1 General Introduction

Plants are an important source of modern pharmaceuticals, some of which may be difficult or impossible to synthesize (Bateman *et al.,* 1998). South Africa is endowed with a large variety of plants, accounting approximately 10% of the word's flowering plant species (Kirsten and Reid, 1982). Many of these plants are used for medicinal purposes. South Africans have been using plants as sources of medicine for a long time, although the first records of Xhosa and Zulu medicinal plants were only published in 1885 (Hutchings, 1989). Watt and Breyer-Brandwijk (1962) initiated research on traditional medicines, which was later expanded by many other workers including Hutchings *et al.* (1989) and van Wyk *et al.* (1997). Nevertheless, it is still believed that many of such plants and knowledge are kept secret by traditional medicine practitioners. Not long ago, Wagner and Bladt (1996) made use of the technique 'Thin layer Chromatography (TLC)' in quality assessment of western medicine. This technique is now also used to assess traditional medicines, e.g. Chinese Traditional Medicines (CTM)

Currently, studies are conducted worldwide in many research areas to determine pharmacological effects of traditional medicine (Rabe and van Staden, 1997). The first efforts made to identify and isolate chemical constituents of plants medicine were performed in the nineteenth century; for example quinine, an anti-malaria agent was isolated from the bark of *Cinchona* species (Lambers *et. al.*, 2002), morphine and codeine were obtained from the opium poppy (*Papaver somniferum*) (Fisher *et. al.*, 1995) and atropine from deadly nightshade (*Atropa belladonna*) (Rothe *et. al.*, 2001). In most instances, compounds isolated were found to be responsible for known uses of traditional medicines (Bell, 1993).

The main chemical difference between isolated pharmaceuticals and traditional medicines is that the former consists of one or more known compounds, whereas traditional remedies have many unknown compounds present in dilute solution (Robbers and Tyler, 2000).

Several methods have been designed to evaluate the biological activity (e.g. bioautography) and chemical composition (e.g. Thin Layer Chromatography) of plant medicines. When assessing plants for biological activity and chemical composition, it should be considered that both these parameters might vary according to the collection time, season, geographical location and the plant part analyzed. Traditional healers generally prefer to use stem bark because its availability is not seasonal, and it is easy to harvest (Tagwireyi *et al*, 2002). Studies have shown that there is a difference in chemical composition between different parts of a plant (Chakraborty and Brantner, 1999). The extensive use of traditional medicines, especially in Africa, has resulted in several problems. The problems include exploitation of bark materials, substitution of rare plant species with plant species looking similar to the rare species and poisoning. The next section deals with the implications of exploitation of such plant medicines.

1.1 Problems with traditional medicines

Over-exploitation of commonly used medicinal plants has become a major problem in the country. The economic exploitation of South Africa's rich natural plant resources is limited. Presently, the indigenous flower industry has relatively successfully established small and medium scale entrepreneurs (Mander *et al.*, 1995). The demand for traditional medicines as alternatives to orthodox medicines has increased. As a consequence of this, unsustainable rate of plant harvesting has frequently resulted in some wild species already being threatened by extinction (Anandhi *et al.*, 2002). The use of plants as medicines may result in extinction of parts

of the valuable flora of South Africa. The result is that popular plant species are becoming scarce to such a degree that the collectors turn to plants in conserved areas for supplies. Illegal and uncontrolled stripping of barks for medicinal purposes has affected the status of natural forests and the natural habitats of South Africa (Kareiva, 2001).

Conservation officials from these sectors are concerned about the uncontrolled utilization of medicinal plants in South Africa's parks and reserves. Certain plant species have become over-exploited resulting in a general shortage of supply. This in turn appears to have led to escalating cost. Plants in very high demand such as *Siphochilus aethiopica* [wild ginger] might have to be produced commercially (Rhainds, *at al.* 2002). Another case involves *Ocotea bullata* (Stinkwood), which is an important traditional medicine in KwaZulu-Natal. This plant species has become over-exploited in the province and throughout the country (Zschocke *et al*, 2000a).

People of South Africa have turned to *Cryptocarya* species namely *C. latifolia* and *C. myrtifolia* as a substitute for *O. bullata*. These species are in turn threatened as their demand increases (van Staden and Zschocke, 2000). Since the country's natural flora of popular plants is facing over-exploitation, the ability to identify popular medicinal plants in any form (i.e. root, bark) is important for enforcing conservation measures and developing policies.

Another problem faced with traditional medicines is poisoning. Toxicity related to traditional medicines and food plants has been a problem in the country for a long time (Savyah, *et al.* 2002). Brandt *et al* (1995) reported poisoning incidents in patients admitted at Ga-Rankuwa Hospitals, in the province of Gauteng, South Africa, that resulted from ingestion of plant medicines. However, there are few incidents reported and recorded. Effects of plant toxins may only become clear after prolonged ingestion of a plant or plant products. Under such circumstances, it may not be easy to relate the physiological effect to the plant or, subsequently,

to identify the specific toxin or plant (Bell, 1993). The implication is that poisoning incidents may also be caused by insufficient ethnobotanical knowledge and law enforcement (Robbers and Tyler, 2000). Some communities have medicinal plants in their private gardens and the South African Government has implemented no restrictive regulations. This may lead to individuals with limited ethnobotanical training collecting plants with a similar appearance to those used for medicinal purposes. These plants may have completely different physiological effects from the desired plants or may even be toxic.

Use of herbal medicines in developed countries is also common (Edgar, *et al.* 2002). Poisoning incidents relating to herbs rarely occur in these countries, since their products undergo a degree of quality control and incidents of toxicity has to be reported.

Aristolochia species contain aristolochic acid, which is one of the natural products known to have potent carcinogenic effects (Ong and Woo, 2001; Stoborova *et al.,* 2001). Consumption of products with aristolochic acid resulted in several life-threatening adverse events e.g. two patients in the United States of America were reported to have developed end-stage renal disease because of the use of botanical preparations containing aristolochic acid (Huang *et al.*, 1997).

In South Africa's big cities like Pretoria, Durban and Cape Town, traditional medicines are openly sold in markets called Muthi markets in the form of bark, roots and leaves etc. These plant medicines do not undergo any quality assessment before commercialization, therefore, no one knows if the correct plant medicines are circulated from traders to consumers. It has been observed that plant medicines demanded by a large population become short in supply and consequently expensive with time. In general, these poisoning incidents were a result of either misidentification, ingestion of incorrectly prescribed medicines or deliberate substitution. There

is a need for a mechanism to determine the authenticity and quality of traditional medicine. This is one of the objectives of the present study.

Adulteration appears to be another problem with traditional medicines or herbal products (Bateman, *et al.,* 1998). "Adulteration" means to make something impure, to contaminate (Collins, 1993). Huang, *et al.* (1997) refers to adulterated products as medicines that do not contain chemical substances labeled as part of the contents.

Dishonest traditional healers and traders may substitute rare medicinal plants with other plant species with a similar appearance. The material used for substitution may contain toxic compounds that can result in fatality.

Chinese Traditional Medicine (CTM) has made a major impact on health issues in East Asia for over the last 5000 years. CTM are grouped into two categories namely: raw Chinese Medicinal Material (CMM) and Chinese Proprietary Medicine (CPM). CMM are medicines used in their natural form and are usually subjected to simple processing (cutting and drying) whereas CPM are plant medicines that have been formulated into tablets, pills, or mixtures (Li *et al.* 2003). The governments of Asia impose minimal quality control on CMM as compared to CPM because formulated medicines are often adulterated with western medicine or other ingredients (Tomlinson *et al.,* 2000).

Adulteration of CTM has been reported in various occasions and is a public health concern in Taiwan. In 1997, Huang *et al.* (1997) conducted a quality control study on 2609 samples collected from eight major general hospitals, following the established standard procedures. The results showed that 23% of the samples contained adulterants. Half of the adulterated samples contained two or more adulterants.

CTM are sometimes found to contain heavy metals or animal parts. This is due to manufacturing problems or to the expense of procuring these species and widespread cultural beliefs in the use of animal parts as tonics (Li *et al.* 2003).

Some traditional medicines are adulterated with undeclared pharmaceutical ingredients such as caffeine, acetaminophen, indomethacin, hydrochlorothiazide, and ephedrine (Koh and Woo, 2000). Adulteration with such ingredients may lead to serious physiological complications, for instance adulteration with mefenamic acid and cadmium is associated with renal failure (Hirshon, 2001).

Wagner and Bladt (1996) developed a thin layer chromatography (TLC) method for drug quality assessment. TLC was found to be useful for assessing CTM quality standards (Jork *et. al.*, 1990). The British Herbal Pharmacopoeia approached adulteration and substitution of drugs by providing monographs of quality standards for 169 commonly used herbs in the United Kingdom for the preparation of botanical drugs. In this study, an attempt is made to assess the level of adulteration in traditional medicines sold in the Pretoria area using a TLC technique.

1.2 Thin layer chromatography (TLC)

Thin layer chromatography (TLC) is a separation method in which uniform thin layers of sorbent or selected media are used as a carrier medium. The first reference to TLC was in 1938 in what was called a drop chromatography on horizontal thin layers (Sgoutas and Kummerow, 1963). Little notice was taken of the method until 10 years later when two American chemists described the separation of terpenes in essential oils by thin layer chromatography (Touchstone and Dobbins, 1983). However, the procedure was not generally accepted in its early years because of the lack of media and apparatus for coating plates (Ulrich, 1966). The effectiveness of

the technique for separation was publicized when Stahl (1969) described equipment and efficient sorbents for preparation of plates in his book "Thin Layer Chromatography". Today, TLC is still one of the most popular and widely used separation techniques.

TLC uses sorbents such as silica gel, which is the most popular layer material and is slightly acidic in nature. A binding agent such as calcium sulfate hemihydrate is used to hold the silica gel (silicic acid) onto the support. Two ultraviolet (UV) indicators, zinc and sodium salts may be incorporated either singly or together with the silica gel to aid in the location of separated substances. Zinc silicate fluoresces when exposed to UV light of 254 nm wavelength, so that substances absorbing this wavelength such as aromatic compounds appear dark with extinguished greenish-yellow fluorescing background (Ziegler *et al.*, 2001). The sodium salts of hydroxypurene sulfonic acids fluoresces at 366 nm and provide a contrast background for substances that absorb at this frequency (Hahn-Deinstrop, 2000; Jork *at al.*, 1990).

Alumina (aluminum oxide) is also widely used as a sorbent and is chemically basic. Silica gel separates large quantities of material as compared to alumina. Alumina is more chemically reactive that silica gel, so care must be exercised with some compounds to avoid decomposition or rearrangement of these substances during sample application (Touchstone, 1983).

Kieselguhr is chemically neutral and does not separate compounds as well as alumina, although it is used mainly as the support for the stationary phase in partition chromatography. The sorbent cellulose is used in paper chromatographic separation. Table 1.1 lists the most common TLC sorbents, the typical compound application for each sorbent and the major chromatographic mechanism.

Table 1.1 Sorbent materials and mode of separation (Touchstone and Dobbins, 1983)

Sorbent	Chromatographic Mechanism	Typical application
Silica gel	Adsorption	Steroids, amino acids, alcohols, hydrocarbons, lipids, aflatoxins, bile acids, vitamins and alkaloids
Silica gel RP	Reverse phase	Fatty acids, vitamins, steroids, hormones and carotenoids
Cellulose, Kieselguhr	Partition	Carbohydrates, sugars, alcohols, amino acids, carboxylic acids and fatty acids
Aluminum oxide	Adsorption	Amines, alcohols, steroids, lipids, aflatoxins, bile acids, vitamins and pyrimidines
Magnesium silicate	Adsorption	Steroids, pesticides, lipids and alkaloids

Adsorption is used to separate highly non-polar, hydrophobic (fat soluble) substances with non-polar mobile phase solvents, whereas partition may be used for polar, hydrophilic (water soluble) substances with polar mobile phase solvents.

Several factors must be considered before development, for example the polarity of both the mobile phase and the sample. In addition, the environment where the experiment is conducted should not be humid or dusty because these will interfere with the development and the visualization of the plates. TLC plates should also be protected from solvent vapours in the laboratory environment (Jork *et al.*, 1990).

In practice, a sample to be separated is applied on the coating 1-2 cm from the one end of the plate. The edge of application is called the starting point or origin. Separation is achieved by passing a solvent, the mobile phase through the coat (layer). The layer, with the sample zone at the bottom is placed on a slight angle from the vertical into a closed tank containing a small

amount of the mobile phase. The nature of the mobile phase is determined by the type of substance to be separated and the type of sorbent to be used for the separation (Jork *et al.*, 1990). The composition of the mobile phase can be as simple as a single, pure solvent (e.g. benzene is used to separate dye on alumina) or as complex as three to four components mixture such as a 9:1:0.1 solution of benzene (B), ethanol (E) and ammonium (A) in the BEA system. The BEA system is used in the present study to separate non-polar compounds.

Capillary action initiates the movement of the mobile phase through the medium in a process called development. Ascending development is the most common, but horizontal, descending and centrifugal methods have been described. After the developed plate is dried, the spots can be visualized in a number of ways such as viewing under ultra violet (UV) light, or spraying with one of the wide variety of reagents. After visualization, many experiments can be considered complete, although other experiments such as bio-autography can be performed on the basis of TLC detection. The distance travelled by the compounds represented as a spot can be calculated in terms of the differential retention (R_f) value.

The R_f value is a convenient way of expressing the position of the substance on a developed chromatogram. It is calculated as the ratio that varies between 0 and 1.0 and it is constant under reproducible conditions. The R_f can be represented mathematically as follows:

$$R_f = \frac{\text{Distance of compound from origin}}{\text{Distance of solvent front from origin}} \qquad (1.1)$$

The distance is measured to the centre of the eluted spot. There are factors affecting the R_f value that should be considered during the development process. For instance, the R_f value can change to a higher range if the chamber is not saturated and the solvent evaporates from the

plate. The concentration and the complexity of the solute applied can also affect the R_f value. Highly concentrated samples may make a smear not a clear spot without any separation. Therefore, the R_f value may become difficult to measure and would be worthless.

1.2.1 Advantages of TLC

TLC has many advantages over other chromatographic methods such as liquid chromatography (LC) and gas chromatography (GC). It uses less solvent for the development of plates compared to LC and the apparatus required are several orders of magnitude cheaper, which makes it a relatively cheap and low technology. The polarity of the solvent can be changed in about 5 minutes. TLC is the easiest chromatographic method to set up because of the short development time and easy change of mobile phase. The most advantageous feature of TLC as opposed to other chromatographic methods is the number of samples that can be handled simultaneously. GC and LC are limited to the analysis of a single sample at a time whereas as many as 22 samples can be applied to a single 20 x 20 cm TLC plate.

TLC is used in the pharmaceutical industry for the identification, purity testing and determination of the concentration of active ingredients, auxiliary substances and preservatives in drugs in synthetic manufacturing processes (Hahn-Deinstrop, 2000). In biochemistry, TLC is used to determine active substances and their metabolites in medical diagnosis. It is also used for the diagnosis of metabolic disorders such as cystinuria, a condition whereby large amounts of cystine are excreted in the urine (Wills, 1985). In cosmetology, TLC is used to analyze constituents of perfumes as well as separating crude material to yield isolated compounds. This technique is widely used for research purposes in food monitoring and environmental analysis (Hahn-Deinstrop, 2000). In environmental monitoring, groundwater and air are analyzed for

pollutants. Ntloedibe (2001) developed a relatively simple TLC technique that has been useful in distinguishing more than 80 western herbal medicines. The same technique will be applied in this study.

1.3 Chemotaxonomy and fingerprinting of plants

Chemotaxonomy of plants is the classification of plants based on their chemical constitution (Smith, 1976). Chemical variation studies were suggested to be one of the principle growing points in the field of taxonomy. There are many factors that give rise to differentiation in a plant's chemical composition. The number and composition of classes of compounds such as alkaloids, flavonoids, essential oils and isoprenoids vary in species and habitats. The variation thus allows the use of chemical composition as a tool for classification of plants to complement botanical classification based mainly on morphology. There are, however, factors that influence the accuracy of classification based on the chemistry of the plants namely: age of the plant, geographic location, ecological habitat and genotypic polymorphism. Influences of these factors on the chemistry of a plant are observed only if the concentration of chemical compounds is sufficient for detection. Plants contain thousands of compounds but the concentration and the polarity of the compounds influence the detection of these compounds by the applicable methods such as TLC (Robbers and Tyler, 2000).

TLC fingerprinting is a good tool for chemotaxonomic classification of plant species, although there are limitations, as some closely related species may be difficult to distinguish. In such cases, the classification based on morphological parameters may be used to differentiate related species. An example is aristolochic acid. Its presence in many plant species and its

toxicology has facilitated the classification of *Aristolochiaceae* species, as a family-characteristic metabolic end product (Hegnauer, 1986).

1.4 Problem statement and objectives

After conducting a literature review, it was established that a large section of South Africa's population depends on traditional medicine (Steyn and Muller, 2000) and that worldwide between 70 to 80% of people use herbs (Griffiths, 1999). This chapter also indicated that the use of traditional medicine is fraught with problems, such as misidentification, over-exploitation, adulteration and substitution of traditional medicines. Based on these problems, this study aims to:

- Identify commonly used traditional medicines in the Pretoria area.
- Develop techniques for authentication and quality assessment of at least 6 plant medicines commonly used in the Pretoria area.
- Determine the magnitude of adulteration and substitution in the Pretoria markets
- Determine the influence of environmental changes on the chemical composition of the *Artemisia afra* species grown in different areas that originated from the same seed source.
- Provide preliminary data on antimicrobial activity of these species
- Compile a TLC fingerprint of the bark samples of over-exploited traditional medicines.

The results of the objectives outlined above are described in Chapter 3 and the approaches followed to accomplish the investigations are given in Chapter 2.

1.5 Conclusion

After a literature review conducted as part of this study, it was found that the use of traditional medicines in South Africa has problems. These include incomplete database of traditional medicines in the Pretoria area, accidental poisoning associated with the intake of such medicines, over-exploitation and substitution of traditional medicines. The next chapter will introduce the methods followed to accomplish the objectives given in Section 1.4

2 Materials and methods

2.1 Introduction

As previously stated in Chapter 1, this study is interested on the identification of medicinal plants that are used in Pretoria area. The aim of this chapter is to give a detailed description of all the experiments followed in this thesis.

2.2 Selection of commonly used medicinal plants in Pretoria area

The information-gathering exercise was conducted by interviewing traditional healers and traders in markets that sell African traditional medicines around Bloed and van der Walt streets in the city of Pretoria. A meeting with a group of traditional healers, traders, collectors and Agricultural Research Council (ARC) conservation officials was attended in Roodeplaat in the year 2000. This assisted in obtaining further information on traditionally used medicines. This meeting discussed a collaborative project between ARC and the traditional healers. During these interviews, information on the identity, use, history of poisoning incidents, cost and plant parts often used, was gathered for plant species commonly used in the Pretoria area. A questionnaire, appearing in the appendix was used during these interviews.

Points were allocated to prioritize the traditionally used medicines. Plant species associated with poisoning or accidental deaths were categorized as most important in this investigation as they pose a threat to the community. These plants were allocated 10 points whereas those that had no such reports were allocated 5 points.

The more difficult it is to obtain a plant part, like digging roots and stripping bark from a tree, the more expensive the materials could be, compared to harvesting leaves. Therefore,

medicines in which the whole plant was used were allocated 10 points, whereas roots, bark, and leaves were allocated 10, 8 and 4 points, respectively.

Only bark materials of the plant samples identified as commonly used, were bought from the markets in the Pretoria city. Reference samples of plants bought from the market were collected from the Pretoria National Botanical Garden (PNBG) and Agricultural Research Council (ARC) in Roodeplaat. These plant samples were used in the laboratory for identification and further investigations.

2.3 Processing of plant materials

All the plant materials were bought around Central Pretoria in the month of May and August 2000 and once in the laboratory, the materials were processed in a similar manner. Fresh plant materials in the form of tree bark collected from the market, ARC and PNBG were cut into small pieces with a side cutter. The pieces were then dried at room temperature in the shade. The insect damaged and contaminated materials were excluded. The sample pieces were then ground to a fine powder in a mill (Kika-labortechnik, Janke and Kunkel). The weights of different materials were determined using a scale (Ohaus corporation) then stored in glass bottles in the dark until required. The powder of the samples was extracted to analyze its chemical composition.

2.4 Thin layer chromatography (TLC) analysis

The powder was divided into three (1.0 gram) and then extracted with three solvents of different polarities: ethanol (polar), acetone (intermediate polarity) and hexane (non-polar). Ten ml of each extracting solvent was added to the powder, which was shaken vigorously (Labotech-

shaking machine) for 5 minutes then centrifuged (Hettich zentifugen) at ± 3500 x g for another 5 minutes. The supernatant was transferred to a clean weighed container. The extraction procedure was repeated two more times on the same plant materials and the marc was discarded.

The supernatant was dried in a laminar flow at room temperature. The weight of the crude extract was determined then redissolved in acetone (Merck) [since it dissolves both polar and non-polar compounds] or where necessary in absolute ethanol [Merck] to make up a final concentration of 10 mg/ml. These stock solutions were used for TLC analysis.

Three freshly made separation systems of various polarities were prepared in advance for use in TLC analysis as shown in Table 2.1 below.

Table 2.1 Separation systems used in TLC analysis

Separation system	Ratio
BEA: non-polar, basic	80 (benzene): 10 (ethanol): 1 (ammonium)
CEF: intermediate polarity, acidic	4 (chloroform): 3 (ethyl acetate): 1 (formic acid)
EMW: polar, neutral	10 (ethyl acetate): 1. 35 (methanol): 1 (water)

Aliquots of 5 µl of concentrated extracts were spotted onto the silica gel plates (Macherey-Nagel, alugram/UV,), 1 cm from the bottom of the plate. The plates were then allowed to develop in the TLC tanks until the mobile phase reached about 1 cm from the top of the plate. The plates were then taken out of the tanks, dried and then visualized under UV at 254 and 350. The visualized plates were then sprayed with one of several spray reagents shown in Table 2.2. In the case of autobiography method, once the plated were developed, and before they were sprayed with visualizing reagents, they were dried overnight and prepared as described in Section 2.5.

Table 2.2. The principles of detection reagents (Stahl, 1969)

Spray reagent	Method of preparation	Detectable compounds
Anisaldehyde-sulphuric acid (H_2SO_4)	1 ml each of sulphuric acid and *p*-anisaldehyde in 18 ml of ethanol.	Sugars, steroids and terpenes
Natural (NP/PEG)		Flavonoids
Phosphoric acid (H_2PO_4)	15 ml 85% phosphoric acid diluted to 100 ml with methanol.	Steroids
Trichloroacetic acid	25% trichloroacetic acid in chloroform.	Steroids
Toluene	20% solution of p-toluenesulphuric acid in chloroform.	Steroids, flavonoids and catechins
Vanillin phosphoric acid (H_2SO_4)	1% solution of vanillin in 50% aqueous phosphoric acid.	Steroids
Vanillin sulphuric acid (H_2SO_4)	1 ml sulphuric acid in a solution of 0.1 g vanillin in 28 ml of methanol.	High alcohols, phenols, steroids and essential oils

2.5 Bio-autography

Bio-autography is a method used to locate antibacterial activity on a chromatogram. This technique has been used widely in the search for new antibiotics (Hamburger and Cordell, 1987). Chemical compounds extracted from the powdered materials of plant species were separated on TLC plate in three mobile systems (see Section 2.4). Developed TLC plates were carefully dried under flow of air overnight for complete removal of the solvents. Any residual solvent from the plates may inhibit the growth of bacteria, therefore, leading to false results.

Internationally accepted strains of all bacteria in Table 2.3 were received from the Medical Microbiology Department at the University of Pretoria and then cultured in nutrient broth every two weeks.

Table 2.3 Details of microorganisms used to test the biological activity of medicines

Micro-organism	Bacterial type	Bacterial strain
Enterococcus faecalis	Gram positive	ATCC 29212
Escherichia coli	Gram negative	ATCC 25922
Staphylococcus aureus	Gram positive	ATCC 29213
Psuedonomas aeruginosa	Gram positive	ATCC 27853

Fresh cultures of the bacteria that were prepared by Laboratory Technician were centrifuged at ± 5300 x g for 20 minutes to concentrate the bacteria. The supernatant was discarded and the bacterial cells re-suspended in fresh nutrient broth. The suspension was dispersed evenly over the TLC plates using a spray gun. The TLC plates were then incubated overnight in a 70% humid atmosphere at 37°C. Dehydrogenase-activity detecting ρ-iodonitrotetralium (INT) violet (Sigma-Aldrich) reagent (2 mg/ml) was evenly sprayed onto the plates and care was taken to minimize airborne contamination at all times. INT is a colourless tetrazolium salt that is converted into a coloured formazan by metabolically active bacteria (Hamburger and Cordell, 1987).

After one-hour incubation, plates sprayed with INT were scanned for later reference. Compounds with anti-bacterial activity appear as clear spots against a pink background.

2.6 Minimum inhibitory concentration (MIC) and total activity

Distilled water (100 µl) was added into each well of the micro-plate, then 100 µl of the extracts (10 mg/ml) was added into the first well and then mixed thoroughly with water. The concentration in these wells was half (5 mg/ml) the stock concentration. Half of the volume (100 µl) from the first well was transferred to the next well and mixed thoroughly, giving it a

concentration of 2.5 mg/ml. The serial dilution was carried on across the plate until the final concentration was 0.04 mg/ml. The last extra volume of 100 µl was discarded from the final well. A volume of 100 µl of each strain of bacteria in Table 2.3 was added into each well, mixed and incubated at 37° C for overnight in a 100% relative humidity. Forty microliters of 0.2 mg/ml INT (Sigma-Aldrich) was added into each well the following day, incubated at 37°C for 30 minutes and then viewed at 30-minute intervals for 2 hours (Eloff, 1998). This experiment was carried in laminar flow cabinet and spills were cleaned with 70% ethanol.

The experimental procedure followed in this study is illustrated as shown in Figure 2.1. This figure gives the order in which the experiments were done. The experiments conducted were: the selection and collection of commonly used traditional medicines in the Pretoria areas; chemical processing of the selected materials; TLC analysis of extract; determination of MIC values and total activity; bio-autography analysis and documentation of results.

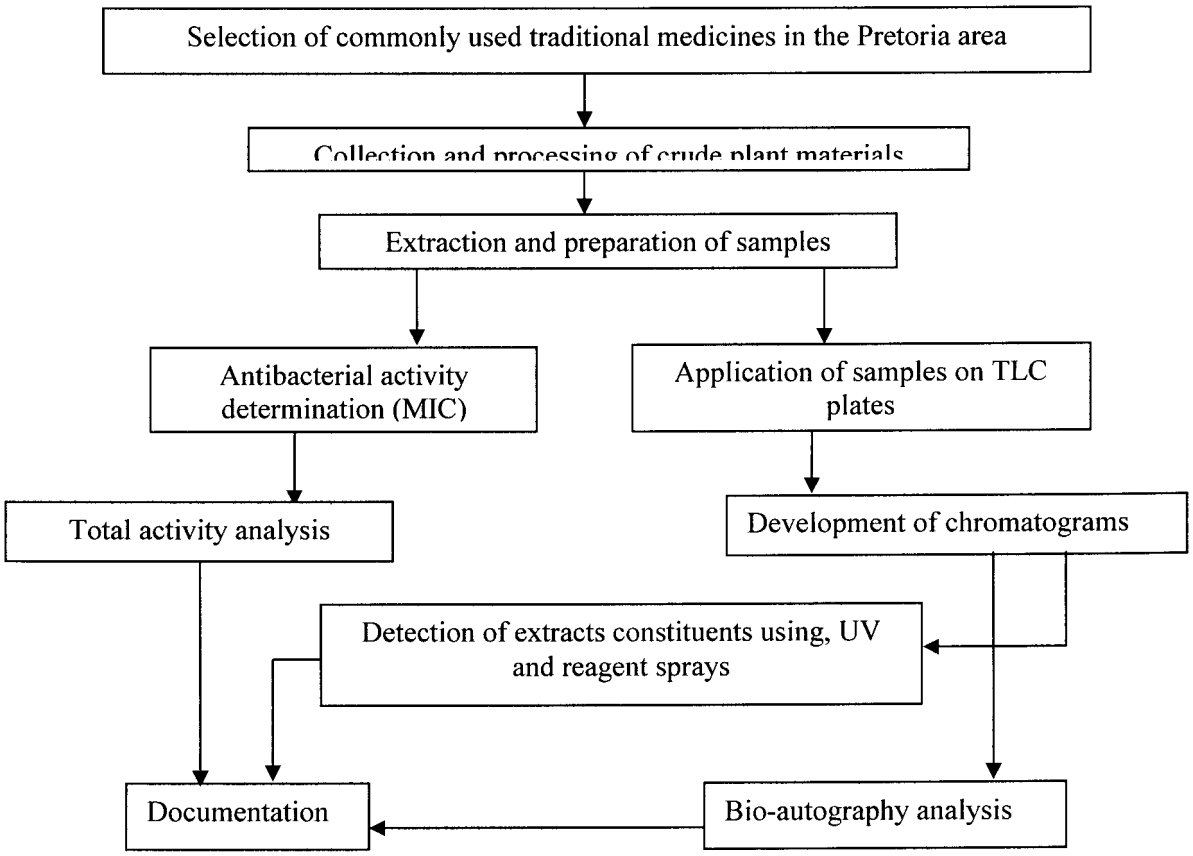

Figure 2.1. The schematic illustration of experimental procedures followed in this study.

2.7 Conclusion

In this chapter, the experiments used in this study are described. The order in which the methods were used is outlined. The use of the experiments was determined by the objectives of each investigation. The next chapters give the objectives and results of the investigations conducted.

3 Selection of commonly used traditional medicines in the Pretoria area

3.1 Introduction

The knowledge of traditional medicine in South Africa is passed orally from one generation to another (Bateman, *et. al., 1998*). Different traditional plants are currently sold at the traditional medicine markets in South African towns and cities. These plants are used for physiological and spiritual disorders. Traditional medicinal plants in Pretoria markets are identified by African names mainly South seSotho and isiZulu and not by scientific names. In many cases, the same plant species are given more than one African name or different species identified by the same African name (Brandt *et al.,* 1995). The multiple naming of traditional medicines frequently leads to confusion.

Some of the traditional medicines in Pretoria street markets are collected from KwaZulu-Natal, Limpopo Province and the Cape by traders or by general collectors in the province of origin. There might be contamination or a mix up of species during their transportation from collector to trader or healer. After exhaustive literature review conducted as part of this study, it was found that studies on whether these medicinal plants are of the correct taxa have not been conducted. This research contributes to the literature by studying the chemical characteristics of traditional medicines so that this information can be used to access the effect of the medicines and be used as package insert.

The aim of this chapter was to collect data on the state of commonly used traditional medicines in the Pretoria area (in order to record all popular traditional medicines in this area). This was achieved by obtaining African and scientific names of common traditional medicines,

their traditional use and geographical distributions in the country through interviewing traditional healers and traders and through published literature. This survey conducted concentrated on species that are in high demand by the community. This demand may cause suppliers to extract unfair profit by adulterating (see Section 1.1). The next section gives the results obtained from investigation conducted in a manner that was explained in Section 2.2.

3.2 Results and discussion

The outcome of the interviews conducted was a variety of 59 plant names; of which 38 were published in literature. The scientific names of the remaining 21, which are listed in Table 3.1 could not be established. Table 3.2 lists the African names of the 38 plant species, their use, the part of the plant often used, scientific name, family names and the total scores allocated to indicate their priority. These species are arranged from high score, indicating high priority to low score indicating low priority. This information has been collected from Hutchings (1989), van Wyk, *et al.* (1997) and van Wyk and van Wyk (1997).

The information in Table 3.1 was obtained from traditional traders and healers. It is possible that the botanical identity of these plant species is known although the African names were not found in available documents. The manner in which the medicines are sold makes it difficult if not impossible to distinguish amongst the materials traded, such that the same plant medicine can be misnamed without notice by the buyer.

Table 3.1 African names and medicinal uses of plant species that could not be identified from African name

No.	African name Sotho/Zulu	Toxicity	Plant part used	Medicinal uses
1	Botlhobotlho (S)	No	Bark	love charm
2	Hlonya (S)	No	Bulb	constipation
3	Ibohlole- mandawu *	No	Bark	dream reminder, treat lung diseases
4	Igobinkosi (Z)	No	Root	good luck
5	Ihlunguhlungu (Z)	No	Bark	heart dysfunctions
6	Imbosisho*	Yes	Roots	diarrhoea
7	Isibhaxha (Z)		Bark	sore throat
8	Kgashi *	Yes	Bark	HIV/AIDS treatment
9	Lehabe (S)	No	Bark	dropsy, sores, HIV
10	Letlhwele (S)	No		menstrual problems, drop, heart and lung problems
11	Loeto latlou (S)	Yes	Bulb	purgative
12	Makgurumetsa (S)	No	Bulb	menstrual problems
13	Maroke (S)	No	Bulb	luck charm
14	Mayime (S)	Yes	Bulbs	urinary problems, constipation administered through the anus, sexual stimulation, swelling and painful feet, erection, and constipation
15	Mokgaloane (S)e	No	Bark	drop and sexual discharge
16	Moruta tsusa (S)	No	Bark	coughs
17	Mpetswa (S)	No	Bulb	bad luck
18	Poha-yabashimana (S)	No	Bark	emetic
19	Sirukulu (S)	No	Root/bark	good luck
20	Umafekufeni (Z)	No	Bark	heart problems
21	Utshongwe (Z) Poo thetlha (S)	No	Bark	coughs

* Neither seSotho (S) nor isiZulu (Z)

Table 3.2 (continues). Traditional medicines commonly used in the Pretoria area

No.	*Scientific name*	Vernacular name	Family mane	Plant part	Medicinal purpose	Total 40
19	*Dierama pendulum,*	Undwendweni	Dierama	Bulb	mixtures for luck remedies.	19
20	*Achyroline stenoptera*	Imphepho	Asteraceae	Whole Plant	burnt and inhaled smoke, believed to relieve headaches and protect against evil spirits. Sold as s ritual incense.	27
21	*Urginea delagoensis*	Umahlanganisa	Hyacinthaceae	Bulb	impotency, tapeworms and roundworms. Reported to be a dangerous and poisonous. All parts of the plant are toxic and lethal, the flowering stems are more toxic than the leave. (Hutchings *et al,* 1996)	26
22	*Hypoxis hemerocallidea*	Monna maledu inkomfe	Hypoxidaceae	Bulb	coughs, asthma, tuberculosis, prostate hypertrophy, anticancer, anti-HIV, anxiety, palpitation, rheumatoid arthritis, can be confused with *Hypoxis cochicifolia.*	26
23	*Glycyrrhiza glabra*	Mlomo-mnandi	Fabaceae	Roots	appendicitis, tuberculosis, spiritual power, antispasmodic, anti-inflammatory, ulcer and luck.	26
24	*Eucomis autumnalis*	Umathunga Mathuba difala	Hyacinthaceae	Bulb	wound, used after operations, to cleanse the digestive system, urinary diseases, stomach ache, fever, colic, flatulence, hangover, coughs, respiratory ailments and to facilitate childbirth.	26
25	*Elephantorrhiza lephantina*	Intolwane	Fabaceae	Bark	menstrual problems, oedema, diarrhea, dysentery, stomach, disorders, emetics, hemorrhoids and perforated peptic ulcer.	26
26	*Callilepsi laureola*	Impila	Asteraceae	Roots	cancer, sore trout, also used for luck, open wounds, tapeworms, infertility, snakebites, cough and traditional pregnancy.	26
27	*Bowiea volubilis*	Igibisila, sekgaga	Hyacinthaceae	Bark	headaches, oedema (dropsy), infertility, sore eyes, sterility, abortions, skin diseases and bladder complaints.	26
28	*Celosia trigya L.*	Velabahleke	Celosia	Roots	luck, love charm, usually mixed with other love remedies.	25
29	*Bersama lucens*	Indiyaza	Melianthaceae	Bark Roots	menstrual pain, headache, stroke, nervous disorders, impotency and infertility.	25
30	*Myrothamnus f labellifolius*	Umafevuka	Myrothamnaceae	Leaves	respiratory ailment, backaches, kidney problems, hemorrhoids and painful menstruation.	24
31	*Vernonia natalensis*	Mokgalo	Asteraceae	Bark	prevent thunder damage and for luck.	23
32	*Lannea edulis*	Mophuroku	Anacardiaceae	Bark	sore eyes, boils and abscesses.	23
33	*Hippobromus pauciflorus*	Umfazi-thethayo	Sapindaceae	Bark	Luck charm	23
34	*Diospyros villosa*	Indodemnyama	Diospyros	Bark	love remedy.	23
35	*Dicoma anomala*	Thlonya	Asteraceae	Leaves	fever, influenza, high blood pressure, diarrhea, cancer, also as snuff for headaches and coughs.	23
36	*Harpephyllum caffrum*	Umgwenya	Anacardiaceae	Bark Roots	love charm, blood purification, emetics, acne and eczema. Roots are used to treat paralysis caused by poison.	22
37	*Allinum dregeanum*	Uphunyuka-bemphethe	Allium	Bulb	protection against evil spirits, used also for luck and to provoke vomiting. Tastes bitter.	21
38	*Nelaginaceae selago*	Umhlabelo	Nidorella	Bark	fungal infections treatment.	20

Table 3.2 Traditional medicines commonly used in the Pretoria area

No.	*Scientific name*	Vernacular name	Family mane	Plant part	Medicinal purpose	Total 40
1	*Croton sylvaticus*	Umahlabekufeni Umahlanganisa	Euphorbiaceae	Bark Leaves	stomachache, fever, epilepsy, toothache, and sore throat as mouthwash.	36
2	*Artemisia afra*	Lengana, wilde als, umhlonyane	Asteraceae	Leaves	fever, colds, flu, sore throat, coughs, asthma, pneumonia, headaches, gastritis, indigestion, poor appetite, flatulence, colic, earaches, malaria and intestinal worms. The roots are known as inyathelo.	33
3	*Warburgia salutaris*	Mlaka, Isibhaha	Canellaceae	Bark	diarrhea icough, ulcer, dyspepsia, diarrhea and diabetes	31
4	*Pentanisia prunelloides*	Icishamililo	Rutaceae	Root Leaves	burns, swellings, sore joints, fever, chest pain, rheumatism, heartburn, vomiting, toothache, tuberculosis, blood impurities, hemorrhoids and snake bites.	31
5	*Peltophorum africanum*	Mosetlha	Fabaceae	Bark	coughs, sore throat, fever, wounds, intestinal parasites, eye complains, dropsy, infertility, venereal diseases, abdominal pain and constipation.	31
6	*Alepidea amatymbica*	Lesokwana Ikhathazo	Apiaceae	Roots	coughs, tuberculosis bronchitis, asthma, fever, colds, influenza, sore eye, smoke is used as cigarette and bee repellent from hive.	31
7	*Acacia caffra*	Mosetlhana	Fabaceae	Leaves	cough, bronchitis, sores and asthma.	31
8	*Urginea sanguinea*	Skanama	Hyacinthaceae	Bulb	anticoagulant, stomach ulcer, hypoxia, asthma, rheumatic arthritis, cubes (kidney), skin complexion after aches, impotency. Bulb is boiled and the extract is used as a drink, overdose is toxic.	30
9	*Knowltonia vesicatonia*	Inkathazo	Ranunculaceae	Leaves	rheumatism, arthritis and gout.	30
10	*Boophane hymanthoides*	Legwama	Hypoxidaceae	Bulb	wounds, diarrhea, and skin diseases and as an anticoagulant.	30
11	*Zanthoxylum capense*	Monokwane	Rutaceae	Whole Plant	stomachache, fever, epilepsy, toothache, and sore throat as mouthwash.	27
12	*Vernonia adoensis*	Inyathelo	Asteraceae	Leaves	given to pregnant women for discomfort, used for abdominal pain, colic. This plant may be confused with *V. natalensis.*	27
13	*Turraea floribunda*	Umadlozana	Meliaceae	Leaves	heart failure, rheumatism and as an emetic. Can be confused with *obtusifolia* and *T. nicotica*.	27
14	*Scabiosa columbaria*	Ibheka	Dipsacaceae	Leaves	colic, sore throat and heartburn.	27
15	*Prunus africana*	Inyazangoma	Rosaceae	Bark Leaves	cough, bronchitis and asthma.	27
16	*Cotyledon orbiculata*	Intelezi	Crassulaceae	Leaves	earache, epilepsy and toothache.	27
17	*Calodendrum capense*	Umemezi omhlophe	Rutaceae	Bark Leaves	skin treatment.	27
18	*Aloe ferox*	Inhlaba	Asphodelaceae	Leaves	indigestion, heartburn nausea, colic, gout, boils, constipation, rheumatism, arthritis, wound, sores, rashes, burns and conjunctivitis.	27

In Table 3.2, the common names gathered from the interviews were matched with published names, and scientific identification of published common names was assumed to be of the investigated plant species. The plant species in Table 3.2 were compared to the traditional medicine recorded as commonly used in KwaZulu-Natal listed in Table 3.3 (Eloff, 1998a). Only 7 traditional medicines commonly used in both KwaZulu-Natal and Pretoria areas were identified from this comparison and they are: *Alepidea amatymbica, Bowiea volubilis, Harpephyllum caffra, Hippobromus pauciflorius, Pentanisia prunelloides, Urginea sp.* and *Warburgia salutaris.* This observation may, however, not represent the true common usage of traditional medicine within the provinces.

There are several possible explanations for this minor overlap between medicinal plants commonly used in these areas. For instance, according to the information on their distribution from the Pretoria National Botanical Institute, all these plant species are found in both Gauteng and KwaZulu-Natal. *Alepidea amatymbica* has two sub-species, one called 'aquatia' and is found in KwaZulu-Natal and another called 'microbracteata' is found in Transvaal.

Table 3.3 (continues). Medicinal plants used in kwaZulu-Natal for medicinal purposes (Eloff, 1998)

No.	Plant	Family	Use
25	*Ocotea bullata*	Lauraceae	urinary complaints, headaches
26	*Pentanisia prunelloides*	Rubiaceae	haemorrhoids, snakebite, rheumatism, burns, stomach pains, tuberculosis, swellings, sore joints, palpitations, boils
27	*Protorhus longifolius*	Anacardiaceae	emeticum, heartburn, bleeding from stomach, depilatory
28	*Rapanea melanophloeos*	Myrsinaceae	heart problems, palpitations, acidity, stomach and muscular pains, expectorant, emetic, enema
29	*Rhoicissus tridentata*	Vitaceae	renal complaints, sterility, epilepsy, stomach ailments, menorrhagia, indigestion
30	*Scilla natalensis*	Liliaceae	enemas, purgative, induce childbirth, boils, veld sores
31	*Sclerocarya birrea*	Anacardiaceae	malaria, diarrhea, heart problems, blood cleansing, abdominal pain, proctitis, fevers, headaches, ulcers, toothache, backache
32	*Senecio gregatus*	Compositae	enema, venereal diseases
33	*Senecio serratuloides*	Compositae	infections, sores, blood purifier, burns, swollen gums, chest pains
34	*Stangeria eriopus*	Cycadales	emetic, headaches, purgative, flatulence, blood pressure
35	*Trichilea dregeana* & *Trichilea emetica*	Meliaceae	stomach complaints, backache, kidney problems, blood cleanser, lumbago, intestinal worms, dysentery
36	*Turbina oblongata*	Convolvulaceae	arthritis, gout, spine pain, sores, abscesses, rheumatism
37	*Urginea sp.*	Liliaceae	rheumatism, gout, bronchitis, asthma, hoarseness, influenza, diuretics, abdominal pains, swellings, demulcent
38	*Warburgia salutaris*	Canellaceae	expectorant, rheumatism, malaria, sores, colds, coughs, venereal diseases, constipation, stomach ulcers
39	*Zanthoxylum davyi*	Rutaceae	coughs, colds, tonic, infected wounds, sore throats, mouth ulcers, boils, toothache, pleurisy, bilharsia

Table 3.3 (continues). Medicinal plants used in kwaZulu-Natal for medicinal purposes (Eloff, 1998)

No.	Plant	Family	Use
25	*Ocotea bullata*	Lauraceae	urinary complaints, headaches
26	*Pentanisia prunelloides*	Rubiaceae	haemorrhoids, snakebite, rheumatism, burns, stomach pains, tuberculosis, swellings, sore joints, palpitations, boils
27	*Protorhus longifolius*	Anacardiaceae	emeticum, heartburn, bleeding from stomach, depilatory
28	*Rapanea melanophloeos*	Myrsinaceae	heart problems, palpitations, acidity, stomach and muscular pains, expectorant, emetic, enema
29	*Rhoicissus tridentata*	Vitaceae	renal complaints, sterility, epilepsy, stomach ailments, menorrhagia, indigestion
30	*Scilla natalensis*	Liliaceae	enemas, purgative, induce childbirth, boils, veld sores
31	*Sclerocarya birrea*	Anacardiaceae	malaria, diarrhea, heart problems, blood cleansing, abdominal pain, proctitis, fevers, headaches, ulcers, toothache, backache
32	*Senecio gregatus*	Compositae	enema, venereal diseases
33	*Senecio serratuloides*	Compositae	infections, sores, blood purifier, burns, swollen gums, chest pains
34	*Stangeria eriopus*	Cycadales	emetic, headaches, purgative, flatulence, blood pressure
35	*Trichilea dregeana* & *Trichilea emetica*	Meliaceae	stomach complaints, backache, kidney problems, blood cleanser, lumbago, intestinal worms, dysentery
36	*Turbina oblongata*	Convolvulaceae	arthritis, gout, spine pain, sores, abscesses, rheumatism
37	*Urginea sp.*	Liliaceae	rheumatism, gout, bronchitis, asthma, hoarseness, influenza, diuretics, abdominal pains, swellings, demulcent
38	*Warburgia salutaris*	Canellaceae	expectorant, rheumatism, malaria, sores, colds, coughs, venereal diseases, constipation, stomach ulcers
39	*Zanthoxylum davyi*	Rutaceae	coughs, colds, tonic, infected wounds, sore throats, mouth ulcers, boils, toothache, pleurisy, bilharsia

Some of the species commonly used in KwaZulu-Natal are found all over the country for example *Scilla natalensis* (Arnold and de Wet, 1993) and are not listed in Table 3.2. Surprisingly, these plants could be listed under the unidentified species in Table 3.1 since they are not listed in Table 3.2. Some plant species like *Stangeria eriopus* are not found in a region formerly known as Transvaal but only in KwaZulu-Natal and South Western Cape. These two areas are also temperate compared to Transvaal since they are along the sea. Because of difference in temperatures, which is one of the variables that affect plant growth, some species will not grow at particular areas.

The plant species in Table 3.2 are organized according to their importance to society and traditional healers. This is based on their demand and unit cost. Plants that are trusted by many people for their purported efficacy might become endangered if action is not taken to preserve them. Plant species on demand may become expensive if their supply is limited. Therefore, these plant medicines have the highest possibility of being adulterated. This thesis, therefore, proposes that the plant species that are on demand should be examined. The characteristics of the selected medicines are explained in the next sections.

3.2.1 *Acacia caffra* (Fabaceae)

Common names: amaquasdoornboom (Afrikaans), hook-thorn (English), umkaya (Ndebele), muguhwa (Shona), morulthana (Tswana), umtoli (Xhosa) and umthole (Zulu)

Acacia caffra is a tree naturally occurring all over South Africa (Arnold and de Wet, 1993). This tree belongs to Group 4 of the acacias, which have hooked pair of thorns and inflorescence (McKinnon–Villa, 1996). The name *Acacia* was derived from "akis " meaning a point or barb and *caffra* means an epiphet frequently bestowed on plants from the eastern parts of South Africa in previous centuries (Thomas, and Grant, 1998). *Caffra* in Hebrew means "person

living on the land" (Van Wyk *et al.*, 1997; Venter, 1996). In Zulu traditional medicine, the bark is used for blood cleansing, the root as love charm emetics and the leaf for infantile abdominal disorders. *Acacia caffra's* young wilted twigs when removed from the tree by storm are associated with prussic acid poisoning. The bark of *Acacia caffra* is known to have tannins (Hutchings *et al.*, 1996) and is often confused with *A. ataxacantha*.

3.2.2 *Acacia karroo* (Fabaceae)

Common names: soetdoring (Afrikaans), sweet/white thorn (English), isingawa (Ndebele), mookana (Northern Sotho), munenje (Shona), moshaoka (Tswana) and umunga (Zulu).

Acacia karroo belongs to the same genus as *Acacia caffra* and is the most widely distributed of all South African trees (van Wyk *et al*, 1997). Its roots are nitrogen fixers and they make this tree ideal for planting on disturbed and poor soils (van Wyk and van Wyk, 1997). Bark decoctions are used as emetics for ailments believed to be caused by sorcery. The bark is also used to relieve stomach ache (Hutchings *et al*, 1996). The whole tree (gum, bark, and leaves) is used for colds, diarrhoea, dysentery, conjunctivitis and haemorrhage. The chemical constituents of this tree are tannins and rhamnose (Hutchings *et al*, 1996; van Wyk *et al*, 1997).

3.2.3 *Artemisia afra* (Asteraceae)

Common names: wilde-als (Afrikaans), African wormwood (English) lengana (Sotho) and umhlonyane (Zulu)

The aromatic *Artemisia afra* is a very common species in South Africa and is used as a multi-purpose medicinal plant. It inherited its name from its ability to eradicate worms. The leaves are the only part mainly used, but the roots may also be used. Fresh leaves are used to treat blocked nose by insertion into the nostrils. Leaf concoctions are used for treatment of

headache, loss of appetite, colic, ear ache, malaria, intestinal worms, fever, cough, colds and influenza. This species is known to have antibacterial and anti-oxidative properties, exerted by the volatile oils [mainly 1, 8 – cineole, α - thujole, β - thujole, camphor and borneol]. The thujoles are associated with harmful effects such as hallucination, when concoctions are overdosed or used over a long period (van Wyk *et al*, 1997 and Hutchings *et al*, 1996).

3.2.4 *Boophane haemanthoides* (Amaryllidaceae)

Common names: Legwama (Sotho)

The *Boophane* genus is made up of geophytes with large bulbs that are thickly tunicated. The *Boophane* consist of six species that are distributed throughout South Africa (Du Plessis and Duncan, 1989). *Boophane disticha* is the most common of all species of this genus and is also known to be poisonous. *Boophane haemanthoides* has been shown to contain alkaloids of the lycorine and crinine type, haemanthine and distichamine (Hutchings *et al*, 1996). This species only occurs in the Cape and few studies have been reported on it (Arnold and de Wet, 1993). This bulb species is often confused with *Boophane disticha*, which is also a medicinal plant. *Boophane disticha* contain toxic alkaloids that have been isolated from the bulb. These alkaloids are: narcissine, boophanine that resemble hydrazine in pharmacological action and exhibit convulsive action similar to colchicines, and haemanthine, which is closely related to atropine (Chan *et al.*, 1994; Perharic *et al.,* 1994).

3.2.5 *Croton sylvaticus* (Euphorbiaceae)

Common names: koorsboom (Afrikaans), forest fever–berry (English) and umanhlanganisa or umahlabekufeni, (Zulu)

Croton sylvaticus share a common Zulu name with *C. gratissimus* and has more than eight Zulu names given to it (Hutchings, 1989). Therefore, possibilities of confusing these two species are high. The medicinal uses of this species include treating abdominal disorders, intestinal inflammations, dropsical swellings and uterine disorders. Its roots are used as purgatives, for pleurisy and indigestion. This species has been reported to be toxic to birds and fish. It is possible that it may be toxic to humans, mostly toxicity is related to dose. The tree bark is known to have tannins whereas the root contains crotin (Hutchings et al, 1996). Croton *sylvaticus* is restricted to the East Coat, Mpumalanga and the Northern Province (van Wyk *et al*, 1997).

3.2.6 *Peltophorum africanum* (Rosaceae)

Common names: huilboom (Afrikaans) weeping wattle (English) and isikhaba-mkhombe (Zulu)

The name 'weeping wattle', is due to the activity of *Ptyelus grossus*, also known as "spittle bug", which occurs in large colonies on the branches of this tree sucking out the sap. They filter out the nutrients, excreting the excess water as a protective froth, which constantly drips to the ground, causing the tree to "weep" (Venter and van den Heever, 1998). This species is one of the versatile trees from South Africa with roots; bark and leaves used in traditional medicine for a variety of ailments e.g. abdominal and chest pain.

It belongs to a genus known to have potential toxins and has anti-inflammatory activity against carrageenan-induced edema in rats. The timber can also be used for furniture and fuel. The roots of this tree are occasionally mixed with other plant species (Bridelia cathartica) to make decoctions for treating infertility and backache (Hutchings *et al.* 1996; Wyk *et al.*, 1997).

3.2.7 *Warburgia salutaris* (Canellaceae)

Common names: koorsboom (Afrikaans), fever tree, pepper-bark tree (English) and isibhaha (Zulu, siSwati and Ndebele), muranga (Shona), or chibaha (Tsonga).

Warburgia salutaris is one of the most popular traditional medicines in South Africa and has become threatened as a result of over-harvesting. It is sold in urban marketplaces in Mozambique, Swaziland, South Africa, Lesotho and Zimbabwe (Low and Rebelo, 1996). This species is highly valued for its activity as commercial herbal medicine, probably due to biologically active drimane sersquiterpinoids, typically warburginal and mannitol, the latter being more widely used as a diuretic and to treat dyspepsia. The bark and roots of this species are the parts mostly used whereas the leaves are the least used. The bark is used as an emetic or purgative, for rheumatism, influenza, malaria, venereal diseases, headache, toothache and gastric ulcers (van Wyk *et al.* 1997).

This plant is often used in mixed remedies. The leaves are used for coughs and to make lotions used for urethral inflammations, sores and irritants. The bark preparations have been found to be harmful, especially if they are extracts of the inner bark. The bark is known to have tannins, mannitol, and sesquiterpenoid dialdehydes. The extracts of *Warburgia salutaris* have broad minimum inhibitory concentration [warburganal, muzugadial and polygodial with polygodial showing potent activity] against *Saccharomyces cerevisae, Candida utilis* and *Sclerotonia libertiana* (Hutchings *et al.* 1996)

In Zimbabwe, *Warburgia salutaris* became locally scarce due to over-harvesting for medicinal purposes, resulting in bark supplies being brought into Zimbabwe from Mozambique. This destruction of *Warburgia salutaris* populations is a widespread problem for this taxon in

Africa (van Wyk *et al.*, 1997). Therefore, this species has a limited distribution in Southern Africa, where it is listed as a vulnerable species in the recent Red Data List for this region (Low and Rebelo, 1996).

3.3 Conclusion

This study has shown that collectors and traders are harvesting numerous plant species. This may lead to over-exploitation of these plant species. The comparison of plant medicines commonly used in KwaZulu-Natal to Pretoria areas has revealed common plant medicines. From the study conducted as part of this thesis, only 7 plant species are commonly used in KwaZulu-Natal and Pretoria.

Traditional medicines collected in Gauteng province were allocated 5 points while those collected elsewhere were allocated 10 points. Traditional medicines collected from KwaZulu-Natal, Northern Province and Cape Town are relatively costly. Expensive medicines were allocated 10 points since the cost is the most important factor in determining whether to replace plant species or not.

SeSotho and isiZulu African names of the traditional medicines traded in Pretoria market areas are the most common trade names. Upon obtaining the African names of the commonly used traditional medicines, the scientific names of those plant species were then retrieved from published documents. Six of the top 10 plant species were selected for TLC identification, based on their availability in Pretoria National Botanical Garden (PNBG) and Agricultural Research Council (ARC). From this chapter, it is recommended for further study that the degree in which plants sold in the market are labeled correctly, is established.