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

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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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KEYWORDS

South Africa

Southern Overberg

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Questionnaires

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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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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.

Tamsyn S.A. Thring

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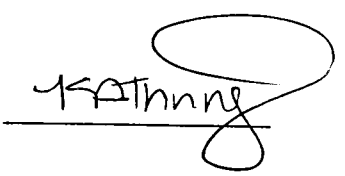

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CHAPTER 1

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