rosemary) (Ind.)	Heart	A leaf infusion is made and drunk as a tea	Diaphoretic, diuretic[a,f] *E. umbellatus* was used by early Cape settlers and the Khoi to treat dropsy, stomachache and heart trouble[a]	Has been claimed to be able to treat heart disease and heart failure[f] Also used in cooking
Vernonia spp. (groenamara) (Ind))	stomach ailments, diabetes, and worms	A tea is made using a small handful of leaves infused in boiling water for these complaints	*V. oligocephala* used to treat stomach disorders[a,d,e,f], rheumatism, dysentery, diabetes[a,f] and ulcerative colitis[f] Other *Vernonia* species used to treat fevers, urinary tract diseases, backbone pain and chronic coughs and to get rid of head lice[e]	Can act as a substitute for *A. afra* in cases where it is used to treat the same ailment
Crassulaceae *Cotyledon orbiculata* L. (kouterie, plakkie) (Ind)	Corns and sores	The leaf sap is smeared on corns or the fleshy inside of the leaf bound to the corn to soften it so it can be removed. Sap is applied to sores	Corns, warts[b], earache[a,b,f], toothache, vermifuge[b,f], boils and epilepsy[a,c] venereal disease[a,d,e], sprains and fractures[d]	

Euphorbiaceae *Ricinus communis* L. (olieblare, kastorolie boom) (I)	Inflammation and rheumatism	Leaves are bound to affected area.	Stomach ache, wounds, sores, boils[a,d,e,f]; epilepsy[c], headache[a],	Leaves not taken internally
Fabaceae *Sutherlandia frutescens* (L.) R. Br. (keurtjies, kankerbossie) (Ind.)	Back pain, bladder and kidneys, cancer, colds, influenza, liver, diabetes, fever and stomach complaints	A tea from 3 teaspoons / small handful of fresh or dried leaves and stems infused in 1L boiling water is made and ±25ml taken morning and evening for all listed complaints	Cough[a] ,washing wounds, fevers[b,g], chicken pox, cancer, eye trouble[b], Old Cape remedy for stomach trouble and internal cancers[a,f], poor appetite, indigestion, peptic ulcer, colds, urinary tract infections are among the many uses for this widely used plant[b,f,g]	Not recommended for pregnant women, need to wait overnight at least for the tea to take effect. Too strong an infusion will cause vomiting
Gentianaceae *Chironia baccifera* L. (aambeibossie) Herbarium specimen no. (TSAT 012) (Ind.)	Stiff muscles	An infusion of the leaves and stems is made and applied to the affected area symptomatically	Boils, hemorrhoids[a,b,f], blood purifier, acne, sores, diarrhea[a,f] and leprosy[f]	
Geraniaceae *Pelargonium species* (malva) (I)	Earache, toothache and pain	Leaves are scrunched up and placed in ear to treat earache or rubbed against sore tooth. Leaves placed on sore area on skin and held in place to alleviate pain	Diarrhea, dysentery[a,b,f], bronchitis[d,f] , fevers[d] coughs[f], *P. betulinum* used to heal wounds and treat stomachache	

Hyacinthaceae *Drimia species* (gifbol) (Ind.)	Wounds and sores,	Peel off the bulb scales and place on sores to draw out infection.	*Drimia* species used to treat stabbing chest pains, stomach ailments, high blood pressure (*D. elata*)[e] and feverish colds (*D. robusta*)[e]	Good for slow healing sores
Lamiaceae *Mentha longifolia* L. (kruisement) (N)	Arthritis, backache, bladder and kidneys, liver, colds, influenza, coughs, fever, headache and stomach trouble	A handful of fresh leaves made into a tea and drunk as needed for adjacent complaints	Diaphoretic, antispasmodic[a,b,f], flatulent colic, hysteria[b,f], coughs, colds, respiratory problems[a,d,f] and wounds[f]	Boiling the leaves with sugar in water makes an excellent syrup for whooping cough
Leonotis leonurus (L.) R. Br. (klipdagga, wilde dagga) (Ind.)	Arthritis, backache, bladder and kidney disorders, cancer, colds, influenza, diabetes, headache, heart, rheumatism, high blood pressure and stomach	A tea is made from a handful or a stems worth of leaves and flowers steeped in boiling water and left to draw in a glass bottle. ±25 ml is drunk morning and night for the adjacent complaints	Coughs, colds, chest problems, piles, boils[b,f], high blood pressure[f], headache[d,e,f], asthma snakebite[a,d,f], epilepsy, emmenagogue, purgative, TB and insect bites[a] include some of the many uses of this plant	Not recommended for pregnant women. Tea should made daily and not be kept. Can use the leaves fresh or dried

Ballota africana (L.) Benth. (kattekruid) (Ind.)	Stomach trouble, colds and liver complaints	A tea is made using 1 teaspoon of herbs in a cup of water for stomach trouble and for the liver. A syrup can be made with boiling water and sugar for colds	Measles[f], colds, influenza, asthma, bronchitis, heart, hysteria, fever, insomnia, typhoid fever[a,f], headaches, liver problems and arthritis[f] and snakebite[a]	
Salvia africana-lutea (L.) (wilde salie) (Ind.)	Cough, sinuses and chest complaints	A tea is made from a small handful of leaves to relieve complaints	Coughs, colds, diaphoretic and female ailments[a]	Often substituted with household sage
Melianthaceae *Melianthus comosus* Vahl. (kruidjie roer my nie) (Ind.)	Wounds and sores	A large handful or bunch of the plant leaves is placed in a bucket of boiling water to draw. This is then used to wash wounds and sores	2 species *M. comosus* and *M. major* used to treat wounds, sores[a,b,d,e,f], bruises, backache and rheumatism[a,b,f] snakebite[a,d,e,f], sprains and fractures[d]. Other uses include root infusions for cancer and leaf decoctions for ringworm[f]	Useful for sores on livestock as well as people. Plant is toxic and should not be taken internally

Mesembryanthemaceae *Carpobrotus spp.* (suurvy, vygies) 3 species found in the area: *C. edulis* (L.) L. Bolus *C. acinaciformis* (L.) L. Bol. *C. murii* (L. Bol.) L. Bol. (endemic) (Ind.)	Stomach trouble, sunburn, mouth ulcers, sore throat, tuberculosis and thrush	Juice squeezed from leaves can be taken either alone or with milk to ease stomach trouble. Sap applied directly to sunburn thrush and mouth ulcers. Used to treat sore throats and TB by chewing the leaves and swallowing the juice but spitting out the skin and residual tissue	Mouth and throat infections using juice as a gargle[a,b,f], Juice swallowed to treat dysentery[a,b,f]; digestive trouble[a,d,f]; TB and as a diuretic[a,f]. Used externally for skin complaints and burns and wounds[a,b,f]. *C. edulis* has also been said to be effective in treating toothache, earache, oral and vaginal thrush[f]	
Myrtaceae *Eucalyptus spp.* (outydse bloukombossie) (I)	Chest complaints and cough	Leaves are scrunched up into a bowl and covered in boiling water and the fumes inhaled	Leaves and oil used as decongestants in cases of chest colds and influenza[a,g]. Also used to treat fevers[d], dysentery and as a wash for pimples or acne[e]	Insect repellent[a]

Rutaceae *Agathosma betulina* (Berg.) Pillans (buchu) (Ind.)	Sprains, pain, arthritis, bladder and kidney ailments, backpain, stomach pain, fever and cancer	For sprains and pains (including arthritic pain) place dried leaves on a cloth and sprinkle with brandy or vinegar and wrap cloth around affected area to relieve the pain. A tea made from 1 handful of fresh leaves (from veld if possible) in boiling water (±1L) and left to stand is used to treat bladder and kidney trouble, back cancer and stomach pain. A small cup should be drunk 3 times daily.	A very popular plant used since 17th and 18th centuries in the Cape to treat numerous complaints such as stomach, kidney and bladder ailments, urinary tract infections, diuretic, rheumatism, wounds, bruises[a,b,f,g]	
Ruta graveolens L. (wynruit, rue) (I)	Bladder and kidneys, convulsions, diabetes, fever, headache, stomach complaints, worms and sinus	A tea is made from the leaves (1 teaspoon in a cup of boiling water) to treat adjacent complaints	Used to treat fever, convulsions[a,c,f], fits (epilepsy and hysteria[c]), respiratory and heart problems[a,f], toothache, earache and to ease childbirth[a,c,f]	
Sapindaceae *Dodonaea angustifolia* L.f. (ysterhouttoppe) Herbarium specimen no. (TSAT 014) (Ind.)	Arthritis, bladder and kidney ailments, colds, influenza, convulsions, fever, inflammation, rheumatism, stomach complaints	The leaf tops (± 3 teaspoons in 1L boiling water) are made into a tea and small amount taken 3 times daily to treat listed problems	Fever, colds, influenza, stomach ailments, sore throat[a,f], pneumonia, arthritis, measles, TB and skin rashes[f]	Leaves can be used dried or fresh but if the dried leaves are a grey colour then should not be used. Fresh leaves are preferable

Solanaceae *Datura stramonium* L. (stinkblaar) (I)	Sores	Place leaf on sore or affected area to draw out the poison	Has many uses in traditional medicine including pain[a], rheumatism, gout, boils, abscesses and wounds by using a poultice[b,e,f]. Fruit has been reported to be used for toothache, tonsillitis, and sore throat[b,f]. To treat asthma, bronchitis[b,f] and headaches[d,e] the leaves are smoked	
Viscaceae *Viscum capense* L.f. (voelënt) (Ind.)	Fever and colds	Whole pieces are placed in brandy and taken to relieve fever and cold symptoms	Used to treat asthma, bronchitis[a,d,f], menstrual problems, fruits used to reduce bleeding and get rid of warts[a,f] diarrhea[d,f]	Is quite difficult to find and is often mixed

References:

a. Watt et al., 1962,
b. Smith 1966
c. Watt 1967
d. Hutchings, 1989
e. Hutchings, 1996
f. van Wyk et al., 1997
g. van Wyk and Gericke 2000

Abbreviations:

(I) - introduced species
(Ind.) - indigenous species
(N) - naturalised species
(E) - endemic species

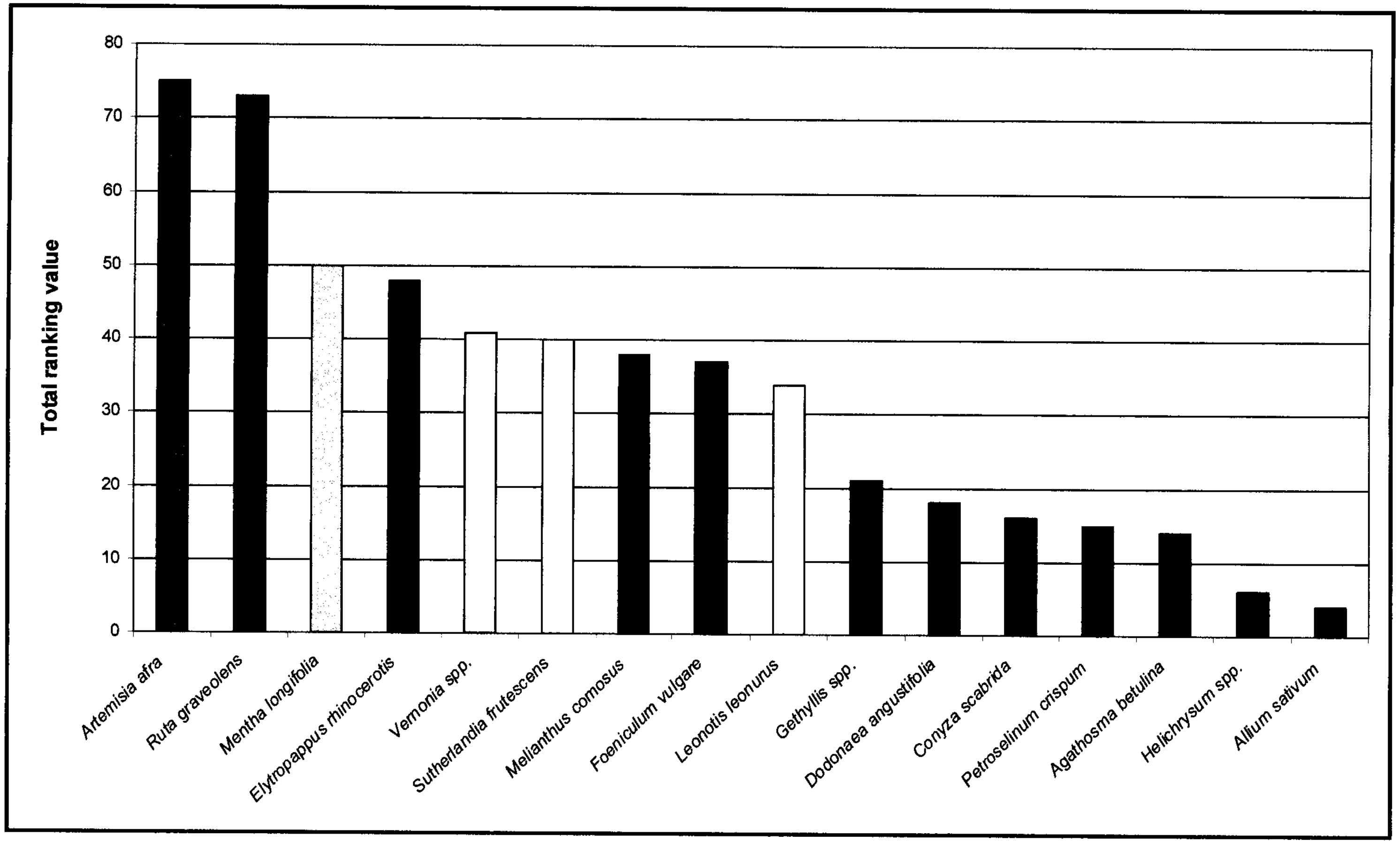

Figure 1. Graph showing the total ranking value for each plant as ranked by order of importance by each informant

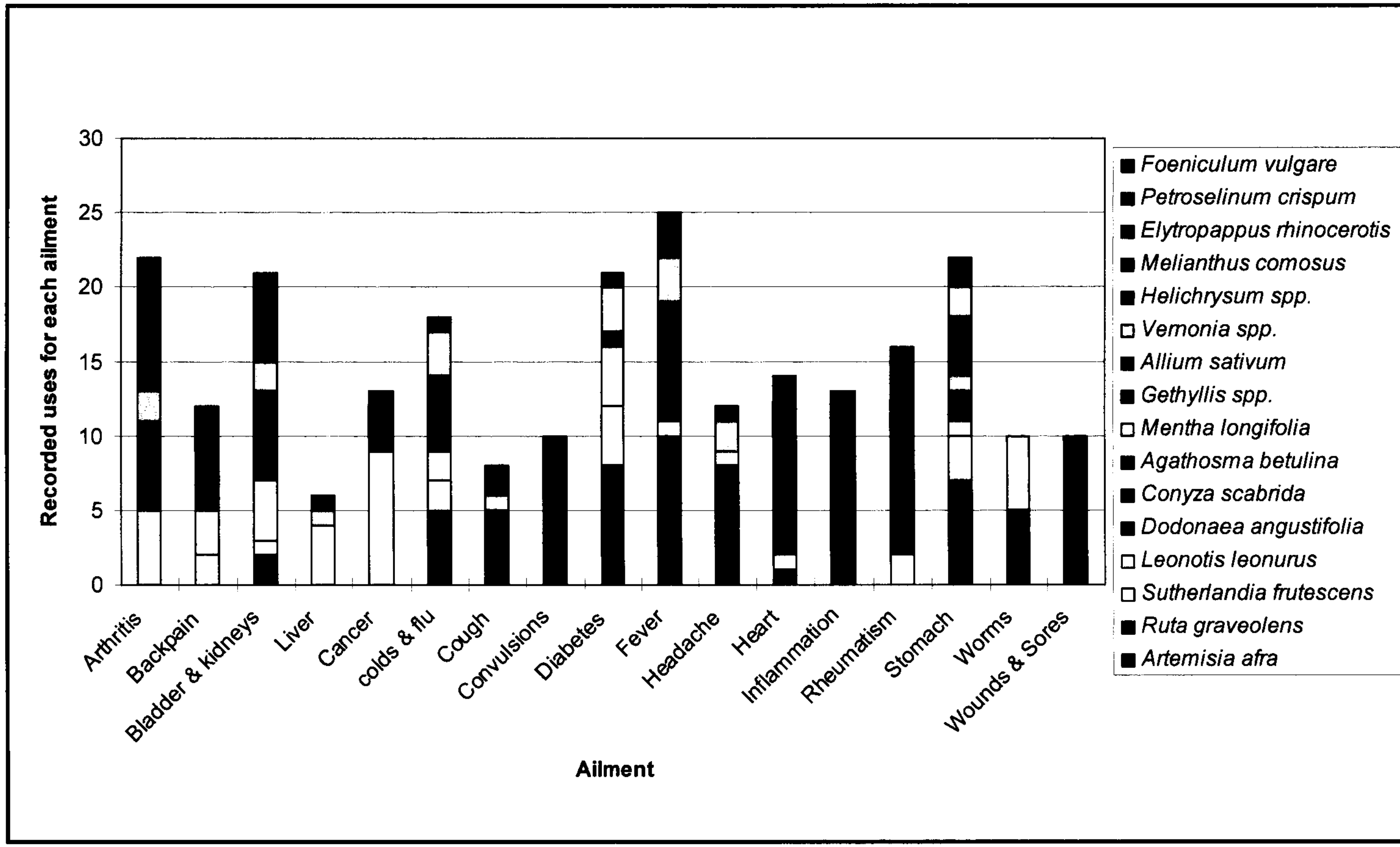

Figure 2. Graph showing which plants are used for specific ailments

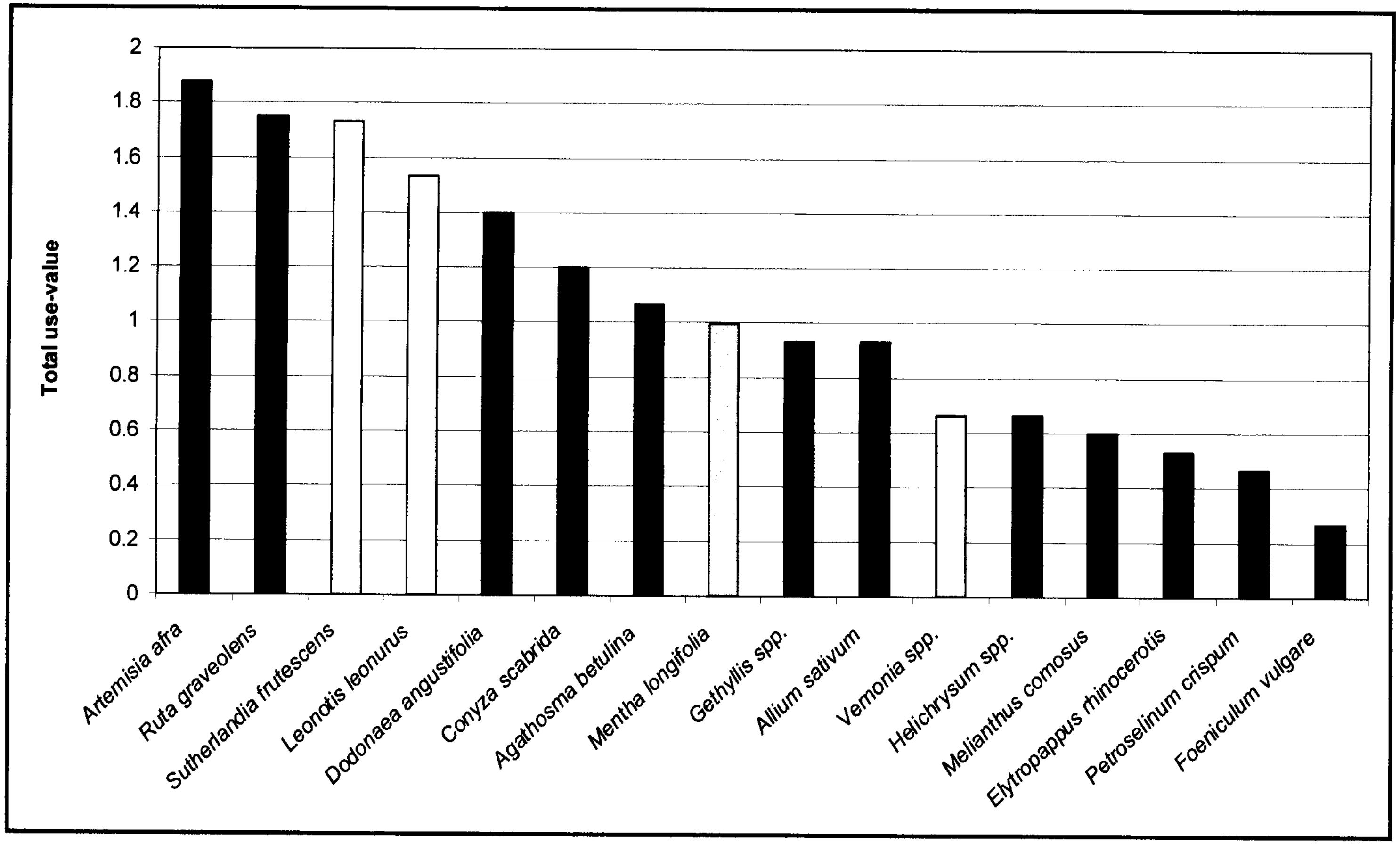

Figure 3. Graph showing the calculated use-values for each plant

4. DISCUSSION

4.1. Outcomes from the interviews

From Table 1 it can be seen that the majority of the plants used in this survey are plants that are commonly used around South Africa and in Zulu, Xhosa and Sotho traditional medicine, for example *Artemisia afra*, *Mentha longifolia* and *Leonotis leonurus*. These popular plants share many of the same uses, for example *A. afra* was found to be used to treat gastrointestinal trouble and as a general tonic (Hutchings, 1989; Hutchings et al., 1996). In the same survey (Hutchings, 1989), *M. longifolia* is used to treat respiratory ailments, headaches and fevers and this is in agreement with the uses for this popular plant in the Bredasdorp / Elim area where these uses are also recorded for this plant. *L. leonurus* is a plant that features frequently in the literature and many uses have been recorded. In Zulu, Xhosa and Sotho medicine it was found to be used to treat gastrointestinal and respiratory disorders and headaches, fevers and snakebite (Hutchings, 1989). Common uses for this plant in this study are headaches, respiratory problems and stomach ailments. So there are common threads between this survey and those results in the literature which suggests that studies like this are important when it comes to choosing a plant species to test in a particular bioassay. If a plant has been reported in many surveys around the country and even in other countries to be used for the treatment of a common ailment then this plant may be a new source of medication for this ailment in the future. Similarly, new uses for a plant may be found and could mean a plant could be re-investigated for new activity which could also lead to new treatments arising.

Some uses have been recorded in this study that have not been found in the literature reviewed such as for *A. betulina* (buchu), a popular plant in current use. In this survey it was mentioned that this plant is useful to treat cancer but the literature consulted does not reflect this. This plant is regarded as a general health tonic and so could be useful in the prevention of cancers and other harmful diseases (van Wyk and Gericke, 2000). Another plant, which does not feature often, in the more current literature, is *C. scabrida*. Although not indigenous to South Africa, it is an African plant which has become naturalized in South Africa. It has a wide distribution and so is surprising that it is not more widely used. In this survey, uses such as for fever, diabetes, rheumatism and inflammation have been recorded as well as those mentioned in the literature: influenza, heart afflictions (Watt and Breyer-Brandwijk, 1962), and respiratory or chest problems (Watt and Breyer-Brandwijk, 1962; Smith, 1966; Hutchings, 1989; Hutchings et al., 1996) Perhaps this plant is one that should be investigated further. However, many bitter tasting herbs appear to be good for treating diabetes. Placing leaves and alcohol in a poultice on the affected area often treats inflammation and Watt and Breyer-Brandwjik (1962) suggest that any plant might have the same effect on inflammation so the relief may not be due to the plant but just to the poultice process. *C. scabrida* has also been reported to be good for convulsions, stomach and pleuritic pains, to hasten the placenta during childbirth (Watt and Breyer-Brandwjik, 1962) and for sprains and fractures (Hutchings, 1989) but none of these uses were mentioned in the study by any of the informants.

4.2. Outcomes from the semi structured questionnaires

The 16 most popular plants can be seen in Figure 1. This graph shows that *A. afra* and *R. graveolens* are the two most popular plants used by the people who participated in this worksheet. However, these two plants are the two plants that were mentioned by every person interviewed in the study, and the remaining plants also had a higher mention of use than any of the other plants in Table 1. Here 15 individuals agreed to participate by completing the worksheet but these were people that were the deemed the most knowledgeable on this matter by their peers and so the information acquired is from reliable sources. All the plants in this graph are easily found with the exception of *Gethyllis*, which perhaps explains why these are the most popular, and most often used plants.

In Figure 2., *A. afra* and *R. graveolens* are seen to treat a wide variety of conditions. This graph shows which plants are recommended to treat the specific conditions. The most widely treated conditions are arthritis, bladder and kidney trouble, diabetes, fever, and stomach trouble. These conditions would therefore have a wide variety of plants to treat them with. Figure 3 shows the calculated use-values generated from the data in figure 2 and shows which plants have the widest range of uses in the study. The two plants with the highest use-values are *A. afra* and *R. graveolens*, corresponding with the two most popular plants in figure 1. However, the ranking values and the use-values often do not correlate with each other such as in the case of *M. comosus* (ranking value of 7th place and use-value 0.6) and *L. leonurus* (ranked 9th with use-value 1.5). This is because *M. comosus* is only used externally and therefore only in the treatment of wounds and sores whereas *L. leonurus* is used for many ailments and so will have a higher use-value because it can be taken internally. A plant such as *M. comosus* was recommended by most people interviewed to treat wounds and sores whereas not everyone necessarily recommends plants such as *E. rhinocerotis*, *P. crispum* and *F. vulgare* to treat the same things and therefore this will affect the use-value. The use-value is therefore dependent not only on how many uses a particular plant has but also on how many people use it for that particular complaint.

Many of the same genera around the world are also used in traditional medicine: *Artemisia absinthium* is widely used in Europe as an appetite stimulant and to treat dyspepsia and gastritis (van Wyk and Wink, 2004). *A. annua* is used in Chinese medicine for a wide variety of ailments such as an antimalarial, a tonic, febrifuge and antibiotic (van Wyk and Wink, 2004). *Conyza sumatrensis* is used in Uganda to treat boils, amoebiosis, fungal infections, and insanity (Tabuti et al., 2003). A different *Conyza* species (*C. floribunda*) in Ecuador has been found to have significant anti inflammatory activity which justified its use in traditional medicine (de las Heras et al., 1998). Perhaps this means that *C. scabrida* used in the Bredasdorp / Elim area also contains the same anti inflammatory compounds which would justify the relevant uses of this plant by the people. Common household plants like potato garlic, thyme, and onions are widely used due to the accessibility of the plants. These are also popular plants used in medicines in places such as England (Brown, 2002). Onions are also used in Uganda but to treat diphtheria and snakebite (Tabuti et al., 2003). Onions have antibiotic and cholesterol lowering activity and are used to treat a wide range of ailments such as prevention of arteriosclerosis, coughs, colds and dysentery (van Wyk and Wink, 2004) and therefore can present a cheaper alternative to a visit to a doctor. Thyme (*Thymus vulgaris*) has antibiotic, expectorant and spasmolytic properties (Brown, 2002; van Wyk and Wink, 2004) which justify its use in treating colds and coughs.

4.3. Other outcomes of the survey

Many of the informants feel that knowledge has been lost because they remember their grandparents and parents using certain plants but cannot remember either the plant name or its use and now wish it had been recorded. This is one of the reasons these individuals wanted to help in this study because they feel that their knowledge is important and should be remembered. Many of the informants get their plant material from plants in their own gardens or along the verges of the main roads. Some plants are difficult to obtain such as *A. betulina* which does not grow in the area. This plant is bought in the supermarket when it is needed or obtained from acquaintances that live in areas where the plant grows naturally. *Gethyllis spp.* are very difficult to find and are very rare. Many of the people interviewed know a farmer who has these plants on his farm and ask permission to collect the material when they need it. The seed pods are usually placed in brandy and can be kept for a long time. The informants generally dry the plant material and store it in brown paper bags or in glass bottles or place material in alcohol. Plants should be collected around August and September after the rains and before flowering, this is when they are said to be at their best. The people interviewed all learned their knowledge of self-care using plants from their parents and / or grandparents and say they would not use the plants if they did not feel relief from the symptoms treated. Most of the information gathered was from women as has been found in similar studies looking at self-care using plant medicines(Gedif and Hahn 2003; Hernández et al., 2003) and all the informants were elderly people as was also found by Malamas and Marselos in 1992.

Many of the people interviewed do visit the local clinic for medication as sometimes they feel the medicine is more effective and that it works faster than some of the plant medicines. None of these people know of any nasty side effects from using the various infusions and treatments except that sometimes if too much is taken then the stomach may be affected. Children are given half the adult dosage (all dosages in the table are adult-sized dosages) but children generally do not like the bitter tasting plants and so are taken to the doctor. The plants are not really recommended for pregnant women, not only because they may prove harmful, but also mostly because the bitter tastes of the medicines can make the woman feel nauseous. The most widely used method of administration of these plant medicines is that of a tea. The teas are generally made with a handful of fresh or whole dried material or 1-2 teaspoons powdered material infused in boiling water

It was interesting that only 21 out of the 36 plant species used are indigenous to South Africa, this is surprising because of the large floral diversity in the study area and one would perhaps expect more fynbos species to be in use. Out of the indigenous plants, *Bulbine lagopus* and *Carpobrotus murii* are endemic to the area but do not appear to be under any collection pressure due to being able to be substituted with other species of their genus. The species of *Gethyllis* and *Drimia* used may also be endemic to the area however, as mentioned, they are hard to find and the former can be kept for a long time in brandy. The latter is not the only plant that can be used for sores and boils and so these plants are not under any collection pressure from the people interviewed. 12 out of the 36 species in use (33%) are species that have been introduced into South Africa. This perhaps has its advantages from a conservation point of view because although the indigenous flora is under threat from increasing agriculture and habitat destruction, it is at least not under threat from being over-collected as well. But it is a shame if past knowledge of local plant species has been forgotten in favour of the uses of the introduced plants.

5. CONCLUSION

These results show that many plants are still in use by mainly elderly women for medicinal purposes, although much of this knowledge has already been lost. Only 58% of the plants in use are indigenous to South Africa, 8.3% are naturalized African species and 33% are introduced species. It is surprising that not more of the indigenous flora is used for medicinal purposes considering the large floral diversity in the area. The main families represented were the Asteraceae, Lamiaceae, Alliaceae and Solanaceae. Families such as the Asteraceae, Lamiaceae and Solanaceae are often well represented in terms of species numbers in other surveys (Leporatti and Ivancheva, 2003; Tabuti et al., 2003). The popular South African medicinal plant *Agathosma betulina* was found to share uses reported in the literature but was also said to be good for treating cancer by several people in the study area. This could provide a reason for testing this plant for anti cancer activity which could prove beneficial should it show activity. Many of the other plants in use are the ones that are commonly used around South Africa and which have been shown to contain phytochemicals which justify their use by the people interviewed. *A. afra*, the most widely used plant, has been found to contain decongestant and antibacterial volatile oils as well as possessing analgesic and antihistamine properties (van Wyk et al., 1997) This justifies the use of the plant for treating many of the conditions listed by the informants such as colds, influenza and headache. This suggests that ethnobotanical surveys can be a reliable source of discovering (and perhaps even re-discovering) plants, as well as new uses of these plants, which deserve further investigation. Ethnobotanical studies can also be useful in preserving this type of knowledge for future generations.

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CHAPTER 4

PRELIMINARY ANTIMICROBIAL STUDIES ON FOUR PLANT SPECIES USED IN THE BREDASDORP / ELIM REGION FOR MEDICINAL PURPOSES

ABSTRACT

Four plant species used in the Bredasdorp / Elim region used for medicinal purposes were screened for their antimicrobial activity. The plant species screened were *Bulbine lagopus* (Asphodelaceae), *Chironia baccifera* (Gentianaceae), *Conyza scabrida* (Asteraceae) and *Dodonaea angustifolia* (Sapindaceae). Plant material was collected and extracted in ethanol, ethyl-acetate, methanol and water. The bioassays used were the disc-diffusion assay, the liquid dilution assay to determine the minimum inhibitory concentration (MIC) and the thin layer chromatography bioautography assay. The microorganisms used were *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans* and *Mycobacterium smegmatis*. In the disc-diffusion assay, 20 out of the 80 extracts showed activity. The best activity was observed with *C. scabrida* against *S. aureus* and *M. smegmatis*. The ethyl-acetate extracts showed the most activity in this assay with the ethyl-acetate extract *of B. lagopus* being the only active extract against *P. aeruginosa*. None of the extracts showed any activity against *C. albicans* in this assay. More activity was observed in the liquid dilution assay with all extracts showing a degree of activity. The best activity was observed in the ethanol extract of *B. lagopus* and the methanol extract of

C. scabrida both having an MIC value of 0.3125mg/ml. Only three extracts were found to be active against *P. aeruginosa* and 13 were active against *C. albicans*. For the TLC bioautography, *M. smegmatis* was chosen as the test organism along with the ethanol, ethyl-acetate and methanol extracts of *C. scabrida* and *D. angustifolia*. This was due to these plants being used to treat tuberculosis symptoms such as coughs and fevers. All six extracts showed at least two zones of inhibition on the TLC plates overlaid with *M. smegmatis*. In general it would seem that these plants are justified in their use in self-care by the people in the study area.

Keywords: Medicinal plants; disc-diffusion assay; minimum inhibitory concentration; Thin Layer Chromatography; bioautography

1. INTRODUCTION

1.1. Background

Due to increasing resistance by microorganisms to antibiotics in current use and the emergence of new infectious diseases, there is a need to look for new antimicrobial sources which have different chemical structures and therefore new modes of action to target these problems (Rojas et al., 2003). Herbal medicines can provide a cheaper and more readily available method of treatment. These treatments are encouraged by organizations such as the WHO (World Health Organisation) provided there is some scientific evidence to support the use of the treatment (Ankli et al., 2002). Developing countries particularly, face high mortality rates due to microbial diseases such as tuberculosis (TB) (Newton et al., 2002) and other microbial infections (Gnanamani et al., 2003). However, TB is becoming an increasing problem in developed countries as well because of the resistant strains of Mycobacterium that are emerging as well as patient non-compliance and people infected with HIV (human immunodeficiency virus) (Newton et al., 2002). Approximately one third of the world's population is infected with TB and an estimated 2-3 million deaths occur worldwide each year (Newton et al., 2002).

In South Africa, TB kills three in every thousand people, making it the highest rate in the world and one out of every 200 people suffer from active TB (Lall and Meyer, 1999). The Western Cape Province alone has one of the highest incidences of TB in the world (Salie et al., 1996). There is a need to find alternatives to treat this and other diseases caused by microbial infections in South Africa. In the literature there are reports where investigations into the ethnopharmacology of plants discovered via ethnobotanical means have shown to be successful in finding new pharmacological activity against TB as well as other microbial infections.

Southern Africa is home to over 30 000 plant species (almost 10% of the world's higher plants) (Cowling and Richardson, 1995; van Wyk and Gericke, 2000) and approximately 3000 of these species are used in medicines (van Wyk et al., 1997). South Africa is rich in floral biodiversity due to the large contribution by the Cape Floral Kingdom which alone contains nearly 9000 species and is said to be the most diverse temperate flora on earth and can compete with the tropical rainforests with regards to species richness (Cowling and Richardson, 1995; van Wyk et al., 1997). It is not surprising, with such a large number of plant species available that there has been a long history of medicinal plant use in South Africa and that there are still many people who rely on plants as a source of medicine. It was estimated in 1998 that there are over 27 million indigenous medicine consumers in South Africa (Mander, 1998) and that 70-80% of black people still consult with traditional medicine practitioners (Donaldson and Scott, 1994). Thus the study of traditional medicine or folk medicine can therefore be a good starting point for pharmaceutical research in Africa.

Many individuals still use plants as a source of medicine, using their own personal recipes which have been passed down from generation to generation. This is true for many people in the Bredasdorp / Elim region of the Western Cape Province in South Africa. From the results of the survey performed in this area, documented in Chapter 3, four plant species were chosen to undergo preliminary antimicrobial tests. In this survey, individuals who use plants in self care were interviewed to find out which plants were in use and the methods regarding their use and preparation. Thirty-six plant species out of 19 families were found to be in general use in the area. Many of these species in use are those which are commonly used in South Africa such as *Artemisia afra*, *Ruta graveolens*, *Elytropappus rhinocerotis* and *Sutherlandia frutescens*. However, some plant species were not found to be scientifically known for their antimicrobial activity and so it was decided to test some of these species based on both their use in the survey and reported uses in the literature. The species chosen were:

Bulbine lagopus (Thunb.) N.E. Br. (Asphodelaceae) (TSAT 011) common name "geel katstert" is used in the study area to treat wounds, sores and skin conditions. *Bulbine species* are reported to be used to treat burns, wounds and skin conditions (Watt and Breyer-Brandwijk, 1962; van Wyk et al., 1997).

Chironia baccifera L. (Gentianaceae) (TSAT 012) common name "aambeibossie" is used in the survey for stiff muscles and sore legs. Reported to be used to treat acne, sores and diarrhea (Watt and Breyer-Brandwijk, 1962; van Wyk et al., 1997).

Conyza scabrida DC. (Asteraceae) (TSAT 0013) common name "paddabossie" is used in the study area to treat chest, heart, fever, diabetes, rheumatism, colds, influenza and inflammation. Reported to be used to treat influenza, chest and stomach afflictions in the literature (Watt and Breyer-Brandwijk, 1962; Smith, 1966).

Dodonaea angustifolia L.f. (Sapindaceae) (TSAT 014) common name "ysterhouttoppe" is used in the study area to treat arthritis, bladder and kidney disorders, colds, influenza, convulsions, fever, inflammation, rheumatism and stomach complaints. Reported to be used in the treatment of fever, colds, influenza, stomach ailments and sore throats (van Wyk et al., 1997; Watt and Breyer-Brandwijk, 1962).

These plants were extracted and tested against the following four pathogens; *Mycobacterium smegmatis* an acid fast gram positive rod often used as a test model organism in the initial screening process before using *M. tuberculosis*. This organism has a similar drug sensitivity response to *M. tuberculosis* (Newton et al., 2002) but is non-pathogenic; *Staphylococcus aureus* a common gram positive pathogen which is responsible for wound infections, boils and a type of food poisoning (Singleton and Sainsbury, 1978); *Pseudomonas aeruginosa* a gram negative aerobic rod isolated from burns (Gnanamani et al., 2003) and urinary tract infections which is often resistant to antibiotics (Singleton and Sainsbury, 1978); and *Candida albicans*, a yeast which causes thrush, vaginitis, bronchocandidiasis and systemic infections (Singleton and Sainsbury, 1978).

The aim of this study was to investigate these plant species to determine whether or not they contained any antimicrobial activity and to determine whether the ethnobotanical approach to finding anti-infectives is a useful approach, as the literature evidence suggests, even in a small-scale survey such as this one.

1.2. Evidence to support plants as a source of antimicrobials

There are many reports in the literature from many countries around the world where researchers are looking at traditional plant use as a platform for pharmacological testing. With regard to TB research, plants are being studied as sources of antimycobacterial drugs. Lall and Meyer (1999) chose 20 plant species reported to be used by South African people to treat TB symptoms such as fever and cough and tested them against drug-resistant and drug-sensitive strains of *Mycobacterium tuberculosis*. Plant extracts from six plant species were found to be active against the resistant strain of TB indicating that some plants may be of use in the future for fighting TB. This study also backs up certain plants being used in traditional medicine for the treatment of TB. In a similar survey, 43 plant species were chosen to be tested for antimycobacterial activity on the basis of being used in traditional medicine to treat TB and leprosy or the symptoms of these diseases (Newton et al., 2002). Two *Mycobacterium* strains were used; *M. smegmatis* and *M. aurum*. Activity was found in five plant species against *M. aurum* and in two species against *M. smegmatis* in the initial screening. This study also isolated some important compounds and highlighted certain plant species which require further investigation using *M. tuberculosis*. Perhaps the reason for only seven plant species showing activity in this study is because although the other plants are used to treat coughs or other TB related symptoms, the symptoms could be caused by other diseases (Lall and Meyer, 1999). Four species of the Asteraceae indigenous to South Africa were screened against *M. smegmatis* in a study in the Western Cape. Out of the four species, two were found to have antimycobacterial activity and one of these plants in particular, *Helichrysum crispum*, is reported to be used to treat coughs, bronchitis and tuberculosis and so is justified in its use in traditional medicine (Salie et al., 1996).

In México, infectious gastrointestinal diseases form one of the ten most important causes of death in the rural areas (Hérnandez et al 2003). Due to Western drugs either being too expensive or not available locally, many people visit traditional medicine practitioners to obtain treatment for these infections (Heinrich et al., 1992; Hérnandez et al., 2003). An ethnobotanical study performed in a village in México (Hérnandez et al., 2003) revealed that 74% of the informants used plants to treat gastrointestinal disorders. Two plants, *Lippia graveolens* and *Lantana achyranthifolia*, were mentioned most frequently as treatments and were found to have the best antibacterial activities with Minimum Inhibitory Concentrations (MIC) less than 0.30mg/ml. These findings justify the use of these plants in traditional medicine (Hérnandez et al., 2003). Another study (Heinrich et al., 1992) found that the criteria for the selection of medicinal plants in a Mixé community in México were not irrational and that many of the plants used did provide the desired effect. Many of the plants used in this study to treat gastrointestinal diseases are plants which have astringent or essential oil components which act in similar ways to the tannin-containing drugs administered to treat diarrhea and dysentery (Heinrich et al., 1992).

Recognition of the need to study traditional knowledge in the hope of finding new compounds is also evident in other countries. In Peru (Rojas et al., 2003; Neto et al., 2002) ethnobotanical surveys have lead to preliminary screening of plant extracts used in traditional medicine which have been found to have antimicrobial activity. Rojas et al. (2003) found antimicrobial activity in 25 out of 36 ethanol extracts. Here the extracts were tested against eight pathogens including *S. aureus* (inhibited by 15 extracts), *P. aeruginosa* (inhibited by 13 extracts) and *C. albicans* (inhibited by 13 extracts). Plants were chosen on the basis of being used in traditional medicine for the treatment of infectious or inflammatory diseases and the results of this study support the folkloric use of these plants (Rojas et al., 2003).

In developing countries, approximately 50-75% of hospital deaths are caused by nosocomial infections (Gnanamani et al., 2003). Injuries such as burns are of great concern because of the susceptibility of these wounds to bacterial infections. In India several plant species chosen for their medicinal uses were tested against eight burn pathogens including *S. aureus* and *P. aeruginosa* and were found to have antimicrobial activity that may help in wound management (Gnanamani et al., 2003). Similarly, in a survey performed in the Eastern Cape province of South Africa found that some plants used by people in the treatment of wounds were justified by testing these plants against microbes (Grierson and Afolayan, 1999). The information was gathered using questionnaires and approaching local people, healers and *Sangomas* (spiritual healers) and four plants were found to be recommended for the treatment of wounds. These were the plants chosen and extracted and tested against 10 microbes including *S. aureus* and *P. aeruginosa*. The results showed that the use of some of these plants in traditional medicine is justified (Grierson and Afolayan, 1999).
There are several other sources of in the literature where African plant extracts have been chosen through ethnobotanical means and which exhibit antimicrobial activity (Baba-Moussa et al., 1999; Sindambiwe et al., 1999). Although traditional medicine is linked with superstition, there can be no doubt that by studying these systems, be it

from healers or from ordinary people, information can be provided which may lead to new developments in the treatments of various diseases.

2. METHODOLOGY

2.1. Plant material

The four plant species were collected in the Bredasdorp area in September 2002 as recommended by the individuals interviewed in the survey. It was said that the plants should be collected after the rain but before flowering and that was when the plants were at their best. The plant species were verified by taxonomist Mr. Frans Weitz and voucher specimens prepared and deposited in the Herbarium at the University of the Western Cape. The plant material was washed with distilled water prior to being dried in an oven at 40°C for approximately 72 hours with the exception of the *Bulbine lagopus* material which was kept fresh and extracted immediately. The dried plant material consisting of leaves and stems from the other three species was coarsely ground in a pestle and mortar in a similar way to how the plant material is used by the people in the survey.

2.2. Solvent extraction

Five different extracts for each plant were prepared using four solvents; methanol, ethanol, ethyl-acetate and distilled water. In each case 10g of dried material in 100ml of solvent or 20g fresh material in 200ml of solvent was extracted. Two aqueous extracts were prepared for each plant, one being a "tea" where the dried material was placed in a flask and boiling water poured over and left to steep. This is a very common way of preparing plant material by the people interviewed. Another method was to place the material in water and boil it on a hotplate for 5-10 minutes as was suggested by some people in the survey. These aqueous extracts were left to cool overnight before being filtered under vacuum the following day. The filtrate was then frozen at -70°C for 48 hours before being freeze-dried to form a powder. For the ethanol, methanol and ethyl-acetate extracts, the plant material was placed in flasks with the solvent and then sonicated in an ultrasound bath for 30 minutes. These extracts were left overnight then sonicated the next day for 30 minutes before being filtered under vacuum. The filtrate was then evaporated to dryness in a rotary evaporator before being frozen at -70°C for 48 hours prior to being freeze dried to produce a powder. The yields of all the extracts could then be determined and stored at 4°C in glass vials for use in the antimicrobial bioassays.

2.3. Antimicrobial screening

2.3.1. The disc-diffusion method

The microorganisms used were *Staphylococcus aureus* (ATCC 29213), *Pseudomonas aeruginosa* (ATCC 27853), *Candida albicans* (ATCC 10231) and *Mycobacterium smegmatis. M. smegmatis* was given as a gift from Professor Paul van Helden of the Department of Biochemistry and Physiology at the University of Stellenbosch Medical School. The microbes were obtained from the Department of Medical Biosciences at the University of the Western Cape. The disc diffusion assay as described in Salie et al (1996) was used as a preliminary screening to observe antimicrobial activity. The dried plant extracts were resuspended to 20mg/ml in their respective solvents and sonicated to dissolve and sterilize the extracts. Then sterile 9mm discs (Schleicher and Schuell) were impregnated with 50µl of extract to yield 1mg extract per disc. The discs were placed in sterile Petri dishes in an incubator (37°C) to allow the solvents to evaporate leaving only plant extract. Negative controls were prepared in the same way but using 50 µl of pure solvent on sterile discs. Ciprofloxacin (40µg/disc) was used as a positive control for *S. aureus*, *P. aeruginosa* and *M. smegmatis* and amphotericin B (25 µg/disc) was the positive control for *C. albicans*. Nutrient agar plates (Bacto nutrient agar) were prepared for *S. aureus*, *P. aeruginosa* and *C. albicans* while Middlebrook 7H11 agar plates were prepared for *M. smegmatis*.

The sterile, dry discs were then placed on the surfaces of the agar plates inoculated with a microbial culture and each extract was tested in triplicate. The plates were incubated at 37°C for 24 hours for *S. aureus*, *P. aeruginosa* and *C. albicans* and for 48 hours for *M. smegmatis*. After incubation the distances of the zones of inhibition were recorded.

2.3.2. Minimum inhibitory concentrations (MIC)

This was based on the liquid dilution method of Rios et al. (1988) and the microplate method by Eloff (1998). Each extract was resuspended to 50mg/ml in sterile distilled water and sonicated to sterilize it. Ninety-six well microtitre plates were used and each extract was made up to 100µl in each well using distilled water to make up six concentrations (5, 2.5, 1.25, 0.625, 0.3125 and 0.15625 mg/well). These were then inoculated with 100µl of a three hour microbial culture grown in nutrient broth at 37°C. Each extract was again tested in triplicate. Distilled water controls inoculated with the test organism were used to ensure the viability of the test organism. To be certain of no other microbial contamination, wells containing only extracts and distilled water were also used as controls. The plates were then sealed with parafilm to prevent evaporation, tapped gently to mix the well contents and incubated for 24 hours (for *S. aureus*, *P. aeruginosa* and *C. albicans*) and for 48 hours for *M. smegmatis*. After the incubation period was over, 40μl of 0.2mg/ml 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT or thiazolyl blue) dissolved in distilled water was added to each well. The extracts were then incubated for a further 30 minutes or until a colour change was noticeable then incubated for another 8 hours. MTT is a tetrazolium salt commonly used in bioassays because it indicates biological activity. The salt acts as an electron acceptor and MTT is reduced to a purple-blue coloured product by biologically active organisms (Eloff, 1998).

2.3.3. Thin layer chromatography bioassay

This bioassay was performed with the help of Evan Springfield in the South African Traditional Medicines Research Group at the School of Pharmacy at the University of the Western Cape. This method is based on that of Slusarenko et al. (1989). Ethanol, methanol and ethyl-acetate extracts of *C. scabrida* and *D. angustifolia* were tested in this bioassay due to having the best overall activity in the above assays. Extracts were resuspended to a concentration of 50mg/ml in methanol and 30μl applied to 5 x 20cm silica F254 (Merck) glass TLC plates. The plates were developed in the organic phase of 1.75M Acetic acid: Ethyl-acetate : Toluene (1:1:1) which was the solvent system that gave the best separation out of the various solvent systems attempted. The plates were dried overnight and then inoculated with the test organism *M. smegmatis* in Middlebrook 7H11 agar. Blank plates were also inoculated to ensure that the test organism was viable and not sensitive to the solvent system. The plates were incubated at 37°C for 48 hours then were sprayed with 0.2mg/ml MTT to observe any zones of inhibition. The Rf values for the zones of inhibition were calculated and the measurements taken from the centre point of each zone. The zone sizes were also measured. From reference plates which were also prepared for each extract, the Rf values of each compound seen under UV light were also calculated (Tables 3a-f).

3. RESULTS

All the plant extracts tested showed a degree of antimicrobial activity. The results from the bioassays are tabulated in Tables 1,2 and in the figures accompanying Table 3.

3.1. The disc-diffusion bioassay

In the disc-diffusion bioassay, the best activity was observed in the ethyl-acetate extracts followed by the methanol extracts. The most activity was found against *S. aureus* and *M. smegmatis* with no activity against *C. albicans* and only the ethyl-acetate extract of *B. lagopus* showed any activity against *P.aeruginosa*. The plant which showed the most activity was *C. scabrida*. This plant showed particularly good activity against *M. smegmatis* with the biggest zones of inhibition being observed for this microorganism (Table 1). It was decided from these results to re-test all the plant extracts using the liquid dilution method which has been found to be more sensitive (Eloff, 1998).

3.2. The Minimum Inhibitory Concentration (MIC) Bioassay

This bioassay is more sensitive as more activity was recorded (Table 2). Each plant extract showed more activity in this assay. In this case, the methanol extracts showed the most activity followed by the ethyl-acetate extracts. The ethanol and aqueous extracts each showed a degree of activity in nine extracts. All but two extracts (*C. baccifera* aqueous extracts) showed activity against *M. smegmatis*. The lowest MIC value was found in the *B. lagopus* ethanol and the *C. scabrida* methanol extracts, both being active at 0.3125 mg/ml against *M. smegmatis*. The second best activity was observed against *S. aureus*, with all but three extracts demonstrating activity. In this bioassay, *C. albicans* was inhibited by 13 out of the 20 extracts. The aqueous infusion of *C. scabrida* showed good inhibition of this yeast with a MIC value of 0.625 mg/ml. *P. aeruginosa* proved to be the most difficult microbe to inhibit with only three extracts showing activity in this bioassay.

3.3. Thin Layer Chromatography (TLC) and bioautography

All six plant extracts tested in this bioassay showed activity by means of white zones of inhibition in the purple background observed after spraying plates with MTT (Figures 1-6 and Tables 3a-f). The plates from *C. scabrida* ethanol, methanol and ethyl-acetate extracts and *D. angustifolia* ethanol extract all showed three zones of inhibition against *M. smegmatis* (Figures 1-4). The remaining two *D. angustifolia* extracts showed two zones of inhibition (Figures 5 and 6). Particularly good activity with regard to zone size was observed on the TLC plate for *C. scabrida* ethanol extract (Figure 1).

Table 1. Results from the disc diffusion assay showing the antibacterial activity of the 20 extracts tested against the four microorganisms

Plant	Extract	Growth inhibition activity			
		S. a	P. a	C.a	M.s
Bulbine lagopus	Aqueous boiled	-	-	-	-
	Aqueous infusion	-	-	-	-
	Ethanol	-	-	-	1+
	Ethyl-acetate	1+	2+	-	1+
	Methanol	-	-	-	-
Chironia baccifera	Aqueous boiled	-	-	-	-
	Aqueous infusion	-	-	-	-
	Ethanol	-	-	-	1+
	Ethyl-acetate	-	-	-	1+
	Methanol	-	-	-	-
Conyza scabrida	Aqueous boiled	1+	-	-	-
	Aqueous infusion	1+	-	-	1+
	Ethanol	2+	-	-	3+
	Ethyl-acetate	1+	-	-	3+
	Methanol	1+	-	-	3+
Dodonaea angustifolia	Aqueous boiled	1+	-	-	-
	Aqueous infusion	1+	-	-	-
	Ethanol	1+	-	-	-
	Ethyl-acetate	-	-	-	-
	Methanol	1+	-	-	1+

S.a – *Staphylococcus aureus*
P.a – *Pseudomonas aeruginosa*
C.a – *Candida albicans*
M.s – *Mycobacterium smegmatis*
- indicates no inhibition, while 1+ = 0.5 – 1mm ; 2+ = 1-2mm ; 3+ = 2-4mm represents the size of the zones

Table 2. Minimum inhibitory concentration (MIC) results of the 20 extracts tested against the four microorganisms

Plant	Extract	MIC (mg/ml)			
		S. a	P. a	C.a	M.s
Bulbine lagopus	Aqueous boiled*	5	-	5	5
	Aqueous infusion*	5	5	5	5
	Ethanol	2.5	-	-	0.3125
	Ethyl-acetate	5	5	-	5
	Methanol	5	-	5	2.5
Chironia baccifera	Aqueous boiled*	-	-	5	-
	Aqueous infusion*	-	-	-	-
	Ethanol	-	-	-	2.5
	Ethyl-acetate	5	-	5	5
	Methanol	1.25	1.25	1.25	1.25
Conyza scabrida	Aqueous boiled*	2.5	-	1.25	1.25
	Aqueous infusion*	1.25	-	0.625	0.625
	Ethanol	2.5	-	5	5
	Ethyl-acetate	0.625	-	2.5	5
	Methanol	5	-	5	0.3125
Dodonaea angustifolia	Aqueous boiled*	5	-	-	5
	Aqueous infusion*	5	-	-	5
	Ethanol	2.5	-	2.5	1.25
	Ethyl-acetate	5	-	-	5
	Methanol	2.5	-	1.25	1.25

S.a – *Staphylococcus aureus*
P.a – *Pseudomonas aeruginosa*
C.a – *Candida albicans*
M.s – *Mycobacterium smegmatis*

*Students "t" test was performed between the two water extracts. There was no significant difference found between the activity of the two types of extracts:
Pooled $s^2 = 5.5436$
$t_{30} = 0.8212 \pm 8.3\%$ (ns) ; $P > 0.05$

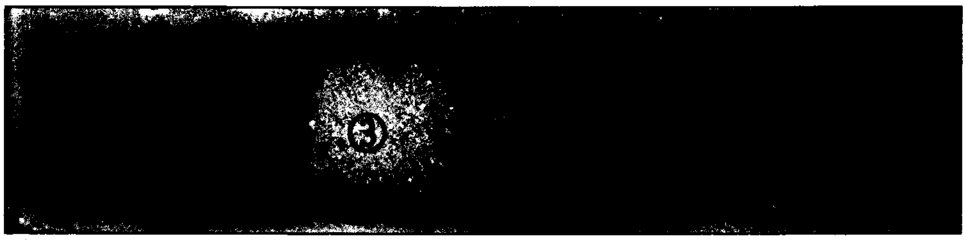

Figure 1. TLC plate of *C. scabrida* ethanol extract showing 3 zones of inhibition

Table 3a. Bioautography results for *C. scabrida* ethanol extract

Rf value of Zones of inhibition	Sizes of zones (LXBmm)	Rf values of bands within zones of inhibition	Band colours under UV 366nm	Visible band colours	Band colours under UV 254nm
1. 0.05	12x15	0.05	N/v	N/v	Light brown
2. 0.40	19x30	0.36 0.48	Light brown Brown	Yellow Yellow	Brown Red
3. 0.57	28x29	0.54 0.58 0.63	Purple/brown Cream N/v	N/v N/v N/v	N/v N/v Grey

N/v: not visible

Figure 2. TLC plate of *C. scabrida* methanol extract showing 3 zones of inhibition

Table 3b. Bioautography results from *C. scabrida* methanol extract

Rf value of Zones of inhibition	Sizes of zones (LXBmm)	Rf values of bands within zones of inhibition	Band colours under UV 366nm	Visible band colours	Band colours under UV 254nm
1. 0.05	10x20	0.05	N/v	N/v	Grey
2. 0.38	20x30	0.35 0.40 0.41	Grey Orange Purple	Cream Cream Yellow	Dark grey N/v Purple/red
3. 0.60	12x20	0.6	N/v	N/v	Light grey

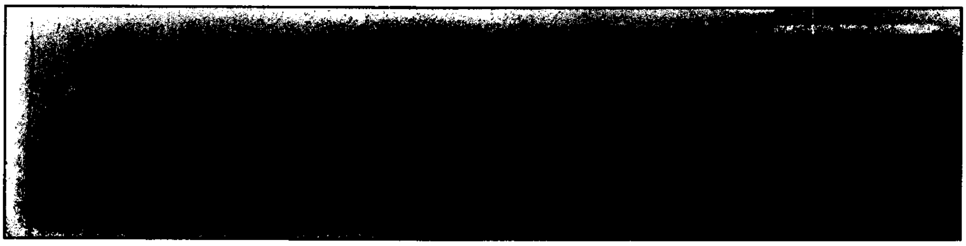

Figure 3. TLC plate of *C. scabrida* ethyl-acetate extract showing 3 zones of inhibition

Table 3c. Bioautography results from *C. scabrida* ethyl-acetate extract

Rf value of Zones of inhibition	Sizes of zones (LXBmm)	Rf values of bands within zones of inhibition	Band colours under UV 366nm	Visible band colours	Band colours under UV 254nm
1. 0.05	12x21	0.05	N/v	Brown	N/v
2. 0.39	25x30	0.33 0.36 0.39 0.43	Brown Orange Brown N/v	Pale yellow N/v Yellow N/v	Purple Red/purple Brown
3. 0.60	7x26	0.6	N/v	N/v	Grey

Figure 4. TLC plate of *D. angustifolia* ethanol extract showing 3 zones of inhibition

Table 3d. Bioautography results from *D. angustifolia* ethanol extract

Rf value of Zones of inhibition	Sizes of zones (LXBmm)	Rf values of bands within zones of inhibition	Band colours under UV 366nm	Visible band colours	Band colours under UV 254nm
1. 0.38	5x25	0.38	Light blue	N/v	Violet
2. 0.42	8x25	0.40 0.42	Red Light blue	Green Cream	N/v Violet
3. 0.61	13x30	0.61	Black	Yellow	Purple

Figure 5. TLC plate of *D. angustifolia* methanol extract showing 2 zones of inhibition

Table 3e. Bioautography results from *D. angustifolia* methanol extract

Rf value of Zones of inhibition	Sizes of zones (LXBmm)	Rf values of bands within zones of inhibition	Band colours under UV 366nm	Visible band colours	Band colours under UV 254nm
1. 0.46	10x30	0.40	Red	N/v	Brown
		0.46	Purple	N/v	Grey
		0.48	Green	Yellow	N/v
2. 0.68	25x34	0.63	Purple	N/v	N/v
		0.68	Orange	Pale yellow	Purple

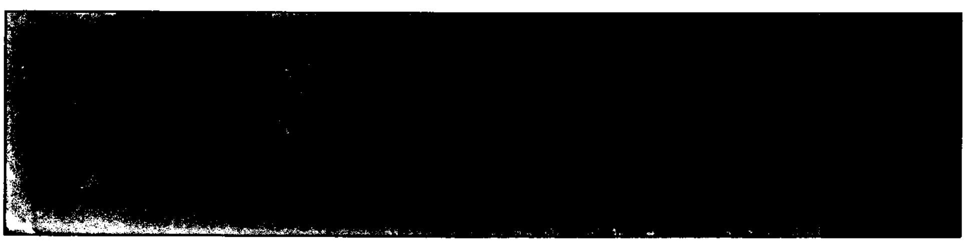

Figure 6. TLC plate of *D. angustifolia* ethyl-acetate extract showing 2 zones of inhibition

Table 3f. Bioautography results from *D. angustifolia* ethyl-acetate extract

Rf value of Zones of inhibition	Sizes of zones (LXBmm)	Rf values of bands within zones of inhibition	Band colours under UV 366nm	Visible band colours	Band colours under UV 254nm
1. 0.45	18x20	0.41	Pink	N/v	N/v
		0.44	Blue	Yellow	N/v
		0.46	Green	Yellow	Brown
2. 0.66	19x20	0.60	N/v	Yellow	Purple
		0.62	Purple	Yellow	Red
		0.69	Red	N/v	Brown

4. DISCUSSION

4.1. The Disc-diffusion and Minimum Inhibitory Concentration Bioassays

4.1.1. Methanol and ethyl-acetate extracts

Methanolic extracts have been shown in the literature to have good antibacterial activity particularly against gram +ve microbes (Rabe and Van Staden, 1997; Grierson and Afolayan, 1999). Martìnez-Vásquez et al. (1999) found that ethyl-acetate extracts showed the best activity in the disc diffusion assay against all the bacteria (both Gram positive and Gram negative) including *P. aeruginosa* and *S. aureus* possibly because of a broad spectrum of antibiotic compounds being present. This is seen in this study for the *S. aureus* MIC results. Here, both the ethyl-acetate and methanol extracts show activity against this organism. However, *P. aeruginosa* was only inhibited by the ethyl-acetate extract of *B. lagopus* and by the methanolic extract of *C. baccifera*. Martìnez-Vásquez et al. (1999) also found that *S. aureus* was the most easily inhibited by ethyl-acetate and methanol extracts. *P aeruginosa* was also among the microbes which were harder to inhibit. This was also observed by Sindambiwe et al. (1999) where none of the plant extracts were active against any of the gram negative bacteria. All the methanol extracts exhibited a degree of activity against *C. albicans* in the MIC or liquid dilution assay. It was found that *C. albicans* was more difficult to inhibit with methanol and ethyl-acetate extracts (Baba-Moussa et al., 1999; Sindambiwe et al., 1999; Rojas et al., 2003). The best activity was shown by the methanol extract of *C. scabrida* which inhibited *Mycobacterium smegmatis* at a concentration of 0.3125 mg/ml. This extract deserves further examination with *M. tuberculosis*.

4.1.2 Aqueous extracts

Due to most of the plants being used in the form of teas or infusions it was valuable to test aqueous extracts of these plants. Although the aqueous extracts did not perform well in the disc-diffusion assay, they did however show better activity in the MIC bioassay. This was true for many extracts which did not show any or hardly any activity in the disc-diffusion assay. This is most likely because the MIC assay is far more sensitive due to being in liquid so that the extract is in contact with all the inoculant at one time, unlike the disc-diffusion assay where it is only able to have an effect on the surrounding microbes. It would seem by looking at the figures (Table 2) that the boiled aqueous extracts show better activity than the extracts made as an infusion but the data was tested using "Students t test" (Table 2 footnotes) and there was no significant difference found between the activity of the two water extracts. Seventy-six percent of the activity observed in the water extracts was at a concentration of 5mg suggesting that a higher concentration of extract is needed to find activity because in the disc diffusion assay only 1mg of extract was present on a disc which perhaps was not strong enough. Aqueous extracts have been found in the literature to demonstrate less activity in these bioassays than extracts such as methanol (Rabe and Van Staden, 1997; Grierson and Afolayan, 1999).

4.1.3. Ethanol extracts

Placing the plant material either in alcohol (such as brandy) or sprinkling the material with alcohol before making a poultice are other ways in which the people in the survey prepare the plants for use. Due to this, it was decided to extract the plant material in ethanol. This is also an extractant reported in the literature (Gnanamani et al., 2003; Rojas et al., 2003). All the ethanolic extracts in the MIC bioassay showed activity against *M. smegmatis* whilst none showed any activity against *P. aeruginosa*. In the disc-diffusion assay, none of the ethanolic extracts was able to inhibit *C. albicans* whereas in the MIC assay, two out of four extracts (*C. scabrida* and *D. angustifolia*) showed activity. This reiterates the sensitivity of the liquid dilution method as opposed to the disc-diffusion method (Eloff, 1998). The lowest MIC value was recorded with the *B. lagopus* ethanol extract (0.3125 mg/ml) against *M. smegmatis*. This plant may be potentially toxic against *M. tuberculosis*. This plant is not used for TB symptoms such as coughing or fevers in the study area, however, if it did prove useful in TB therapy, this would confirm that testing plants used medicinally (as opposed to random screening), is a good way of finding new compounds.

4.2. Thin layer chromatography and bioautography

Ethanol, methanol and ethyl-acetate extracts were chosen in this assay. The solvent system used here did not give adequate separation of the aqueous extracts and the solvent systems which did, were not suitable for this assay because the test organism, *M. smegmatis*, was sensitive to them. In the thin layer chromatography bioassay, the methanol and ethyl-acetate extracts gave very similar results for both *C. scabrida* and *D. angustifolia*. The results showed that there are at least three compounds present in the *C. scabrida* methanol and ethyl-acetate extracts (Tables 3b and c). Three zones of inhibition could be seen on both plates and in the bigger zones more than one compound can be found. Similarly, for *D. angustifolia* the methanol and ethyl-acetate plates (Tables 3e and f) show two zones of inhibition with more than one compound within each zone. The ethanol extracts from both *C. scabrida* and *D. angustifolia* both showed three zones of inhibition (Tables 3a and d). The large zone (zone number 3) in Figure 1 appears to be made up of three compounds one or more of which could be responsible for the activity. These results suggest that these plants be investigated further as possible candidates for the treatment of TB and TB related symptoms.

4.3. Possible explanations for activity

Dodonaea angustifolia and *Conyza scabrida* are used to treat symptoms associated with colds and influenza such as chest conditions and the plant material is made into a tea and drunk. All the aqueous extracts tested against *M. smegmatis* were active in the liquid dilution assay. Due to being active against *M. smegmatis,* it is possible that *C. scabrida* and *D. angustifolia* may show activity against other Mycobacteria such as *M. tuberculosis* which would justify the use of these plants by the people in the study due to one of the symptoms of TB being coughing. *D. angustifolia* contains diterpenoids (van Wyk et al., 1997) which are compounds which exhibit antimicrobial and antifungal activity (van Wyk and Wink, 2004) which may explain the activity seen in the extracts of this plant. Saponins are found in expectorant drugs for treating chest conditions such as bronchitis (Bruneton, 1995; van Wyk and Wink, 2004) and it is possible that this plant contains saponins (foaming was observed during extraction procedure suggesting the presence of saponins) which maybe contribute to the plant being used for chest afflictions. Flavonoids have been reported in *C. scabrida* (El-Karemy et al., 1987) and these compounds have been found to be the active ingredients in many plant-based drugs (Bruneton, 1995; van Wyk and Wink, 2004). They are protein modifying compounds because they interact with proteins to form hydrogen bonds and can therefore control a number of molecular targets (van Wyk and Wink, 2004). Flavonoids have antibacterial activity as well as anti-inflammatory, antiviral, antioxidant and anti-allergenic properties (Bruneton, 1995; van Wyk et al., 1997). The antimicrobial activity exhibited by *C. scabrida* in the bioassays could be attributed to these flavonoids which are present in this plant.

P. aeruginosa proved to be the most difficult of the bacteria to inhibit with only one extract (*B lagopus* ethyl-acetate) being active against it in the disc diffusion assay and three extracts (ethyl-acetate and aqueous infusion of *B lagopus* and the methanolic extract of *C. baccifera*) showing inhibition in the microplate assay. Perhaps this helps to justify the use of these plants externally by people considering that this is a bacterium which is found in wounds such as burns. However this would only apply to the one active aqueous extract of *B. lagopus* because the people do not use ethyl-acetate or methanol in the preparation of the plant material and so those compounds are not necessarily present in their decoctions. *B. frutescens* and other *Bulbine* species are said to contain anthraquinones such as chrysophanol which has antibacterial properties (van Wyk et al., 1997) and so these compounds could be responsible for the antibacterial activity exhibited *in vitro*. The healing effect is said to be likely due to glycoproteins such as aloctin A and B (van Wyk et al., 1997). So perhaps just by cleansing the wound and then applying leaf sap directly is enough to heal the wound because the layer of leaf gel helps to keep the wound clean while the glycoproteins aid in the healing process. *C. baccifera* can be used externally in the treatment of sores and is a bitter plant but not much is know about the healing or antibacterial properties of this plant (van Wyk et al., 1997). *C. krebsii* has antiviral activity against the *Herpes simplex* virus (van Wyk and Gericke, 2000). This is one of the plants that also showed activity against *C. albicans* and, as antifungal drugs are very expensive to make, this plant is perhaps worth investigating further.

5. CONCLUSION

The plants tested here all showed activity against the various organisms and deserve further examination. It could be beneficial to isolate the active principles of the two species tested in the TLC bioassay and perhaps to take this bioassay further and use the remaining microbial strains. It may also be useful to test *B. lagopus* and *C. baccifera* in the TLC bioautography assay and isolate the active compounds. *In vivo* testing should also be considered to observe the activity of the extracts in a living system. *C. scabrida* in particular showed the most activity particularly against *M. smegmatis*. As this plant is used to treat chest related conditions and fever (amongst other uses) which are symptoms relating to TB, this plant is a potential candidate for testing against *M. tuberculosis* and the people in the study area are certainly justified in its use. From the results shown, it can be seen that antimicrobial activity can be discovered via ethnobotanical means and that plant uses in traditional medicine can be justified in certain cases.

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CHAPTER 5

DISCUSSION AND CONCLUSION

5.1. DISCUSSION

The discovery of useful compounds from plants entails three disciplines; ethnobotany, ethnomedicine and ethnopharmacology. In Chapter 3, the plants used by the people in the study area were studied (ethnobotany) and their uses, dosages and preparations (ethnomedicine) were documented. Then in Chapter 4, preliminary antimicrobial testing found antimicrobial activity in four plant species chosen from the survey (ethnopharmacology). These three disciplines have formed the basis for this thesis.

In Chapter 3, the survey determined that 36 plant species from 19 families are currently in use in self-care in the study area. This information was gathered using questionnaires and the uses, dosages and preparations of each plant was documented (Chapter 3, Table 1). From the results of the survey, it is evident that knowledge regarding medicinal plants is being lost and that this could affect the discovery of new antimicrobial compounds. Knowledge regarding the medicinal uses of plants is being lost because it is no longer being passed down from generation to generation because younger generations are not interested in learning these things. This has been conveyed by many of the informants in the interviews who say that their children and grandchildren would rather visit a western doctor than take a herbal remedy. These people also mentioned that they had forgotten a great deal of information because they had not written it down. It was also felt that a great deal of knowledge had been lost because particular individuals who were renowned for their plant remedies had died. Many of these people expressed that this survey should perhaps have been performed earlier to gather information from these people before they had passed away. It is possible, then, that these deceased individuals may have held valuable information which could have provided new clues for discovering new treatments. This provided the answer "yes" to the research question which asked whether medicinal plant knowledge systems were declining and if this could impede new antimicrobial compounds from plants being discovered.

Having answered the research question, the hypothesis can also be confirmed. The hypothesis stated that the knowledge of these medicinal plants was declining due to not being passed down and that the plant species used medicinally contain potential antimicrobial compounds. Many of the plants in use in this area have been found to have antimicrobial activity such as *Artemisia afra*, *Carpobrotus edulis*, *Helichrysum* species, *Tulbaghia violacea* and *Vernonia* species (van Wyk et al, 1997). The four plants *Bulbine lagopus*, *Chironia baccifera*, *Conyza scabrida* and *Dodonaea angustifolia* were tested in this study as potential antimicrobials and all four plants exhibited a degree of antimicrobial activity (Chapter 4). This helps to confirm the hypothesis because a plant such as *B. lagopus* is an endemic plant and there is no mention of this plant and its antimicrobial properties in the consulted literature which suggests the knowledge of this plant is also confined to this area of study. If the information from the people who use this plant is not documented, and these individuals die, then this knowledge is lost.

The majority of the knowledge was found to be known by the women in the community as has been found in similar studies (Gedif and Hahn, 2003; Hernández et al., 2003). Most of these women were elderly and had learnt their knowledge of plants from their parents and grandparents as was also noticed by Malamas and Marselos (1992). Most of the plants in use in the study area are ones that are popular in plant based medicines around South Africa such as *Artemisia afra*, *Mentha longifolia* and *Ruta graveolens* and these share common uses found in the literature. These common threads can also aid in the justification of plants used in herbal medicines if the same uses for certain plants match up with the uses in the literature. If a researcher is looking for a plant to investigate for a specific purpose and it can be seen in several independent studies that a particular plant is used repeatedly to treat that condition then this plant may turn out to be a worthy candidate for investigation. In the same instance, there is reason to promote the use of this plant in traditional medicine because it is widely used to treat the same condition or conditions.

The families comprising the largest number of plant species were the Asteraceae (six species) and the Lamiaceae (six species) and the Alliaceae and Solanaceae (both with three species each). The Asteraceae has been found to contain many plants used in traditional medicine (Amusan et al., 2002; Tabuti et al., 2003) as have the Lamiaceae and Solanaceae (Gedif and Hahn., 2003). Plants from the Alliaceae such as garlic are widely used around the world particularly in Europe for medicinal as well as culinary purposes (Brown, 2000). If certain plant families appear to be used more for particular conditions then surveys such as this one may act as a guide not only for choosing plants used medicinally to be screened but also for the random screening of plants.

Chapter 4 dealt with this aspect of the thesis. Four plants; *Bulbine lagopus*, *Chironia baccifera*, *Conyza scabrida* and *Dodonaea angustifolia* were chosen for preliminary screening against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans* and *Mycobacterium smegmatis* using the disc-diffusion and liquid dilution assays. The chosen plants were extracted in water, ethanol, ethyl-acetate and methanol. Water was used because most of the people use the plants in water as a tea and ethanol because in some cases the plant material is placed in alcohol. Methanol and ethyl-acetate extracts were also prepared because of good activity reported in the literature. The best activity was found in the methanol and ethyl acetate extracts. This has been seen in other experiments for example; methanol (Rabe and Van Staden, 1997; Grierson and Afolayan, 1999) and ethyl-acetate (Martínez-Vázquez et al., 1999).

Some of the uses of the plants chosen can begin to be justified with the preliminary bioassays used. *Conyza scabrida* is the plant which gave the best activity in the bioassays and was especially active against *Mycobacterium smegmatis*. The aqueous infusion (made as a tea) extract for this plant showed good activity against this bacterium. This plant is drunk as a tea and is used to treat chest conditions and fever, among other uses, which are symptoms associated with tuberculosis. It is a plant which perhaps deserves further investigation and testing against *Mycobacterium tuberculosis*. This plant was reported to be a remedy for the 1918 influenza epidemic and has also been used to treat chest complaints (Smith, 1966) which also suggests it may be useful in the treatment of TB.

Similarly, this study has shown that there may be justification of the uses of the tested plants in self-care in the area. *B. frutescens* and other *Bulbine* species are said to contain anthraquinones such as chrysophanol which has antibacterial properties and glycoproteins such as aloctin A and B also found in *Bulbine* species may be attributed to the healing process (van Wyk et al, 1997). The anthraquinones may be responsible for the *in vitro* activity observed. *C. scabrida* and *D. angustifolia* contain diterpenoids (van Wyk et al, 1997) which are compounds which exhibit antimicrobial and antifungal activity (van Wyk and Wink, 2004). Flavonoids are also present in *C. scabrida* (El-Karemy et al., 1987) and in *D. angustifolia* (van Wyk et al., 1997). These compounds are often the active ingredients in many plant-based drugs (Bruneton, 1995; van Wyk and Wink, 2004). These compounds are prominent in the Asteraceae and have the ability to decrease capillary permeability and fragility and are prescribed for minor circulatory disorders (Bruneton, 1995). Flavonoids have antibacterial activity as well as anti-inflammatory, antiviral, antioxidant and anti-allergenic properties (Bruneton, 1995; van Wyk et al, 1997) so the activity seen in the bioassays could also be attributed to these molecules. From the activity seen in this study, these plants should be further studied in terms of their phytochemistry to reach a more substantial conclusion about the efficacy of these plants in plant remedies.

However, the *in vitro* bioassays used have their limitations in that they cannot represent conditions in a living system. These plants may not necessarily exhibit activity when taken by a person, conversely, some plants may need some biochemical change in composition which occurs in the body and causes the plant to have the desired effect. But, for preliminary testing, bioassays can provide a useful starting point and save the expense of perhaps testing on animals unnecessarily. From this study, it can be seen that the liquid dilution bioassay used to determine the minimum inhibitory concentration (MIC) is a better method for observing antimicrobial activity. It has been tested and found to be far more sensitive than the disc-diffusion assay (Eloff, 1998).

Another factor which should perhaps be considered is that of geographical variation. Plants which are found in the Southern Overberg and which grow in other parts of South Africa may have differing chemical compositions. This was seen in a study of *Aloe ferox* where overall the composition of the major compounds was invariable over the many populations studied, but that the proportion of aloin to the other compounds varied considerably in and between populations (van Wyk et al., 1994). Seasonal variation could also affect activity but in the case of this study, the plants were collected during September after the rains but before flowering. This was the time that the informants recommended that the plants be collected. Further research would reveal whether this does affect the properties of these plants as mentioned by the informants and whether this is the best time to collect the material or not.

5.2. CONCLUSION

In conclusion, the aims of this study were achieved. Thirty-six plants from 19 families were found to be in use and an inventory was compiled regarding the uses, preparations and dosages of these plants. Antimicrobial activity was found in the four species tested and therefore there is the possibility of finding a new compound from these lesser studied plants. This study has helped to document some of the important knowledge held by many people in the study area and this may be useful for future generations should they wish to find out more about plants used in the area.

Ethnobotanical surveys do provide an efficient way of discovering new drug activities than does the random screening of plants (Huxtable, 1992). It has been said that "Natural products are an irreplaceable source of novel compounds" and that "man is a poor organic chemist compared to nature" (Huxtable, 1992). With increased urbanization and modernization much of the knowledge of many plants will be forgotten if efforts are not made to collect it now. There is still perhaps a degree of skepticism in Western society surrounding the uses of plants in medicines though, due to there still being a degree of mystery and superstition involved with many traditional medicine systems. There still appears to be a large gap between science and society. It has been said "western ideology has largely underestimated the inordinate belief in traditional medicine by many people, particularly those from developing countries" (Bye and Dutton, 1991). Although traditional medicine systems are often linked with sorcery and magic it cannot be coincidence that so many plants used in traditional medicine have been proven to be effective in the treatment of so many illnesses.

5.3. FURTHER RECOMMENDATIONS

The study area is part of the Southern Overberg, which, as already mentioned, has a huge floral biodiversity and warrants further investigation in other communities around this area. By covering a wider area in the Southern Overberg, it may be possible to find evidence of more local or endemic flora being used which could help in discovering new platforms for drug development. With regard to the antimicrobial studies, it would be beneficial to examine those compounds seen in the thin layer chromatography experiments more thoroughly using techniques such as HPLC (high performance liquid chromatography). These compounds could then be isolated and re-tested for activity to see which are the most active fractions. It may also be beneficial to look at *in vivo* testing to observe the biochemistry of these compounds within a living system. Another species, *C. floribunda*, in Ecuador has been found to have significant anti-inflammatory activity (de las Heras et al., 1998) and so with regard to *Conyza scabrida*, seeing as it is used also for inflammation, it may be worth performing some preliminary anti-inflammatory tests to investigate this claim.

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APPENDIX A

Preliminary questionnaire

Interviewee: ....

Date: ...../...../.....

1. What are your most widely used herbs?
2. What conditions are these plants used to treat?
3. Are these herbs easy to find? If not why?
4. Where do you find these herbs? (grow, buy, collect?)
5. How long have you been using plants as medicine?

   ....yrs.........months

6. How or from whom has your knowledge of these herbs been obtained?
7. Which parts of the plants do you use?
8. Specify method/s or recipe of preparation e.g. extraction methods (burnt, boiled,

Alcohol, tea) and how much of the plant is used?

9. What is the dosage of the plant drug? And how should it be taken (inhaled, orally etc)

10. When should the medicine be taken and for how long should its use be continued?

11. Are there any side effects? (dizziness, headache, vomiting?)

12. Can any of these herbs be combined

13. Can these plant drugs be taken by pregnant women?

14. Can these plants be stored? If so for how long and in what condition are they stored (dried, extractions in alcohol...) if not why?

15. How do you tell if the plant is still potent and capable of treating the conditions it is meant to treat? (smell, leaf colour?)

16. Can any other plant(s) be substituted when the above plants are not available?

APPENDIX B

Semi structured questionnaire:

Name:____________________________

Date: ___________

1. Plants which have been mentioned by all informants

Wilde Als	Buchu
Paddabossie	Klipdagga / Wilde Dagga
Suurvy, Vygie	Keurtjie
Ysterhouttoppe	Renosterbos
Kruitjie-roer-my-nie	Malva
Kattekruid	Kruisement
Kooigoedbos	Wynruit
Koekmakranka	Voelent
Plakkie / Kouterie	Groenamara
Gifbol / Maerman	Wilde roosmaryn
Vinkel	Stinkblaar
Wilde salie	Olieblare

Other plants not on this list

Please could you put your top 10 most used plants in the space below. Number one being the most used plant by you and number 10 the least used. If you do not use 10 plants please write down the names of the ones you do use starting with the one you use the most.

1.________________________________

2.________________________________

3.________________________________

4.________________________________

5.________________________________

6.________________________________

7.________________________________

8.________________________________

9.________________________________

10._______________________________

2. Please give your personal recommendations for the best plant(s) to treat the following conditions:

Arthritis____

Backache____

Bladder and Kidneys____

Liver____

Cancer____

Colds and flu____

Coughs____

Convulsions____

Diabetes____

Fever____

Headache____

Heart____

Inflammation____

Rheumatism____

Stomach ____

Worms____

Wounds____

Thank you very much for your contribution

APPENDIX C

List of all informants who contributed to the survey in Chapter 3.

Mrs. Katrina Wyngaard	Mrs. Lea Hans	Mr. Jordaan
Mrs. Paulina M Newman	Mrs. Mary Daniels	Mrs. L Giljomee
Mrs. Dora Jacobs	Mrs. May Maarman	Mrs. S. Odendaal
Mrs, Rachel Pietersen	Mr. Leonard Johannes	Mrs. Maxi Giljomee?
Mrs. Sophia Skenjana	Mr. AB Carolus	Ms. Andree Joorst
Mrs. Fatima Visagie	Mrs. E. Swart	Ms. Christina Afrika
Sister. Paulina Prins	Mr. M. Klaasen	Ms. Caroline Apollis
Mrs. Johanna Jantjies	Mrs. B. Baaitjies	Ms. M. Schippers
Mrs. Freda Jantjies	Mrs. K. Swart	Mrs. Petru Swart
Mrs. Jo-ann Carels	Mrs. Joey Swart	Mrs. D. Murtz
Mrs. Stiena Souls	Mrs. E. Lazarus	Mrs. Minnie Jappta
Mrs. Lien Kies	Mrs. Matty Lourens	Mrs. Sarah Stols
Mrs. Stienie Baatjies	Mrs. Johanna Gertze	Mr. J. Swart
Miss. Ann Engel	Mrs. Paulina Newman	Mrs. P. Visser
Mrs. Sarah Pail	Mrs. Annie Norton	

APPENDIX D

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Examples:

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Multiauthor

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