THE ANTIMICROBIAL ACTIVITY AND CHEMICAL PROFILE OF TRADITIONAL MEDICINAL PLANTS INDIGENOUS TO SOUTHERN AFRICA USED TO TREAT RESPIRATORY TRACT INFECTIONS

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DECLARATION

I, Anisa Suliman declare that this research report is my own work. It is being submitted for the degree of Masters of Science in Medicine (Pharmaceutical Affairs) at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

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DEDICATION

To my precious Uthmaan.

I hope that this research report will serve to demonstrate that hard work, dedication and most importantly discipline goes a long way.

ABSTRACT

Fifteen traditional medicinal plants, indigenous to southern Africa, that are used to treat various respiratory ailments were screened for their antimicrobial activity and their chemical profiles were documented. Acetone:methanol (1:1) extracts were prepared from the leaves, stems, roots, barks and thorns of the investigated plant species.

The antimicrobial activity was determined against pathogens associated with respiratory conditions *i.e. Moraxella catarrhalis, Bacillus cereus, Enterococcus faecalis, Klebsiella pneumoniae, Staphylococcus aureus, Candida albicans* and *Cryptococcus neoformans*. The MIC values ranged from 0.08 mg/ml to 12 mg/ml. The two pathogens against which the most number of extracts obtained MIC values that were ≤ 1 mg/ml were *Moraxella catarrhalis* (68% of the extracts) and *Bacillus cereus* (56% of the extracts). The plant extracts that obtained the five lowest average MIC values against the respiratory pathogens were the root extracts of *Terminalia sericea* (0.69 mg/ml), leaf and stem extract of *Chenopodium ambrosioides* (1.04 mg/ml), leaf, stem and flower extract of *Leucas martinicensis* (1.10 mg/ml), leaf extract of *Zanthoxylum davyi* (1.29 mg/ml) and the leaf and stem extracts of *Lantana rugosa* (1.32 mg/ml).

For the bioautographic assays, clear zones of inhibition were recorded for *Lantana rugosa* (leaves and stems) and *Vitex rehmannii* (leaves) against *Staphylococcus aureus* and *Moraxella catarrhalis*. The root extract of *Ziziphus mucronata* had a clear zone of inhibition against *Staphylococcus aureus.* The leaf and stem extracts of *Chenopodium ambrosioides* had a clear zone of inhibition against *Moraxella catarrhalis*.

The chemical profiles that were recorded for the plant extracts comprised of HPLC and TLC chromatograms. The HPLC and TLC profiles resulted in the separation of the chemical constituents thus providing a chemical fingerprint for the plant extracts. Flavonoids were tentatively identified for *Acacia sieberiana* (leaves), *Alepidea amatymbica* (roots), *Clematis oweniae* (leaves), *Clerodendrum glabrum* (leaves), *Heteromorpha arborescens* (bark), *Peucedanum caffrum* (roots B), *Vitex rehmannii* (leaves) and *Ziziphus mucronata* (leaves). The TLC chromatograms qualitatively displayed good separation of the compounds present in the plant extracts.

The antimicrobial activity recorded for the plant extracts validates their traditional uses to treat various respiratory infections and the chemical profiles provide a reference of the chemical profiles of the plant extracts that can be used in future investigations.

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LIST OF ABBREVIATIONS

ATCC	American Type Culture Collection
CFU	Colony forming units
DMSO	Dimethyl sulphoxide
HPLC	High performance liquid chromatography
INT	*p*-iodonitrotetrazolium violet
mg	milligrams
MIC	Minimum inhibitory concentration
min	Minutes
ml	Millilitres
NCCLS	National Committee for Clinical Laboratory Standards
NHLS	National Health Laboratory Services
PDA	Photodiode array
R_f	Retention factor
SANBI	South African National Biodiversity Institute
TLC	Thin layer chromatography
TMD	Thermabeam mass selective detector
TSA	Tryptone Soya agar
TSB	Tryptone Soya broth
µg	Microgram
µl	Microlitre
UV	Ultraviolet
WHO	World Health Organisation

CHAPTER 1

Introduction

1.1 Introduction

Globally, one of the major causes of deaths are infectious diseases both in developing, and surprisingly enough, also in developed countries. Two main reasons for this is the increase in deaths due to respiratory track infections and HIV/AIDS (Iwu *et al.*, 1999). In South Africa, of the top 20 leading causes of deaths, lower respiratory infections ranked third highest of the infectious diseases causing mortality (Norman *et al.*, 2006). Mortality statistics related to deaths caused by unspecified acute lower respiratory infections has shown South Africa to have the highest number of deaths due to acute respiratory infections as compared to other countries (Nation Master, 2004).

Traditional medicine has a very deep rooted and rich cultural heritage in South Africa with approximately 200 000 traditional healers being consulted by about 30 million people for their healthcare needs (Taylor *et al.*, 2001; Pefile, 2005). The main reasons for this are, firstly, for the majority of the South African population, traditional medicine is very important from a cultural point of view. Secondly, and more significantly, the majority of the people using traditional medicine live in the rural areas, where access to conventional healthcare is very limited due to their high cost and the poor dissemination of appropriate healthcare facilities in these areas (Fennell *et al.*, 2004; Light *et al.*, 2005; Pefile, 2005).

Respiratory infections are ranked quite high as causing mortality in South Africa and since the majority of the South African population rely on traditional medicine for their healthcare needs, it was thought appropriate to investigate plant species that are indigenous to South Africa, so as to verify their traditional uses to treat various respiratory diseases.

1.2 Prevalence of respiratory tract infections in developing countries

Investigation of alternative therapies such as traditional medicinal plants for their effectiveness in treating respiratory infections could prove valuable, especially in developing countries where acute respiratory infections are very prevalent. Acute respiratory tract infections have a major impact on the mortality of children. In developing countries, 50% of deaths occur in children who are five years or younger and 25-33% of these deaths are attributed to acute respiratory infections (WHO, 1986; von Schirnding *et al.*, 1991; Shapiro, 1998).

An in-depth analysis of the estimates of acute respiratory infections on child deaths was carried out by Williams *et al.* (2002). They found that world-wide, for the year 2000, 1.9 million childhood deaths were due to acute respiratory infections, and 70% of these deaths were in Africa and Southeast Asia. Estimates of annual death rates for acute respiratory infections in infants ranged from 1.5 deaths per 1000 in North America compared to 11 - 15 deaths per 1000 in Central and South America and Africa (Shapiro, 1998). Similarly the annual mortality rates for children 1 to 4 years caused by acute respiratory infections were highest in Africa (5 deaths per 1000) compared to 1-1.5 deaths per 1000 in central and South America with North America having the lowest rates of 0.08 deaths per 1000 children (Shapiro, 1998).

In developing countries, deaths caused by acute respiratory tract infections, are more specifically due to lower respiratory tract infections (WHO, 1986; Klig and Chen, 2003). Pneumonia is currently the leading cause of death in developing countries in children less than five years of age wherein it is responsible for 20% of these childhood deaths (Zar and Madhi,

2006). Singh (2005) reports that African and Asian countries record 2-10 times more children suffering from pneumonia (7-40/100 annually) as compared to the United States.

In sub-Saharan Africa, the impact of HIV infection has further intensified the impact of pneumonia on the mortality of children less than five years of age. Of the 2.3 million children infected by HIV worldwide, the great majority of these children live in sub-Saharan Africa. In this region, pneumonia has been recorded as the most frequent reason for hospitalisations and deaths in HIV-infected children. In Africa, HIV infected children have poor access to antiretroviral therapy, thus increasing the rate of these children developing lung diseases as a result of their compromised immunity. In sub-Saharan Africa it is reported that 90% of children infected with HIV develop a respiratory disease. (Zar and Madhi, 2006).

Figure 1.1, as adapted from the studies carried out by Williams *et al.* (2002) shows the estimated percentages of childhood deaths worldwide, for the year 2000, due to lower acute respiratory infections. In South Africa, the childhood deaths ranged between 15-20%. For the rest of Africa, as well as the Far East and some parts of South America, the percentages of childhood deaths ranged from 15% to greater than 25% which is much higher as compared to the more industrialised and developed countries. Therefore acute lower respiratory tract infections have a major impact on childhood mortality especially in developing countries.

A few studies have also been carried out to explore the impact of acute respiratory tract infections on childhood morbidity and mortality in South Africa. von Schirnding *et al.* (1991) showed that acute respiratory infections was an important cause of childhood deaths in South Africa. They examined national data for the years 1968 to 1985 and data for greater Cape

Town for the year 1987. The data revealed that 90% of deaths were due to pneumonia. Of these deaths there was a great disparity amongst the race groups where more deaths were recorded amongst blacks and coloureds as compared to whites and Asians. The study also showed that deaths due to acute respiratory infections in South Africa were 7 to 270 times greater than that recorded in Western European countries.

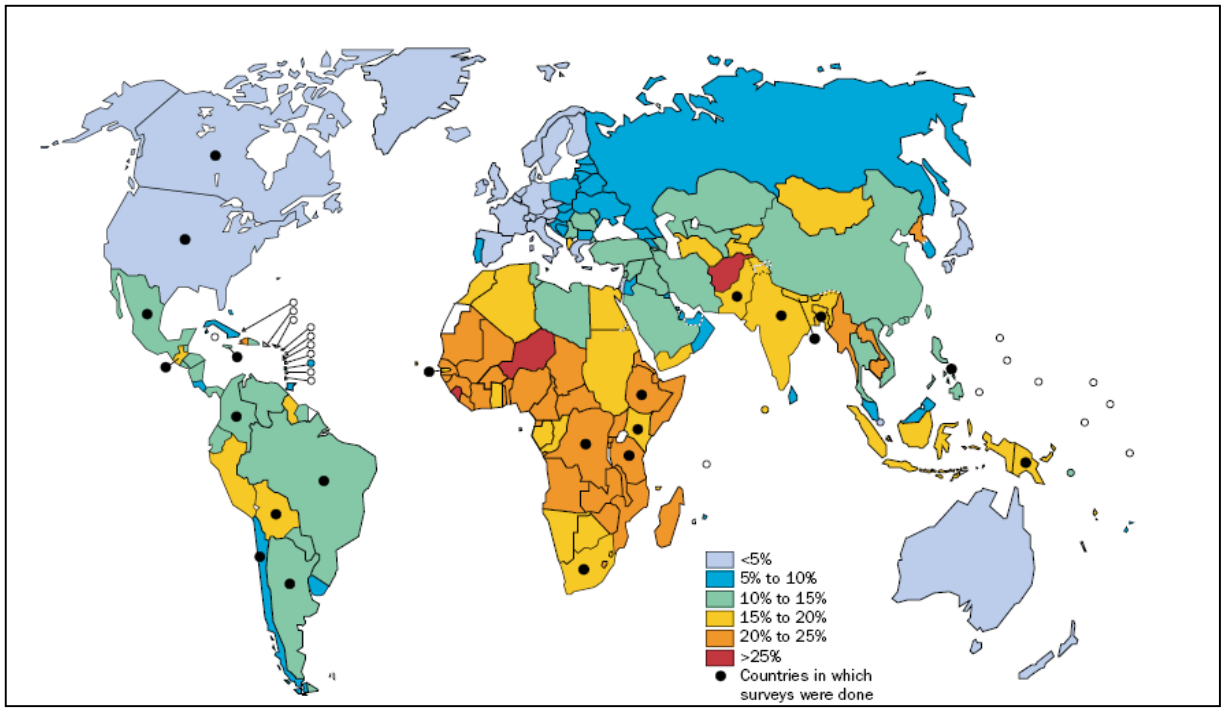


Figure 1.1 Percentage estimates of child deaths due to acute lower respiratory tract infections in the year 2000 (Adapted from Williams *et al.*, 2002).

The impact of lower respiratory tract infections on South African children was also demonstrated by a study carried out by Hla (2001). The study clearly demonstrated that acute lower respiratory infections are very prevalent amongst children in the age group of one month babies to children of 12 years. During the course of the study over 200 children were referred to the Chris Hani Baragwanath Hospital from primary health care facilities every month presenting with symptoms of acute lower respiratory infections. Of these referrals, 40% to

65% were admitted to the hospital to be treated for acute respiratory infections. Pillay *et al.* (2001) in a study to determine the impact of HIV infection on the admission rates of children in a South African academic hospital showed that 40.6% of the admissions were due to acute respiratory infections where 29.7% of the primary diagnosis was due to pneumonia.

Therefore there is a high prevalence of acute respiratory infections in young children under five years of age especially in the developing countries. Furthermore, the high impact of the HIV infection on young children in these developing countries further intensifies the effect that acute respiratory infections, especially pneumonia, has on the mortality of these young children. Thus, the motivation for this study was to investigate the chosen plant species for their effect on respiratory tract infections.

1.3 Increasing resistance of respiratory pathogens to conventional antibiotics

Another important reason as to why this research topic was chosen is the ever increasing emergence of resistant micro-organisms to conventional antimicrobial therapy.

Specifically related to respiratory tract infections, there is a rapid increase in resistant pathogens that cause both community and hospital acquired infections. In South Africa, resistance to the respiratory pathogen, *Streptococcus pneumoniae*, is recorded as having first emerged in 1978 (Klugman, 1998). Resistance patterns to the usual antimicrobial agents are very alarming. It is estimated that eighty percent of the worldwide antibiotic usage takes place in the community instead of in hospitals, and surprisingly enough, 80% of this usage is for respiratory tract infections. (Liebowitz *et al.*, 2003). Liebowitz *et al.* (2003) also recorded relatively high rates of antimicrobial resistance to antibiotics such as penicillins, macrolides

and cotrimoxazole for respiratory pathogens *viz. Streptococcus pneumoniae*, *Haemophilus influenza*, *Moraxella catarrhalis*, *Klebsiella pneumoniae* and *Streptococcus pyogenes*.

1.4 Respiratory infections caused by the pathogens used in this research study

The respiratory pathogens used in this research study were Gram-positive *Bacillus cereus, Enterococcus faecalis* and *Staphylococcus aureus*. The Gram-negative pathogens were *Klebsiella pneumoniae* and *Moraxella catarrhalis.* The yeast pathogens were *Candida albicans* and *Cryptococcus neoformans*. Below is a brief overview of the respiratory infections that these pathogens are responsible for.

Candida albicans is a yeast pathogen that forms part of the normal flora of the mucous membranes in the respiratory, gastrointestinal and female genital tracts. The primary infection caused by *Candida albicans* is candidiasis, which is an overgrowth of this pathogen and it most commonly affects the mouth, skin, nails and female genital organs (Jawetz *et al.*, 1982). Pharyngitis, an upper respiratory tract infection, can be caused by fungal infections. Oral candidiasis as a result of *Candida albicans* can involve the pharynx, leading to pain and inflammation (Dasaraju and Liu, 2002).

Cryptococcus neoformans, a yeast organism, can become airborne and as a result enters the respiratory tract and causes infection in humans. *Cryptococcus neoformans* causes infections that are asymptomatic or associated with nonspecific pulmonary signs and symptoms. However, cryptococcosis is an opportunistic infection and as a result can pose a serious problem for immunosuppressed patients. It can cause serious pneumonia and the infection in

the lungs can spread systemically to the central nervous system and other organs (Jawetz *et al.*, 1982; Dasaraju and Liu, 2002).

Staphylococcus aureus is a group of Gram-positive staphylococci bacteria. It is commonly found on the mucous membranes and the skin. Being part of the skin, it can be a cause of otitis externa, which is an infection of the ear involving the external auditory canal (Dasaraju and Liu, 2002). *Staphylococcus aureus* also forms part of the normal flora of the nose (Jawetz *et al.*, 1982) and is thus also one of the bacterial causes of sinusitis (Dasaraju and Liu, 2002). In certain lower respiratory tract infections such as bronchiolitis and influenza, *Staphylococcus aureus* is a cause of secondary bacterial infections. Influenza can lead to serious *Staphylococcus aureus* pneumonia which develops quickly wherein purulent sputum is produced and lung function deteriorates. Methicillin-resistant strains of *Staphylococcus aureus* are a common cause of lung infections in hospitals (Bannister *et al.*, 2000).

Klebsiella pneumoniae is a Gram-negative pathogen of the Enterobacteriacea family (Boyd and Hoerl, 1981). It is also found as part of the normal flora of the intestinal tract and occurs freely in the soil and water (Airborne Pathogen Data Base, 2002). *Klebsiella pneumoniae* causes infections such as pneumonia, septicaemia and wound infections. It is also a common cause of lung infections in hospitals. Lobar pneumonia caused by *Klebsiella pneumoniae* can occur in patients who are suffering from asthma for a long time, or in debilitated patients or in those who are suffering from alcoholism (Bannister *et al.*, 2000).

Enterococcus faecalis is a Gram-positive bacterium which mainly colonises the large intestine and also the urinary tract (Rollins and Joseph, 2000). On occasion, *Enterococcus faecalis* has

been known to cause empyema, which is an infection of the pleural space (Behnia *et al.,* 2002). A study by Patterson *et al.* (1995) also found *Enterococcus faecalis* to cause pleuropulmonary infections.

Bacillus cereus is a Gram-positive bacterium that has the ability to form spores (Kotiranta *et al.*, 2000). Food poisoning is a common result of *Bacillus cereus* infections. However, *Bacillus cereus* is now also being recognised as causing serious systemic infections especially in high risk patients such as the immunocompromised patient, the severely ill patient, neonates and patients that have artificial devices inserted for e.g. ventricular shunts and intravascular catheters. Pneumonia caused by *Bacillus cereus* occurs in patients whose immune systems are weak and are thus more susceptible (Gaur and Shenep, 2001). *Bacillus cereus* was found to be the cause of a necrotising pneumonia that caused the deaths of two premature neonates (Jevon *et al.*, 1993). Gray *et al.* (1999) have also reported respiratory tract infections in preterm neonates that were caused by *Bacillus cereus*. In another study, *Bacillus cereus* was also isolated from the respiratory tissue of patients that were ventilated in a paediatric intensive care unit. The re-usable ventilation tubes and the alcohol solution that was used to disinfect the equipment were all found to be colonised with *Bacillus cereus* (Kalpoe *et al.*, 2008). Two other cases have also been reported where respiratory tract infection by *Bacillus cereus* occurred due to the ventilation equipment being colonised by this pathogen as a result of inadequate disinfecting practices (Bryce *et al.*, 1993 and van der Zwet *et al.*, 2000 in Kalpoe *et al.*, 2008).

Moraxella catarrhalis is a Gram-negative organism. It is commonly found as part of the normal flora of the nasopharynx (Verduin *et al.*, 2002). Verduin *et al.* (2002) have reported

that *Moraxella catarrhalis*, over the past 20 to 30 years, has become a true pathogen, responsible for various respiratory tract infections. It causes upper respiratory tract infections in healthy children and elderly people. These infections include sinusitis, otitis media, tracheitis, bronchitis and pneumonia. *Moraxella catarrhalis* very rarely causes lower respiratory tract infections in children however these do occur in children under the age of one year (Verduin *et al.*, 2002). In adults, *Moraxella catarrhalis* very often causes laryngitis. *Moraxella catarrhalis* is not a common cause of lower respiratory tract infections (LRT) in healthy adults, however, patients suffering from chronic obstructive pulmonary disease (COPD), elderly patients with pneumonia and patients with compromised immune systems are more susceptible to *Moraxella catarrhalis* infections. It is also classified as a nosocomial pathogen (Verduin *et al.,* 2002).

1.5 Outline of the study

Fifteen plant species were investigated in this research study. The plant species were selected based on their documented traditional uses to treat various respiratory ailments (Table 1.1). This research study focused mainly on the broad scale screening of these plant species. Their antimicrobial activity was investigated against respiratory pathogens whereby the minimum inhibitory concentrations (MIC) of the extracts were determined and bioautographic assays were carried out against the respiratory pathogens.

High performance liquid chromatography (HPLC) and thin layer chromatography (TLC) profiles were also recorded for each of the plant extracts to act as a fingerprint for future investigations. HPLC allows for the separation of the chemical compounds that possess varying polarities (Phillipson, 2003) thereby allowing for the profiling of the compounds

Table 1.1 Plant species used traditionally to treat specific respiratory ailments.

Plant species	Respiratory ailment
Acacia sieberiana (Fabaceae)	Leaf, bark and resin are used for their astringent and expectorant effect on upper respiratory tract infections [a]. The exudates, bark and leaf are used in the Congo for colds [c].
Alepidea amatymbica (Apiaceae)	The roots are used to treat coughs, colds or influenza. Either the roots are eaten raw or cooked. Children are given a root infusion or a snuff of the root [b, c]. The Zulu use the plants to treat colds. Coryza and cough in children are treated with a root infusion enema. Influenza is treated by drinking a root decoction by the Zulus of Gauteng and the Swati. The South Sotho chew the root for chest ailments [c].
Chamaecrista mimosoides (Fabaceae)	It is reported that in East Africa the plant is used for chest conditions including pneumonia [a].
Chenopodium ambrosioides (Chenopodiaceae)	In Western medicine the plant is made into a cold and cough remedy. The steam of the boiled plant can be inhaled or a steam bath can be used for healing colds, coughs and general body pains [a]. The Sotho use an infusion for colds and a green leaf tincture is used by the Xhosa as a cough suppressant [c].
Clematis oweniae (Ranunculaceae)	A root decoction is taken for coughs by the indigenous people of southern Africa. Finely ground leaves are inhaled to relieve colds and headaches [a, c].
Clerodendrum glabrum (Verbenaceae)	Leaves are used for coughs [b, c], as well as for colds, sore throats and chest complaints [b].
Heteromorpha arborescens (Apiaceae)	The Xhosa use the roots to treat coughs [b, c]. In Zimbabwe the roots are used to treat coughs, chest pain and fever [b].
Lantana rugosa (Verbenaceae)	A tea from stems and leaves is taken for bronchitis. The Xhosa take a leaf extract to treat bronchitis and as a lavage for catarrh and ear ache [a]. An infusion of the leaf is used to aid bronchial conditions. For the treatment of coryza, the Pedi use the crushed leaves as a snuff or cold infusion [c].
Leucas martinicensis (Lamiaceae)	It has been shown that the leaves are used to make tea to treat colds and flu [a]. The Europeans drink a hot decoction for colds and the steam is inhaled occasionally also for colds [c].
Peucedanum caffrum (Apiaceae)	The roots are used in traditional medicine. Zulu medicinal usage of this plant is as an enema for chest complaints [b].
Schkuhria pinnata (Asteraceae)	Crushed leaves that are soaked in water are used for upper and lower respiratory tract infections and influenza [a, c].
Terminalia sericea (Combretaceae)	Hot poultices are made with an extract of the root's outer layer for pneumonia. The roots are also used in a cough remedy [a].
Vitex rehmannii (Verbenaceae)	Unspecified plant parts are used to treat pleurisy [b] (inflammation of the membrane that lines the lungs and the pleural cavity).
Zanthoxylum davyi (Rutaceae)	Powdered bark that is cooked is chewed for severe coughs and colds. The leaves are used for chest pains, the roots for sore throats and the bark for chronic coughs [b].
Ziziphus mucronata (Rhamnaceae)	A decoction or tea of the root, bark or leaves is used for chest complaints and coughs [a, c]. A hot-pulp poultice of burnt root is sometimes used together with leaves for swollen glands in tuberculosis [a, c]. For chest complaints, the Zulu take a mixture of the bark and leaf in water as an emetic [c].

[a.] von Koenen (2001); [b.] Hutchings *et al*. (1996); [c.] Watt and Breyer-Brandwijk (1962)

(Molnár-Perl and Füzfai, 2005) found in the plant species and thus serving as a "fingerprint" for the chemical constituents of the plant species (Springfield *et al.*, 2005).

HPLC and TLC analysis of plant species have been utilised in many studies (Scott *et al.*, 2004; Fennell *et al.*, 2004; Springfield *et al.*, 2005) to provide a means for the correct identification of the plant species and also to profile the chemical composition of the plants.

According to Marston and Hostettmann (1999), the use of HPLC together with a detector e.g. a UV photodiode array detector allows one to determine what type of chemical compound is present in the plant extract. In this study, the HPLC system was coupled with a UV photodiode array detector in order to analyse the UV spectra of the compounds so as to tentatively identify flavonoids. Furthermore, when HPLC is coupled with a photodiode array detector, flavonoids can be detected and identified with a high degree of accuracy (Molnár-Perl and Füzfai, 2005). Previous studies (Palomino *et al.*, 1996; Keinänen and Julkunen-Tiitto, 1998; Ye *et al.*, 2002) have utilised HPLC coupled with a photodiode array detector in order to detect flavonoids present in plant species.

Figure 1.2 is a diagrammatic flowchart which describes the various steps that were carried out during this research study. Monographs of the 15 plant species have also been drawn up and are presented in Appendix A. These monographs provide information on the botanical description, geographical distribution and traditional medicinal uses of the plant species. The HPLC chromatograms for each of the extracts together with the flavonoids that were tentatively identified for the extracts are also presented in the monographs.

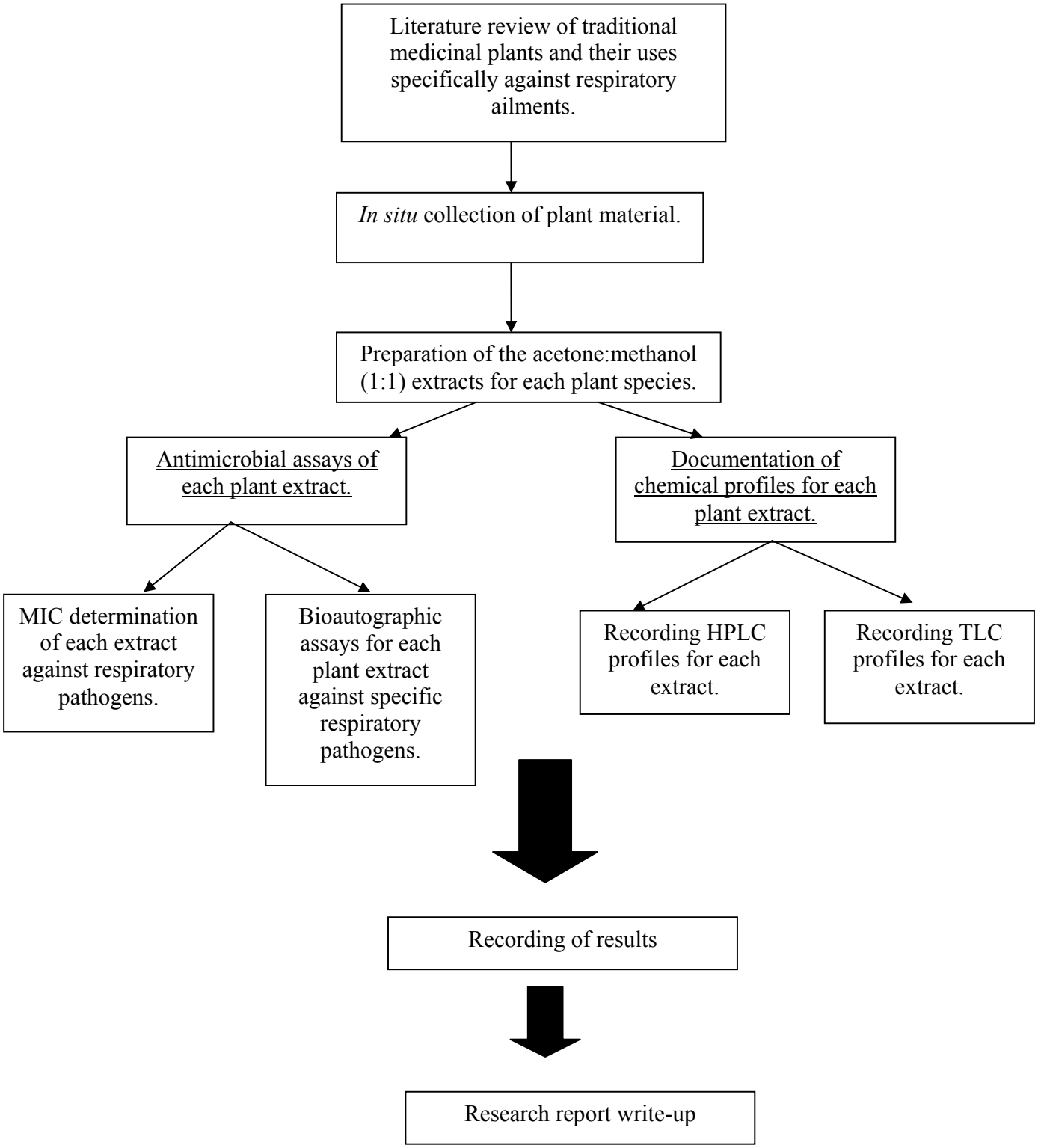


Figure 1.2 Diagrammatic flow chart of the research project.

The monographs also include a literature review of previous studies carried out on the antimicrobial activity, chemistry and toxicity of the plant species. These monographs serves as a means of providing information on these traditional medicines which falls in line with other research studies that were aimed at providing much needed information into the safety, efficacy and quality of South African medicinal plants (South African Traditional Medicines Research Group, 1999; Scott *et al.*, 2004; Springfield *et al.*, 2005).

Thus this research study was aimed at contributing to the ever increasing research into South African traditional medicines (Light *et al.*, 2005), focusing primarily on providing a scientific justification for their uses to treat various respiratory ailments.

1.6 Objectives of the study

The objectives of the study were to find a scientific rationale for the use of African traditional medicine, specifically those indigenous to southern Africa, against respiratory tract pathogens, by investigating the plants for their antimicrobial activity, which the traditional healers claim they have. This was further subdivided into the following objectives:

- To determine the possible antimicrobial activity of the plant species investigated by determining the minimum inhibitory concentrations (MIC) required to inhibit the growth of the selected respiratory pathogens
- By performing bioautographic assays of the plant extracts against specific respiratory pathogens to determine whether individual or groups of compounds show inhibitory activity.

- To document the phytochemical profiles of these traditional medicinal plants using thin layer chromatography (TLC) and high performance liquid chromatography (HPLC).

CHAPTER 2

Materials and Methods

2.1 Collection of plant material

Table 2.1 lists the plant species, their locality, plant parts used to prepare the extracts and voucher numbers of the specimens that were collected from various locations in the wild around southern Africa. Voucher specimens are retained at the Department of Pharmacy and Pharmacology, Faculty of Health Sciences, University of the Witwatersrand. Identification of the plant species was carried out at the South Africa National Biodiversity Institute (SANBI) in Pretoria.

2.2 Preparation of the plant extracts

Approximately 5 g of dried plant material was ground up into a fine powder and placed into a flat bottom flask. The grounded plant material was then soaked in approximately 10 to 15 ml (a quantity sufficient to just cover the powder) of a methanol:acetone (Merck) (1:1) mixture. The flasks were then sealed with polyfilm and placed overnight in a water bath set at a temperature of 40°C. The extract was filtered and washed with a fresh mixture of acetone:methanol (1:1) and the filtrate air dried. Thereafter the dried filtrate was used to prepare various concentrations of plant extract to be used for minimum inhibitory concentration (MIC) determination, bioautographic assays, thin layer chromatography and high performance liquid chromatography.

Table 2.1 Summary of plant material used.

Species	Locality	Plant part used	Voucher number
Acacia sieberiana DC. var. *woodii* (Burt Davy) Keay & Brenan	Near Sudwala (Mpumalanga)	Bark Leaves	B & F 16
Alepidea amatymbica Eckl. & Zeyh. var. *amatymbica*	Sabie District (Mpumalanga)	Roots	P.M Burgoyne 9523
Chamaecrista mimosoides (L.) Greene	Wilge River pumpstation near Balmoral (Gauteng)	Leaves Roots	B & F 13
Chenopodium ambrosioides L.	Near Rayton (Gauteng)	Leaves and stems Roots	B & F 14
Clematis oweniae Harvey	Near Sudwala (Mpumalanga)	Leaves	B & F 15
Clerodendrum glabrum E. Meyer var. *glabrum*	Abel Erasmus Pass (Mpumalanga)	Leaves Bark	PMB 9527
Heteromorpha arborescens (Spreng.) Cham & Schltdl. var. *abyssinica* (A.Rich) H. Wolff *	272 Mooiplaats district of Cullinan (Gauteng)	Leaves Bark Stems	B & F 18
Lantana rugosa Thunb.	Rayton (Gauteng)	Leaves and stems	B & F 10
Leucas martinicensis (Jacq.) R. Br.	Mooiplaats (Mpumalanga)	Leaves, stems and flowers	B & F 11
Peucedanum caffrum (Meisn.) Phill.	Roossenekal, 16 km north of Roossenekal (Mpumalanga)	Roots A Roots B Roots C	P.M Burgoyne 9550
Schkuhria pinnata (Lam.) Kuntze ex Thell.	Rhenosterfontein (Northern Province)	Leaves	B & F 11
Terminalia sericea Burchell ex DC.	Swaziland	Roots	AdCAV21
Vitex rehmannii Guerke	Waterberg (Northern Province)	Leaves	PMB 9520
Zanthoxylum davyi (Verdoorn) Waterm.	Nelspruit (Mpumalanga)	Leaves Roots Bark Thorns	PMB 9525
Ziziphus mucronata Willd. subsp. *mucronata*	Melville Koppies (Gauteng)	Bark Leaves Roots	AdCAV362

* The roots are used traditionally as shown in Table 1.1. However, it was decided to study the leaves, bark and stems so as to avoid destructive harvesting of the roots.

2.3 Phytochemistry

2.3.1 Thin layer chromatography (TLC)

Thin layer chromatography (TLC) was used as a qualitative means of separating the various compounds present in the plant extracts and to produce a chemical fingerprint for the various species.

The dried filtrate as described in Section 2.2 was dissolved in acetone to a concentration of 64 mg/ml and 3 µl of this extract was spotted onto silica gel TLC plates using a calibrated micro-capillary tube. The TLC plate was developed in a mobile phase comprising of toluene:dioxan:acetic acid (90:25:10). Approximately 15 to 20 minutes was allowed for the mobile phase to move up the TLC plate. The plates were removed from the developing tank and air dried, after which they were viewed under UV lights (254 nm and 366 nm). The TLC plates were also sprayed with 0.5 % anisaldehyde sulphuric acid spray reagent (Fluka) comprising glacial acetic acid, methanol and concentrated sulphuric acid (10:85:5). Colour development was performed by heating the plates for approximately 10 minutes in an oven at 105°C.

2.3.2 High performance liquid chromatography (HPLC)

The plant extracts were analysed using high performance liquid chromatography (HPLC) for the purposes of documenting the chromatograms as well as for the tentative identification of flavonoids.

The HPLC analysis was performed using a Waters 2695 HPLC system equipped with both a 2996 photodiode array (PDA) and a Thermabeam electron impact (EI) mass selective detector

(TMD). The TMD detector was operated in EI mode with the ionizer at 70 eV (fixed) with a gain of 10 and scanning mass range of 50-550 amu.

A Phenomenex Aqua C18 column (250 x 2.1 mm, 5 µm) thermostated at 40°C was used. The flow rate of the HPLC was 0.2 ml/min and the gas flow through the nebuliser 30 l/h. The nebuliser temperature was 80°C, the expansion region 90°C and the source temperature 225°C.

The TMD was operated in positive ion mode with no flow splitting, thereby utilizing the full HPLC eluent at a flow rate of 0.2 ml/min. The mobile phase started with 10% acetonitrile, 90% water containing 10 mM formic acid. The solvent ratio was changed through a linear gradient to 90% acetonitrile, 10% water (with 10 mM formic acid) at 40 minutes. This ratio was maintained at 10 minutes, where the solvent ratio was changed back to the initial conditions.

The dried filtrate of the plant extract was dissolved in acetone (Merck) to a concentration of 64 mg/ml and 10 µl of this preparation was used as the injection volume.

The HPLC chromatograms for each of the plant extracts were recorded using the Empower® software program. For each peak, the retention time, % area and the absorbance maxima of the corresponding UV spectra, was recorded in table format.

Flavonoids were tentatively identified by analysing the UV spectra of each peak. As described by Markham (1982), two criteria were used to determine whether the UV spectrum was a

flavonoid or not. Firstly, the shape of the UV spectra was compared to that of standard UV spectra for various flavonoids. The second criterion was the wavelength of the peaks of the UV spectra. The flavonoid types that were searched for were isoflavones, flavanones, flavones and flavonols. The UV spectra for each of these flavonoids have two peaks and each peak has absorbance maxima that falls within a specific range that is characteristic for that flavonoid type. Table 2.2 lists the wavelength range for each of the flavonoid types for peak 1 and peak 2. In this way the flavonoids were tentatively identified.

Table 2.2 Wavelength ranges for the different flavonoid types.

Flavonoid type	Absorbance maxima for peak 1 (nm)	Absorbance maxima for peak 2 (nm)
Flavone	240-270	304-350
Flavonol	240-270	352-385
Isoflavone	245-270	300-340
Flavonone	270-295	330

2.4 Antimicrobial assays

2.4.1 Test organisms

Pathogens that are known to cause various respiratory diseases were chosen to test the antimicrobial activity of the plants extracts, since these plant species are used traditionally to treat various respiratory ailments (Table 1.1).

The following pathogens were used:

- Two yeast pathogens; *Candida albicans* (ATCC 10231) and *Cryptococcus neoformans* (ATCC 90112).
- Three Gram-positive bacteria; *Bacillus cereus* (ATCC 11778), *Enterococcus faecalis* (ATCC 29212) and *Staphylococcus aureus* (ATCC 12600).

- Two Gram-negative bacteria; *Klebsiella pneumoniae* (ATCC 13883) and *Moraxella catarrhalis* (ATCC 23246).

The stock cultures of the above organisms, except for *Candida albicans,* were obtained from the National Health Laboratory Services (NHLS). *Candida albicans* was obtained from the South African Bureau of Standards. The stock cultures were kept at a temperature of -20°C. Inoculum preparation from the stock culture was performed as per the NCCLS (2003) guidelines.

Tryptone soya (Oxoid) broth (TSB) was the medium used to prepare the inoculum. The TSB was prepared by transferring 40 g of the TSB powder to a sterile bottle wherein one litre of distilled water was added and the bottle swirled to dissolve the powder. The prepared broth was then autoclaved at 121°C for 15 minutes. Sterility of the broth was confirmed by incubating the broth and thereafter visually examining the broth to confirm that there was no growth of any organisms.

Inoculum was prepared whereby colonies from the stock culture were transferred into a tube containing the Tryptone soya (Oxoid) broth. The inoculated tubes were then incubated at 37°C for the bacterial pathogens and at 25°C for the yeast pathogens. After the incubation period, the purity was verified by inoculation onto agar and examining colonies.

2.4.2 Minimum inhibitory concentration (MIC)

The antimicrobial activity was established by determining the minimum inhibitory concentrations (MIC) of the plant extracts that was required to inhibit the growth of the test

pathogens. The MIC's were determined using the micro-dilution technique. This technique involves the use of a 96-well microplate and tetrazolium salts which indicate bacterial growth. According to Eloff (1998), the micro-dilution technique has many advantages. It is a robust method that is inexpensive and capable of achieving reproducible results. It is one of the more sensitive methods that requires only a small amount of sample and can be carried out on a large number of extracts.

The starting concentration of the plant extracts was 64 mg/ml. The dried plant extracts were dissolved in acetone (Merck). However, dimethyl sulphoxide (DMSO) (Merck) was used for those dried plant extracts which did not adequately dissolve in acetone. Table 2.3 lists those plant extracts that were dissolved in acetone and Table 2.4 lists those plant extracts dissolved in DMSO.

Table 2.3 Plant extracts dissolved in acetone.

Plant extract dissolved in acetone
Lantana rugosa (leaves and stems)
Terminalia sericea (roots)
Chenopodium ambrosioides (leaves and stems)
Acacia sieberiana (leaves)
Leucas martinicensis (leaves, stems and flowers)
Schkuhria pinnata (leaves)
Chamaecrista mimosoides (leaves)
Ziziphus mucronata (leaves)
Clematis oweniae (leaves)
Zanthoxylum davyi (leaves)
Zanthoxylum davyi (thorns)
Vitex rehmannii (leaves)
Clerodendrum glabrum (leaves)

Sterile 96-well microtitre plates were used and they were prepared aseptically under a laminar flow unit. Firstly 100 µl of sterile water was placed into each well. Thereafter 100 µl of stock

plant extract was placed into the first row of wells to yield a final concentration of 16 mg/ml in acetone or DMSO. Serial dilutions were then performed from one row to another using a multi-channel pipette, whereby 100 µl aliquots were transferred from one row to another. The last 100 µl aliquot from the last row was discarded.

Table 2.4 Plant extracts dissolved in DMSO.

Plant extract dissolved in DMSO
Acacia sieberiana (bark)
Chenopodium ambrosioides (roots)
Ziziphus mucronata (bark)
Ziziphus mucronata (roots)
Chamaecrista mimosoides (roots)
Zanthoxylum davyi (roots)
Zanthoxylum davyi (bark)
Clerodendrum glabrum (bark)
Heteromorpha arborescens (leaves)
Heteromorpha arborescens (bark)
Heteromorpha arborescens (stems)
Peucedanum caffrum (roots A)
Peucedanum caffrum (roots B)
Peucedanum caffrum (roots C)
Alepidea amatymbica (roots)

The microplate was removed from the laminar airflow unit and 100 µl (approximately 1 x 10^8 CFU/ml) of culture was added to the wells. One ml of inoculum from the subcultures as described under section 2.4.1 was transferred into 100 ml of sterile broth. It is from this dilution that 100 µl of culture was added to each well.

Contamination between wells was avoided by not allowing the tips of the pipette containing the culture to touch the diluted plant extract in the well. The microplate was then sealed with a sterile sealing film and incubated at 37°C for 24 hours for the bacterial pathogens and at 25°C

for 48 hours for the yeasts. The final serial dilution of the wells with the culture resulted in a concentration range of the plant extract from 16 mg/ml to 0.125 mg/ml.

The MIC's were determined in duplicate for each extract and where replicates were not consistent, the MIC was redetermined.

Ciprofloxacin (0.01 mg/ml) (Sigma-Aldrich) and amphotericin B (Sigma-Aldrich) (0.01 mg/ml) were used as the positive controls for the bacteria and fungi respectively. DMSO (64 mg/ml) was also used as a solvent control so as to ensure that the solvent did not have a negative effect on organism growth.

After the incubation period, 40 µl of a 0.2 mg/ml *p*-iodonitrotetrazolium violet (Sigma-Aldrich) solution (INT) was added into all the inoculated wells. The tetrazolium compounds in the INT binds to the living organism and changes from colourless to red thus indicating the presence of bacterial growth (Eloff, 1998). The plates that were inoculated with bacteria were visually examined after six hours and the plates inoculated with the yeasts were examined after 24 hours. The minimum inhibitory concentration for a particular plant extract was determined by recording the lowest concentration of the well which did not exhibit a red colour.

Some of the 64 mg/ml plant extracts examined using this method did not show a red colour in any of the wells for that particular column i.e. no growth of organism detected. Thus an MIC value in the dilution range of 16 mg/ml to 0.125 mg/ml could not be determined. Therefore a 5

mg/ml concentration of the extract was prepared and the MIC was re-determined. The serial dilution for a 5 mg/ml extract ranged from 1.25 mg/ml to 0.0097 mg/ml.

2.4.3 Thin layer chromatography (TLC) bioautographic assays

Thin layer chromatography (TLC) bioautographic assays were done for two of the pathogens *viz. Staphylococcus aureus* (ATCC 12600) and *Moraxella catarrhalis* (ATCC 23246). The purpose of conducting a bioautographic assay was to qualitatively show which compound within the plant extract was actually responsible for the antimicrobial activity.

The agar-overlay technique was used to conduct the bioautographic assay. This method produces clear zones of inhibition and there is minimal contamination (Marston and Hostettmann, 1999). The active compound, as obtained from the TLC analysis of the plant extract, is able to diffuse from the TLC plate into the inoculated agar layer. The plate is then incubated after which it is sprayed with a tetrazolium salt. In the presence of microorganism growth the tetrazolium salt binds to the pathogen and is displayed as a red/purple colour. Clear spots against a red/purple background are indicative of zones of inhibition (Marston and Hostettmann, 1999).

The TLC plates for the plant extracts were prepared as per the method described under Section 2.3.1. Once the plates were developed and dried, they were sterilized under 254 nm UV spectra light for one hour.

Since the bioautographic assays were performed on all the plant extracts against a particular pathogen, large bioassay plates were used. A base layer of 100 ml Tryptone soya agar (Oxoid)

was poured into the plates and allowed to set. The irradiated TLC plate was then transferred aseptically with autoclaved forceps onto the set base layer of TSA. The TLC plate was gently pressed onto the agar so as to ensure that it sticks to the agar.

Subcultures of the relevant pathogen were prepared as described under Section 2.4.1. Thereafter 1 ml of this subculture was pipetted into 100 mls of Trytone Soya Agar (TSA). This 100 ml inoculated TSA was then poured over the TLC plate. The entire plate was then swirled to evenly distribute the culture and allowed to set. Once set, the bioassay plate was refrigerated for one hour to allow pre-diffusion of the compounds from the TLC plate into the agar. Thereafter the plate was incubated at 37°C for 24 hours.

After the incubation period, the entire plate was sprayed with INT and left at room temperature for six hours. Clear zones around a particular band of the TLC plate indicated that those particular compounds within the plant extract were responsible for antibacterial activity.

CHAPTER 3

Antimicrobial Activity

3.1 Introduction

In order to scientifically verify the traditional uses of the plant species for treating various respiratory ailments, their antimicrobial activity was investigated. The antimicrobial activity was determined by obtaining the minimum inhibitory concentrations (MIC) of the plant extracts against a particular respiratory pathogen using the micro-dilution technique (Eloff, 1998). This allowed for the quantitative determination of the antimicrobial activity of the plant extracts. The method of determining the MIC and the list of pathogens used are stated in Chapter 2, Section 2.4.

3.2 Results

The MIC values for the plant extracts are presented as bar charts against a particular pathogen (Figures 3.1 to 3.7). Due to the scale of the bar charts, the MIC values for the antibiotic and antifungal controls were not depicted on the graph but are given as a footnote at the bottom of the graph. The MIC values for those plant extracts that were equal to or greater than the DMSO (dimethyl sulphoxide) control value, are not shown on the bar charts because one cannot conclusively establish whether the antimicrobial activity is due to the plant extract or the DMSO in which it was dissolved.

Antimicrobial activity was classified as follows: (Gibbons, 2004; Ríos and Recio, 2005):

- MIC ≤ 1 mg/ml : good antimicrobial activity
- MIC > 1mg/ml or < 4 mg/ml: moderately good antimicrobial activity
- MIC = 4 mg/ml or < 6 mg/ml: moderate antimicrobial activity
- MIC ≥ 6 mg/ml: poor antimicrobial activity

Figure 3.1 represents the MIC values for the plant extracts against *Candida albicans* (ATCC 10231). The lowest MIC value against *Candida albicans* was 1.0 mg/ml which was obtained by *Terminalia sericea* (roots) and *Chenopodium ambrosioides* (leaves and stems), representing good antimicrobial activity. The next lowest MIC was 1.5 mg/ml for *Leucas martinicensis* (leaves, stems and flowers) and *Heteromorpha arborescens* (leaves). Six of the plant extracts displayed moderately good antimicrobial activity. Moderate antimicrobial activity was displayed by 12 plant extracts and six extracts displayed poor activity. Two extracts had MIC values greater than the DMSO control value (Table 3.2) and were thus excluded from Figure 3.1.

Figure 3.2 represents the MIC values for the plant extracts against *Cryptococcus neoformans* (ATCC 90112). *Terminalia sericea* (roots) displayed the lowest MIC value of 0.16 mg/ml. The next lowest MIC value was 0.25 mg/ml, displayed by *Chenopodium ambrosioides* (leaves and stems), *Zanthoxylum davyi* (leaves) and *Zanthoxylum davyi* (bark). Fourteen plant extracts displayed good antimicrobial activity having MIC values ≤ 1 mg/ml. Moderately good activity was displayed by nine extracts, two plant extracts showed moderate activity and a further two extracts displayed poor antimicrobial activity. No extract obtained an MIC value that was greater than or equal to the DMSO control value (Table 3.2).

In Figure 3.3 the MIC values of the plant extracts against *Klebsiella pneumoniae* (ATCC 13883) are displayed. The *Ziziphus mucronata* leaf extract did not show any sensitivity and thus was not represented in Figure 3.3. *Terminalia sericea* (roots) had the lowest MIC of 0.25 mg/ml, followed by *Zanthoxylum davyi* (leaves) having an MIC of 0.5 mg/ml. These two plant extracts displayed good antimicrobial activity. Thereafter, *Chenopodium ambrosioides* (leaves

and stems) had the next lowest MIC of 1.5 mg/ml representing moderately good activity which was also displayed by 10 other plant extracts. Five extracts showed moderate activity and one extract showed poor activity. Eight plant extracts obtained MIC values that were greater than or equal to the DMSO control value (Table 3.2), and were thus excluded from Figure 3.3.

The MIC values of the plant extracts against *Bacillus cereus* (ATCC 11778) are presented in Figure 3.4. The lowest MIC value against *Bacillus cereus* was 0.08 mg/ml displayed by both *Vitex rehmannii* (leaves) and *Alepidea amatymbica* (roots). *Terminalia sericea* (roots) followed with a value of 0.13 mg/ml. The next lowest MIC value, 0.25 mg/ml, was for *Heteromorpha arborescens* (bark). *Zanthoxylum davyi* (bark) had an MIC value of 0.38 mg/ml and *Zanthoxylum davyi* (leaves) had an MIC value of 0.31 mg/ml. Both *Schkuhria pinnata* (leaves) and *Peucedanum caffrum* (roots A) had an MIC value of 0.5 mg/ml. *Zanthoxylum davyi* (thorns) had the next lowest MIC of 0.75 mg/ml. In total 15 plant extracts showed good antimicrobial activity, nine had moderately good activity and three extracts had moderate antimicrobial activity. One extract had an MIC value equal to the DMSO control value (Table 3.2) and was thus excluded from Figure 3.4.

In Figure 3.5, the MIC values for the plant extracts against *Enterococcus faecalis* (ATCC 29212) are shown. The lowest MIC value against *Enterococcus faecalis* was 0.5 mg/ml for extracts *Lantana rugosa* (leaves and stems) and *Leucas martinicensis* (leaves, stems and flowers). The next lowest MIC value was 1 mg/ml displayed by *Chenopodium ambrosioides* (leaves and stems), *Acacia sieberiana* (leaves) and *Zanthoxylum davyi* (leaves). Therefore five plant extracts showed good antimicrobial activity. Moderately good activity was also

displayed by five plant extracts. Four plant extracts showed moderate activity and four plant extracts showed poor activity. Nine plant extracts obtained MIC values that were greater than or equal to the DMSO control value (Table 3.2) and were thus excluded from Figure 3.5.

Figure 3.6 represents the MIC values for the plant extracts against *Staphylococcus aureus* (ATCC 12600). *Lantana rugosa* (leaves and stems) exhibited the lowest MIC (0.25 mg/ml) against this pathogen. The next lowest MIC was 0.5 mg/ml for *Chenopodium ambrosioides* (leaves and stems). *Schkuhria pinnata* (leaves) had an MIC value of 0.75 mg/ml. An MIC value of 1 mg/ml was displayed by *Terminalia sericea* (roots), *Leucas martinicensis* (leaves, stems and flowers) and *Chamaecrista mimosoides* (leaves). Thus these plant extracts have shown good antimicrobial activity against *Staphylococcus aureus*. Seven plant extracts showed moderately good activity, nine showed moderate activity and five plant extracts showed poor activity. One extract had an MIC value equal to the DMSO control value (Table 3.2) and was thus excluded from Figure 3.6.

In Figure 3.7, the MIC values of the plant extracts against *Moraxella catarrhalis* (ATCC 23246) are shown. MIC values less than or equal to 1 mg/ml were displayed by 19 of the plant extracts. The lowest MIC value against *Moraxella catarrhalis* was 0.31 mg/ml displayed by *Terminalia sericea* (roots) and *Vitex rehmannii* (leaves). *Zanthoxylum davyi* (thorns) had an MIC value of 0.38 mg/ml and the MIC value for *Lantana rugosa* (leaves and stems) was 0.47 mg/ml. An MIC value of 0.5 mg/ml was exhibited by *Acacia sieberiana* (leaves), *Ziziphus mucronata* (bark, leaves and roots), *Clematis oweniae* (leaves), *Clerodendrum glabrum* (bark) and *Heteromorpha arborescens* (stems). The next lowest MIC value was 0.63 mg/ml for *Chamaecrista mimosoides* (leaves). *Heteromorpha arborescens* (bark) had an MIC value of

0.75 mg/ml. One extract (leaves of *Clerodendrum glabrum*) showed moderately good activity, five extracts (*Schkuhria pinnata* (leaves), *Zanthoxylum davyi* (roots and bark), *Heteromorpha arborescens* (leaves) and *Peucedanum caffrum* (roots C)) had moderate activity and three extracts (*Acacia sieberiana* (bark), *Peucedanum caffrum* (roots B) and *Alepidea amatymbica* (roots)) had poor activity. No extract obtained an MIC value that was greater than or equal to the DMSO control value (Table 3.2).

3.3 Discussion

3.3.1 Sensitivities of the tested pathogens

In order to determine which of the pathogens were the most sensitive, two criteria were considered. The first was the number of plant extracts that showed good antimicrobial activity against that pathogen i.e. the number of extracts that obtained MIC values of ≤ 1 mg/ml (Table 3.1). The second criterion was the average MIC value obtained by the plant extracts against that particular pathogen. However, the number of plant extracts having MIC values greater than or equal to the DMSO control value against each pathogen, was also considered because for these extracts one cannot be certain whether the antimicrobial activity is due to the extract or the DMSO it was dissolved in (Table 3.1). Table 3.2 gives the names of those plant extracts that obtained MIC values greater than or equal to the DMSO control value for that particular pathogen.

The most sensitive pathogens were *Cryptococcus neoformans, Bacillus cereus* and *Moraxella catarrhalis*. For these pathogens, the numbers of extracts showing good antimicrobial activity i.e. having MIC values of ≤ 1 mg/ml were 14, 15 and 19 extracts respectively (Table 3.1).

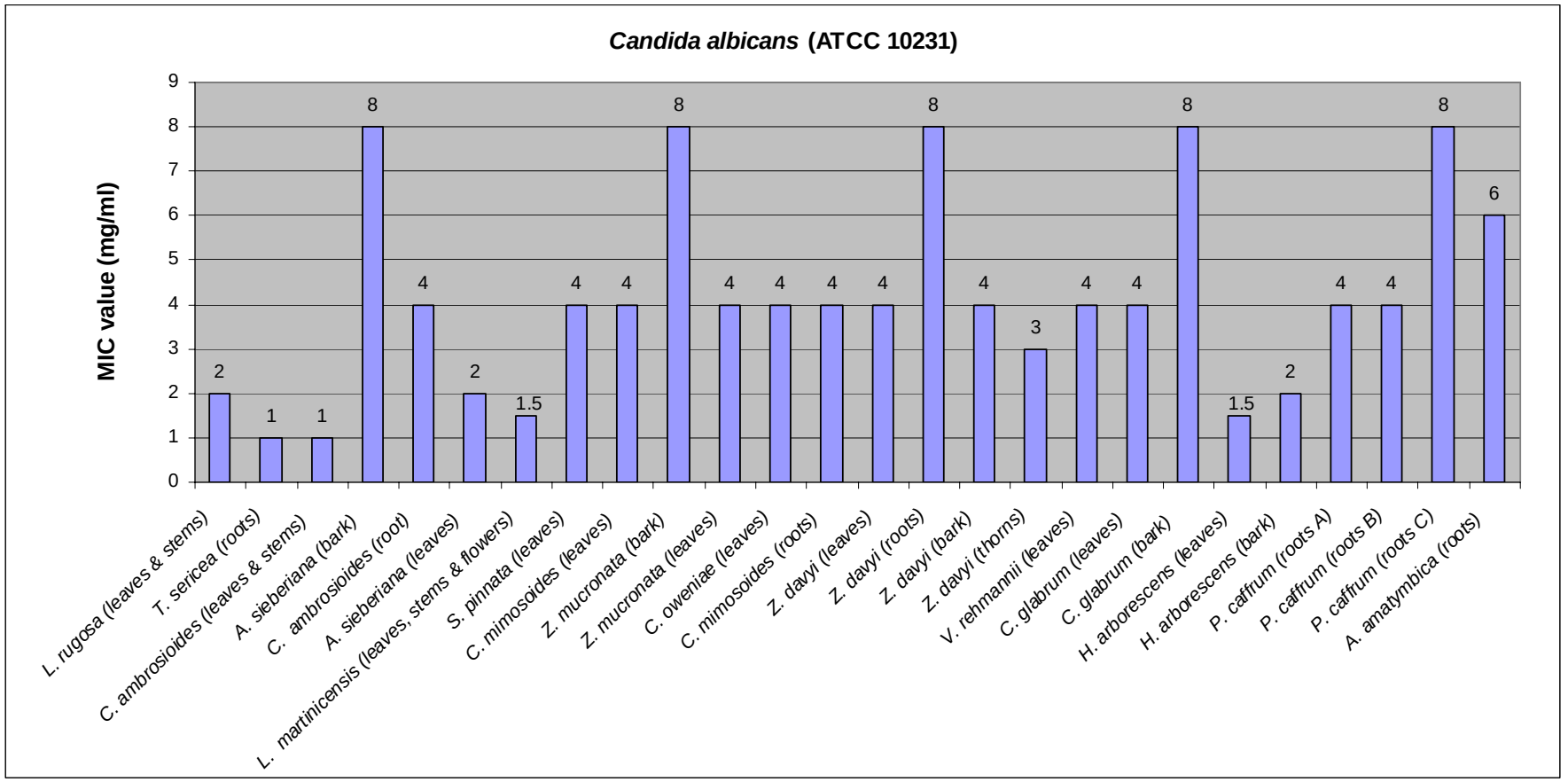


Figure 3.1 MIC values of plant extracts against *Candida albicans* (ATCC 10231). Controls: Amphotericin B (MIC=1.25 µg/ml), DMSO (MIC=9 mg/ml) and Acetone (MIC ≥ 16 mg/ml).

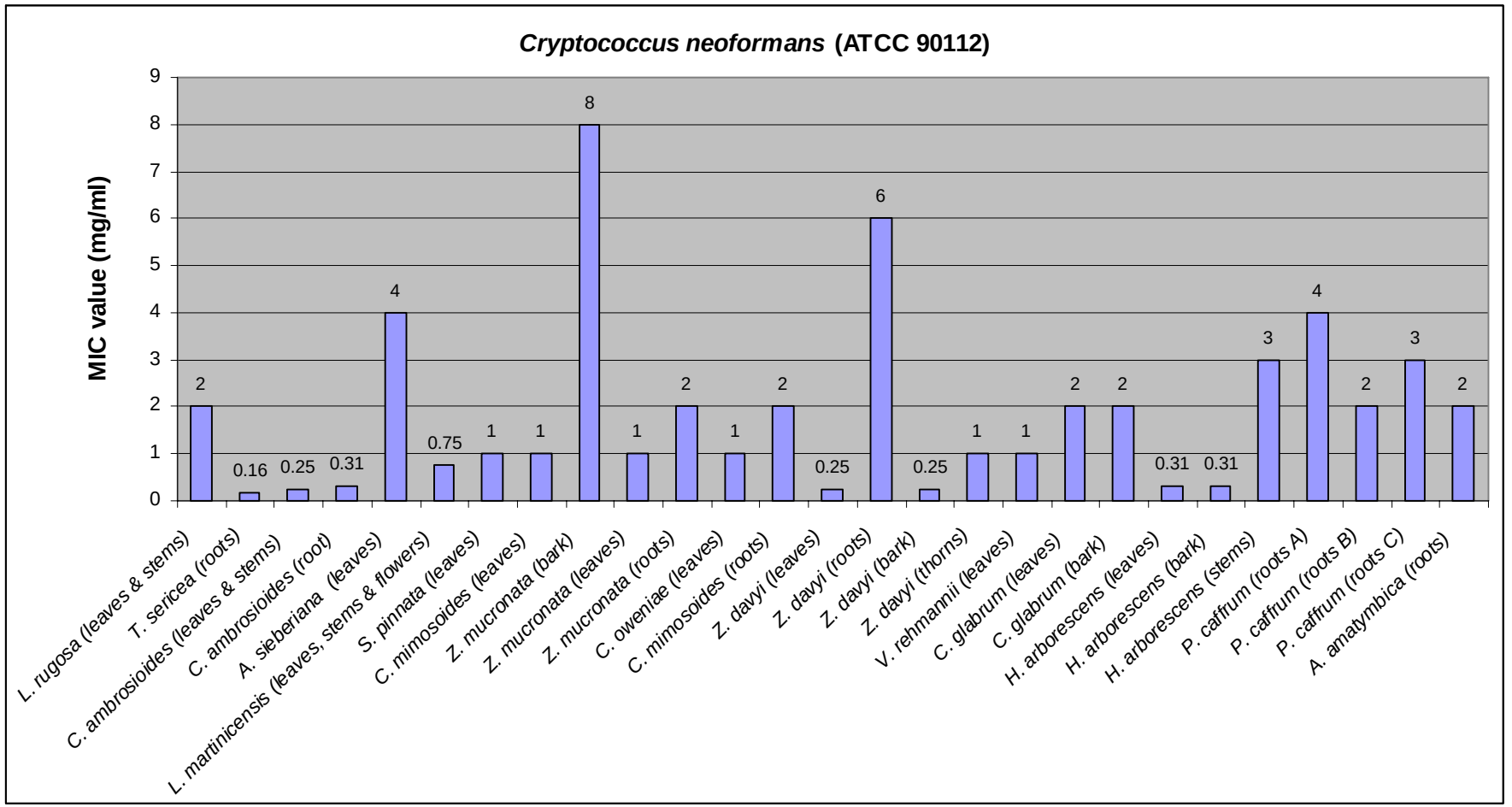


Figure 3.2 MIC values of plant extracts against *C. neoformans* (ATCC 90112). Controls: Amphotericin B (MIC=1.5 µg/ml), DMSO (MIC=9 mg/ml) and Acetone (MIC ≥ 16 mg/ml). No results were obtained for *Acacia sieberiana* (bark) extract due to insufficient sample.

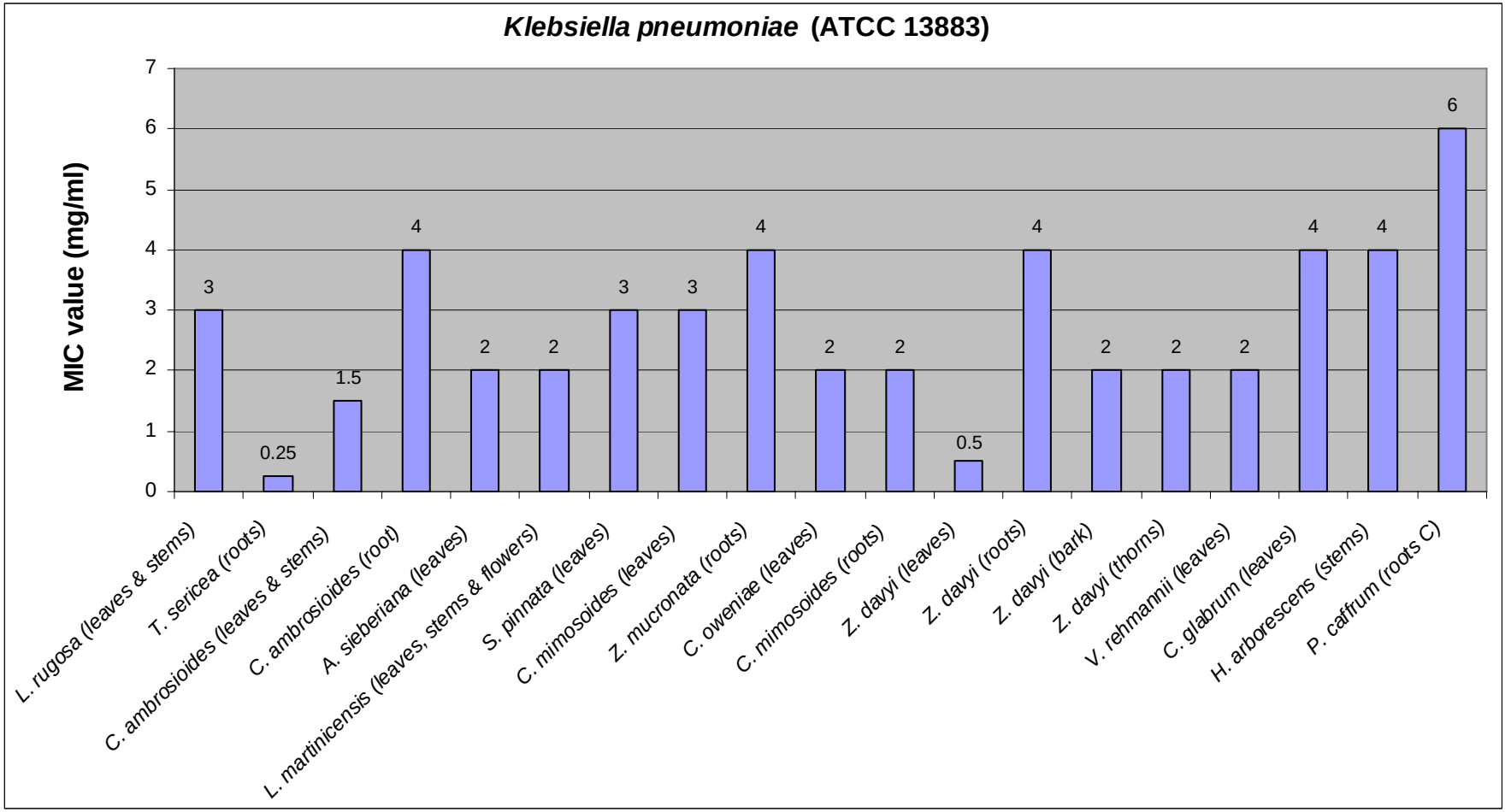


Figure 3.3 MIC values of plant extracts against *Klebsiella pneumoniae* (ATCC 13883). Controls: Ciprofloxacin (MIC=0.625 µg/ml), DMSO (MIC = 8 mg/ml) and Acetone (MIC ≥ 16 mg/ml).

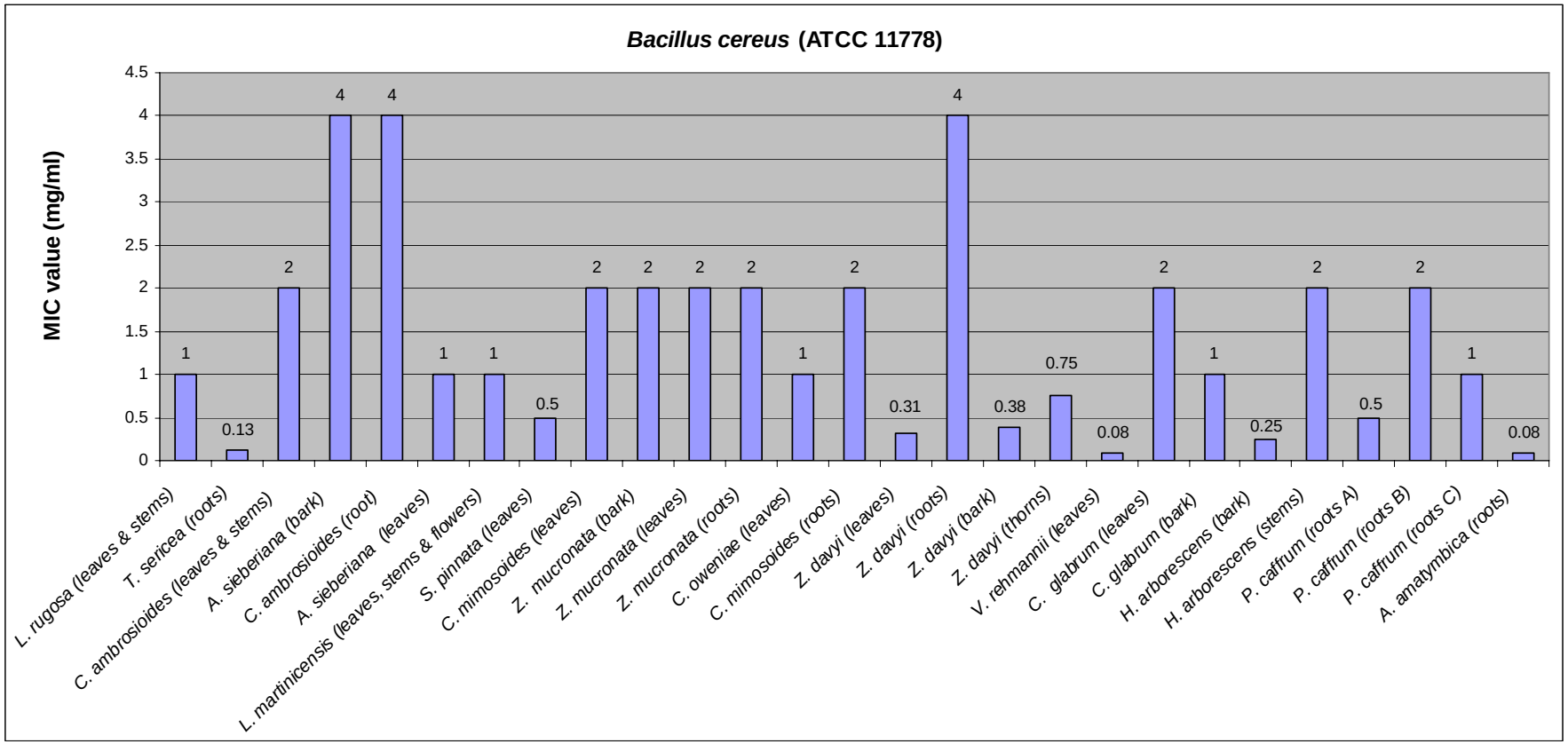


Figure 3.4 MIC values of plant extracts against *Bacillus cereus* (ATCC 11778). Controls: Ciprofloxacin (MIC=0.039 µg/ml), DMSO (MIC=8 mg/ml) and Acetone (MIC ≥ 16 mg/ml).

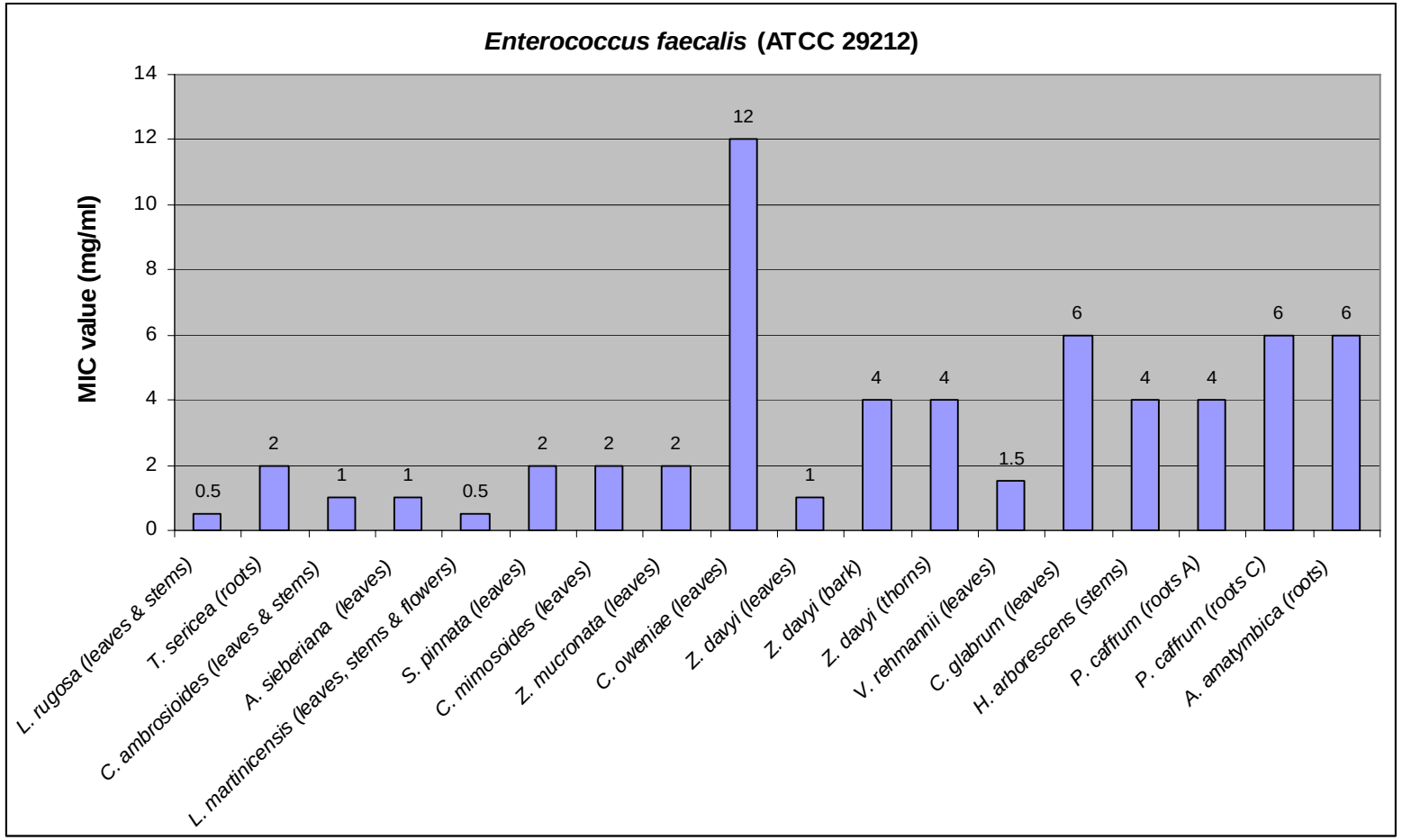


Figure 3.5 MIC values of plant extracts against *Enterococcus faecalis* (ATCC 29212). Controls: Ciprofloxacin (MIC=0.938 µg/ml), DMSO (MIC = 8 mg/ml) and Acetone (MIC ≥ 16 mg/ml). No results were obtained for *Acacia sieberiana* (bark) extract due to insufficient sample.

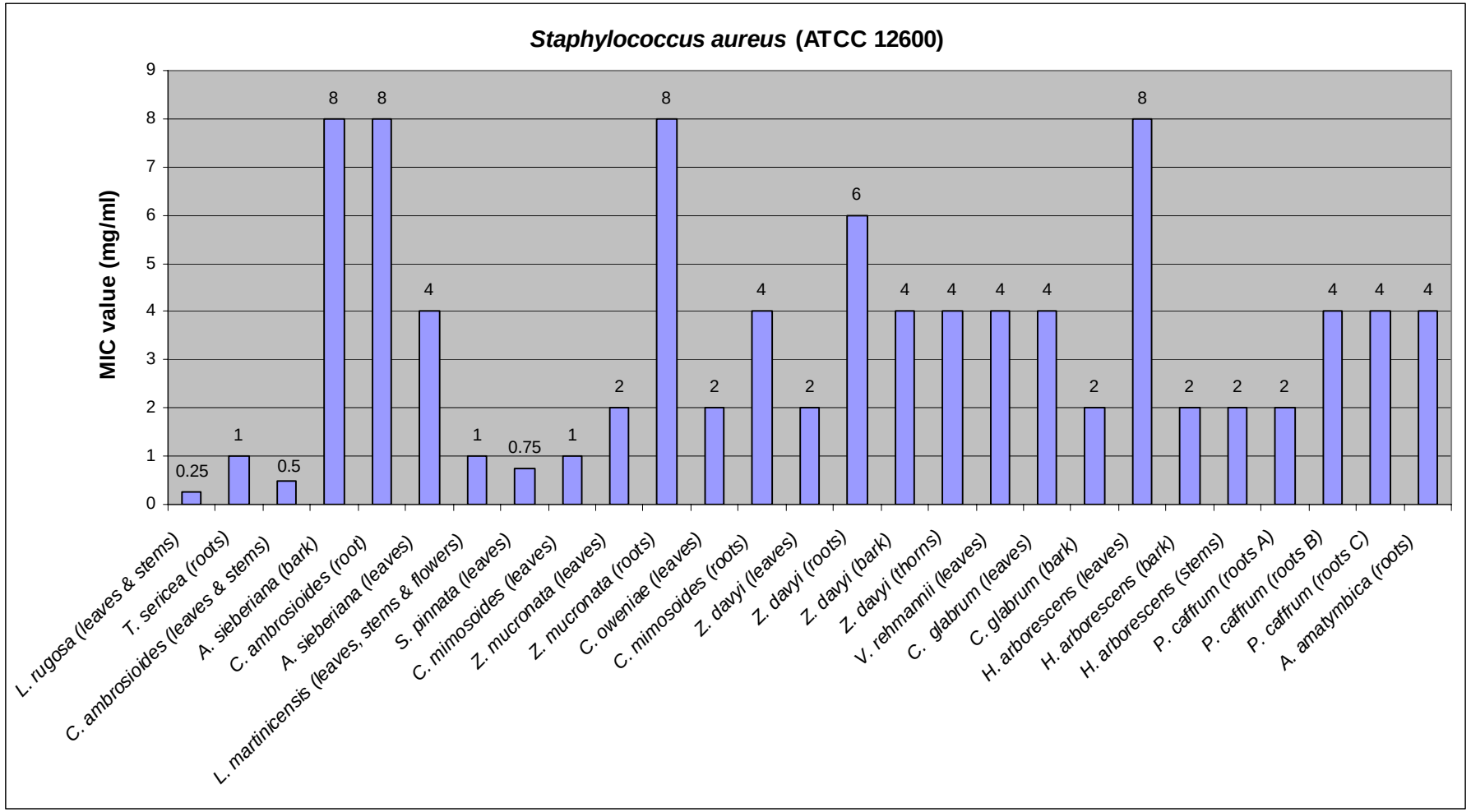


Figure 3.6 MIC values of plant extracts against *Staphylococcus aureus* (ATCC 12600). Controls: Ciprofloxacin (MIC=0.313 µg/ml), DMSO (MIC=16 mg/ml) and Acetone (MIC ≥ 16 mg/ml).

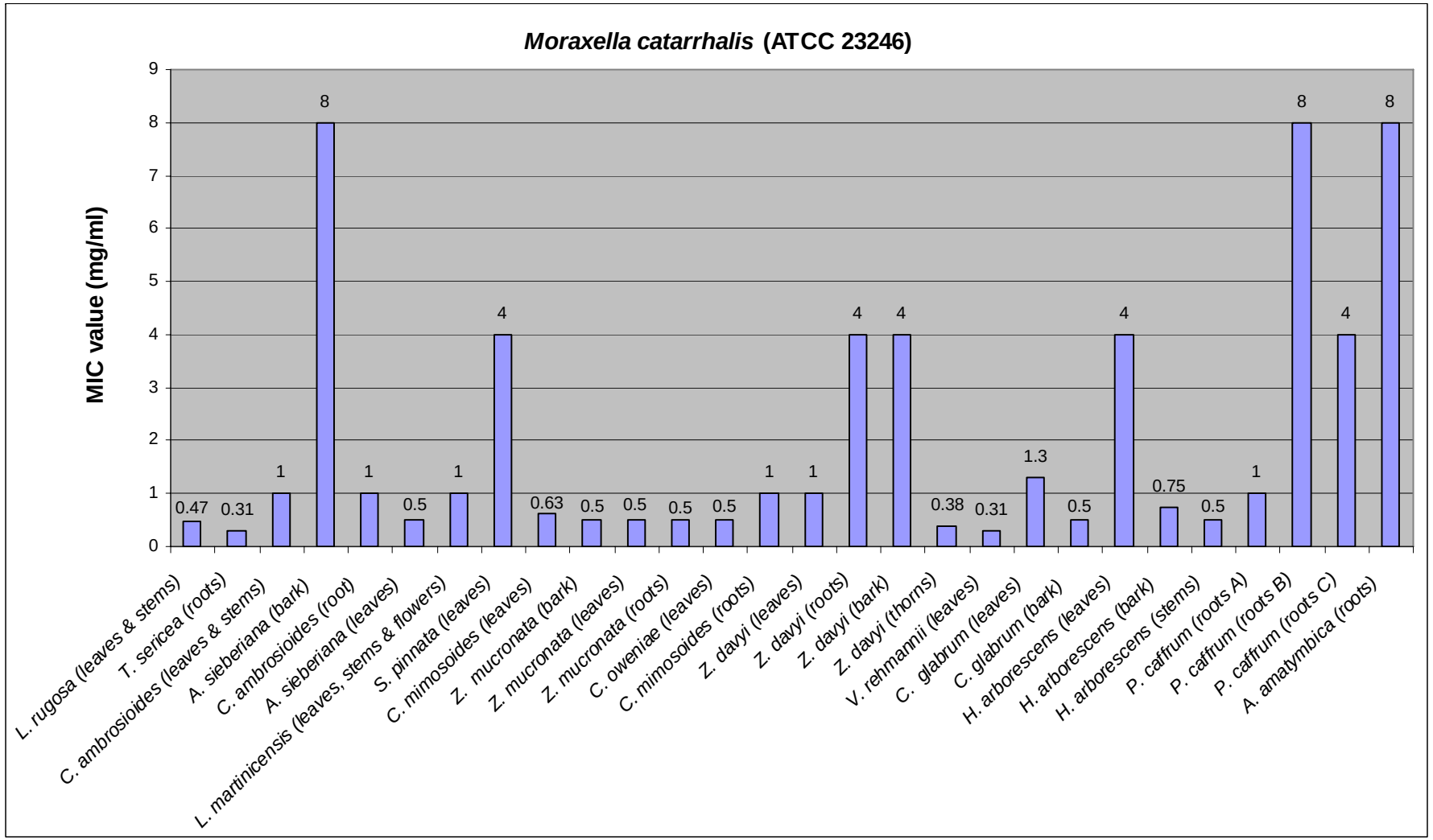


Figure 3.7 MIC values of plant extracts against *M. catarrhalis* (ATCC 23246). Controls: Ciprofloxacin (MIC=0.235 µg/ml), DMSO (MIC=16 mg/ml) and Acetone (MIC ≥ 16 mg/ml).

From the average MIC values that the extracts obtained against the pathogens, the lowest average MIC value (1.44 mg/ml) was obtained against *Bacillus cereus* (Table 3.1). The second lowest average MIC value (1.87 mg/ml) was observed for *Cryptococcus neoformans*. *Moraxella catarrhalis* had the highest number of extracts (19 out of 28 extracts) that obtained MIC values of ≤ 1 mg/ml (Table 3.1). For *Cryptococcus neoformans* and *Moraxella catarrhalis* there were no extracts that had MIC values greater than or equal to that of the DMSO control, thus all the extracts showed activity against these two pathogens (Table 3.1 and 3.2).

Table 3.1 Summary of the antimicrobial activity of the plant extracts against the respiratory pathogens.

Pathogen	**Number of extracts with MIC ≤ 1 mg/ml (Good antimicrobial activity)**	**Average MIC value (mg/ml) (Range of MIC values)**	**Number of extracts with MIC ≥ DMSO control**
Candida albicans (ATCC 10231)	2	4.15 (1.00-8.00 mg/ml)	2
Cryptococcus neoformans (ATCC 90112)	14	1.87 (0.16-8.00 mg/ml)	None
Klebsiella pneumoniae (ATCC 13883)	2	2.70 (0.25-6.00 mg/ml)	8
Bacillus cereus (ATCC 11778)	15	1.44 (0.08-4.00 mg/ml)	1
Enterococcus faecalis (ATCC 29212)	5	3.30 (0.50-12.00 mg/ml)	9
Staphylococcus aureus (ATCC 12600)	6	3.43 (0.25-8.00 mg/ml)	1
Moraxella catarrhalis (ATCC 23246)	19	2.08 (0.31-8.00 mg/ml)	None

Table 3.2 Plant extracts that have MIC values greater than or equal to the DMSO control value.

Pathogen	DMSO control value (mg/ml)	Plant extracts with MIC value ≥ to DMSO control
Candida albicans (ATCC 10231)	9 mg/ml	• *Ziziphus mucronata* (roots) 12 mg/ml • *Heteromorpha arborescens* (stems) 16 mg/ml
Cryptococcus neoformans (ATCC 90112)	9 mg/ml	None
Klebsiella pneumoniae (ATCC 13883)	8 mg/ml	• *Acacia sieberiana* (bark) 8 mg/ml • *Ziziphus mucronata* (bark) 8 mg/ml • *Clerodendrum glabrum* (bark) 8 mg/ml • *Heteromorpha arborescens* (leaves) 12 mg/ml • *Heteromorpha arborescens* (bark) 8 mg/ml • *Peucedanum caffrum* (roots A) 8 mg/ml • *Peucedanum caffrum* (roots B) 8 mg/ml • *Alepidea amatymbica* (roots) 8 mg/ml
Bacillus cereus (ATCC 11778)	8 mg/ml	*Heteromorpha arborescens* (leaves) 8 mg/ml
Enterococcus faecalis (ATCC 29212)	8 mg/ml	• *Chenopodium ambrosioides* (roots) 8 mg/ml • *Ziziphus mucronata* (bark) 8 mg/ml • *Ziziphus mucronata* (roots) 8 mg/ml • *Chamaecrista mimosoides* (roots) 8 mg/ml • *Zanthoxylum davyi* (roots) 8 mg/ml • *Clerodendrum glabrum* (bark) 16 mg/ml • *Heteromorpha arborescens* (leaves) 16 mg/ml • *Heteromorpha arborescens* (bark) 16 mg/ml • *Peucedanum caffrum* (roots B) 8 mg/ml
Staphylococcus aureus (ATCC 12600)	16 mg/ml	*Ziziphus mucronata* (bark) 16 mg/ml
Moraxella catarrhalis (ATCC 23246)	16 mg/ml	None

Nine extracts obtained MIC values that were greater than or equal to the DMSO control value for *Enterococcus faecalis* (Table 3.1 and 3.2). These extracts were thus considered not active

against *Enterococcus faecalis*, making this pathogen not particularly sensitive to the plant extracts.

The average MIC values exhibited by the plant extracts against the respiratory pathogens ranged between 1.44 mg/ml to 4.15 mg/ml. Thus, on average and according to the classification of the antimicrobial activity (Section 3.1), the plant extracts exhibited moderate antimicrobial activity against the respiratory pathogens.

3.3.2 Plant species that showed best activity

The lowest MIC value of each extract and the pathogen against which this value was obtained is listed in Table 3.3. The leaf extract of *Vitex rehmannii* and the root extract of *Alepidea amatymbica* both obtained the lowest MIC value (0.08 mg/ml) from all the extracts against *Bacillus cereus*. The next lowest MIC value was 0.13 mg/ml obtained for *Terminalia sericea* root extracts, also against *Bacillus cereus*. The next lowest MIC value (0.25 mg/ml) was obtained by *Lantana rugosa* (leaves and stems), *Chenopodium ambrosioides* (leaves and stems), *Zanthoxylum davyi* (leaves) and *Heteromorpha arborescens* (bark) against *Staphylococcus aureus*, *Cryptococcus neoformans* or *Bacillus cereus.*

Another criterion that was used to establish the most active plant extracts was the number of pathogens against which the plant extract obtained MIC values of ≤ 1 mg/ml. These pathogens are listed in Table 3.3 for each plant extract. This represented the pathogens against which the plant extract obtained noteworthy antimicrobial activity.

Table 3.3 Lowest MIC values obtained by the plant extracts and the list of pathogens against which MIC values of ≤ 1 mg/ml were obtained.

Plant extract	Lowest MIC value (mg/ml)	Pathogen against which the lowest MIC value was obtained	Pathogens against which the extract obtained an MIC value of ≤ 1 mg/ml
Lantana rugosa (leaves and stems)	0.25	*S. aureus*	• *Bacillus cereus* • *Enterococcus faecalis* • *Staphylococcus aureus* • *Moraxella catarrhalis*
Terminalia sericea (roots)	0.13	*B. cereus*	• *Candida albicans* • *Cryptococcus neoformans* • *Klebsiella pneumoniae* • *Bacillus cereus* • *Staphylococcus aureus* • *Moraxella catarrhalis*
Chenopodium ambrosioides (leaves and stems)	0.25	*C. neoformans*	• *Candida albicans* • *Cryptococcus neoformans* • *Enterococcus faecalis* • *Staphylococcus aureus* • *Moraxella catarrhalis*
Acacia sieberiana (bark)	4.00	*B. cereus*	None
Chenopodium ambrosioides (roots)	0.31	*C. neoformans*	• *Cryptococcus neoformans* • *Moraxella catarrhalis*
Acacia sieberiana (leaves)	0.50	*M. catarrhalis*	• *Bacillus cereus* • *Enterococcus faecalis* • *Moraxella catarrhalis*
Leucas martinicensis (leaves, stems and flowers)	0.50	*E. faecalis*	• *Cryptococcus neoformans* • *Bacillus cereus* • *Enterococcus faecalis* • *Staphylococcus aureus* • *Moraxella catarrhalis*
Schkuhria pinnata (leaves)	0.50	*B. cereus*	• *Cryptococcus neoformans* • *Bacillus cereus* • *Staphylococcus aureus*
Chamaecrista mimosoides (leaves)	0.63	*M. catarrhalis*	• *Cryptococcus neoformans* • *Staphylococcus aureus* • *Moraxella catarrhalis*
Ziziphus mucronata (bark)	0.50	*M. catarrhalis*	• *Moraxella catarrhalis*
Ziziphus mucronata (leaves)	0.50	*M. catarrhalis*	• *Cryptococcus neoformans* • *Moraxella catarrhalis*
Ziziphus mucronata (roots)	0.50	*M. catarrhalis*	*Moraxella catarrhalis*

Table 3.3 continued.

Plant extract	Lowest MIC value (mg/ml)	Pathogen against which the lowest MIC value was obtained	Pathogens against which the extract obtained an MIC value of ≤ 1 mg/ml
Clematis oweniae (leaves)	0.50	*M. catarrhalis*	• *Cryptococcus neoformans* • *Bacillus cereus* • *Moraxella catarrhalis*
Chamaecrista mimosoides (roots)	1.00	*M. catarrhalis*	*Moraxella catarrhalis*
Zanthoxylum davyi (leaves)	0.25	*C.neoformans*	• *Cryptococcus neoformans* • *Klebsiella pneumoniae* • *Bacillus cereus* • *Enterococcus faecalis* • *Moraxella catarrhalis*
Zanthoxylum davyi (roots)	4.00	*K. pneumoniae, B. cereus* and *M. catarrhalis*	None
Zanthoxylum davyi (bark)	0.25	*C. neoformans*	• *Cryptococcus neoformans* • *Bacillus cereus*
Zanthoxylum davyi (thorns)	0.38	*M. catarrhalis*	• *Cryptococcus neoformans* • *Bacillus cereus* • *Moraxella catarrhalis*
Vitex rehmannii (leaves)	0.08	*B. cereus*	• *Cryptococcus neoformans* • *Bacillus cereus* • *Moraxella catarrhalis*
Clerodendrum glabrum (leaves)	1.30	*M. catarrhalis*	None
Clerodendrum glabrum (bark)	0.50	*M. catarrhalis*	• *Bacillus cereus* • *Moraxella catarrhalis*
Heteromorpha arborescens (leaves)	0.31	*C. neoformans*	*Cryptococcus neoformans*
Heteromorpha arborescens (bark)	0.25	*B. cereus*	• *Cryptococcus neoformans* • *Bacillus cereus* • *Moraxella catarrhalis*
Heteromorpha arborescens (stems)	0.50	*M. catarrhalis*	*Moraxella catarrhalis*
Peucedanum caffrum (roots A)	0.50	*B. cereus*	• *Bacillus cereus* • *Moraxella catarrhalis*
Peucedanum caffrum (roots B)	2.00	*C. neoformans* and *B. cereus*	None
Peucedanum caffrum (roots C)	1.00	*B. cereus*	*Bacillus cereus*
Alepidea amatymbica (roots)	0.08	*B. cereus*	*Bacillus cereus*

In this regard, *Terminalia sericea* root extracts demonstrated the most broad-spectrum activity, whereby it obtained MIC values that were ≤ 1 mg/ml against six of the seven pathogens *viz. Candida albicans, Cryptococcus neoformans, Klebsiella pneumoniae, Bacillus cereus, Staphylococcus aureus* and *Moraxella catarrhalis* (Table 3.3). *Chenopodium ambrosioides* (leaves and stems), *Leucas martinicensis* (leaves, stems and flowers) and *Zanthoxylum davyi* (leaves) followed, in that they obtained MIC values of ≤ 1 mg/ml against five of the respiratory pathogens (Table 3.3). Thereafter, the leaf and stem extracts of *Lantana rugosa* followed, wherein it obtained MIC values of ≤ 1 mg/ml against four of the pathogens (Table 3.3).

Below is a more in-depth discussion on the active plant extracts in terms of correlating the findings in this research study to any previous antimicrobial studies carried out on the plant species.

3.3.2.1 Terminalia sericea (Combretaceae)

The root extracts for *Terminalia sericea* displayed good antimicrobial activity. It exhibited the lowest MIC values out of all the extracts (Table 3.4) against four of the seven pathogens tested *viz. Candida albicans*, *Cryptococcus neoformans*, *Klebsiella pneumoniae* and *Moraxella catarrhalis*. Figure 3.8 shows that the root extracts of *Terminalia sericea* having good antimicrobial activity against six of the pathogens and moderately good activity against the remaining one pathogen.

Table 3.4 Plant extracts that obtained the lowest MIC values against each pathogen.

Pathogen	Plant species	MIC (mg/ml)
Candida albicans (ATCC 10231)	• *Terminalia sericea* (roots) • *Chenopodium ambrosioides* (leaves and stems)	1.00
Cryptococcus neoformans (ATCC 90112)	*Terminalia sericea* (roots)	0.16
Klebsiella pneumoniae (ATCC 13883)	*Terminalia sericea* (roots)	0.25
Bacillus cereus (ATCC 11778)	• *Vitex rehmannii* (leaves) • *Alepidea amatymbica* (roots)	0.08
Enterococcus faecalis (ATCC 29212)	• *Lantana rugosa* (leaves and stems) • *Leucas martinicensis* (leaves, stems and flowers)	0.50
Staphylococcus aureus (ATCC 12600)	*Lantana rugosa* (leaves and stems)	0.25
Moraxella catarrhalis (ATCC 23246)	• *Terminalia sericea* (roots) • *Vitex rehmannii* (leaves)	0.31

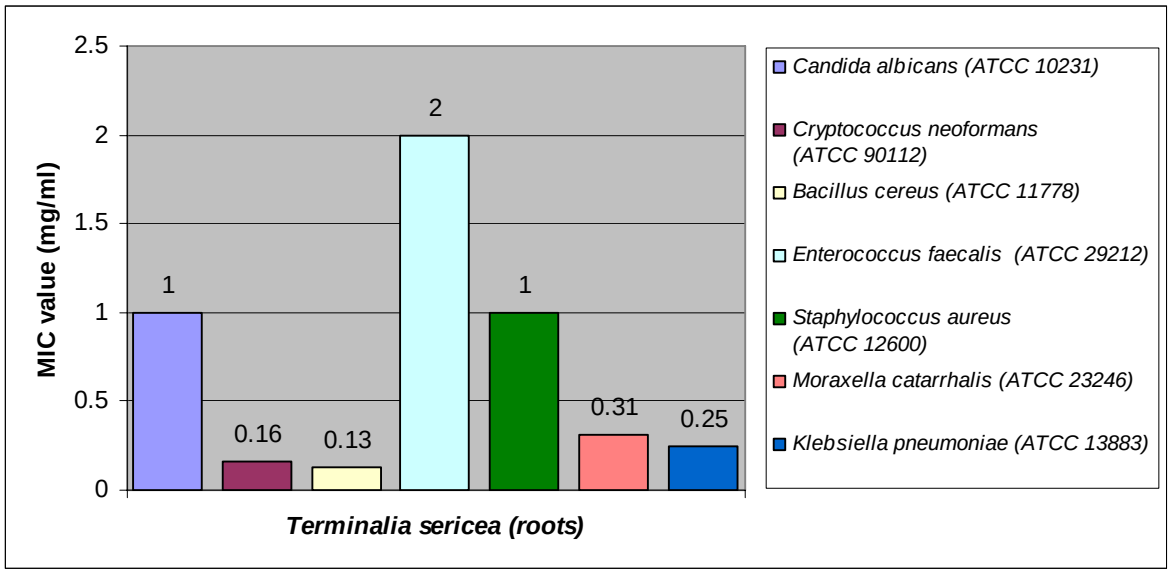


Figure 3.8 MIC values for pathogens when tested against *Terminalia sericea* root extract.

This antimicrobial activity of *Terminalia sericea* has also been demonstrated in studies carried out by Eloff (1999), Fyhrquist *et al.* (2002) and Moshi and Mbwambo (2005). Eloff (1999) has

shown that the acetone extracts of the leaves of *Terminalia sericea* has antibacterial activity and it was also one of the five best species active against Gram-positive organisms, *Staphylococcus aureus* and *Enterococcus faecalis*. In this research report, the acetone:methanol (1:1) root extracts of *Terminalia sericea* showed good activity against both the Gram-positive and Gram-negative pathogens (Figure 3.8).

Fyhrquist *et al.* (2002) carried out an ethnobotanical study on some of the *Terminalia* and *Combretum* species, which both belong to the Combretaceae family. They showed that the methanol, ethanol, acetone and hot water extracts of the *Terminalia sericea* roots have good antimicrobial activity as these extracts obtained one of the larger zones of inhibition in the agar diffusion method. Fyhrquist *et al.* (2002) also determined the MIC values of the methanol root extract of *Terminalia sericea* by the cylinder agar diffusion method which produced an MIC value of 5.3 mg/ml against *Staphylococcus aureus*. The micro-dilution technique (Eloff, 1998) used in this study yielded a lower MIC value against this pathogen for the acetone:methanol (1:1) root extract (Figure 3.8). This may suggest superiority of determining MIC via the micro-dilution technique. It could also suggest that the solvent system of using acetone together with methanol favours better extraction of the antimicrobial compounds.

The acetone:methanol (1:1) root extract of *Terminalia sericea* in this research study obtained the lowest MIC values from all the extracts against yeast pathogens *Candida albicans* and *Cryptococcus neoformans* (Table 3.4). This good antimicrobial activity against the yeast pathogens is also corroborated by Fyhrquist *et al.* (2002) who showed that the methanolic leaf extract of *Terminalia sericea* has the highest activity against *Candida albicans* by the hole-plate agar diffusion method wherein it obtained the largest zone of inhibition from all the

extracts including the antifungal controls. This antifungal activity could be due to the chemical compounds, tannins and saponins. These compounds were shown to be in high quantities in seven west African Combretaceae species that were studied for their antifungal activities (Baba-Moussa *et al.,* 1999).

Antimicrobial tests carried out by Moshi and Mbwambo (2005) on intermediate and polar extracts of *Terminalia sericea* showed the root extract as having best activity against *Candida albicans* and *Staphylococcus aureus*. The Moshi and Mbwambo (2005) study established antimicrobial activity qualitatively by the disc-diffusion method. Also, with this method there was no inhibition of *Klebsiella pneumoniae*. The MIC values obtained in this research study quantified the antimicrobial activity, whereby MIC values of 1 mg/ml was obtained against *Candida albicans* and *Staphylococcus aureus* and 0.25 mg/ml was obtained against *Klebsiella pneumoniae*. Thus these low MIC values agree with the good antimicrobial activity reported by Moshi and Mbwambo (2005).

The antifungal activities of six South African *Terminalia* species were also studied by Masoko *et al.* (2005). In this study, acetone, hexane, dichloromethane and methanol leaf extracts were prepared and tested against five fungal pathogens, two of which were *Candida albicans* and *Cryptococcus neoformans*. The antimicrobial activity was also determined by the serial microplate dilution method. Masoko *et al.* (2005) showed the *Terminalia sericea* leaf extracts to be one of the most active species against the fungal pathogens. The leaf extracts displayed one of the lowest average MIC values after 24 hours. Similarly, in this research report, the acetone:methanol (1:1) root extracts of *Terminalia sericea* obtained the lowest MIC values from all the extracts for both *Candida albicans* and *Cryptococcus neoformans* (Table 3.4). In

the Masoko *et al*. (2005) study, both the acetone and methanol leaf extracts obtained an MIC value of 0.16 mg/ml against *Cryptococcus neoformans*. This study also resulted in an MIC value of 0.16 mg/ml for the acetone:methanol (1:1) root extracts. Thus, the leaf and root extracts of *Terminalia sericea* show similar activity against *Cryptococcus neoformans*.

Steenkamp *et al.* (2004) studied the antibacterial activity of the water and methanol extracts of the bark of *Terminalia sericea*. They also used the micro-dilution technique to determine the MIC values and obtained the same MIC value (1 mg/ml) against *Staphylococcus aureus* as was obtained in this research study. Steenkamp *et al.* (2004) have also reported that *Terminalia sericea* was more active than the other plant species against the bacteria tested.

The ethanol root extracts of *Terminalia sericea* demonstrated the highest inhibition of the bacteria tested in the disc diffusion assay carried out by Eldeen *et al.* (2005). The zones of inhibition ranged between 1.2-2.7 mm for Gram-negative bacteria and between 0.6-2.3 mm for Gram-positive bacteria (Eldeen *et al.*, 2005). In this research study, the MIC values obtained by the acetone:methanol (1:1) root extracts of *Terminalia sericea*, also shows this plant species to have good antimicrobial activity. The MIC values ranged between 1.3-2.0 mg/ml for the Gram-positive bacteria and were less than 1 mg/ml against the Gram-negative bacteria (Figure 3.8).

The antibacterial activity of *Terminalia sericea* roots could be attributed to a bioactive compound, Anolignan B, which was isolated from the ethyl acetate root extracts of this plant (Eldeen *et al.*, 2006). The MIC values of this compound showed antibacterial activity against

both Gram-positive and Gram-negative bacteria, having better activity against the Gram-positive bacteria.

Terminoic acid is another compound that has been isolated from *Terminalia sericea* by bioassay-guided fractionation (Kruger, 2004 in Eloff *et al*., 2008). MIC values of 1.56 and 0.33 mg/ml were obtained in an *in vitro* antibacterial test against *Staphylococcus aureus* for terminoic acid and an acetone extract of *Terminalia sericea* respectively. An *in vivo* study was also carried out, wherein a mixture of either terminoic acid or the crude acetone extract of *Terminalia sericea* was made with aqueous cream and applied to lesions on the skin of the backs of rats that were infected with *Staphylococcus aureus*. Both the creams containing terminoic acid and the acetone extract of *Terminalia sericea* obtained far better results in healing the wounds than the gentamycin cream (Kruger, 2004 in Eloff *et al*., 2008).

3.3.2.2 Chenopodium ambrosioides (Chenopodiaceae)

The leaf and stem extract of *Chenopodium ambrosioides* in this research study has shown good to moderate antimicrobial activity as it obtained MIC values between 0.25 – 2.00 mg/ml against the respiratory pathogens (Figure 3.9). Together with *Terminalia sericea* root extracts, the leaf and stem extracts of *Chenopodium ambrosioides* obtained the lowest MIC value (1 mg/ml) from all the extracts against *Candida albicans* (Table 3.4). MIC values of ≤ 1 mg/ml were obtained against *Candida albicans*, *Cryptococcus neoformans, Enterococcus faecalis, Staphylococcus aureus* and *Moraxella catarrhalis*. Antimicrobial activity against the Gram-negative pathogens was also promising in that good to moderately good activity was demonstrated (Figure 3.9).

In a study carried out by Yasunaka *et al.* (2005), a methanol extract of the ground parts of *Chenopodium ambrosioides* obtained an MIC value of 1.02 mg/ml against *Staphylococcus aureus*. The aqueous leaf extracts of *Chenopodium ambrosioides* also showed notable antimicrobial activity against *Staphylococcus aureus* in that the extract obtained a zone of inhibition that was greater than that of the antibiotic control (Desta, 1993). The good antimicrobial activity of *Chenopodium ambrosioides* has also been demonstrated in other studies wherein it has shown the potential of having very good antimycobacterial (Lall and Meyer, 1999) and antimalarial activity (Pollack, *et al.*, 1990).

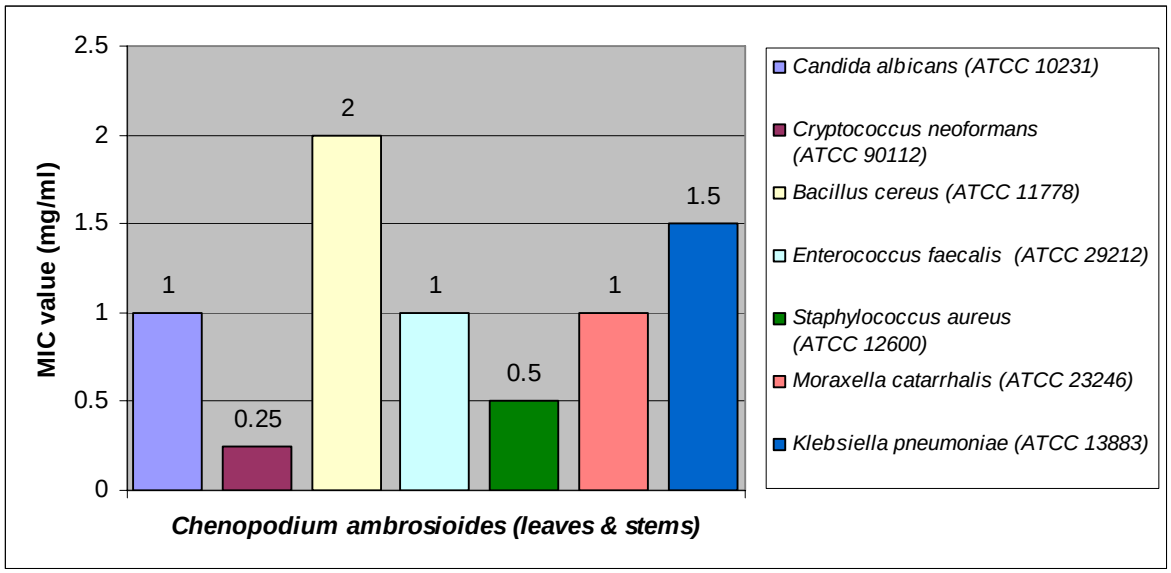


Figure 3.9 MIC values for pathogens when tested against *Chenopodium ambrosioides* leaf and stem extract.

3.3.2.3 Leucas martinicensis (Lamiaceae)

The MIC values recorded for the extract of the leaf, stem and flowers of *Leucas martinicensis* also shows this extract to have promising antimicrobial activity. It obtained MIC values that were ≤ 1 mg/ml against five of the seven respiratory pathogens *viz. Cryptococcus neoformans,*

Bacillus cereus, Enterococcus faecalis, Staphylococcus aureus and *Moraxella catarrhalis* (Figure 3.10). Also, *Leucas martinicensis* (leaves, stems and flowers) together with *Lantana rugosa* (leaves and stems) obtained the lowest MIC value (0.50 mg/ml) from all the extracts against *Enterococcus faecalis* (Table 3.4). The MIC values against the respiratory pathogens ranged between 0.25 mg/ml and 2.00 mg/ml. Thus, the antimicrobial activity of *Leucas martinicensis* (leaves, stems and flowers) ranged between good to moderately good against these pathogens.

Very little research has been conducted on *Leucas martinicensis*. The antimicrobial activity of *Leucas martinicensis* was studied by Vlietinck *et al.* (1995) wherein two pathogens that are common to this research study *viz. Staphylococcus aureus* and *Candida albicans* were also used. Vlietinck *et al.* (1995) used the hole-plate agar diffusion method and the aqueous extract of the leaf and the stem showed no inhibition zone against these two pathogens. In contrast, the micro-dilution technique (Eloff, 1998) used in this research study appeared to be more sensitive in that it showed the acetone:methanol (1:1) extract of the leaves, stems and flowers of *Leucas martinicensis* to have MIC values of 1.00 and 1.50 mg/ml respectively against these two pathogens.

Other studies conducted on *Leucas martinicensis* showed the dichloromethane:methanol (1:1) extract of the whole plant to have *in vitro* antiplasmodial activity (Clarkson *et al.*, 2004). In another study, Maïkere-Faniyo *et al.* (1989) used the agar dilution streak method and recorded the methanolic leaf extract of *Leucas martinicensis* to completely inhibit the growth of *Salmonella typhi* and *Shigella dysenteriae*, which are two bacteria that cause diarrhoea.

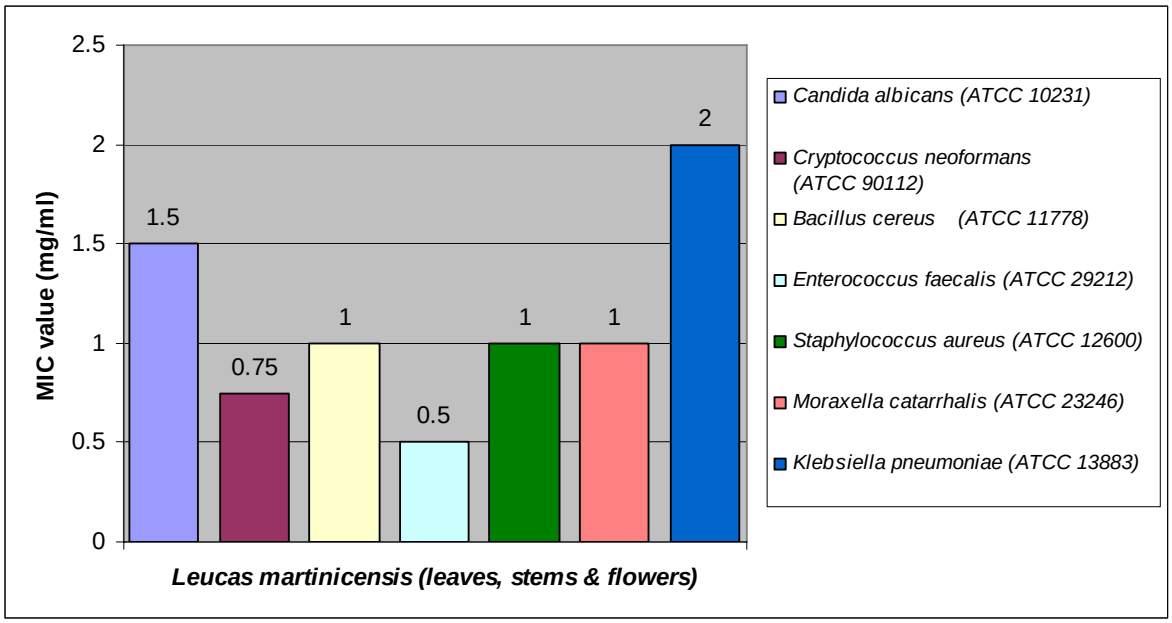


Figure 3.10 MIC values for pathogens when tested against *Leucas martinicensis* leaves, stems and flower extract.

3.3.2.4 Lantana rugosa (Verbenaceae)

The extract of the leaves and stems of *Lantana rugosa* yielded the lowest MIC values (Table 3.4) from all the extracts against *Enterococcus faecalis* (0.5 mg/ml) and *Staphylococcus aureus* (0.25 mg/ml). McGaw and Eloff (2005) also recorded MIC values that were ≤ 1 mg/ml against these two pathogens. They investigated the antimicrobial activity of the acetone leaf extracts of *Lantana rugosa* wherein they reported that the extract inhibited the growth of all the tested pathogens and in particular, obtained MIC values of 0.78 mg/ml and 0.39 mg/ml against *Enterococcus faecalis* and *Staphylococcus aureus* respectively.

In previous studies, antimicrobial activity has been recorded for two other *Lantana* species *viz.* *Lantana camara* (Kumar *et al.*, 2006) and *Lantana balansae* (Salvat *et al.*, 2004). Kumar *et al.* (2006), prepared dichloromethane:methanol (1:1) extracts of the plant species and established

the antimicrobial activity qualitatively using the agar dilution-streak method. The leaf extract of *Lantana camara* showed complete inhibition of *Bacillus cereus*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Enterococcus faecalis* and *Candida albicans*.

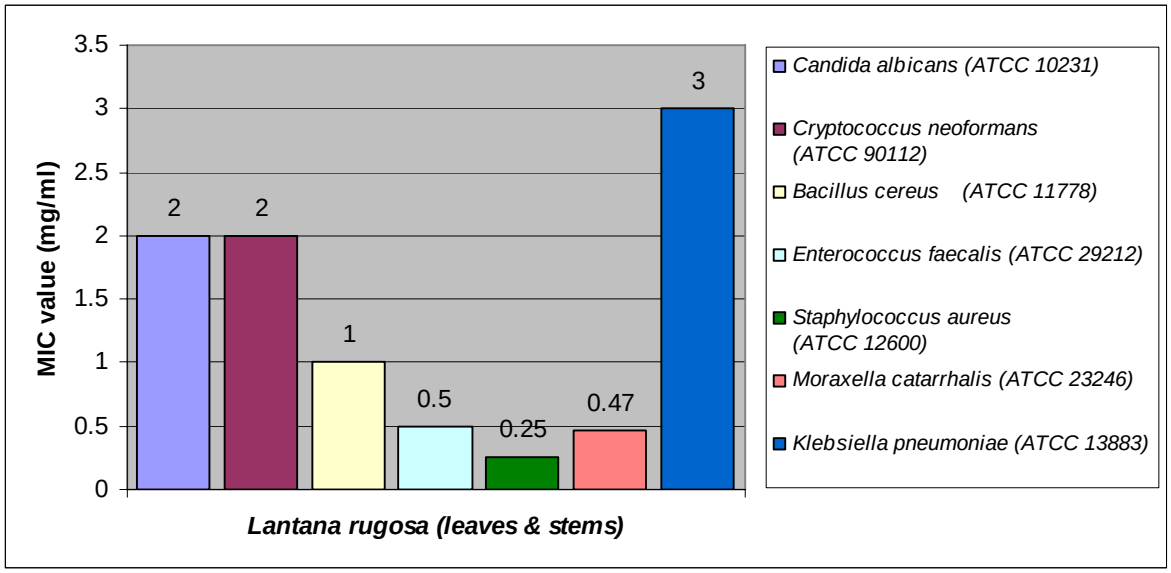


Figure 3.11 MIC values for pathogens when tested against *Lantana rugosa* leaves and stem extract.

In the study conducted by Salvat *et al.* (2004), the MIC values obtained for the methanolic extracts of the branches of *Lantana balansae* were 0.25 mg/ml for *Staphylococcus aureus* and greater than 1 mg/ml for *Klebsiella pneumoniae*.

In this research report, similar antimicrobial activity was shown by the acetone:methanol (1:1) extracts of the leaves and stems of *Lantana rugosa*. Figure 3.11 shows the leaves and stems of *Lantana rugosa* to have good antimicrobial activity (MIC ≤ 1 mg/ml) against Gram-positive *Bacillus cereus, Enterococcus faecalis, Staphylococcus aureus* and Gram-negative *Moraxella*

catarrhalis. Moderately good activity was shown against *Candida albicans* and *Cryptococcus neoformans* and moderate antimicrobial activity was shown against *Klebsiella pneumoniae.*

3.3.2.5 Vitex rehmannii (Verbenaceae)

The acetone:methanol (1:1) leaf extracts of *Vitex rehmannii* had the lowest MIC values out (Table 3.4) of all the extracts against *Bacillus cereus* (0.08 mg/ml) and *Moraxella catarrhalis* (0.31 mg/ml). Good antimicrobial activity was shown against *Cryptococcus neoformans* where an MIC value of 1 mg/ml was obtained (Figure 3.12).

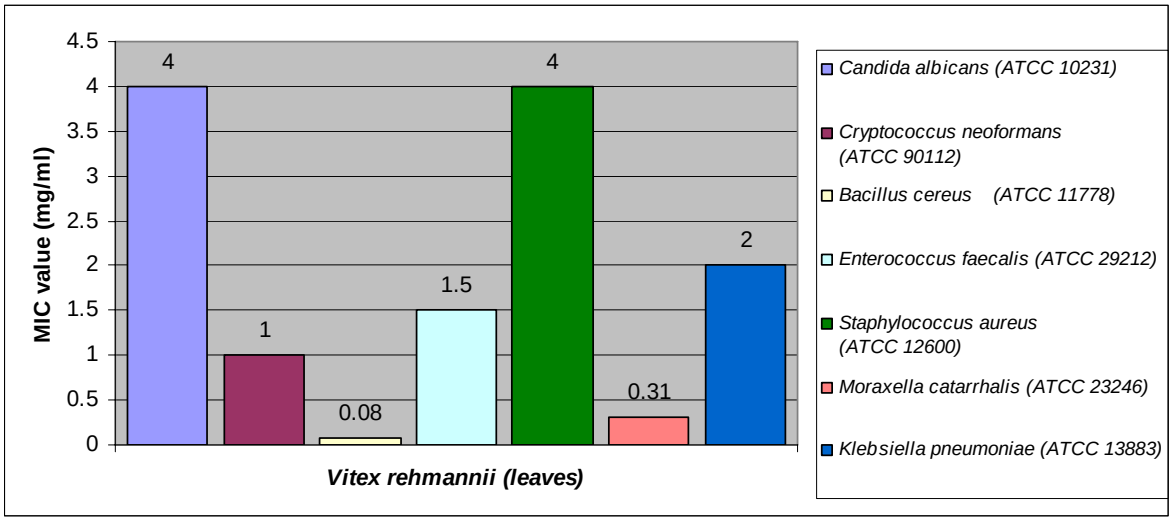


Figure 3.12 MIC values for pathogens when tested against *Vitex rehmannii* leaf extract.

Nyiligira (2004a) determined the MIC values for the acetone extracts of the aerial parts of five *Vitex* species. The *Vitex rehmannii* extracts demonstrated good antimicrobial activity where MIC values of < 1 mg/ml were obtained against *Staphylococcus aureus, Bacillus cereus, Enterococcus faecalis* and *Cryptococcus neoformans*. Nyiligira (2004a) isolated 12*S*,16*S*/*R*-dihydroxy-*ent*-labda-7,13-dien-15,16-olide from *Vitex rehmannii* and showed that this compound was responsible for the antimicrobial activity.

Therefore the antimicrobial activity of *Vitex rehmannii* shown in this research report could also be attributed to this compound. Figure 3.12 shows the MIC values obtained by the acetone:methanol (1:1) leaf extracts of *Vitex rehmannii*.

3.3.2.6 Zanthoxylum davyi (Rutaceae)

The antimicrobial activity was determined for the extracts of the leaves, roots, bark and thorns of the plant species *Zanthoxylum davyi* belonging to the Rutaceae family. The leaf extract of *Zanthoxylum davyi* showed the best antimicrobial activity compared to the other plant parts for this species (Table 3.5). The leaf extract displayed good antimicrobial activity i.e. MIC values of ≤ 1 mg/ml for five out of the seven pathogens *viz. Cryptococcus neoformans*, *Bacillus cereus*, *Enterococcus faecalis*, *Moraxella catarrhalis* and *Klebsiella pneumoniae*. In addition, the leaf extract exhibited the second lowest MIC value from all the plant extracts against *Cryptococcus neoformans*, *Klebsiella pneumoniae* and *Enterococcus faecalis*. The average MIC values obtained by each extract (Table 3.5) also shows the leaf extracts to be the most active and the roots the least active. This is a positive finding in terms of plant part substitution so as to preserve this plant species. Previous studies have been carried out to provide evidence to support plant part substitution so as to promote the use of the aerial parts of the plant instead of the bark or roots which is more destructive to the plant when harvested (Zschocke *et al.*, 2000; Lewu *et al*., 2006). Zschocke *et al.* (2000) showed that the aerial parts of *Siphonochilus aethiopicus* to be more active pharmacologically than the underground parts of the plant and Lewu *et al*. (2006) showed that the roots and the leaves of *Pelargonium sidoides* to have very similar antimicrobial activity.

Table 3.5 MIC values for pathogens when tested against the leaves, roots, bark and thorns extracts of *Zanthoxylum davyi*.

Pathogen	MIC value (mg/ml)				
	Zanthoxylum davyi (leaves)	*Zanthoxylum davyi* (roots)	*Zanthoxylum davyi* (bark)	*Zanthoxylum davyi* (thorns)	Average MIC value (mg/ml) against each pathogen
Candida albicans (ATCC 10231)	4.00	8.00	4.00	3.00	4.75
Cryptococcus neoformans (ATCC 90112)	0.25	8.00	0.25	1.00	2.38
Bacillus cereus (ATCC 11778)	0.31	4.00	0.38	0.75	1.36
Enterococcus faecalis (ATCC 29212)	1.00	8.00[a.]	4.00	4.00	3.00[a.]
Staphylococcus aureus (ATCC 12600)	2.00	6.00	4.00	4.00	4.00
Moraxella catarrhalis (ATCC 23246)	1.00	4.00	4.00	0.38	2.34
Klebsiella pneumoniae (ATCC 13883)	0.50	4.00	2.00	2.00	2.13
Average MIC value for each extract (mg/ml)	1.29	ND[b.]	2.66	2.16	

[a.] MIC value against *E. faecalis* is equal to DMSO control value (Table 3.2), thus antimicrobial activity cannot be conclusively attributed to the extract. This value was therefore excluded when calculating the average MIC value for all the extracts against this pathogen.
[b.] ND=Not determined. The average MIC value for the root extract against the respiratory pathogens was not determined. See footnote a. above.

In a study investigating the antibacterial activity of Venda medicinal plants (Steenkamp *et al.*, 2007a), *Zanthoxylum davyi* was one of the most active plants. The methanol extract of the bark obtained an MIC value of 1 mg/ml against *Staphylococcus aureus*. The acetone:methanol (1:1) bark extract in this research study obtained an MIC value of 4 mg/ml (Table 3.5) against *Staphylococcus aureus*, however the leaf extract obtained a lower MIC value of 2 mg/ml.

The antifungal activity of *Zanthoxylum davyi* has been studied by Steenkamp *et al.* (2007b). The methanol bark extracts had an MIC value of < 1 mg/ml against *Candida albicans*. In this research report the acetone:methanol (1:1) bark extract obtained an MIC value of 4 mg/ml against this pathogen. Steenkamp *et al.* (2007b) determined the MIC value using the macro-broth tube dilution method. The same ATCC strain of *Candida albicans* was used by Steenkamp *et al.* (2007b) as was used in this research report. The solvent used for extraction in this research report *viz.* acetone:methanol, and the determination of the MIC by the micro dilution method (Eloff, 1998), could be possible reasons for the differences in the MIC values obtained in this research report and the Steenkamp *et al.* (2007b) study.

A phytochemical analysis of the stem bark of *Zanthoxylum davyi* was conducted by Tarus *et al.* (2006). This study yielded five benzo[c]phenanthridine alkaloids; chelerythrine, dihydrochelerythrine, bocconoline, 6-hydroxydihydrochelerythrine and 6-methoxy-7-demethyldihydrochelerythrine. Two other compounds that were also isolated were 4-methoxy-1-methyl-2(1H)-quinolinone and *meso*-sesamin. Of these compounds, chelerythrine had good antimicrobial activity and therefore might be the active component in *Zanthoxylum davyi*.

In a study carried out by Matu and van Staden (2003), the antibacterial activity of 12 plants used as traditional medicines in Kenya were studied. Two of these plant species were *Zanthoxylum chalybeum* and *Zanthoxylum usambarense*. The antibacterial activity was examined using disc-diffusion assays wherein the methanol and hexane extracts of the roots and stem bark of these two *Zanthoxylum* species displayed high antibacterial activity against Gram-positive pathogens, one of which was *Staphylococcus aureus*.

The antifungal activity of two Cameroonian *Zanthoxylum* species, *Zanthoxylum leprieurii* and *Zanthoxylum xanthoxyloides*, has also been investigated (Ngane *et al.*, 2000). Using disc diffusion assays, the aqueous-ethanol (90%) extracts of the leaves, roots and stem barks of *Zanthoxylum leprieurii* and *Zanthoxylum xanthoxyloides* showed inhibition of *Candida albicans* and *Cryptococcus neoformans*. Ngane *et al.* (2000) also obtained an MIC value of 1.00 mg/ml for *Zanthoxylum xanthoxyloides* against both *Candida albicans* and *Cryptococcus neoformans*.

Therefore, the antimicrobial activity of *Zanthoxylum davyi* obtained in this research report falls in line with the antimicrobial activity displayed by other *Zanthoxylum* plant species. The antimicrobial activity of the four extracts of *Zanthoxylum davyi* displayed in this research report can be summarised by considering the average MIC values obtained by the extracts against each pathogen (Table 3.5). The extracts showed the best activity against *Bacillus cereus.* Moderately good activity was obtained against *Moraxella catarrhalis*, *Klebsiella pneumoniae* and *Cryptococcus neoformans*. Moderate antimicrobial activity was shown against *Candida albicans, Enterococcus faecalis* and *Staphylococcus aureus.* Thus the leaves, roots, bark and thorn extracts of *Zanthoxylum davyi* displayed good, moderately good and moderate antimicrobial activity against the respiratory pathogens, obtaining average MIC values ranging between 1.36 mg/ml – 4.75 mg/ml.

3.4 Conclusion

- The MIC values determined for the plant extracts (Figures 3.1-3.7) demonstrates that these plant species do show antimicrobial activity against the respiratory pathogens, thus supporting their traditional uses to treat various respiratory ailments (Table 1.1).

- On average the antimicrobial activity of the plant extracts ranged between moderately good to moderate activity against the respiratory pathogens (Table 3.1).

- The most sensitive pathogens were *Cryptococcus neoformans,* Gram-positive *Bacillus cereus* and Gram-negative *Moraxella catarrhalis. Enterococcus faecalis* was the least sensitive of the pathogens.

- The antimicrobial activity demonstrated by *Terminalia sericea* is congruent with data presented in previous studies (Eloff, 1999; Fyhrquist *et al.,* 2002; Steenkamp *et al.*, 2004; Eldeen *et al.*, 2005; Masoko *et al.,* 2005; Moshi and Mbwambo*,* 2005).

- The leaf and stem extract of *Chenopodium ambrosioides* obtained relatively low MIC values (0.25-2.0 mg/ml) against the respiratory pathogens thus displaying promising antimicrobial activity (Figure 3.9).

- The MIC values obtained by the leaves, stems and flowers of *Leucas martinicensis* ranged between 0.5 and 2.0 mg/ml (Figure 3.10) representing good to moderately good antimicrobial activity.

- The antimicrobial activity of *Lantana rugosa* shown in this research report (Figure 3.11), ranged between good to moderately good activity (0.25 – 3.00 mg/ml). The MIC values of ≤ 1 mg/ml recorded for *Enterococcus faecalis* and *Staphylococcus aureus* are

in agreement with those obtained by McGaw and Eloff (2005) against these same two pathogens.

- The antimicrobial activity demonstrated by the leaf extracts of *Vitex rehmannii* supports the antimicrobial activity as was also shown by Nyiligira (2004a).

- The leaves, roots, bark and thorn extracts of *Zanthoxylum davyi* displayed good, moderately good and moderate antimicrobial activity against the respiratory pathogens with the leaf extract displaying the best activity (Table 3.5).

CHAPTER 4

Thin Layer Chromatography

and

Bioautographic assay

4.1 Introduction

4.1.1 Thin layer chromatography (TLC)

Thin layer chromatography (TLC) was carried out on the plant extracts as part of the phytochemical profiling of the species. TLC just like HPLC, also allows for the separation of the compounds present within the plant extract. Thus the TLC chromatogram also serves as a chemical fingerprint of the plant extract (Scott *et al.*, 2004). According to Marston and Hostettmann (1999), TLC is an easy and inexpensive method for determining which chemical compounds are present in the plant extract and may contribute to the biological activity. TLC requires minimal equipment, is simple to conduct and can be repeated without much difficulty.

The method used to develop the TLC chromatograms for each of the extracts is described in Chapter 2, Section 2.3.1. When the TLC plate is viewed under the UV light, the chemical compounds are seen as bands each with a unique colour and R_f value. The R_f value is the retention factor and represents the position that the chemical compound is retained on the TLC plate as it is separated from all the other chemical constituents of the plant extract.

4.1.2 Thin layer chromatography (TLC) bioautographic assays

As part of the screening process of these traditional medicinal plants, the antimicrobial activity of the chemical compounds present in the plant extracts were also studied by conducting TLC bioautographic assays. The method used was the agar-overlay technique and it is described in Chapter 2, Section 2.4.3.

Marston and Hostettmann (1999) describe bioautography as a convenient and easy method of examining the specific effect that plant extracts have on microorganisms. If a compound is

separated by TLC and shows up as having a clear zone on the bioautographic assay plate, it means that the compound inhibits the growth of the pathogen and thus it is an active compound. Thus the purpose of performing bioautographic assays on the plant extracts was to serve as a preliminary identification of the active compounds that have antimicrobial activity.

4.2 Results and discussion of the TLC analysis

Figure 4.1 is the photograph of the TLC plate for extracts 1-15 and Figure 4.2 is the photograph of the TLC plate for extracts 16 – 28. Tables 4.1 and 4.2 list the R_f values and the colours of the major bands.

TLC chromatograms were obtained for all the plant extracts except for the bark extracts of *Acacia sieberiana* and *Ziziphus mucronata* as there was insufficient sample for these two extracts. Good separation of compounds was obtained for the remainder of the extracts (Figure 4.1 and 4.2) and the number of major bands that were identified for the plant extracts ranged between one and five (Tables 4.1-4.2). The only extract that did not show any separation of compounds was the root extract of *Terminalia sericea* (extract 2: Figure 4.1).

The most characteristic feature when viewing the TLC plates is a consistency in the bands of the leaf extracts which appears in the top region of the plate (extracts 1, 3, 6, 7, 8, 9, 11, 13, 15: Figure 4.1 and extracts 19, 20, 22: Figure 4.2). There is a very prominent purple spot (Figure 4.1 and 4.2) that is common to all the leaf extracts which has an R_f value of 0.79 (Figure 4.1 and Table 4.1) and an R_f value of 0.75 (Figure 4.2 and Table 4.2). This consistent band amongst the leaf extracts suggests that common compounds exist amongst the leaf extracts of different plant species. These compounds that are common to all the leaf extracts

could possible be chlorophyll and its degradation products as was described by Zschocke *et al.* (2000) who obtained the TLC chromatograms of the leaf extracts of four medicinal plants in a study that was conducted to compare the chemical composition and biological activity of the different plant parts of the same plant species.

A compound with R_f value of 0.99 having a blue colour is common to almost all the extracts. It appears for all the leaf extracts (extracts 1, 3, 6, 7, 8, 9, 11, 13, 15: Figure 4.1 and Table 4.1 and extracts 19, 20, 22: Figure 4.2 and Table 4.2) and for the root extracts it appears for extracts 5, 14 (Figure 4.1 and Table 4.1), 16, 25, 26, 27, 28 (Figure 4.2 and Table 4.2) with exceptions for the root extracts of *Terminalia sericea* and *Ziziphus mucronata*. This compound also appears for the bark and thorn extracts of *Zanthoxylum davyi* (extracts 17 and 18: Figure 4.2 and Table 4.2).

The TLC chromatogram for the leaf and stem extracts of *Lantana rugosa* has a prominent compound. It is turquoise in colour and has R_f value of 0.58 (extract 1: Figure 4.1 and Table 4.1). *Leucas martinicensis* (leaves, stems and flowers) has a yellowish compound with R_f value of 0.63 (extract 7: Figure 4.1 and Table 4.1). *Schkuhria pinnata* leaf extracts has a compound towards the bottom of the plate that is light blue in colour and has R_f value of 0.21 (extract 8: Figure 4.1 and Table 4.1).

Figure 4.1 shows a light blue compound that is common to extracts 12 and 14. This compound has a R_f value of 0.71 (Table 4.1) and appears for the root extracts of *Ziziphus mucronata* and *Chamaecrista mimosoides*. When examining the chromatograms for the leaf and root extracts of *Ziziphus mucronata* (extracts 11 and 12: Figure 4.1 and Table 4.1), there appears to be

compounds that are present in the leaves but not in the roots. The different chemical compositions of the leaves and roots could support their traditional use in combination (Table 1.1). A study by van Vuuren and Viljoen (2008) have also shown enhanced *in vitro* antimicrobial activity when the different plant parts of *Croton gratissimus* are combined.

The TLC chromatograms of the bark and thorn extracts of *Zanthoxylum davyi* show some prominent compounds that stand out on the TLC plate (extracts 17 and 18: Figure 4.2 and Table 4.2). Both these extracts have a common compound at R_f value of 0.50 and have a bright blue colour. The thorn extract has a bright yellow compound at R_f value of 0.74 (extract 18: Figure 4.2) and the bark extract also appears to have a compound at this R_f value but having a blue colour (extract 17: Figure 4.2). The root, bark and thorn extracts of *Zanthoxylum davyi* (extracts 16, 17, 18: Figure 4.2) appears to have a band of bluish compounds that are clustered together between the compounds that have R_f values 0.50 and 0.74 (Table 4.2). However, these bands appear clearer for the thorn extract and have R_f values of 0.65 and 0.69 (Table 4.2).

The bark and stem extracts of *Heteromorpha arborescens* also have a common compound that is prominent on the TLC plate (extracts 23, 24: Figure 4.2 and Table 4.2). This compound has an R_f value of 0.61 and is bright blue in colour. The stem extract also has an additional compound that stands out on the TLC plate which is bright blue in colour and has R_f value of 0.49.

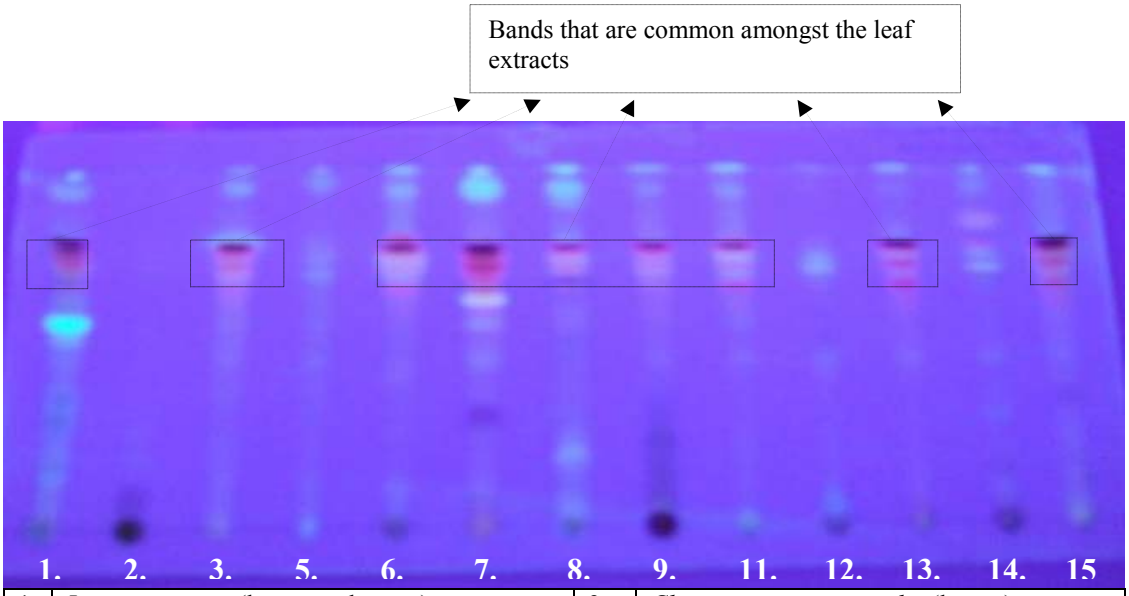
A difference is seen in the TLC chromatograms between the leaves and the woody parts of the same plant species such as the roots, stems, barks and thorns. The plant species that have

extracts for both the leaves and the roots are *Chenopodium ambrosioides* (extracts 3 and 5: Figure 4.1), *Chamaecrista mimosoides* (extracts 9 and 14: Figure 4.1) and *Ziziphus mucronata* (extracts 11 and 12: Figure 4.1). *Clerodendrum glabrum* has extracts for the leaf and the bark (extracts 20 and 21: Figure 4.2) and for *Zanthoxylum davyi* there are extracts for the leaf, root, bark and thorns (extract 15: Figure 4.1; extracts 16, 17, 18: Figure 4.2). For *Heteromorpha arborescens* there are extracts for the leaf, bark and stems (extracts 22, 23, 24: Figure 4.2). The bands that are common to the leaf extracts have been described above. The root extracts have compounds that are mainly blue in colour. In addition, the leaf extracts have more compounds as compared to the extracts of the roots, stems, barks and thorns (Table 4.1 and 4.2). Differences in the chemical composition between the leaves and roots were also seen in the TLC chromatograms of *Eucomis autumnalis* (Zschocke *et al.*, 2000). Zschocke *et al.* (2000) described the compounds in the TLC chromatograms of the leaves to be chlorophyll and its degradation products. Zschocke *et al.* (2000) also described the compounds seen in the TLC chromatograms of the root extracts as bright blue fluorescence bands when the TLC plate was viewed under 365 nm UV light. This is consistent with the findings of the compounds in the TLC chromatograms of the leaves and root extracts in this research study.

Due to the TLC chromatograms displaying the different chemical compositions of the different plant parts for the same plant species, it is suggested that studies similar to those conducted by van Vuuren and Viljoen (2008) also be carried out on these plant species in order to verify the efficacy of the individual plant parts and their combinations and also to demonstrate whether the plant parts act synergistically or antagonistically.

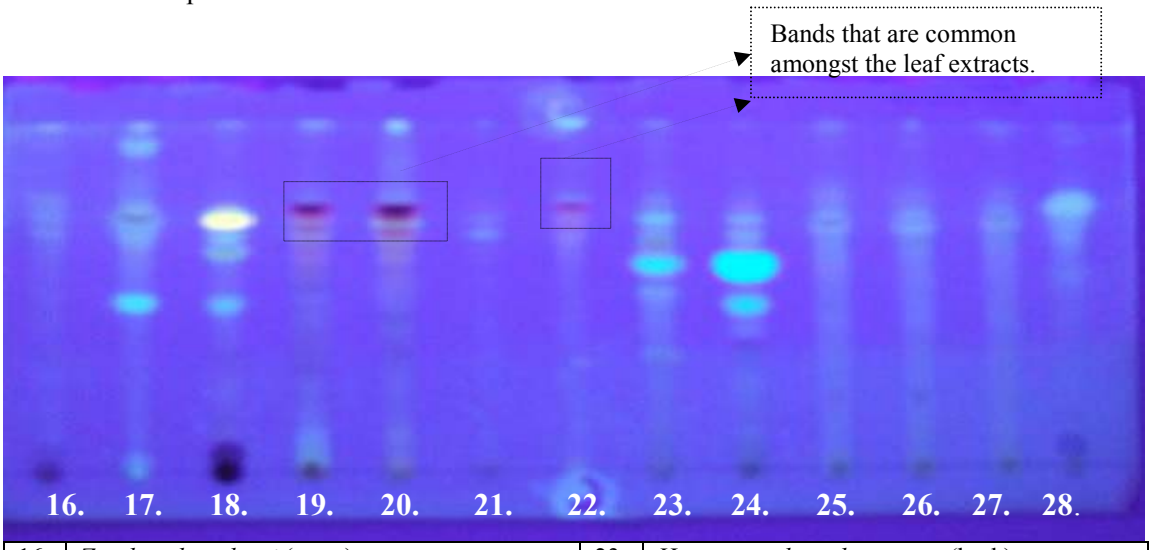
The acetone:methanol (1:1) root extract of *Terminalia sericea* (extract 2: Figure 4.1) did not show any visible bands on the TLC plate. However in a study conducted by Masoko *et al.* (2005), wherein the antifungal activities of six *Terminalia* species were studied, TLC was also used to analyse the chemical composition of the extracts. In this study, the leaves of *Terminalia sericea* were analysed and they were extracted with acetone, hexane, dichloromethane and methanol. The TLC plates were developed using three eluent systems *viz.* ethyl acetate:methanol:water (polar/neutral), chloroform:ethyl acetate:formic acid (intermediate polarity/acidic) and benzene:ethanol:ammonia hydroxide (non-polar/basic). The TLC chromatograms of the acetone and methanol leaf extracts displayed very faint and not very noticeable bands. However, for the hexane and dichloromethane leaf extracts, very clear bands were seen in all three eluent systems. Therefore, the eluent system used (toluene:dioxan:acetic) and the solvents used to prepare the root extracts (methanol:acetone (1:1)) in this research study, could be possible reasons for the lack of separation of the compounds of *Terminalia sericea* on the TLC plate. It might also be possible that the root extract does not easily display bands on TLC chromatograms as compared to that of the leaf extract.

TLC chromatograms also allows for the detection of compounds that are common to different plant species which belong to the same plant family. The leaf extracts for *Vitex rehmannii* and *Clerodendrum glabrum*, which belong to the Verbenaceae family, have almost identical TLC chromatograms (extracts 19 and 20: Figure 4.2). The same major compounds were noted for these two extracts (Table 4.2). The first compound has R_f value of 0.75 (purple colour) and the second compound has R_f value of 0.99 and is light blue in colour.



1.	*Lantana rugosa* (leaves and stems)	9.	*Chamaecrista mimosoides* (leaves)
2.	*Terminalia sericea* (roots)	11.	*Ziziphus mucronata* (leaves)
3.	*Chenopodium ambrosioides* (leaves & stems)	12.	*Ziziphus mucronata* (roots)
5.	*Chenopodium ambrosioides* (roots)	13.	*Clematis oweniae* (leaves)
6.	*Acacia sieberiana* (leaves)	14.	*Chamaecrista mimosoides* (roots)
7.	*Leucas martinicensis* (leaves, stems & flowers)	15.	*Zanthoxylum davyi* (leaves)
8.	*Schkuhria pinnata* (leaves)		

Figure 4.1 TLC plate for plant extracts 1 to 15 viewed under 366 nm UV light. No results were obtained for extracts 4 and 10, bark extracts for *Acacia sieberiana* and *Ziziphus mucronata,* due to insufficient sample.



16.	*Zanthoxylum davyi* (roots)	23.	*Heteromorpha arborescens* (bark)
17.	*Zanthoxylum davyi* (bark)	24.	*Heteromorpha arborescens* (stems)
18.	*Zanthoxylum davyi* (thorns)	25.	*Peucedanum caffrum* (roots A)
19.	*Vitex rehmannii* (leaves)	26.	*Peucedanum caffrum* (roots B)
20.	*Clerodendrum glabrum* (leaves)	27.	*Peucedanum caffrum* (roots C)
21.	*Clerodendrum glabrum* (bark)	28.	*Alepidea amatymbica* (roots)
22.	*Heteromorpha arborescens* (leaves)		

Figure 4.2 TLC plate for plant extracts 16 to 28 viewed under 366 nm UV light.

Heteromorpha arborescens and *Peucedanum caffrum* both belong to the Apiaceae family. The bark and stem extracts of *Heteromorpha arborescens* and the three root extracts of *Peucedanum caffrum* all have a compound with R_f value of 0.73 and having a light blue colour (extracts 23, 24, 25, 26, 27: Figure 4.2 and Table 4.2).

Table 4.1[*] R_f values and the colours of major bands present in plant extracts 1 to 15 for TLC plate viewed under 366 nm UV light.

R_f values and colours of the major bands as they eluted up the TLC plate.													
	0.99 blue	No bands recorded	0.99 blue	0.99 blue	0.99 blue	0.99 blue	0.99 blue	0.99 blue	0.99 blue		0.99 blue	0.99 blue	0.99 blue
				0.98 light blue									
	0.95 blue		0.95 blue		0.95 blue	0.95 blue	0.95 blue	0.95 blue	0.95 blue			0.95 blue	
												0.86 orange	
			0.84 blue										
	0.79 purple		0.79 purple		0.79 purple	0.79 purple	0.79 purple	0.79 purple	0.79 purple		0.79 purple		0.79 purple
				0.75 light blue									
										0.71 light blue		0.71 light blue	
				0.70 light blue									
												0.68 blue	
					0.66 orange								
						0.63 yellow							
	0.58 turquoise												
									0.48 light blue				
							0.21 light blue						
										0.09 blue			
Extract number	**1.**	**2.**	**3.**	**5.**	**6.**	**7.**	**8.**	**9.**	**11.**	**12.**	**13.**	**14.**	**15.**
Plant extract	*Lantana rugosa* (leaves and stems)	*Terminalia sericea* (roots)	*Chenopodium ambrosioides* (leaves and stems)	*Chenopodium ambrosioides* (roots)	*Acacia sieberiana* (leaves)	*Leucas martinicensis* (leaves, stems and flowers)	*Schkuhria pinnata* (leaves)	*Chamaecrista mimosoides* (leaves)	*Ziziphus mucronata* (leaves)	*Ziziphus mucronata* (roots)	*Clematis oweniae* (leaves)	*Chamaecrista mimosoides* (roots)	*Zanthoxylum davyi* (leaves)

[*] The columns corresponding to the leaf extracts are shaded in.

Table 4.2[*] R_f values and the colours of major bands present in plant extracts 16 to 28 for TLC plate viewed under 366 nm UV light.

R_f values and colours of the major bands as they eluted up the TLC plate.													
	0.99 blue	0.99 blue	0.99 blue	0.99 blue	0.99 blue		0.99 blue			0.99 blue	0.99 blue	0.99 blue	0.99 blue
		0.95 light blue											
	0.80 blue												
				0.75 purple	0.75 purple		0.75 purple						0.75 blue
	0.74 blue	0.74 blue	0.74 bright yellow										
								0.73 blue	0.73 blue	0.73 blue	0.73 blue	0.73 blue	
	0.69 blue	0.69 blue	0.69 blue			0.69 light blue							
			0.65 light blue										
								0.61 bright blue	0.61 bright blue				
		0.50 bright blue	0.50 bright blue										
									0.49 bright blue				
Extract number	**16.**	**17.**	**18.**	**19.**	**20.**	**21.**	**22.**	**23.**	**24.**	**25.**	**26.**	**27.**	**28.**
Plant extract	*Zanthoxylum davyi* (roots)	*Zanthoxylum davyi* (bark)	*Zanthoxylum davyi* (thorns)	*Vitex rehmannii* (leaves)	*Clerodendrum glabrum* (leaves)	*Clerodendrum glabrum* (bark)	*Heteromorpha arborescens* (leaves)	*Heteromorpha arborescens* (bark)	*Heteromorpha arborescens* (stems)	*Peucedanum caffrum* (roots A)	*Peucedanum caffrum* (roots B)	*Peucedanum caffrum* (roots C)	*Alepidea amatymbica* (roots)

[*] The columns corresponding to the leaf extracts are shaded in.

4.3 Results and discussion of bioautographic assay

Bioautographic assays were carried out against two pathogens *viz.* Gram-positive *Staphylococcus aureus* (ATCC 12600) and Gram-negative *Moraxella catarrhalis* (ATCC 23246).

Figure 4.3, 4.5, 4.7 and 4.9 are the photographs of the bioautographic assay plates and Figures 4.4, 4.6, 4.8 and 4.10 are diagrammatic representations of the bioautographic plates showing the clear zones of inhibition recorded once the plates were sprayed with INT. Due to the poor resolution of the photographs, diagrams were drawn so as to more clearly display the position of the clear zones of inhibition in correlation to the extract numbers.

4.3.1 Staphylococcus aureus (ATCC 12600)

The bioautography assay plate for extracts 1-15 against *Staphylococcus aureus* (ATCC 12600) exhibited clear zones of inhibition for extracts 1 and 12 (Figure 4.3 and 4.4). Extract one corresponds to the leaf and stem extract of *Lantana rugosa*. The region where this clear inhibition zone appears seems to correspond to the area on the TLC plate where a group of bands are clustered together (extract 1: Figure 4.1 and Table 4.1).

Extract 12 corresponds to the root extract of *Ziziphus mucronata* (Figure 4.4). The region where the zone of inhibition appears corresponds to a region on the TLC plate (Figure 4.1 and Table 4.1) where a compound could not be seen when the TLC plate was viewed.

A distinct zone of inhibition is seen for extract number 19 (Figure 4.5 and 4.6) on the bioautographic assay plate for extracts 16-28. Extract 19 is the leaf extract of *Vitex rehmannii*.

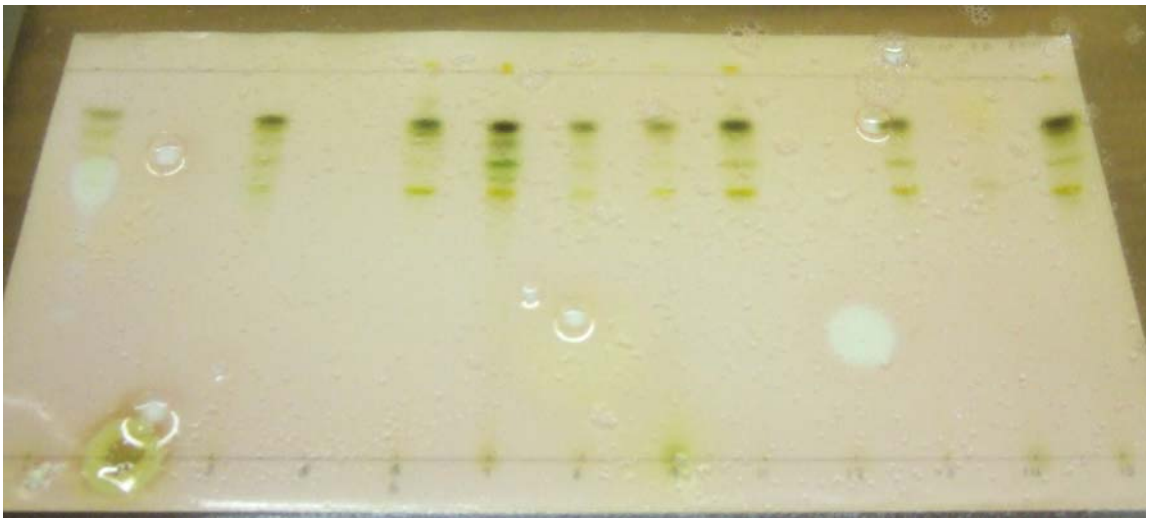
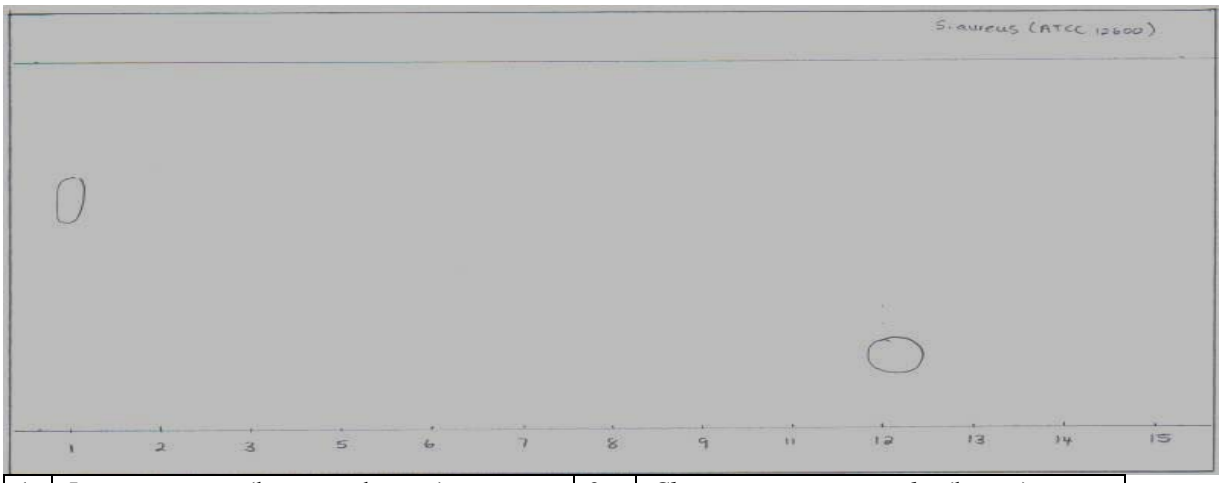


Figure 4.3 Bioautographic assay plate of plant extracts 1 to 15 against *Staphylococcus aureus* (ATCC 12600).



1.	*Lantana rugosa* (leaves and stems)	9.	*Chamaecrista mimosoides* (leaves)
2.	*Terminalia sericea* (roots)	11.	*Ziziphus mucronata* (leaves)
3.	*Chenopodium ambrosioides* (leaves and stems)	12.	*Ziziphus mucronata* (roots)
5.	*Chenopodium ambrosioides* (roots)	13.	*Clematis oweniae* (leaves)
6.	*Acacia sieberiana* (leaves)	14.	*Chamaecrista mimosoides* (roots)
7.	*Leucas martinicensis* (leaves, stems and flowers)	15.	*Zanthoxylum davyi* (leaves)
8.	*Schkuhria pinnata* (leaves)		

Figure 4.4 Diagrammatic representation of the clear zones of inhibition for the bioautographic assay for extracts 1-15 against *Staphylococcus aureus* (ATCC 12600). No results were obtained for extracts 4 and 10, the bark extracts of *Acacia sieberiana* and *Ziziphus mucronata,* due to insufficient sample.

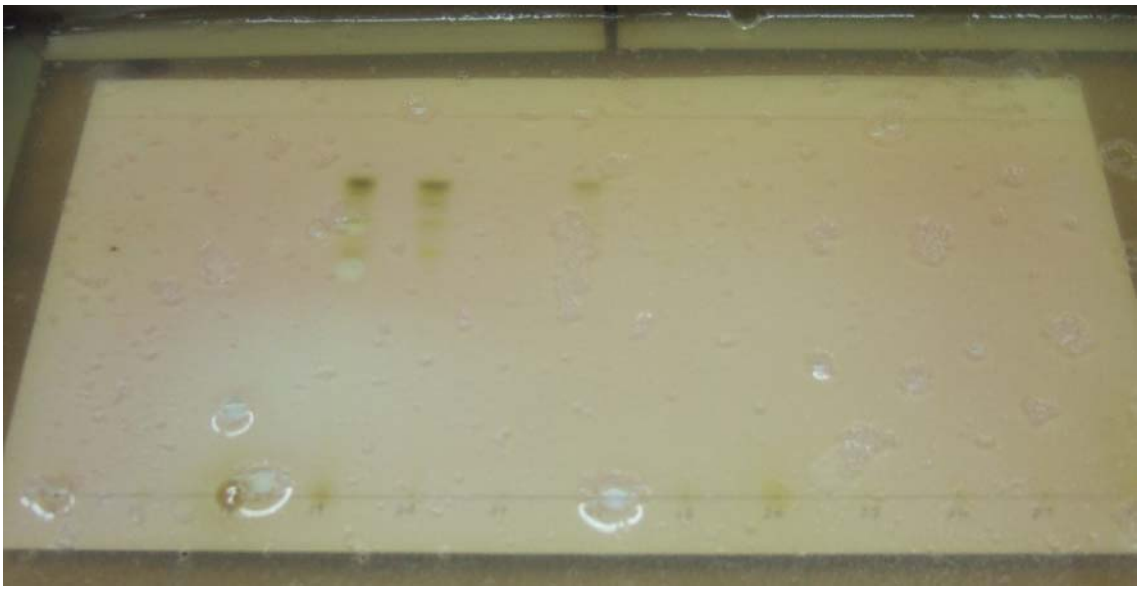


Figure 4.5 Bioautographic assay plate of plant extracts 16 to 28 against *Staphylococcus aureus* (ATCC 12600).

16.	*Zanthoxylum davyi* (roots)	23.	*Heteromorpha arborescens* (bark)
17.	*Zanthoxylum davyi* (bark)	24.	*Heteromorpha arborescens* (stems)
18.	*Zanthoxylum davyi* (thorns)	25.	*Peucedanum caffrum* (roots A)
19.	*Vitex rehmannii* (leaves)	26.	*Peucedanum caffrum* (roots B)
20.	*Clerodendrum glabrum* (leaves)	27.	*Peucedanum caffrum* (roots C)
21.	*Clerodendrum glabrum* (bark)	28.	*Alepidea amatymbica* (roots)
22.	*Heteromorpha arborescens* (leaves)		

Figure 4.6 Diagrammatic representation of the clear zones of inhibition for the bioautographic assay for extracts 16-28 against *Staphylococcus aureus* (ATCC 12600).

This zone of inhibition appears in the area where no bands were recorded during the TLC analysis when the TLC plate was viewed under UV light of 366 nm. However, it would appear that the clear zone of inhibition is located just below the group of compounds that are clustered together (Figure 4.2 and Table 4.2).

As diagrammatically represented in Figure 4.6, a clear inhibition zone is also seen spanning across an area between extracts 18 and 19 (Figure 4.5). Extract 18 is the thorn extract of *Zanthoxylum davyi*. The clear region for extract 18 seems to correspond to the area on the TLC plate (Figure 4.2 and Table 4.2) where the compound with R_f value of 0.50 was recorded.

4.3.2 Moraxella catarrhalis (ATCC 23246)

In the bioautographic assay for extracts 1-15, against Gram-negative *Moraxella catarrhalis* (ATCC 23246), three clear zones of inhibition were recorded (Figure 4.7 and Figure 4.8). The first clear zone corresponds to extract one being the leaves and stems of *Lantana rugosa* (Figure 4.7). This clear zone appears in approximately the same region as the clear zone recorded in the bioautographic assay against *Staphylococcus aureus* (ATCC 12600) (Figure 4.3), for the same plant extract, thus suggesting that it is possibly the same compound inhibiting the growth of these two pathogens. It is therefore recommended that this compound be further investigated in terms of isolation and recording its quantitative antimicrobial activity. When the MIC values were determined against the respiratory pathogens, the leaves and stem extract of *Lantana rugosa* also displayed good to moderately good antimicrobial activity having MIC values ranging between 0.25 – 3 mg/ml (Figure 3.11).

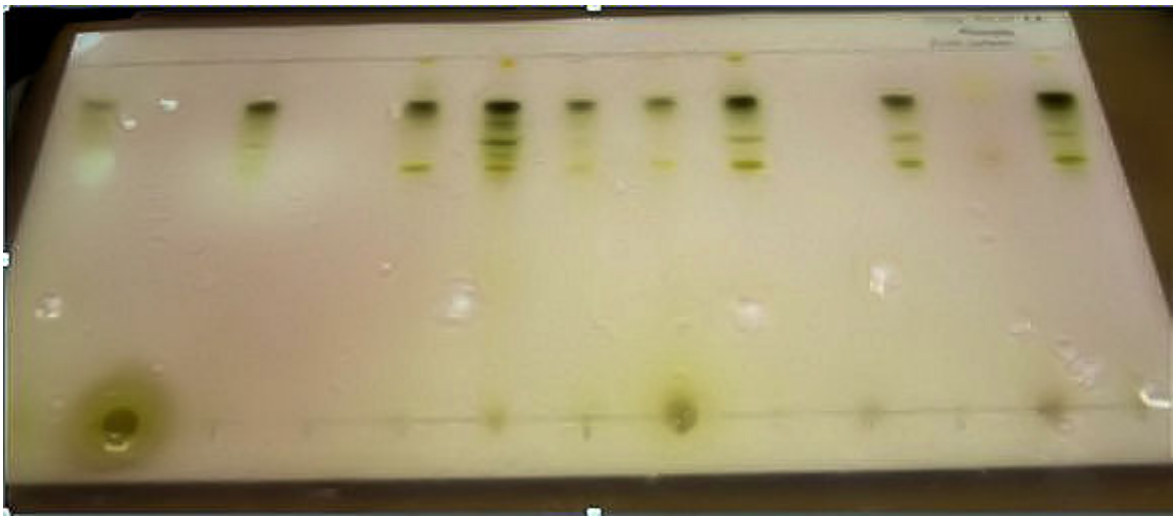
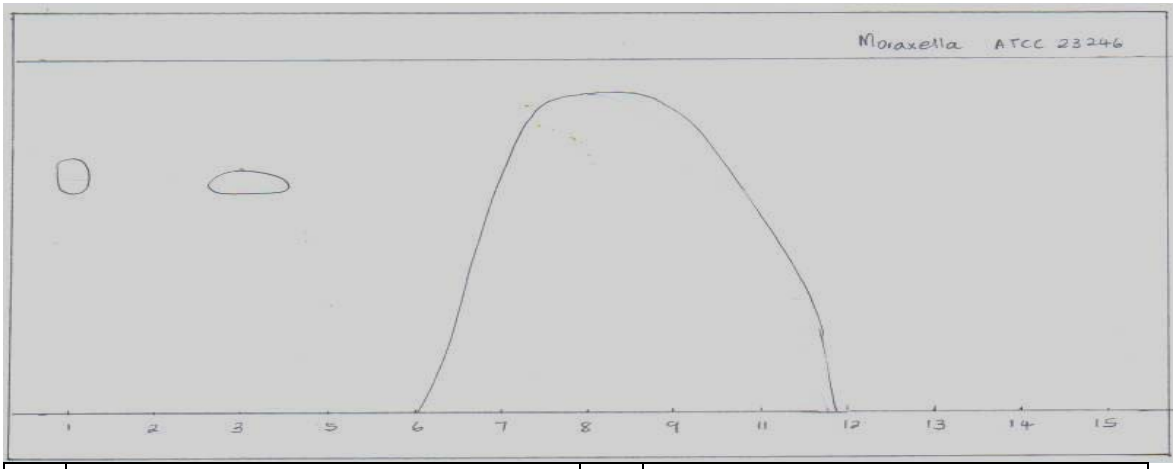


Figure 4.7 Bioautographic assay plate of plant extracts 1 to 15 against *Moraxella catarrhalis* (ATCC 23246).



1.	*Lantana rugosa* (leaves and stems)	9.	*Chamaecrista mimosoides* (leaves)
2.	*Terminalia sericea* (roots)	11.	*Ziziphus mucronata* (leaves)
3.	*Chenopodium ambrosioides* (leaves & stems)	12.	*Ziziphus mucronata* (roots)
5.	*Chenopodium ambrosioides* (roots)	13.	*Clematis oweniae* (leaves)
6.	*Acacia sieberiana* (leaves)	14.	*Chamaecrista mimosoides* (roots)
7.	*Leucas martinicensis* (leaves, stems and flowers)	15.	*Zanthoxylum davyi* (leaves)
8.	*Schkuhria pinnata* (leaves)		

Figure 4.8 Diagrammatic representation of the clear zones of inhibition for the bioautographic assay for extracts 1-15 against *Moraxella catarrhalis* (ATCC 23246). No results were obtained for extracts 4 and 10, the extracts of the bark for *Acacia sieberiana* and *Ziziphus mucronata,* due to insufficient sample.

The second clear zone as seen in Figure 4.7 and diagrammatically drawn in Figure 4.8 corresponds to extract three which is the leaves and stem extract of *Chenopodium ambrosioides*. This clear zone appears in the region of the TLC plate where a group of bands are clustered together (extract 3: Figure 4.1 and Table 4.1). It is recommended that this plant extract be further investigated with the aim of determining which compound is the active compound. Additional evidence that this extract most likely has active antimicrobial compounds is shown by the MIC values that were obtained against the respiratory pathogens (Figure 3.9). Antimicrobial activity ranged between good and moderately good (0.25 – 2 mg/ml). MIC values of ≤ 1 mg/ml representing good antimicrobial activity was obtained against *Candida albicans, Cryptococcus neoformans, Enterococcus faecalis, Staphylococcus aureus and Moraxella catarrhalis*. Moderately good activity was obtained against *Bacillus cereus* and *Klebsiella pneumoniae.*

The third zone of inhibition spreads across a broad area covering extracts 7, 8, 9, and 11 (Figure 4.7 and 4.8). These extracts are for the leaves, stems and flowers of *Leucas martinicensis,* and the leaf extracts of *Schkuhria pinnata, Chamaecrista mimosoides* and *Ziziphus mucronata* respectively. Due to this zone of inhibition being so broad, it is difficult to give the R_f value for one specific compound that might possess antimicrobial activity. Therefore it is recommended that further investigation into the isolation of these compounds be conducted so as to determine exactly which compounds are active.

In the bioautographic assay plate for extracts 16-28 against *Moraxella catarrhalis* (ATCC 23246), a clear zone of inhibition is seen for extract 19 which is the leaf extract of *Vitex rehmannii* (Figures 4.9 and 4.10). The region where this clear zone appears seems to be in the

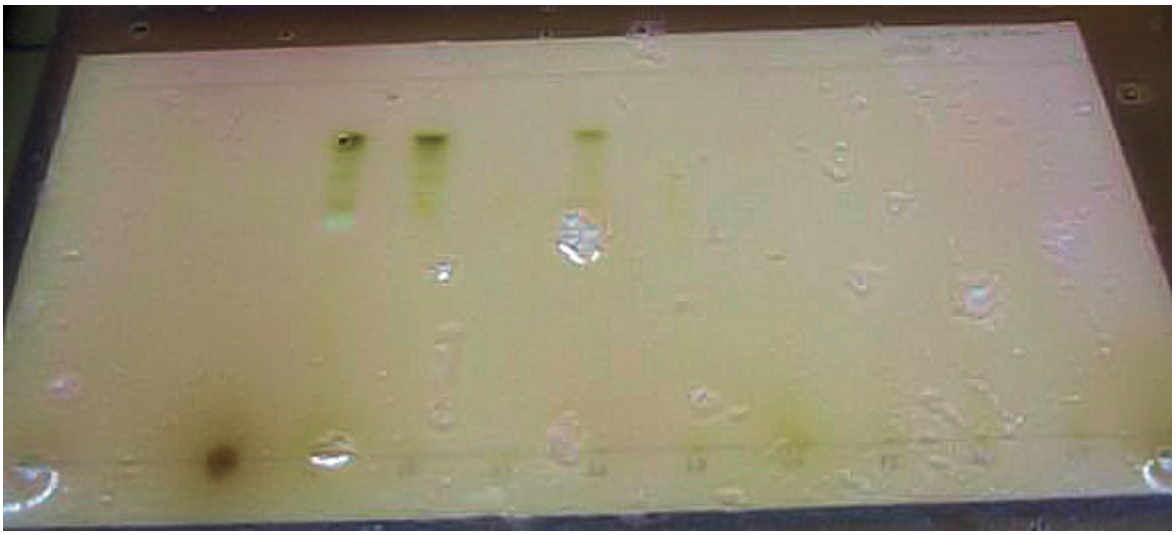
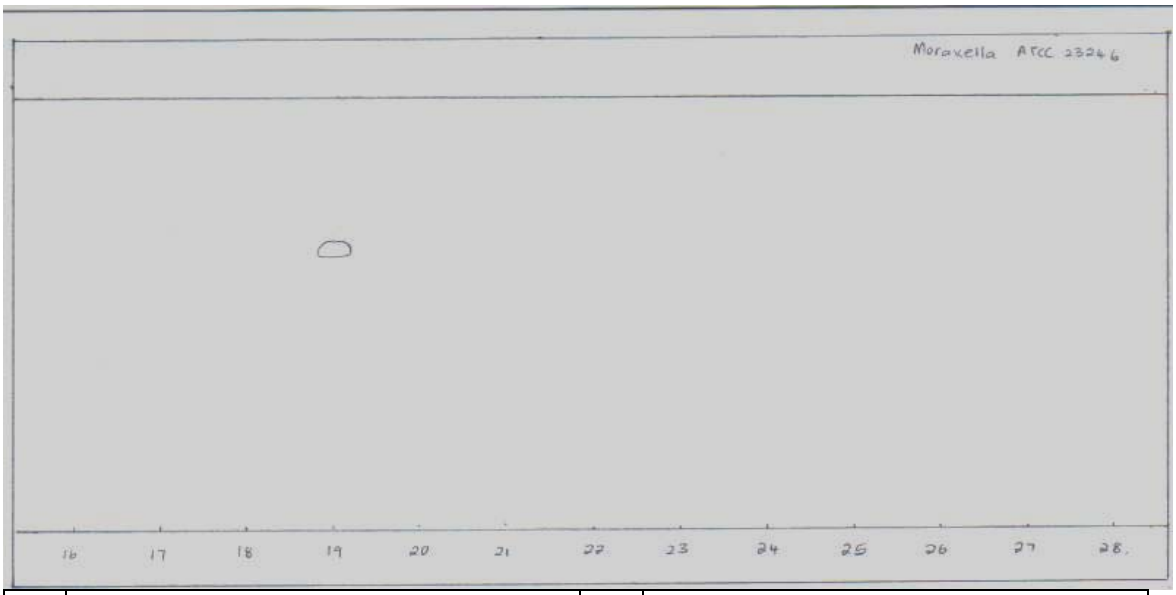


Figure 4.9 Bioautographic assay plate of plant extracts 16 to 28 against *Moraxella catarrhalis* (ATCC 23246).



16.	*Zanthoxylum davyi* (roots)	23.	*Heteromorpha arborescens* (bark)
17.	*Zanthoxylum davyi* (bark)	24.	*Heteromorpha arborescens* (stems)
18.	*Zanthoxylum davyi* (thorns)	25.	*Peucedanum caffrum* (roots A)
19.	*Vitex rehmannii* (leaves)	26.	*Peucedanum caffrum* (roots B)
20.	*Clerodendrum glabrum* (leaves)	27.	*Peucedanum caffrum* (roots C)
21.	*Clerodendrum glabrum* (bark)	28.	*Alepidea amatymbica* (roots)
22.	*Heteromorpha arborescens* (leaves)		

Figure 4.10 Diagrammatic representation of the clear zones of inhibition for the bioautographic assay for extracts 16-28 against *Moraxella catarrhalis* (ATCC 23246).

same area as that seen in the bioautographic assay for *Staphylococcus aureus* (ATCC 12600). As stated above, an R_f value for this compound could not be recorded because no compound was seen in this area of the chromatogram when the TLC plate was viewed under the 366 nm UV light. However, since this clear zone appeared in the bioautographic assays for both *Staphylococcus aureus* and *Moraxella catarrhalis* it is likely that this is an active compound and further investigation to determine the nature of this compound is recommended. Previous research on *Vitex rehmannii* (Nyiligira, 2004a) has resulted in the isolation of a compound *viz.* 12*S*,16*S/R*-dihydroxy-*ent*-labda-7,13-dien-15,16-olide which was noted to have antimicrobial activity. Therefore, the compound having the clear zone of inhibition in this research report should be further investigated so as to confirm whether it is the same compound as isolated by Nyiligira (2004a) or whether it is a different compound.

It is also interesting to note that both *Lantana rugosa* and *Vitex rehmannii* both belong to the Verbenaceae family. Thus it would be interesting to further investigate these two plant species so as to determine whether the active compound, as shown on the bioautographic plates, is the same between these two plant species.

In summary, the bioautographic assay of the plant extracts against *Moraxella catarrhalis* (ATCC 23246) displayed four clear zones of inhibition (Figure 4.7, 4.8, 4.9 and 4.10), thus showing that the plant extracts have notable antimicrobial activity against this Gram-negative pathogen. A similar observation was noted in Chapter 3, Section 3.3.1 where *Moraxella catarrhalis* (ATCC 23246) was recorded to be one of the more sensitive respiratory pathogens against the plant extracts.

This is an unusual observation against a Gram-negative pathogen. In many studies wherein the antimicrobial activity of various plant species is screened against a range of Gram-positive and Gram-negative pathogens (Rabe and van Staden, 1997; Matu and van Staden, 2003; Eldeen *et al*., 2006), poor activity is usually seen against the Gram-negative pathogens. Reasons cited for this phenomenon are that Gram-negative bacteria are in general more resistant than Gram-positive bacteria (Rabe and van Staden, 1997). The cell walls of Gram-negative bacteria have a thick murein layer (Matu and van Staden, 2003) as well as an outer layer composed of lipopolysaccharides (Eldeen *et al.*, 2006) which prevents the entry of antimicrobial agents.

Therefore, the antimicrobial activity displayed in this bioautographic assay, for these plant extracts against *Moraxella catarrhalis* suggests two possibilities. The one is that *Moraxella catarrhalis* may possibly have a weaker mechanism of resistance as compared to other Gram-negative pathogens. The other possibility is that the plant extracts in this study display very promising antimicrobial activity against this pathogen and thus warrants further investigation of these traditional medicinal plants.

4.4 Conclusion

4.4.1 Summary of the thin layer chromatography results

- The TLC analysis of the plant extracts showed good separation of the compounds providing a valuable tool for the qualitative profiling of the chemical constituents of the plant extracts.

- Prominent bands were seen for the leaf and stem extract of *Lantana rugosa* (R_f value of 0.58, turquoise colour), the leaves, stems and flowers of *Leucas martinicensis* (R_f

value of 0.63, yellowish colour) and the leaf extract of *Schkuhria pinnata* (R_f value of 0.21, blue colour).

- All the leaf extracts had a common purple compound suggesting that the leaf extracts amongst different plant species have certain common compounds.

- TLC chromatograms allowed for the identification of common compounds in different plant parts of the same plant species *viz.* the bark and thorn extracts of *Zanthoxylum davyi* (R_f value=0.50, bright blue colour) and the bark and stem extracts of *Heteromorpha arborescens* (R_f value=0.61, bright blue colour).

- The TLC chromatograms also allowed for the noting of common compounds in extracts of plant species belonging to the same plant family *viz. Vitex rehmannii* and *Clerodendrum glabrum* (Verbenaceae) and *Heteromorpha arborescens* and *Peucedanum caffrum* (Apiaceae).

- The TLC analysis of the plant species in this report demonstrated that there are distinct differences in the chemical compositions of the leaf and the woody parts of the plant such as the root, barks, stems and thorns.

- The differences in the chemical compositions of the different plant parts of the same plant species could provide an explanation for their traditional use in combination,

therefore additional studies to verify whether the plant parts actually work synergistically or antagonistically is recommended.

4.4.2 Summary of the bioautographic assay results

- The bioautographic assays proved very beneficial as clear zones of inhibition were recorded for certain of the plant extracts thus demonstrating qualitatively that these extracts do contain active compounds that have antimicrobial activity.

- Clear zones of inhibition were recorded for the following plant extracts:
    - *Lantana rugosa* (leaves and stems) against both *Staphylococcus aureus* and *Moraxella catarrhalis.*
    - *Ziziphus mucronata* (roots) against *Staphylococcus aureus*.
    - Broad inhibition zone across *Zanthoxylum davyi* (thorns) and *Vitex rehmannii* (leaves) against *Staphylococcus aureus.*
    - *Vitex rehmannii* (leaves) against both *Staphylococcus aureus* and *Moraxella catarrhalis.*
    - Broad inhibition zone across *Leucas martinicensis* (leaves, stems and flowers), *Schkuhria pinnata* (leaves), *Chamaecrista mimosoides* (leaves) and *Ziziphus mucronata* (leaves) against *Moraxella catarrhalis.*

- The four clear inhibition zones recorded against *Moraxella catarrhalis* shows that this pathogen is particularly sensitive to the active compounds present in the plant extracts.

- It is recommended that further investigation into the compounds where clear zones of inhibition were recorded, be conducted. This would encompass isolation of the compounds and determining the quantitative antimicrobial activity.

CHAPTER 5

Conclusion

5.1 General conclusions

The main objective of this research study was to document the scientific rationale for the use of African traditional medicine, specifically those indigenous to southern Africa, against respiratory tract pathogens.

5.1.1 OBJECTIVE 1 and 2: Antimicrobial activity of the plant species

Minimum inhibitory concentrations were obtained for the plant extracts against the respiratory pathogens, thus scientifically investigating their traditional uses to treat respiratory infections. The MIC values quantified the antimicrobial activity of the extracts against the respiratory pathogens. The most sensitive pathogens were *Cryptococcus neoformans, Bacillus cereus* and *Moraxella catarrhalis* where the average MIC values that were obtained against these pathogens ranged between 1.44 mg/ml to 2.08 mg/ml.

The plant extracts that displayed the best antimicrobial activity are listed in Table 5.1 wherein the range of the MIC values as well as the average MIC values are summarised. *Terminalia sericea* root extracts obtained the lowest average MIC value (0.69 mg/ml) which is in agreement with the promising antimicrobial activity that this species has shown in many previous studies (Table A27).

Good to moderately good antimicrobial activity (Table 5.1) is shown by the leaf and stem extract of *Chenopodium ambrosioides* and this extract also showed a clear zone of inhibition in the bioautographic assay against *Moraxella catarrhalis*. These findings suggest that this extract does have active antimicrobial compounds.

Table 5.1 Plant extracts that showed the best antimicrobial activity.

Plant extract	Range of MIC values obtained against the respiratory pathogens (mg/ml)	Average MIC value (mg/ml)
Terminalia sericea (roots)	0.13-2.00	0.69
Chenopodium ambrosioides (leaves and stems)	0.25-2.00	1.04
Leucas martinicensis (leaves, stems and flowers)	0.50-2.00	1.10
Zanthoxylum davyi (leaves)	0.25-4.00	1.29
Lantana rugosa (leaves and stems)	0.25-3.00	1.32

The MIC values obtained for the leaves, stems and flowers of *Leucas martinicensis* quantifies the antimicrobial activity of this species as compared to previous studies where the antimicrobial activity has been demonstrated qualitatively via diffusion methods (Table A20).

The leaf extract of *Zanthoxylum davyi* showed the best antimicrobial activity when compared to the roots, bark and thorns. Thus the results obtained in this research study provide evidence as to the efficacy of the aerial parts of *Zanthoxylum davyi* which is beneficial to promote the sustainable harvesting of this plant species.

The MIC values obtained for the studies on the leaf and stem extract of *Lantana rugosa* as well as the zones of inhibition recorded in the bioautographic assays against both *Staphylococcus aureus* and *Moraxella catarrhalis* serves as evidence that this extract possibly has antimicrobial compounds of notable activity.

5.1.2 OBJECTIVE 3: Documentation of the chemical profiles for the plant extracts

The chemical profiles that were documented for the plant extracts comprised of HPLC and TLC chromatograms. The chromatograms provided a chemical fingerprint for the extracts which can be used for future investigations. A study carried out by Foukaridis *et al.* (1994) is a good example of how HPLC chromatograms of traditional medicinal plants were used for identification purposes. The urine or blood samples of patients that were suspected to be poisoned by medicinal plants were analysed using HPLC coupled with UV spectra and then compared to the HPLC chromatograms of the actual plant species so as to confirm whether the poisoning was caused by the plant.

The flavonoids that were tentatively identified in this research report (Appendix A) represent compounds within the extract that have potential antimicrobial activity. The types of flavonoids that were tentatively identified were flavones, flavonols and isoflavones and previous research wherein these flavonoids were isolated from plant species such as *Elaeagnus glabra, Calliandra californica, Erythrina latissima* and *Sophora* and *Euchresta* species, have shown them to possess antimicrobial activity (Mori *et al.*, 1987; Encarnacion *et al.*, 1994; Dastidar *et al.*, 2004; Chacha *et al.*, 2005).

The TLC chromatograms of the plant extracts showed good separation of the chemical compounds thereby providing a basis for the qualitative documentation of the chemical profiles of the extracts. The TLC chromatograms also showed that the leaf extracts amongst different plant species to possess common compounds while the leaf and the woody parts of the plant extracts have different chemical compositions. The differences in the chemical compositions could preliminarily support the traditional uses of the plant parts either alone or

in combination because certain compounds can act together to enhance biological activity or they can act antagonistically and not provide the desired therapeutic effect. Evidence whereby chemical compounds within a plant act synergistically for an improved antimicrobial effect was shown for *Osmitopsis asteriscoides* (Viljoen *et al.*, 2003).

5.2 Possible further investigations

This research study was a broad scale screening of the chosen plant species for their antimicrobial activity and the documentation of their chemical profiles. Therefore, the results obtained in this research study provide a basis for possible future investigations. This could be followed by more in-depth investigations involving the isolation of the active compounds and thereafter determining its actual antimicrobial activity. Based on the plant species traditional uses, additional pharmacological studies, such as anti-inflammatory activity and toxicity studies could also be conducted.

5.2.1 Further studies based on the antimicrobial activity results

The relatively low MIC values and the clear zones of inhibition recorded for the following plant extracts warrants further investigation;

- Leaf and stem extracts of *Lantana rugosa*.
- Leaf and stem extracts of *Chenopodium ambrosioides*.
- Leaves, stems and flowers of *Leucas martinicensis*.

In addition, limited previous studies are available for *Lantana rugosa* and *Leucas martinicensis*, thus further investigations of these plant species could be beneficial in validating their traditional uses.

5.2.2 Further phytochemical studies

The HPLC and TLC chromatograms recorded in this study merely documented the chemical profiles of the plant extracts. Further investigations could encompass in-depth phytochemical analysis involving the identification of the types of major compounds and determining the concentrations at which they are present in the extracts.

Additional analytical studies where HPLC can be coupled with other types of detectors for e.g. fluorescent detectors and refractive index detectors can also be conducted so as to identify other types of chemical compounds apart from flavonoids.

In conclusion, the work carried out in this research report assists in supporting the declaration made by Taylor *et al.* (2001) which states that the "pharmacological screening of plants for medicinal compounds is of vital importance, both to provide a scientific basis for the continued use of the plants, thereby validating their historical utilisation by traditional healers and herbalists, and to provide society with sources of new, effective and safe drugs."

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APPENDIX A

Monographs of plant species investigated

Summary of the monographs

A monograph for each of the investigated plant species is presented in alphabetical order. Each monograph comprises the following sections:

i. Botanical description

ii. Geographical distribution

iii. Traditional medicinal uses for respiratory ailments

iv. HPLC profiles wherein the HPLC chromatograms are presented for each extract together with the type of flavonoid that was tentatively identified for that extract.

v. Literature review which consists of a summary of previous studies found on the antimicrobial activity, chemistry and toxicity of the plant species.

A1. *Acacia sieberiana* DC. var. *woodii* (Burt Davy) Keay & Brenan (Fabaceae)

A1.1 Botanical description

Acacia sieberiana grows to a tree approximately 18 m tall with a large crown that is flat and spreads widely. The bark has a light brown to yellow colour and it is able to peel off into big flat portions. Younger branches are covered by many long yellow hairs. The leaves are bipinnately compound i.e. there are many leaflets arranged side by side and are attached to a main stem which is attached to the branch (Figure A1). The flowers are cream-coloured balls that have a fragrance. The fruit is a creamy-brown pod (Venter and Venter, 2002).

Figure A1 Leaves and young branches of *Acacia sieberiana* (S.v.Vuuren).

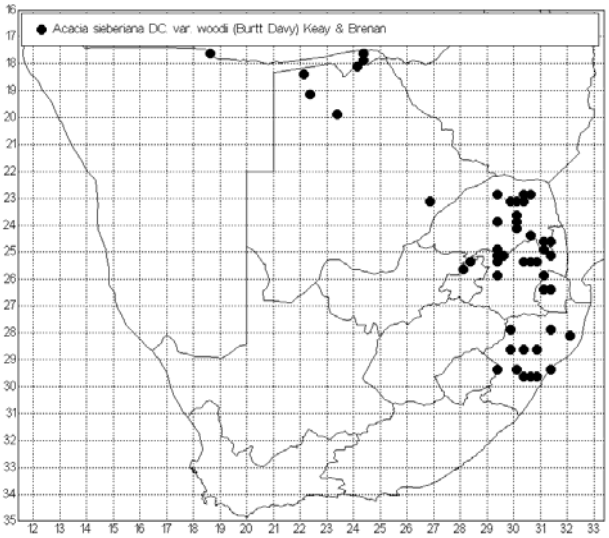

Figure A2 Distribution map of *Acacia sieberiana* (SANBI).

A1.2 Geographical distribution

Acacia sieberiana has a distribution within South Africa in the Northern Province, Gauteng, Mpumalanga, Swaziland and KwaZulu-Natal. It is also found in Botswana and Namibia

(Figure A2). The northern African distribution includes Ethiopia and Sudan (Venter and Venter, 2002).

A1.3 Traditional medicinal uses for respiratory ailments

The leaf, bark and resin are used for their astringent and expectorant actions to relieve the symptoms in upper respiratory tract infections (von Koenen, 2001). Watt and Breyer-Brandwijk (1962) report more specifically that in the Congo the exudates, bark and leaf are used to treat colds by acting as an astringent.

A1.4 HPLC profiles

Figure A3 is the HPLC chromatogram for the leaf extract of *Acacia sieberiana*. Data for the chromatogram comprising retention time, % area and the absorbance maxima of the corresponding UV spectra is given in Table A1. The flavonoids that were tentatively identified include two flavonols and a flavone (Table A1).

A1.5 Literature review

A1.5.1 Antimicrobial activity

A summary of the antimicrobial studies that have been carried out on *Acacia sieberiana* is given in Table A2.

A1.5.2 Chemistry

- A glucoside, 2-β-D-glucopyranosyloxy-2-methylpropanol has been isolated from *Acacia sieberiana* (Brimer *et al.*, 1982).

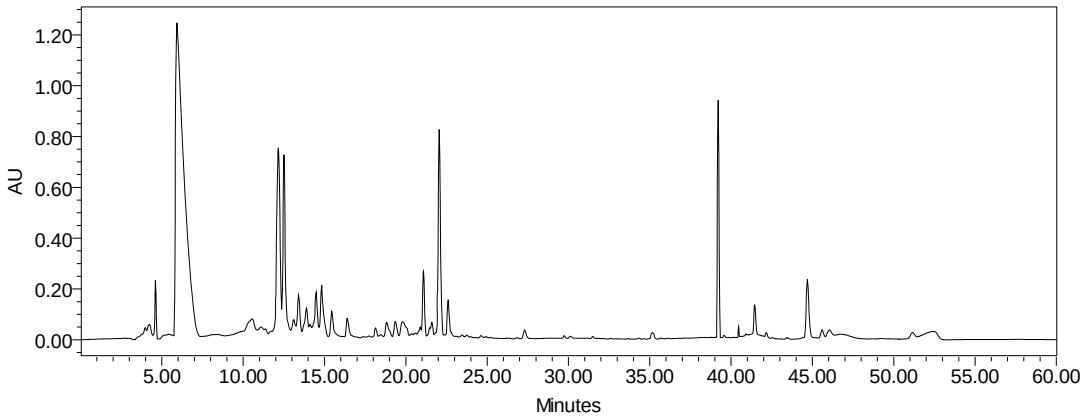

Figure A3 HPLC chromatogram of the leaves of *Acacia sieberiana*.

Table A1 Data for the HPLC chromatogram of *Acacia sieberiana* (leaves).

Retention time	**% Area**	**Absorbance maxima (flavonoid tentatively identified)**
4.611	1.25	267.4
5.927	45.56	265.0
12.165	10.34	278.0
12.511	6.81	206.3
13.102	0.55	266.2; 387.2
13.408	1.47	281.5
13.891	0.89	215.6; 275.6
14.486	1.38	233.2; 285.1; 329.0
14.826	2.24	202.8; 278.0
15.448	0.90	229.7; 327.8
16.398	0.76	203.9; 255.6; 352.8
18.141	0.31	206.3; 278.0
18.825	0.44	201.6; 281.5
19.358	0.68	254.4; 352.8 **(flavonol)**
19.797	1.25	269.7; 343.2
21.085	1.95	201.6; 229.7; 278.0
21.557	0.31	255.6; 345.6
21.602	0.36	254.4; 352.8 **(flavonol)**
22.054	8.02	252.0; 348.0 **(flavone)**
22.601	1.19	250.9; 348.0
27.305	0.40	253.2; 348.0
35.148	0.35	241.4; 326.6; 365.6
39.204	6.71	278.0; 326.6
40.462	0.16	249.7; 274.4; 327.8
41.452	1.23	247.3; 278.0; 325.4
44.692	2.66	268.5; 332.5
46.057	1.85	269.7; 332.5

Table A2 Summary of antimicrobial studies conducted on *Acacia sieberiana*.

Reference	Plant part	Type of extract	Antimicrobial activity	Results
Eldeen and van Staden (2007)	Leaf, bark and roots	Dichloromethane, ethyl acetate and ethanol	Antimycobacterial	The ethyl acetate and ethanol extracts of the leaf and bark obtained an MIC value of 3.12 mg/ml against *Mycobacterium aurum* A+. The dichloromethane, ethyl acetate and ethanol root extracts obtained MIC values of 6.25, 3.12 and 0.78 mg/ml respectively.
Eldeen *et al.* (2005)	Leaf, bark and root	Ethyl acetate, ethanol and aqueous	Antibacterial	MIC values were obtained against *B. subtilis*, *S. aureus*, *E. coli* and *K. pneumoniae*. Ethyl acetate bark extract obtained MIC value of 0.780 mg/ml against all the bacteria tested. Low MIC value of 0.390 mg/ml was obtained against *K. pneumoniae* by the aqueous leaf extract and the ethyl acetate root extract obtained an MIC value of 0.092 mg/ml against *S. aureus*, and *E. coli*.
Rabe and van Staden (1997)	Bark	Water and methanol	Antibacterial	The water and methanol extracts of the bark of *A. sieberiana* displayed zones of inhibition in the agar diffusion method against *S. epidermis* and *B. subtilis*. MIC values of > 2.0, 2.0 and > 2.0 mg/ml were obtained against *S. aureus, S. epidermis* and *B. subtilis* respectively for the methanolic bark extract.

- A cyanogenic glucoside of the chemical name (2*R*)-2-(β-D-glucopyranosyloxy)-3-hydroxy-3-methylbutanenitrile was isolated from the pods of *Acacia sieberiana* (Brimer *et al.*, 1981).
- Another cyanogenic glycoside was isolated from the pods of *Acacia sieberiana viz.* (2*S*)-2-[(6-*O*-α-L-arabinopyranosyl-β-D-glucopyranosyl)oxy]-3-methylbut-3-enenitrile (Nartey *et al.*, 1981).

- Acacipetalin, a cyanogenic glucoside was shown to be present in *Acacia sieberiana* var. *woodii* (Rimington, 1935 in Seigler *et al.*, 1976).
- Dihydro-acacipetalin was isolated from the leaves of *Acacia sieberiana* (Butterfield *et al.*, 1975).

A1.5.3 Toxicity

- In two bacterial mutagenicity tests i.e. the Ames test and the VITOTOX® test the dichloromethane and 90% methanol extracts of the bark of *Acacia sieberiana* displayed no genotoxic effects (Elgorashi *et al.*, 2003).
- No genotoxicity was recorded for the dichloromethane and methanol extracts of the bark of *Acacia sieberiana* in the micronucleus test (used to detect any breakages in chromosome) and the alkaline comet assay (indicates DNA damage occurs) (Taylor *et al.*, 2003).

A2. *Alepidea amatymbica* Eckl. & Zeyh. var. *amatymbica* (Apiaceae)

A2.1 Botanical description

The plant is a perennial herb that typically grows in the form of a rosette with the leaves arranged close to the ground (Figure A4). The leaves are oblong in shape with a characteristically toothed margin. They originate from a single or branched rhizome which is an underground stem. The plant consists of a flowering stalk that is almost two meters in height with numerous small white flowers arranged in dense, rounded heads. There are two forms of *Alepidea amatymbica.* The plant found in the Eastern Cape has leaves which taper towards its base and the leaves of the plant found in the Northern regions are heart-shaped. (van Wyk *et al*., 2000).

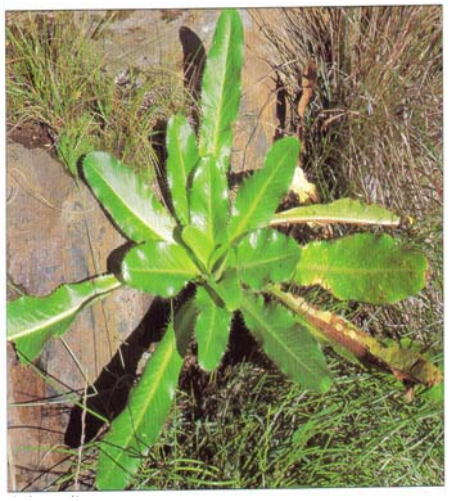

Figure A4 Rosette shaped *Alepidea amatymbica* (van Wyk *et al*., 2000).

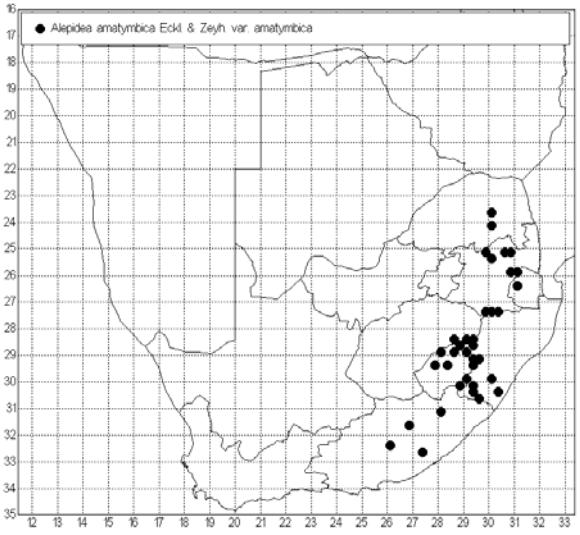

Figure A5 Distribution map of *Alepidea amatymbica* (SANBI).

A2.2 Geographical distribution

Alepidea amatymbica grows typically in grassland areas (van Wyk *et al.*, 2000). The distribution of *Alepidea amatymbica* spans from the Eastern Cape northwards through Lesotho, KwaZulu-Natal, Swaziland, Mpumalanga and the Northern Province (Figure A5).

A2.3 Traditional medicinal uses for respiratory ailments

Traditionally the rhizomes and the roots are used and they are also sold commercially in many parts of South Africa (van Wyk *et al.*, 2000). The Zulu use the roots which they either eat raw or cook for coughs, colds and influenza (Hutchings *et al.*, 1996). Children with coughs and colds are given root infusions. Snuff is made from the powdered root. The smoke of burning roots is also inhaled (Watt and Breyer-Brandwijk, 1962; Hutchings *et al.*, 1996). The Sotho and Xhosa commonly use the rhizomes for respiratory ailments and they also chew the rhizomes for sore throats (Hutchings *et al.*, 1996). Influenza is treated by the Zulus of Gauteng and the Swati by drinking a root decoction (Watt and Breyer-Brandwijk, 1962).

A2.4 HPLC profiles

Figure A6 is the HPLC chromatogram for the root extract of *Alepidea amatymbica* and data for the chromatogram comprising retention time, % area and the absorbance maxima of the corresponding UV spectra is given in Table A3. Four flavones were tentatively identified for the root extract of *Alepidea amatymbica* (Table A3).

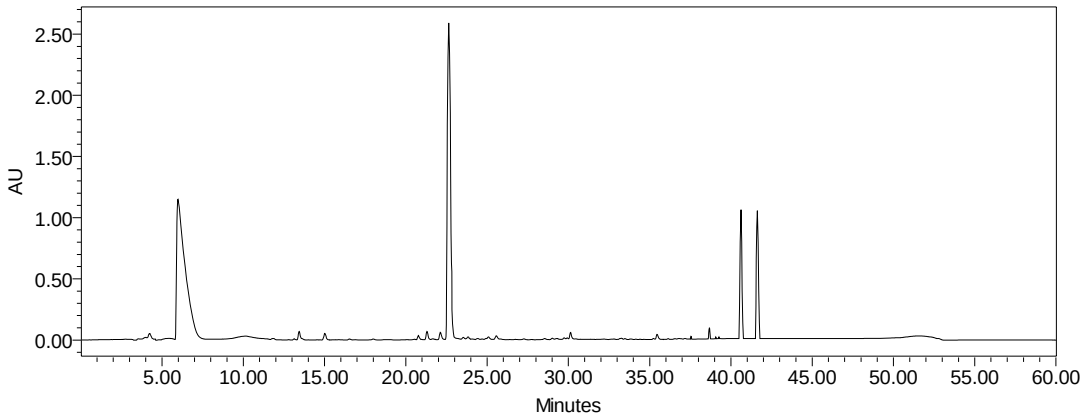

Figure A6 HPLC chromatogram of the roots of *Alepidea amatymbica*.

Table A3 Data for the HPLC chromatogram of *Alepidea amatymbica* (roots).

Retention time	**% Area**	**Absorbance maxima (flavonoid tentatively identified)**
3.549	0.13	278.0; 308.8; 345.6
3.994	0.27	203.9; 274.4; 332.5
4.233	0.59	206.3; 278.0; 295.7
5.987	41.68	265.0
13.130	0.05	234.4; 313.5
13.437	0.52	220.3; 241.4; 325.4
15.015	0.49	239.1; 323.0
20.773	0.24	235.6; 325.4
21.302	0.54	242.6; 327.8 **(flavone)**
22.126	0.53	243.8; 327.8 **(flavone)**
22.640	35.02	208.6; 325.4
23.537	0.12	242.6; 326.6
23.825	0.17	243.8; 325.4
25.085	0.13	245.0; 329.0 **(flavone)**
25.567	0.26	242.6; 327.8
29.007	0.18	248.5; 337.3
29.741	0.09	250.9; 336.1
29.930	0.08	245.0; 318.3
30.134	0.42	242.6; 325.4 **(flavone)**
35.458	0.33	254.4; 398.0
37.544	0.07	308.8
38.674	0.42	308.8
39.071	0.03	308.8
39.268	0.06	308.8
40.616	8.47	308.8
41.622	9.08	308.8

A2.5 Literature review

A2.5.1 Antimicrobial activity

A summary of the antimicrobial studies that have been carried on *Alepidea amatymbica* is given in Table A4.

Table A4 Summary of antimicrobial studies conducted on *Alepidea amatymbica*.

Reference	Plant part	Type of extract	Antimicrobial activity	Results
Mulaudzi *et al.* (2009)	Rhizome	Petroleum ether, dichloromethane, ethanol and water	Antibacterial and antifungal	MIC values were obtained against *B. subtilis, E. coli, K. pneumoniae, S. aureus* and *C. albicans*. The petroleum ether and dichloromethane extract obtained an MIC value of 0.39 mg/ml against *B. subtilis*. The ethanol extract obtained an MIC value of 0.78 mg/ml against *E. coli* and *K. pneumoniae*. The water extract obtained an MIC value of 0.20 mg/ml against *C. albicans.*
Stafford *et al.* (2005)	Leaf	Ethanol	Antibacterial activity of fresh and stored material (90 days, 1 year)	MIC values (1.56 mg/ml) remained the same for fresh and stored material against *B. subtilis*, *S. aureus* and *K. pneumoniae*. MIC value against *E. coli* was 3.13 mg/ml for fresh material and decreased to 1.56 mg/ml upon storage.
	Rhizome	Ethanol	Antibacterial activity of fresh and stored material (90 days, 1 year)	Antibacterial activity against *B. subtilis* improved after storage with MIC values of 3.13 mg/ml for fresh material and 1.56 mg/ml for stored material. No change in MIC values (1.56 mg/ml) for fresh and stored material against *S. aureus, E. coli* and *K. pneumoniae.*
Clarkson *et al.* (2004)	Whole plant	Dichloromethane :methanol (1:1)	Antiplasmodial	An IC_{50} value of 12.5 was recorded against a chloroquine-sensitive strain (D10) of *Plasmodium falciparum*.

A2.5.2 Chemistry

- Phenolic acids, rosmarinic acid and caffeic acid have been isolated from the leaves of *Alepidea amatymbica*. Rosmarinic acid, its glucoside ((*R*)-3'-*O*-β-D-glucopyranosylrosmarinic acid) and caffeic acid has been isolated from the roots (Olivier *et al.*, 2008).
- Several kaurene-type diterpenoids have been isolated from the roots and aerial parts (Rustaiyan and Sadjadi, 1987) and from the lipophilic extracts of the rhizomes (Holzapfel *et al.*, 1995) of *Alepidea amatymbica*.
- The flavonols, quercetin and kaempferol, were identified in the leaves of *Alepidea amatymbica* (Crowden *et al.*, 1969).

A2.5.3 Toxicity

- No mutagenic effects were recorded for the petroleum ether, dichloromethane or ethanol extracts of the rhizome in the Ames test against *Salmonella typhimurium* strain TA98 (Mulaudzi *et al.*, 2009).
- In the brine shrimp test on *Artemia salina*, the hexane extract of the rhizomes of *Alepidea amatymbica* showed low toxicity (LC_{50} = 0.2 ng/ml). A kaurene derivative of *Alepidea amatymbica*, wedelia seco-kaurenolide, also showed low toxicity having an LC_{50} value of 0.5 ng/ml (Somova *et al.*, 2001).

A3. *Chamaecrista mimosoides* (L.) Greene (Fabaceae)

A3.1 Botanical description

Chamaecrista mimosoides is an annual plant which grows to a height of approximately 50 cm (von Koenen, 2001). The leaves are pinnate comprising of numerous rows of leaflets which grow in a feather like pattern (PIER, 2008). The leaflets are attached to the main stem which is pilose i.e. it has long soft straight hairs (von Koenen, 2001). The flowers have yellow petals (Figure A7).

Figure A7 *Chamaecrista mimosoides* plant (PIER, 2008).

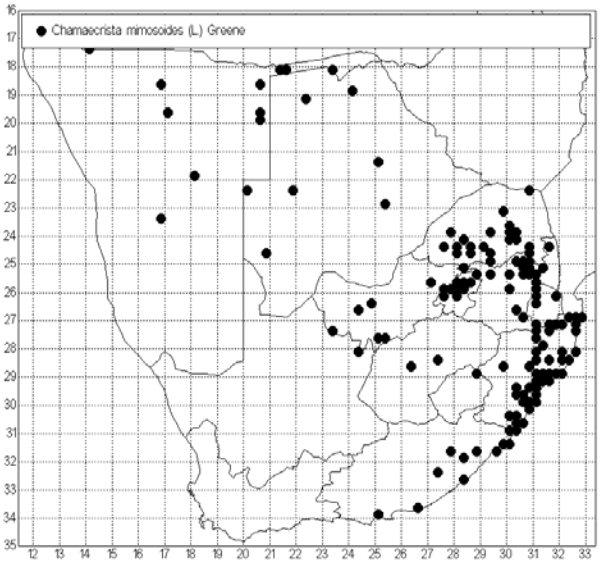

Figure A8 Distribution map of *Chamaecrista mimosoides* (SANBI).

A3.2 Geographical distribution

Chamaecrista mimosoides is found in Namibia, Botswana and Swaziland. Within South Africa it is distributed in the North West, Northern Province, Gauteng, Mpumalanga, Free State, KwaZulu-Natal and Eastern Cape (Figure A8).

A3.3 Traditional medicinal uses for respiratory ailments

In East Africa coughs are treated by using the roots. The roots are also used in this part of Africa to treat various chest ailments as well as pneumonia (Hutchings *et al.*, 1996; von Koenen, 2001).

A3.4 HPLC profiles

The HPLC chromatograms for the leaf extract and the root extract are presented in Figures A9 and A10 respectively. Data for the chromatograms comprising retention time, % area and the absorbance maxima of the corresponding UV spectra is given in Tables A5 and A6. No flavonoids were tentatively identified for this plant species.

A3.5 Literature review

A3.5.1 Antimicrobial activity

No studies were found reporting on the antimicrobial activity of *Chamaecrista mimosoides*.

A3.5.2 Chemistry

- The leaves contain a tannin. From a plant in India, chrysophanol has been isolated (Ganguly *et al.*, 1985 in Hutchings *et al.*,1996).
- The compound responsible for the leaf-opening of *Chamaecrista mimosoides* was isolated and identified as calcium 4-*O*-β-D-glucopyranosyl-(Z)-*p*-coumarate (Ueda *et al.*, 1998).
- The leaves and stems have yielded a yellow anthrquinone pigment. (Mukkerjee *et al.*, 1987 in Hutchings *et al.*,1996).

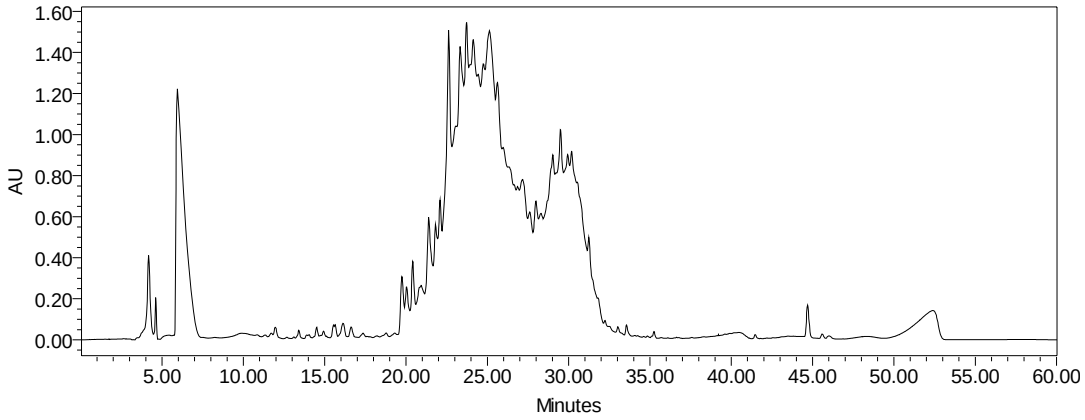

Figure A9 HPLC chromatogram of the leaves of *Chamaecrista mimosoides*.

Table A5 Data for the HPLC chromatogram of *Chamaecrista mimosoides* (leaves).

Retention time	% Area	Absorbance maxima
4.173	3.22	278
4.613	0.82	268.5
5.948	30.82	265.0
14.506	0.53	268.5; 351.6
15.640	0.63	270.9; 320.9
19.748	2.22	254.4; 348.0
20.035	1.53	203.9; 278.0; 343.2
20.413	1.86	201.6; 287.4
20.830	0.95	203.9; 270.9; 349.2
21.394	2.74	205.1; 340.9
21.815	1.02	202.8; 278.0; 349.2
22.091	1.16	202.8; 269.7; 377.6
22.625	8.93	206.3; 269.7; 351.6
23.008	2.92	203.9; 270.9; 350.4
23.329	6.39	206.3; 272.1; 349.2
23.720	5.82	206.3; 279.2; 349.2
24.136	2.51	203.9; 270.9; 350.4
24.751	1.15	203.9; 281.5; 348.0
25.130	8.14	203.9; 273.3; 343.2
25.617	2.60	203.9; 281.5; 344.4
27.986	0.84	281.5; 348.0
28.758	0.32	274.4; 350.4
29.027	3.65	202.8; 281.5; 346.8
29.498	3.47	202.8; 270.9; 348.0
29.943	1.89	202.8; 270.9; 348.0
30.180	1.13	202.8; 270.9; 348.0
31.243	0.64	269.7; 348.0
33.558	0.34	270.9; 348.0
44.691	1.75	268.5

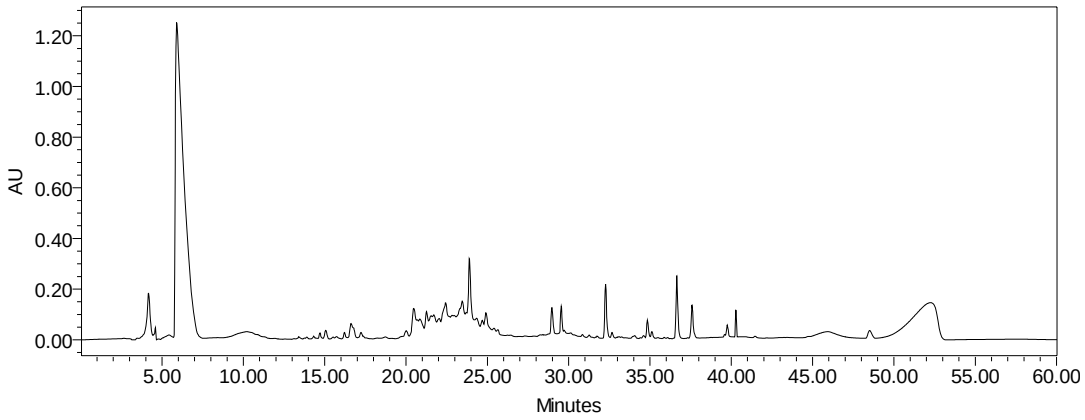

Figure A10 HPLC chromatogram of the roots of *Chamaecrista mimosoides*.

Table A6 Data for the HPLC chromatogram of *Chamaecrista mimosoides* (roots).

Retention time	% Area	Absorbance maxima
4.588	0.39	262.6; 337.3; 363.4
5.902	55.45	265.0
14.708	0.20	207.4; 278.0
15.064	0.41	221.5; 293.4
16.214	0.27	205.1; 281.5
16.623	1.32	205.1; 279.2; 380.0
17.232	0.47	229.7; 272.1; 314.7; 383.6
20.012	0.41	205.1; 278.0
20.477	1.03	202.8; 281.5
21.261	0.69	323.0
21.546	1.05	201.6; 279.2; 323.0
22.039	0.17	229.7; 273.3; 380.0
22.433	1.11	279.2
23.459	1.12	229.7; 327.8
23.898	2.85	216.8; 304.0
24.689	0.26	232.0; 285.1; 323.0
24.914	0.71	232.0; 283.9; 323.0
28.969	1.07	229.7283.9
29.544	0.81	232.0; 307.6
32.271	2.15	229.7; 272.1; 317.1; 394.4
32.667	0.20	252.0; 324.2
34.844	0.66	288.6
35.115	0.24	280.3
36.651	2.38	225.0; 257.9; 287.4
37.586	1.41	225.0; 266.2; 285.1
39.754	0.36	276.8; 361.4
40.284	0.66	273.3; 324.2
52.246	19.31	247.3; 321.8

- Anthracene derivatives are reported to be found in *Chamaecrista mimosoides* and appear to be responsible for its laxative action (Gbile and Adesina, 1987).

A3.5.3 Toxicity

No studies were found reporting on the toxicity of *Chamaecrista mimosoides*.

A4. *Chenopodium ambrosioides* L. (Chenopodiaceae)

A4.1 Botanical description

Chenopodium ambrosioides is an annual or perennial plant. It possesses small "glandular trichomes" i.e. the plant has small outgrowths that produce a liquid substance. The trichomes have an aromatic fragrance. The leaves are elongate to ovate in shape i.e. they are quite long but can be broad and coming to a point at both ends (Figure A11). The aromatic odour of the trichomes would explain the commercial use of the extracts of *Chenopodium ambrosioides* to add fragrances to lotions and creams (Paré *et al.*, 1993).

Figure A11 *Chenopodium ambrosioides* plant (Bromilow, 1995).

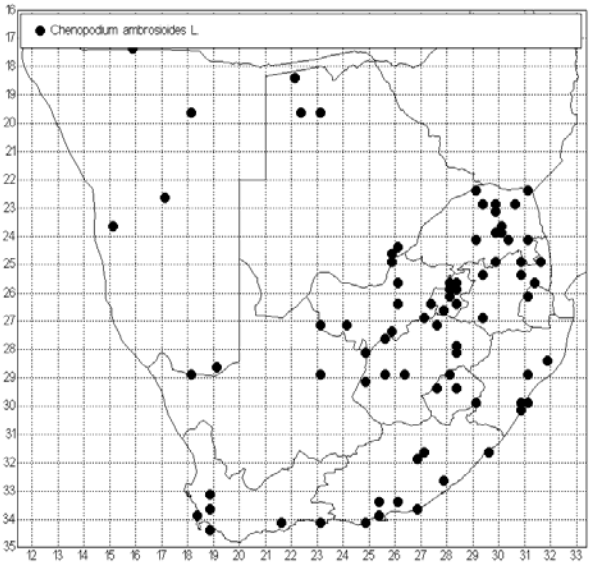

Figure A12 Distribution map of *Chenopodium ambrosioides* (SANBI).

A4.2 Geographical distribution

Chenopodium ambrosioides is originally from Central America, but now it is found in many regions of southern Africa (Hutchings *et al.*, 1996). It is sporadically distributed across all of South Africa's provinces (Figure A12). *Chenopodium ambrosioides* occurs naturally in Gauteng, Free State, KwaZulu-Natal, Eastern, Western and Northern Cape, Swaziland, Lesotho and Botswana (von Koenen, 2001). The various species of the Chenopodiaceae are found plentiful in the tropical and warm regions such as tropical America and Africa (Paré *et al.*, 1993).

A4.3 Traditional medicinal uses for respiratory ailments

Chenopodium ambrosioides is used in Western medicine to treat colds and coughs. The steam from the boiled plant can be inhaled for its therapeutic effect on colds, coughs and body aches (von Koenen, 2001). The Sotho are also recorded to use this plant species for colds where it is said to cause perspiration. The Xhosa use tinctures of the green leaves to suppress coughs (Watt and Breyer-Brandwijk, 1962; Hutchings *et al.*, 1996). It is also used to relieve the symptoms of asthma (Caceres *et al.*, 1991; von Koenen, 2001).

A4.4 HPLC profiles

The HPLC chromatograms for the leaf and stem extract and the root extract are presented in Figures A13 and A14 respectively. Data for the chromatograms comprising retention time, % area and the absorbance maxima of the corresponding UV spectra is given in Tables A7 and A8.

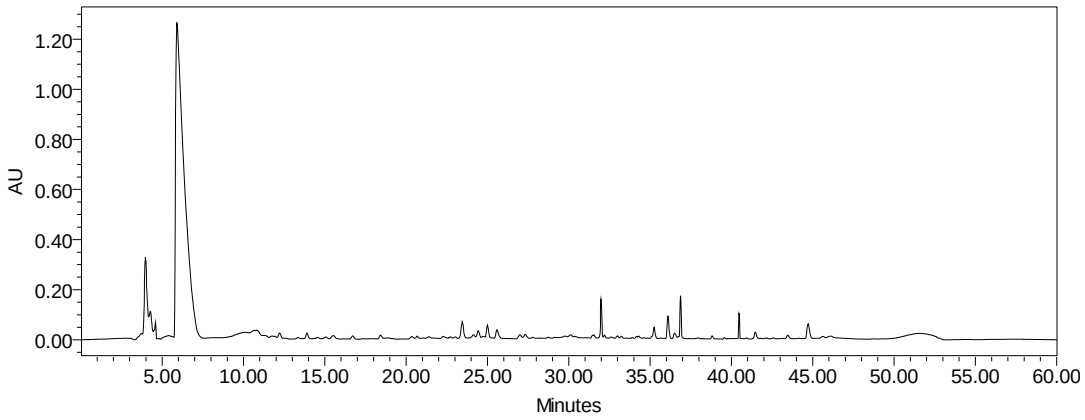

Figure A13 HPLC chromatogram of the leaves and stems of *Chenopodium ambrosioides*.

Table A7 Data for the HPLC chromatogram of *Chenopodium ambrosioides* (leaves and stems).

Retention time	% Area	Absorbance maxima
3.970	6.08	273.3
4.269	2.00	259.1; 325.4
4.584	0.80	259.1; 363.4
5.909	78.90	265.0
12.219	0.33	213.3; 257.9; 374.0
13.902	0.34	255.6; 343.2; 374.0
18.426	0.54	233.2; 308.8; 393.2
23.451	1.19	241.4
24.432	0.40	237.9
24.998	0.73	237.9; 292.2; 318.3
25.586	0.58	236.7; 287.4; 318.3
31.985	1.49	236.7
32.193	0.12	240.3; 308.8
35.247	0.57	237.9; 263.4
36.095	1.23	236.7; 363.4; 293.2
36.508	0.37	278.0; 363.4; 393.2
36.872	1.68	221.5; 273.3; 393.2
38.816	0.16	254.4; 374.0; 399.2
40.473	0.84	249.7; 272.1; 308.8
41.468	0.34	273.3; 325.4
43.462	0.22	274.4; 325.4
44.715	1.10	267.4; 332.5

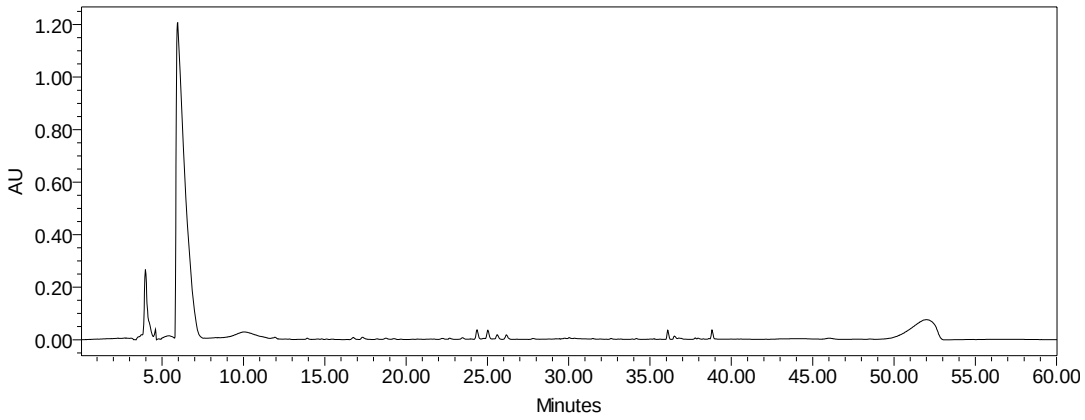

Figure A14 HPLC chromatogram of the roots of *Chenopodium ambrosioides*.

Table A8 Data recorded for the HPLC chromatogram of *Chenopodium ambrosioides* (roots).

Retention time	% Area	Absorbance maxima
3.622	0.19	201.6; 266.2; 338.5
3.976	6.96	201.6; 301.6
4.587	0.45	261.5; 363.4
4.786	0.05	257.9; 301.6; 332.5
5.372	0.84	201.6; 266.2; 278.0
5.954	76.49	265.0
13.925	0.05	254.4; 338.5
16.752	0.16	283.9; 368.0
17.321	0.15	235.6; 292.2; 368.0
18.757	0.18	234.4; 278.0; 308.8
22.685	0.36	242.6; 308.8
23.474	0.12	243.8; 308.8
24.362	0.57	232.0; 289.8
25.028	0.52	240.3;292.2;318.3
25.605	0.27	240.3;288.6;318.3
26.171	0.30	241.4; 314.7
30.029	0.05	243.8; 283.9
36.092	0.44	239.1; 323.0
36.507	0.21	278.0; 368.0
36.785	0.14	275.6; 368.0
37.779	0.06	256.7; 342.0
37.966	0.05	263.8; 324.2; 368.0
38.625	0.02	255.6; 308.8
38.816	0.44	253.2; 308.8; 323.0

A4.5 Literature review

A4.5.1 Antimicrobial activity

A summary of the antimicrobial studies that have been carried out on *Chenopodium ambrosioides* is given in Table A9.

Table A9 Summary of antimicrobial studies conducted on *Chenopodium ambrosioides*.

Reference	Plant part	Type of extract	Antimicrobial activity	Results
Patrício *et al.* (2008)	Leaves	Hydroalcoholic crude extract	Leishmanicidal	The hydroalcoholic extract of the leaves of *C. ambrosioides* were shown to kill *Leishmania amazonensis* when injected into lesions on mice that were infected with this parasite.
Monzote *et al.* (2006)	Aerial parts	Essential oil	Leishmanicidal	*In vitro* and *in vivo* tests on the essential oil of *C. ambrosioides* had a leishmanicidal effect against *Leishmania amazonensis.*
Yasunaka *et al.* (2005)	Ground parts	Methanol	Antibacterial	MIC value of >1024 µg/ml against *E. coli* and 1024 µg/ml against *S. aureus*.
Wang and Huang (2005)	Leaf and stem	95% ethanol extracts	Anti-*Helicobacter pylori*	*C. ambrosioides* showed moderate anti-*H.pylori* activity where the zones of inhibition against eight of the ten strains tested ranged between 8-15 mm in diameter.
MacDonald *et al.* (2004)	Leaves, fruit and flowers	Aqueous infusion	Nematocidal	The aqueous infusion was extracted three times with hexane resulting in a hexane layer which contained ascaridole and an aqueous layer. Close to 90% of the nematocidal activity was recorded for the aqueous layer.
Lall and Meyer (1999)	Aerial parts	Acetone	Antimycobacterial	*C. ambrosioides* obtained an MIC value of 0.5 mg/ml against a drug sensitive strain of *M. tuberculosis* and an MIC value of 0.1 mg/ml against a drug resistant strain.

Table A9 continued.

Reference	Plant part	Type of extract	Antimicrobial activity	Results
Kishore *et al.* (1996)	Leaves	Essential oil	Antidermatophytic	The essential oil of *C. ambrosioides* completely inhibited the growth of *Trichophyton mentagrophytes* and *Microsporum audouinii* which are dermatophytes that cause skin infections. In an *in vivo* study carried out on guinea-pigs that were infected with *T. mentagrophytes* and *M. audouinii*, an ointment made from the oil of *C. ambrosioides* completely cleared the infection after 15 and 13 days for *T. mentagrophytes* and *M. audouinii* respectively.
Desta (1993)	Leaves	Petroleum ether, Dichloromethane, Methanol, Residual aqueous, Direct aqueous.	Antibacterial and antifungal	All the extracts recorded a zone of inhibition against *S. aureus*. In addition, the petroleum ether extract also had an inhibition zone against *P. aeruginosa* and *K. pneumoniae* and the direct aqueous extract obtained zones of inhibition against *E. coli* and *P. aeruginosa.*

A4.5.2 Chemistry

- The ethyl acetate extract of the aerial parts of *Chenopodium ambrosioides* resulted in the isolation of four monoterpene hydroperoxides which also showed *in vitro* trypanocidal activity against *Trypanosoma cruzi* (Kiuchi *et al.*, 2002). The structures of these four compounds were reported as (-)-(2*S*,4*S*)- and (-)-(2*R*,4*S*)-*p*-mentha-1(7),8-dien-2-hydroperoxide and (-)-(1*R*,4*S*)- and (-)-(1*S*,4*S*)-*p*-mentha-2,8-dien-1-hydroperoxide.
- Three monoterpenes were isolated from the leaves of *Chenopodium ambrosioides* which were extracted with *n*-hexane:ethanol:methanol (1:1:1) and there chemical names were

reported as (-)(1*R*,4*S*)-1,4-dihydroxy-*p*-menth-2-ene, (-)(1*R*,2*S*,3*S*,4*S*)-1,2,3,4-tetrahydroxy-*p*-menthane and chenopanone (Ahmed, 2000).

- Limonene and trans-pinocarveol were found as the two major compounds in the essesntial oil of the leaves of *Chenopodium ambrosioides* (Sagrero-Nieves and Bartley, 1995).
- Two flavonol glycosides *viz.* kaempferol 3-rhamnoside-4'-xyloside and kaempferol 3-rhamnoside-7-xyloside were isolated from the fruits of *Chenopodium ambrosioides* (Jain *et al.*, 1990).
- Ascaridole is a major constituent of the essential oil of *Chenopodium ambrosioides* (Johnson and Croteau, 1984; Kliks, 1985).
- In addition to ascaridole, Johnson and Croteau (1984) also showed the that the essential oil of *Chenopodium ambrosioides* consists of α-terpinene, limonene, γ-terpinene, *p*-cymene, hydrocarbons, isoascaridole, *trans*-diol and *cis*-diol.
- Saponins are found to be contained in all the plant parts, particularly the roots (Watt and Breyer-Brandwijk, 1962).
- Fresh leaves contain kaempferol flavonoids (Ariswa *et al.*, 1971 in Hutchings *et al.*, 1996).
- Various other compounds isolated from the plants include quercetin, oxalic, malic and succinic acid, glucose and two triterpenoid glycosides, chenopodoside A and B (Gupta *et al.,* 1971; Bogacheva *et al.,* 1972; Bahrman *et al.,* 1985 in Hutchings *et al.*, 1996).

A4.5.3 Toxicity

- The methanolic extract of the whole plant of *Chenopodium ambrosioides* was inactive in the brine shrimp lethality test and it did not cause any aberrations in rat lymphocyte chromosomes *in vivo* (Sowemimo *et al.,* 2007).

- The essential oil component of *Chenopodium ambrosioides* was shown to have potential genotoxic and cytotoxic effects on human lymphocyte cell cultures as it was shown to cause cellular damage (recorded from chromosomal aberrations and sister chromatid exchanges) and cellular death (recorded from the mitotic index) (Gadano *et al.*, 2006).
- An aqueous extract of *Chenopodium ambrosioides* which was extracted with hexane resulted in a hexane layer and an aqueous layer. The hexane layer contained ascaridole which caused relaxation of gastrointestinal smooth muscle of rats, thus showing that ascaridole, which is contained in the essential oil of *Chenopodium ambrosioides,* could be toxic to mammalian smooth muscle (MacDonald *et al.*, 2004).
- The aqueous extract of *Chenopodium ambrosioides* prepared from the decoction and infusion of the aerial parts, was separated to yield a methylene chloride fraction. The methylene chloride fraction was shown to cause genotoxicity and cytotoxicity (Gadano *et al.*, 2002).

A5. *Clematis oweniae* Harvey (Ranunculaceae)

A5.1 Botanical description

Clematis oweniae is classified as a climber. The leaves have a bipinnate arrangement comprising of numerous leaflets. The flowers are white and occur as panicles i.e. they occur in groups that branch out and each flower has its own stem. The flowers produce seeds that have a very hard coating (von Koenen, 2001).

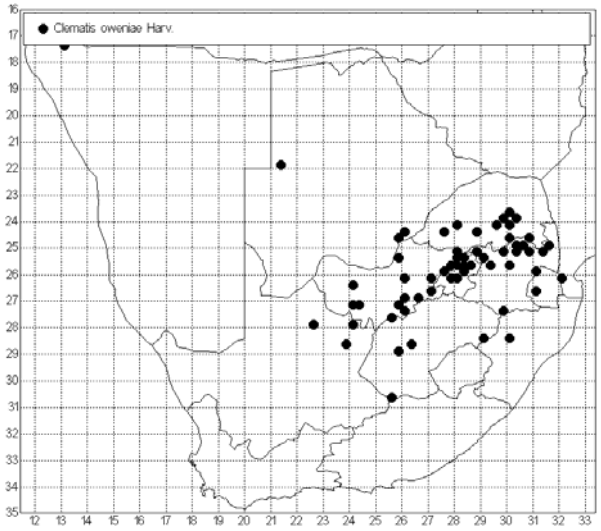

Figure A15 Distribution map of *Clematis oweniae* (SANBI).

A5.2 Geographical distribution

Clematis oweniae is found in the following provinces of South Africa *viz.* North West, Northern Province, Gauteng, and sparsely distributed in Mpumalanga, Free State, KwaZulu-Natal and Northern Cape. It is also found in Namibia, Botswana and Swaziland (Figure A15).

A5.3 Traditional medicinal uses for respiratory ailments

In southern Africa coughs are treated with a root decoction. Finely ground leaves are inhaled to relieve colds and headaches (Watt and Breyer-Brandwijk, 1962; von Koenen, 2001).

A5.4 HPLC profiles

Figure A16 is the HPLC chromatogram of the leaf extract of *Clematis oweniae*. Data for the chromatogram comprising retention time, % area and the absorbance maxima of the corresponding UV spectra is given in Table A10. A flavone and a flavonol were tentatively identified for the leaf extract (Table A10).

A5.5 Literature review

A5.5.1 Antimicrobial activity

No studies were found reporting on the antimicrobial activity of *Clematis oweniae*.

A5.5.2 Chemistry

No studies were found reporting on the chemistry of *Clematis oweniae.*

A5.5.3 Toxicity

No studies were found reporting on the toxicity of *Clematis oweniae.*

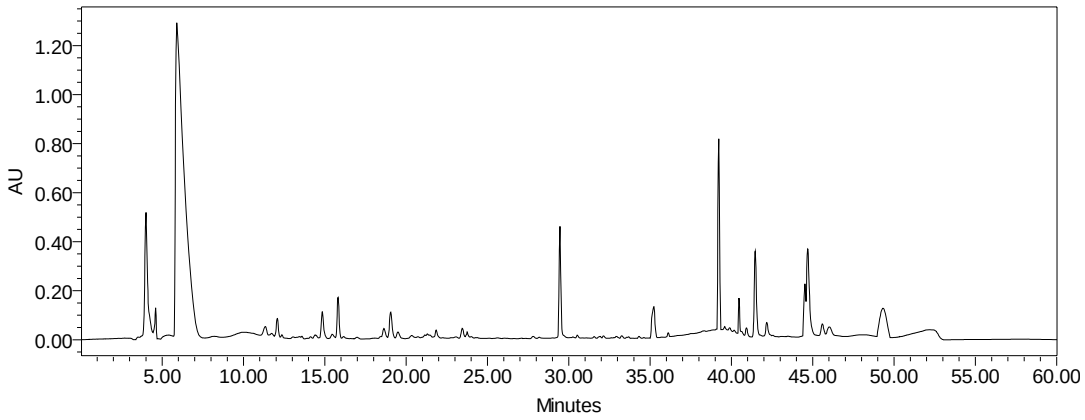

Figure A16 HPLC chromatogram of the leaves of *Clematis oweniae*.

Table A10 Data for the HPLC chromatogram of *Clematis oweniae* (leaves).

Retention time	**% Area**	**Absorbance maxima (flavonoid tentatively identified)**
4.002	7.27	210.9
4.595	0.99	267.4
5.904	54.69	265.0
11.339	0.51	201.6; 260.3; 289.8
12.076	0.75	219.1; 330.1
14.843	1.20	219.1; 323.0
15.811	1.59	210.9;269.7;348.0 **(flavone)**
18.633	0.42	214.5; 269.7; 336.1
19.048	1.32	203.9;255.6;354.0 **(flavonol)**
19.496	0.30	203.9;255.6;354.0
21.845	0.25	223.8; 289.8; 327.8
23.453	0.49	241.4
23.754	0.41	236.7; 301.6
29.453	3.83	214.5; 272.1; 318.3
35.225	1.90	236.7; 326.6
36.113	0.13	241.4; 279.2
39.215	6.58	279.2; 327.8
40.469	0.85	245.0; 272.1
40.924	0.30	273.3
41.463	3.63	243.8; 273.3; 325.4
42.174	0.54	273.3
44.519	1.95	267.4; 332.5
44.691	4.92	267.4
45.589	0.63	267.4; 332.5
46.020	0.63	267.4; 332.5
49.324	3.94	267.4

A6. *Clerodendrum glabrum* E. Meyer var. *glabrum* (Verbenaceae)

A6.1 Botanical description

Clerodendrum glabrum is a shrub which grows to a height of just over a meter. The leaves are attached to the stem with a short stalk and their shape is described as ovate to elliptic i.e. the leaf is long and slightly narrow with the bottom region being broad and tapering to a point at the top (Figure A17). Many flowers appear in clusters and the petals are white. The plant produces a fleshy fruit which has a hard outer covering and a hard seed on the inside (von Koenen, 2001).

Figure A17 Leaves and flowers of *Clerodendrum glabrum* (S.v.Vuuren).

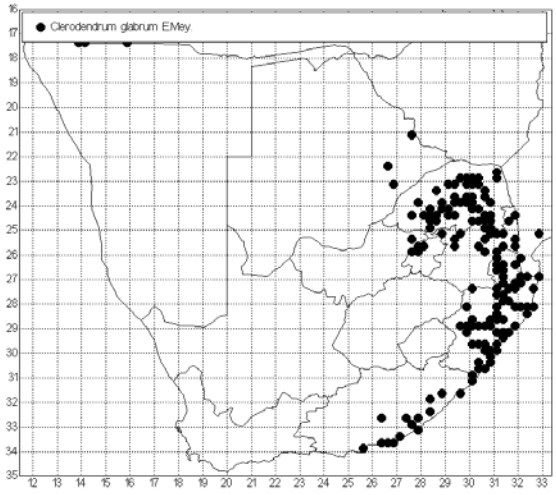

Figure A18 Distribution map of *Clerodendrum glabrum* (SANBI).

A6.2 Geographical distribution

Clerodendrum glabrum is mainly distributed in the eastern regions of southern Africa (Figure A18). It occurs in Namibia, Botswana and Swaziland. Within South Africa it is found in the Northern Province, North West, Gauteng, Mpumalamga, KwaZulu-Natal and Eastern Cape.

A6.3 Traditional medicinal uses for respiratory ailments

The Zulu take leaf infusions for coughs and fevers (Watt and Breyer-Brandwijk, 1962; Hutchings *et al.*, 1996; von Koenen, 2001). Leaf infusions are also taken by the Vhavenda for colds, sore throats and chest complaints (Hutchings *et al.*, 1996). von Koenen (2001) reports that the Zulu make an extract of the root and it is suspected that this extract is used to assist fever and coughs.

A6.4 HPLC profiles

The HPLC chromatograms for the leaf extract and the bark extract are presented in Figures A19 and A20 respectively. Data for the chromatograms comprising retention time, % area and the absorbance maxima of the corresponding UV spectra is given in Tables A11 and A12. A flavone was tentatively identified for the leaf extract of *Clerodendrum glabrum* (Table A11).

A6.5 Literature review

A6.5.1 Antimicrobial activity

The methanol:dichloromethane (1:1) extract of the twigs of *Clerodendrum glabrum* was recorded to have an IC_{50} value of 19 against a chloroquine-sensitive strain (D10) of *Plasmodium falciparum* (Clarkson *et al.*, 2004).

A6.5.2 Chemistry

No studies were found reporting on the chemistry of *Clerodendrum glabrum.*

A6.5.3 Toxicity

No studies were found reporting on the toxicity of *Clerodendrum glabrum.*

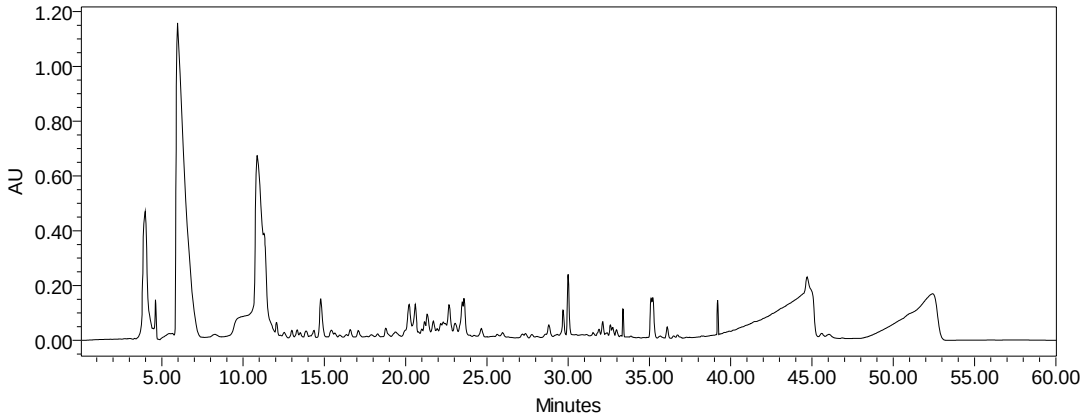

Figure A19 HPLC chromatogram of the leaves of *Clerodendrum glabrum*.

Table A11 Data for the HPLC chromatogram of *Clerodendrum glabrum* (leaves).

Retention time	**% Area**	**Absorbance maxima (flavonoid tentatively identified)**
3.975	10.19	202.8
4.617	1.00	266.2
5.971	46.23	265.0
10.864	20.50	226.2; 270.9
12.058	0.32	234.4; 386.0
14.776	1.80	242.6
16.591	0.25	230.9; 293.4; 325.4
18.781	0.75	229.7; 289.8; 327.8
20.213	1.87	234.4; 327.8
20.594	1.28	229.7; 281.5
21.167	0.25	229.7; 279.2
21.330	0.70	203.9; 229.7; 278.0
21.709	0.42	225.0; 282.7; 326.6
22.165	0.44	232.0; 289.8
22.673	2.11	228.5; 283.9; 325.4
23.047	0.56	233.2; 294.5
23.492	1.37	229.7
23.592	1.12	230.9; 278.0
28.811	0.39	232.0; 289.8
29.690	0.75	267.4; 336.1 **(flavone)**
29.999	1.87	216.8; 273.3; 336.1
32.124	0.37	312.3
32.599	0.57	268.5; 327.8
32.971	0.22	272.1; 329.0
33.375	0.57	270.9; 324.2
35.204	2.50	236.7; 324.2
36.094	0.37	237.9; 282.7; 371.6
39.199	0.60	279.2; 324.2
44.700	0.66	279.2; 329.0

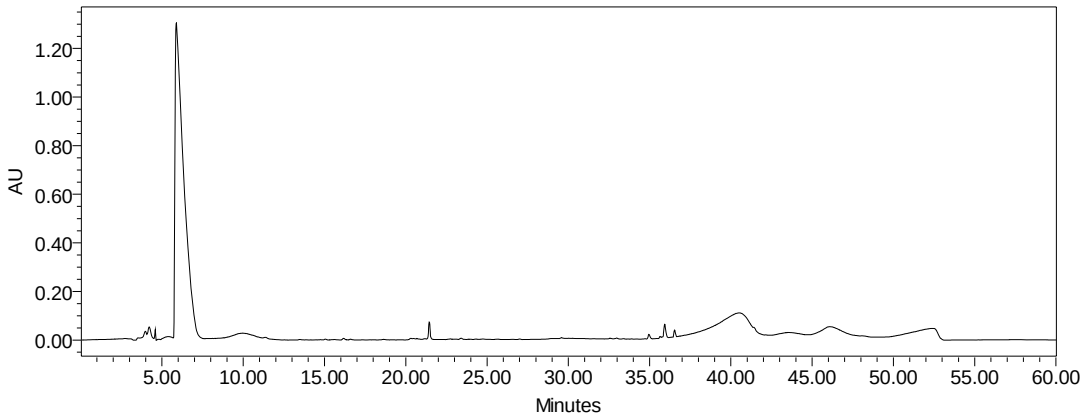

Figure A20 HPLC chromatogram of the bark of *Clerodendrum glabrum*.

Table A12 Data for the HPLC chromatogram of *Clerodendrum glabrum* (bark).

Retention time	% Area	Absorbance maxima
3.542	0.16	203.9; 278.0; 321.8
3.977	0.81	203.9; 268.5; 286.3
4.217	1.22	206.3; 308.8; 344.4
4.587	0.29	278.0; 337.3; 363.4
4.791	0.08	257.9; 301.6; 332.5
5.403	0.76	205.1; 278.0; 321.8
5.890	75.65	265.0
9.974	1.11	206.3; 278.0; 344.4
15.052	0.04	261.5
16.171	0.10	227.4; 268.5
16.602	0.06	265.0; 351.6
20.317	0.10	232.0; 286.3; 325.4
21.447	0.74	203.9; 234.4; 280.3
23.400	0.07	242.6; 278.0; 308.8
29.577	0.19	242.6; 281.5; 326.6
32.575	0.06	278.10
32.978	0.06	278.0; 326.6
34.960	0.25	242.6; 288.6; 365.6
35.647	0.07	253.2; 286.3; 342.0
35.930	0.78	237.9; 287.4
36.538	0.29	250.9; 285.1; 339.7
40.512	13.84	248.5; 274.4; 326.6
46.090	3.28	278.0

A7. *Heteromorpha arborescens* (Spreng.) Cham & Schltdl. var. *abyssinica* (A.Rich) H. Wolff (Apiaceae)

A7.1 Botanical description

Heteromorpha arborescens is a tree that can grow to a height of approximately three meters. The bark has a characteristic appearance wherein thin pieces appear to be peeling off (Figure A21). The leaves appear to have a lanceolate shape where it is long becoming wider towards the base and tapering towards the top end. They are a rich green colour and have one main vein running across the middle of the leaf. The flowers have green to yellow petals and they occur in umbels i.e. they are grouped together and the stems of each flower originate from the same point. The fruits appear to be a capsule having a thick green skin. The fruit (Figure A21) occurs closed and when it is open there is a berry like structure on the inside (von Koenen, 2001).

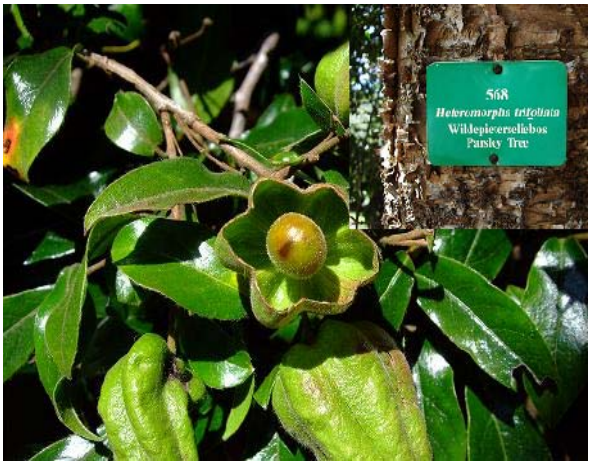

Figure A21 Bark, leaves and fruit of *Heteromorpha arborescens* (S.v.Vuuren).

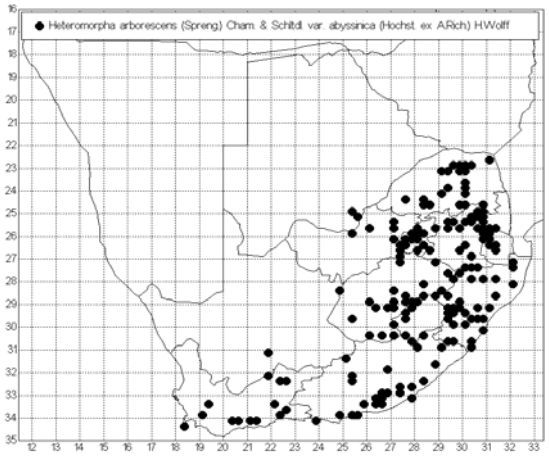

Figure A22 Distribution map of *Heteromorpha arborescens* (SANBI).

A7.2 Geographical distribution

The distribution of *Heteromorpha arborescens* is concentrated to the northern, central and eastern regions of the country with some occurrence in the Western Cape (Figure A22). The provinces in which it is found are North West, Northern Province, Mpumalamga, Gauteng, Free State, KwaZulu-Natal, Eastern Cape, Western Cape and Northern Cape. It also occurs in Swaziland and Lesotho.

A7.3 Traditional medicinal uses for respiratory ailments

The Zulu traditionally use the leaves and the bark to treat scrofula which is a form of tuberculosis that affects the lymph nodes. The Xhosa also use the roots to treat scrofula, as well as for shortness of breath and coughs. In Zimbabwe the roots are used for asthma, coughs and pain in the chest (Hutchings *et al.*, 1996).

A7.4 HPLC profiles

The HPLC chromatograms for the leaf, bark and stem extracts are presented in Figures A23 to A25. Data for the chromatograms comprising retention time, % area and the absorbance maxima of the corresponding UV spectra is given in Tables A13 to A15. A flavone was tentatively identified for the bark extract of *Heteromorpha arborescens* (Table A14).

A7.5 Literature review

A7.5.1 Antimicrobial activity

A summary of the antimicrobial studies that have been carried on *Heteromorpha arborescens* is given in Table A16.

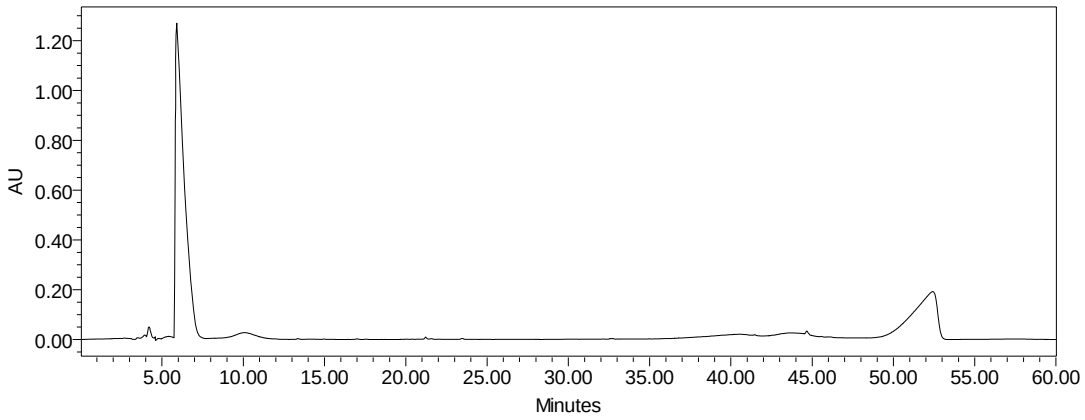

Figure A23 HPLC chromatogram of the leaves of *Heteromorpha arborescens*.

Table A13 Data for the HPLC chromatogram of *Heteromorpha arborescens* (leaves).

Retention time	% Area	Absorbance maxima
2.674	0.02	202.8; 280.3; 372.8
3.496	0.12	205.1; 278.0; 319.4
3.946	0.41	207.4; 332.5; 372.8
4.200	1.10	207.4; 280.3; 308.8
4.563	0.07	278.0; 307.6
4.795	0.14	257.9; 301.6; 332.5
5.416	0.71	207.4; 278.0; 326.0
5.908	64.78	265.0
10.065	2.35	207.4; 278.0; 336.1
13.352	0.03	237.9; 323.0; 393.2
14.949	0.01	237.9; 278.0; 295.7
17.003	0.02	265.0; 337.3
21.218	0.10	242.6; 329.0
21.568	0.03	242.6; 265.0; 329.0
23.463	0.05	245.0; 326.6; 368.0
32.595	0.02	276.8; 329.0; 369.2
32.730	0.02	259.1; 282.7; 337.3
36.793	0.01	276.8; 329.0
38.238	0.09	273.3; 326.6
40.438	0.70	270.9; 326.6
41.455	0.04	273.3; 326.6
42.655	0.03	278.0
43.188	0.27	279.2
43.855	1.29	278.0
44.672	0.51	269.7
52.417	27.07	246.1; 273.3; 325.4

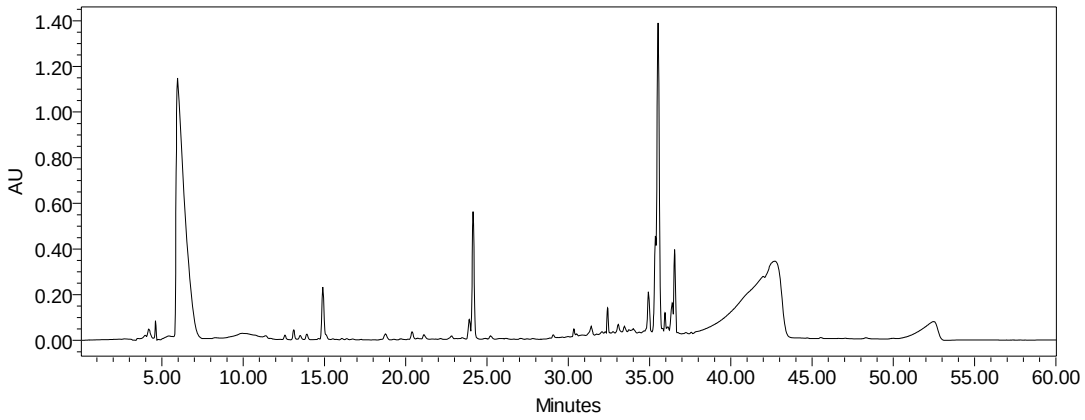

Figure A24 HPLC chromatogram of the bark of *Heteromorpha arborescens*.

Table A14 Data for the HPLC chromatogram of *Heteromorpha arborescens* (bark).

Retention time	**% Area**	**Absorbance maxima (flavonoid tentatively identified)**
4.195	0.47	206.3; 278.0; 308.8
4.610	0.49	278.0; 337.3; 372.8
5.966	48.27	265.0
12.573	0.17	229.7; 266.2; 393.2
13.111	0.40	202.8; 226.2; 333.7
13.507	0.21	229.7; 283.9; 325.4
13.916	0.25	227.4; 291.0; 332.5
14.899	2.67	219.1; 239.1; 324.2
18.752	0.35	232.0; 278.0; 308.8
20.392	0.36	233.2; 325.4
21.118	0.23	257.9; 315.9
23.910	0.91	255.6
24.147	5.76	229.7; 294.5; 343.2
25.218	0.18	257.9; 319.4
29.075	0.29	257.9
30.346	0.19	242.6; 323.0 **(flavone)**
31.409	0.34	253.0; 333.7
32.417	0.75	255.6
33.072	0.41	250.9
33.457	0.25	256.7
34.934	1.78	246.1
35.365	3.57	255.6
35.524	16.02	245.0
35.951	0.45	243.8
36.396	1.28	240.3
36.542	3.01	243.8
42.860	10.94	243.8; 273.3; 325.4

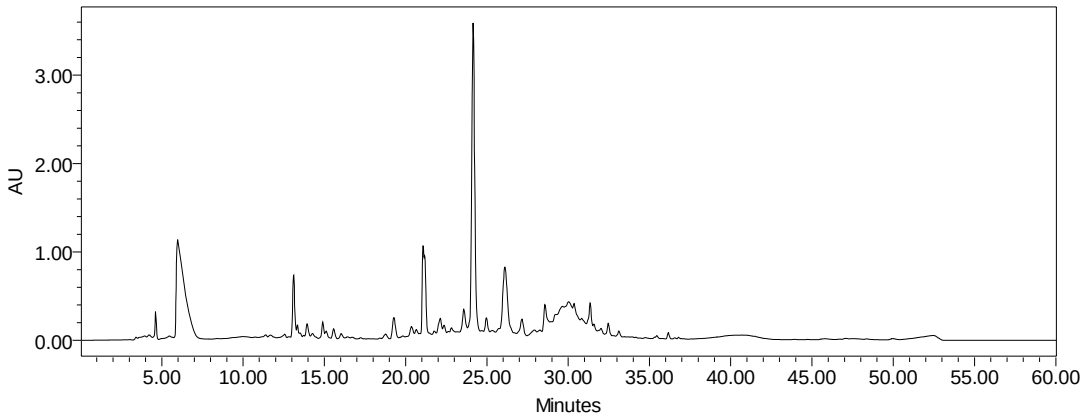

Figure A25 HPLC chromatogram of the stems of *Heteromorpha arborescens*.

Table A15 Data for the HPLC chromatogram of *Heteromorpha arborescens* (stems).

Retention time	% Area	Absorbance maxima
4.620	1.15	none
5.977	24.10	265.0
13.111	3.94	202.8; 225.0; 336.1
13.343	0.38	201.6; 219.1; 325.4
13.940	0.80	255.6
14.902	0.69	219.1; 323.0
15.571	0.70	201.6; 255.6
16.038	0.32	202.8; 226.2; 283.9
19.278	1.96	228.5; 294.5; 343.2
20.365	0.81	229.7; 287.4; 323.0
20.657	0.45	325.4
21.077	8.93	219.1; 243.8; 327.8
22.130	1.31	330.1
22.363	0.50	232.0; 332.5
22.821	0.27	236.7; 336.1
23.582	2.08	230.9; 283.9; 324.2
24.157	28.97	233.2; 293.4; 334.9
24.969	0.96	247.3; 293.4
26.108	8.96	229.7; 281.5; 321.8
27.156	1.25	233.2; 281.5; 338.5
28.573	1.65	232.0; 281.5
29.227	0.53	233.2; 280.3
29.666	2.14	233.2; 280.3
30.037	3.19	233.2; 280.3
30.361	1.11	235.6; 323.0
31.351	1.46	247.3; 288.6
32.464	0.75	250.9
33.119	0.30	250.9
36.163	0.36	236.7

Table A16 Summary of antimicrobial studies conducted on *Heteromorpha arborescens*.

Reference	Plant part	Type of extract	Antimicrobial activity	Results
McGaw *et al.* (2000)	Leaf	Ethanol	Antibacterial	MIC value of 0.78 mg/ml recorded against *B. subtilis*.
		Ethanol and water	Anthelmintic	Anthelmintic activity against the free-living nematode *Caenorhabditis elegans* were shown whereby the percentage of living worms were less than that of the control and the movement of the worms were slowed down when exposed to the extract.
Beuscher *et al.* (1994)	Root bark	Ethanol and dichloromethane	Antiviral	The ethanol extract was active against poliovirus and the dichloromethane extract showed activity against rhinovirus. Antiviral activity was also measured whereby the aqueous ethanol extract was able to cause the induction of interferon (IFN-α and IFN-β).
Desta (1993)	Milky exuate	Dichloromethane, methanol, residual aqueous, direct aqueous	Antibacterial and antifungal	Zones of inhibition were recorded against *S. aureus*, *Salmonella gallinarum*, *E. coli*, *P. aeruginosa* and *C. albicans*.
Heyndrickx *et al.* (1992)	Leaf	Ethanol	Anti-scabies	Anti-scabies activity was recorded when 100% mortality was caused by the leaf extract against the mite *Psoroptes cuniculi*.

A7.5.2 Chemistry

- Petroleum extracts of the leaves have yielded two antifungal compounds *viz.* falcarindiol and sarisan (Villegas *et al.*, 1988 in McGaw *et al.*, 2008b).
- The major compounds of the essential oil are α-pinene, germacrene D and sabinene (Mwangi *et al.*, 1994).
- The methanol extracts of the leaves resulted in the isolation of two saikosaponins which were shown to have anti-inflammatory activity (Recio *et al.*, 1995).

A7.5.3 Toxicity

- In two bacterial mutagenicity tests i.e. the Ames test and the VITOTOX® test the dichloromethane and 90% methanol extracts of the leaves and the twigs/bark of *Heteromorpha arborescens* displayed no genotoxic effects (Elgorashi *et al.*, 2003).
- DNA damage and chromosomal aberrations were recorded for the methanol and dichloromethane extracts of the leaves and the twigs/bark in the micronucleus and alkaline comet assays carried out on human white blood cells (Taylor *et al.*, 2003).

A8. *Lantana rugosa* Thunb. (Verbenaceae)

A8.1 Botanical description

Lantana rugosa grows as shrubs with stems that point upwards. The leaves are dark green and have a wrinkled appearance. The shape of the leaves are ovate i.e. they are broad at the base and pointed at the top, and it is covered with fine hairs on the underside. The flowers are flower spikes i.e. each flower has its own stalk and the flowerhead is attached directly to the stalk. The petals are pale pink to deep violet (Figure A26). The fruit is a berry with two stone pits. (von Koenen, 2001). The fruit is light purple when ripe and is approximately the size of a lentil. It is sweet and juicy having a pleasing flavour (Watt and Breyer-Brandwijk, 1962).

Figure A26 *Lantana rugosa* shrub (S.v.Vuuren).

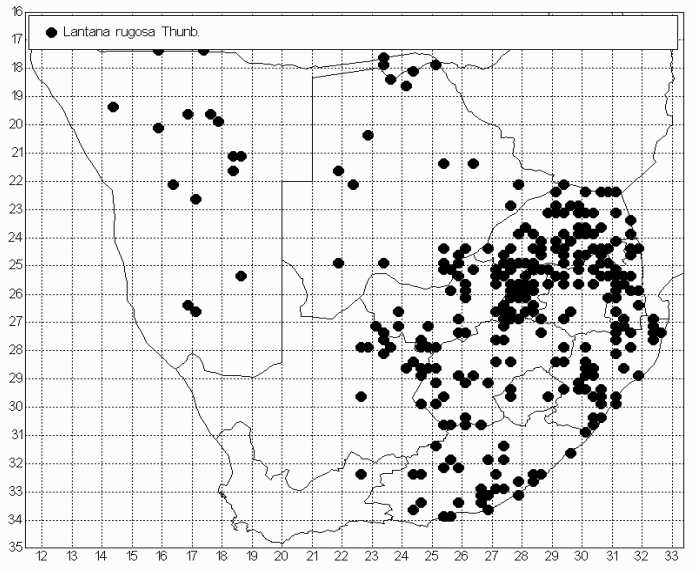

Figure A27 Distribution map of *Lantana rugosa* (SANBI).

A8.2 Geographical distribution

Lantana rugosa is sparsely distributed in Namibia and Botswana (Figure A27). It is readily distributed over Gauteng, Mpumalanga and the Northern Province. There is scattered distribution over North West Province, the Free State, KwaZulu-Natal, Eastern Cape, Swaziland and Lesotho.

A8.3 Traditional medicinal uses for respiratory ailments

Bronchitis is treated by drinking a tea from the stems and leaves and the South African Xhosa also treat bronchitis by using an extract of the leaves (Watt and Breyer-Brandwijk, 1962; von Koenen, 2001). Catarrh, which is an inflammation of the nasal passages, is treated by using the leaf extract as a lavage (von Koenen, 2001). For the treatment of coryza (acute inflammation of the nasal mucous membrane), the Pedi use the leaves as a snuff by crushing them or they make a cold infusion of the leaves (Watt and Breyer-Brandwijk, 1962). Hutchings *et al.* (1996) has also recorded that in various parts of Africa the leaves are taken for coughs and colds.

A8.4 HPLC profiles

Figure A28 is the HPLC chromatogram of the leaf and stem extract of *Lantana rugosa*. Data for the chromatogram comprising retention time, % area and the absorbance maxima of the corresponding UV spectra is given in Table A17.

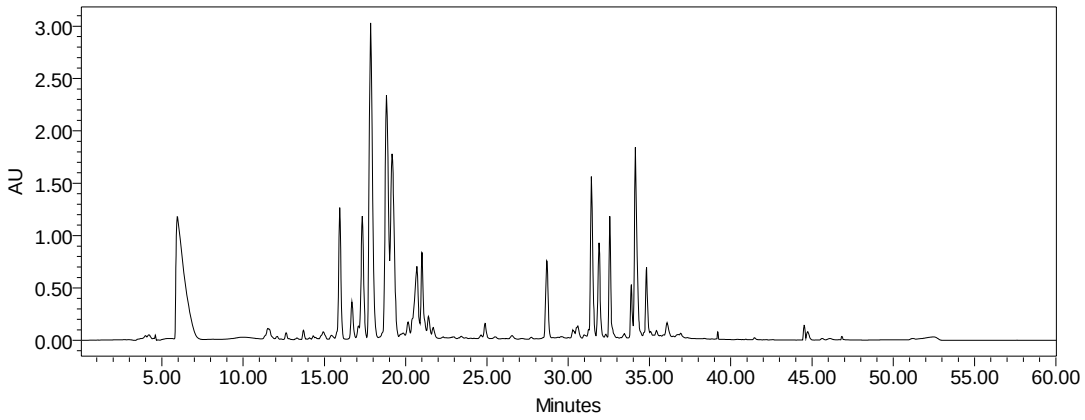

Figure A28 HPLC chromatogram of the leaves and stems of *Lantana rugosa*.

Table A17 Data for the HPLC chromatogram of *Lantana rugosa* (leaves and stems).

Retention time	% Area	Absorbance maxima
5.958	14.62	265
11.522	0.40	278.0
12.647	0.17	236.7; 398.0
13.712	0.24	225.0; 278.0
15.945	4.52	329
16.697	1.30	330.1
17.331	5.28	329.0
17.850	15.45	327.8
18.821	11.84	330.1
19.169	10.04	330.1
20.148	0.47	205.1; 281.5; 327.8
20.689	4.13	327.8
21.006	2.95	327.8
21.404	0.76	233.2; 278.0
21.694	0.35	230.9; 288.6; 327.8
24.886	0.43	219.1; 286.3; 348.0
28.687	2.85	216.8; 285.1; 345.6
30.288	0.30	240.3; 273.3; 343.2
30.587	0.54	241.4; 302.8
31.427	5.56	234.4; 308.8
31.902	3.14	235.6; 308.8
32.560	3.23	216.8; 268.5; 318.3
33.881	1.34	216.8; 278.0; 327.8
34.132	7.02	228.5; 304.0
34.815	1.86	229.7; 308.8
36.083	0.49	247.3

A8.5 Literature review

A8.5.1 Antimicrobial activity

A summary of the antimicrobial studies that have been carried out on *Lantana rugosa* is given in Table A18.

Table A18 Summary of antimicrobial studies conducted on *Lantana rugosa*.

Reference	Plant part	Type of extract	Antimicrobial activity	Results
McGaw and Eloff (2005)	Leaf	Acetone	Antibacterial	MIC values of 1.6 mg/ml, 0.78 mg/ml, 1.6 mg/ml and 0.39 mg/ml obtained against *E. coli*, *E. faecalis*, *P. aeruginosa* and *S. aureus* respectively.
			Anthelmintic	At a concentration of 1 mg/ml, *L. rugosa* had some effect on the growth of the nematode *Caenorhabditis elegans*.

A8.5.2 Chemistry

A volatile oil and lantanin, an alkaloid, are constituents of the leaves (Watt and Breyer-Brandwijk, 1962; Hutchings *et al.,* 1996). Study of the plants in India have resulted in the isolation of a hydrocarbon (Hutchings *et al.,* 1996).

A8.5.3 Toxicity

The cytotoxicity of *Lantana rugosa* was investigated using the brine shrimp lethality test wherein it obtained an LC_{50} value of 0.69 mg/ml (McGaw and Eloff, 2005).

A9. *Leucas martinicensis* (Jacq.) R. Br. (Lamiaceae)

A9.1 Botanical description

Leucas martinicensis is an upright herb reaching a height of about one meter. The leaves are crenate to serrate in shape which narrows towards the base i.e. the margins of the leaves have teeth that are sometimes rounded and blunt or pointed and sharp (Figure A29). The flowers are plentiful and appear to grow from the same point on the stem and point out in different directions. Thus they appear like small round green balls on the stem. The petals of the flowers are light lilac in colour (von Koenen, 2001).

Figure A29 Flowers and leaves of *Leucas martinicensis* (Wursten, 2005).

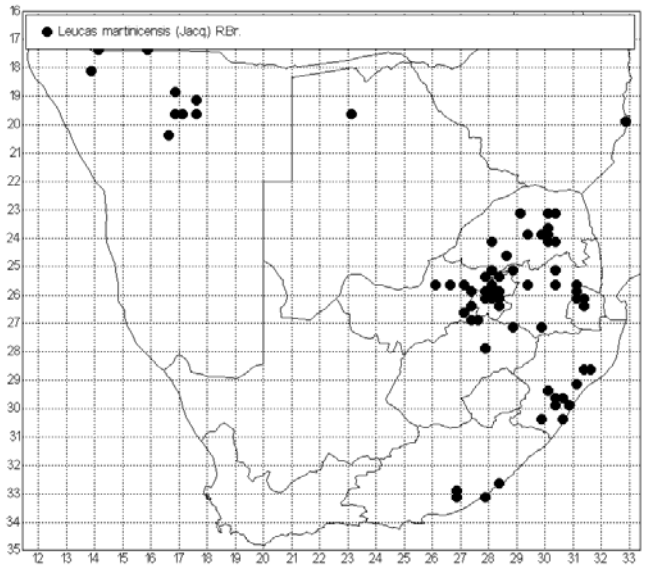

Figure A30 Distribution map of *Leucas martinicensis* (SANBI).

A9.2 Geographical distribution

Leucas martinicensis is found sparsely in Namibia, Botswana, Swaziland and Zimbabwe. Within South Africa it is found in the North West, Northern Province, Mpumalanga, Gauteng, Free State, KwaZulu-Natal and Eastern Cape (Figure A30).

A9.3 Traditional medicinal uses for respiratory ailments

It is reported by von Koenen (2001) and Huthchings *et al.* (1996) that a tea of the leaves is used to treat colds and flu. The steam is also occasionally inhaled for colds (Watt and Breyer-Brandwijk, 1962).

A9.4 HPLC profiles

Figure A31 is the HPLC chromatogram of the leaves, stems and flowers of *Leucas martinicensis*. Data for the chromatogram comprising retention time, % area and the absorbance maxima of the corresponding UV spectra is given in Table A19. No flavonoids were tentatively identified for this plant species.

A9.5 Literature review

A9.5.1 Antimicrobial activity

A summary of the antimicrobial studies that have been carried out on *Leucas martinicensis* is given in Table A20.

A9.5.2 Chemistry

- The essential oil of the leaves and flowers was found to contain 1-hepten-3-ol and germacrene D (Muhayimana *et al.*, 1998).

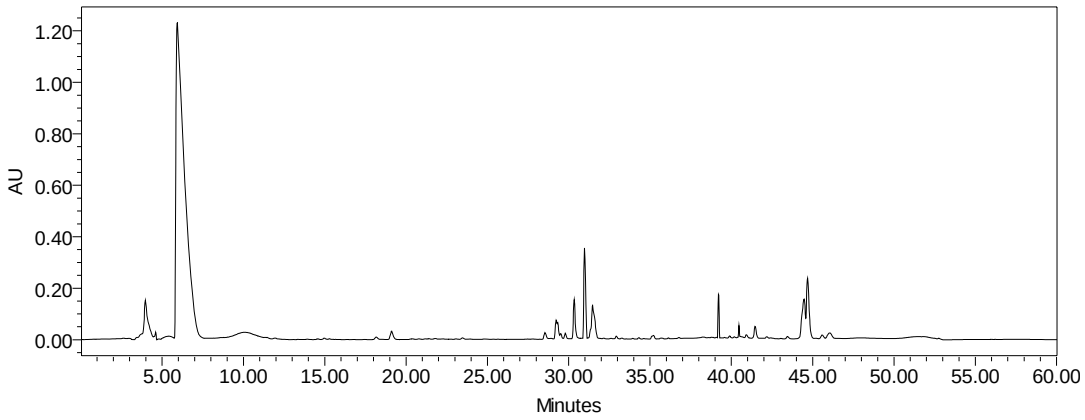

Figure A31 HPLC chromatogram of the leaves, stems and flowers of *Leucas martinicensis*.

Table A19 Data for the HPLC chromatogram of *Leucas martinicensis* (leaves, stems and flowers).

Retention time	% Area	Absorbance maxima
3.522	0.12	202.8; 265.0; 339.7
3.755	0.37	265.0; 332.5; 350.4
3.972	4.22	201.6; 278.0
4.602	0.33	255.6; 337.3
5.937	71.25	265.0
18.164	0.13	242.6; 327.8
19.112	0.57	234.4; 330.1
28.543	0.37	293.4
29.230	1.19	269.7; 318.3
29.355	0.23	230.9; 286.3; 314.7
29.508	0.25	269.7; 318.3
29.791	0.26	285.1
30.336	1.97	216.8; 254.4; 273.3; 345.6
30.977	4.31	247.3; 278.0
31.470	2.91	232.0; 314.7
32.925	0.12	247.3; 308.8
34.311	0.06	253.2; 280.3; 308.8
35.208	0.26	245.0; 326.6; 365.6
39.213	1.23	281.5; 326.6
39.898	0.08	254.4; 280.3
40.471	0.25	249.7; 291.0
40.926	0.15	276.8
41.462	0.64	272.1; 325.4
42.181	0.15	268.5
43.451	0.12	276.8; 325.4

Table A19 continued.

Retention time	% Area	Absorbance maxima
44.469	3.36	293.4; 332.5
44.689	4.24	268.5; 337.3
45.585	0.25	268.5; 332.5
46.053	0.60	268.5; 332.5

Table A20 Summary of antimicrobial studies conducted on *Leucas martinicensis*.

Reference	Plant part	Type of extract	Antimicrobial activity	Results
Clarkson *et al.* (2004)	Whole plant	Dichloromethane: methanol (1:1)	Antiplasmodial	An IC_{50} value of 13.3 was recorded for *L. martinicensis* against a chloroquine-sensitive strain (D10) of *Plasmodium falciparum*.
Vlietinck *et al.* (1995)	Leaf	Aqueous residue	Antibacterial and antifungal	A zone of inhibition was recorded against *P. aeruginosa* in the agar diffusion method and partial inhibition was recorded against fungal pathogen *Microsporum canis.*
Maïkere-Faniyo *et al.* (1989)	Leaf	Methanol	Antibacterial	The methanolic leaf extract of *L. martinicensis* completely inhibited the growth of *Salmonella typhi* and *Shigella dysenteriae*, which are two bacteria that cause diarrhoea, in the agar dilution method.

A9.5.3 Toxicity

No studies were found reporting on the toxicity of *Leucas martinicensis*.

A10. *Peucedanum caffrum* (Meisn.) Phill. (Apiaceae)

A10.1 Botanical description

The species within this genus comprise of perennial herbs or undershrubs with taproots and usually are acaulescent i.e. do not have any stems. (Hutchings *et al.*, 1996). As per examination of samples kept at the herbarium in Pretoria, the collectors of this species all document that the plant has a height of approximately 60-90 cm (Figure A32). The flowers have a light yellow, green colour. The leaves smell of parsley.

Figure A32 *Peucedanum caffrum* (A.R. Magee).

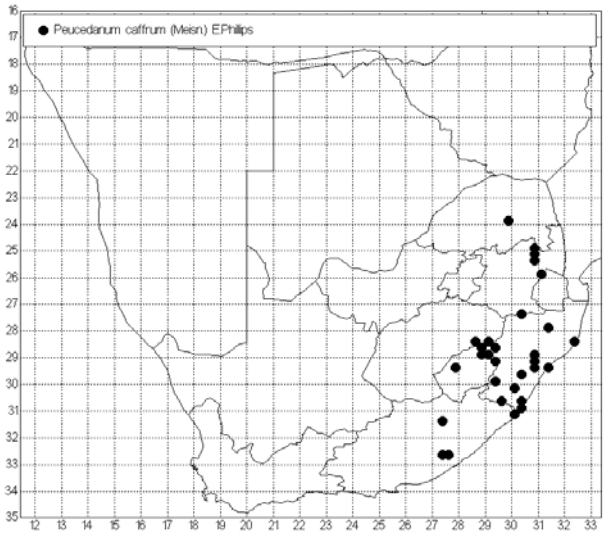

Figure A33 Distribution map of *Peucedanum caffrum* (SANBI).

A10.2 Geographical distribution

There is a very sparse distribution of *Peucedanum caffrum* within South Africa (Figure A33). It is found in KwaZulu-Natal, Lesotho, Free State, some parts of the Eastern Cape, Mpumalamga and Northern Province.

A10.3 Traditional medicinal uses

Peucedanum caffrum is used by the Zulus as enemas to treat chest complaints. (Hutchings *et al*., 1996).

A10.4 HPLC profiles

The HPLC chromatograms for the three root extracts are given in Figures A34 to A36. Data for the chromatograms comprising retention time, % area and the absorbance maxima of the corresponding UV spectra is given in Tables A21 to A23. An isoflavone was tentatively identified for the root extract (location B) of *Peucedanum caffrum* (Table A22).

A10.5 Literature review

A10.5.1 Antimicrobial activity

No studies were found reporting on the antimicrobial activity of *Peucedanum caffrum.*

A10.5.2 Chemistry

No studies were found reporting on the chemistry of *Peucedanum caffrum.*

A10.5.3 Toxicity

No studies were found reporting on the toxicity of *Peucedanum caffrum.*

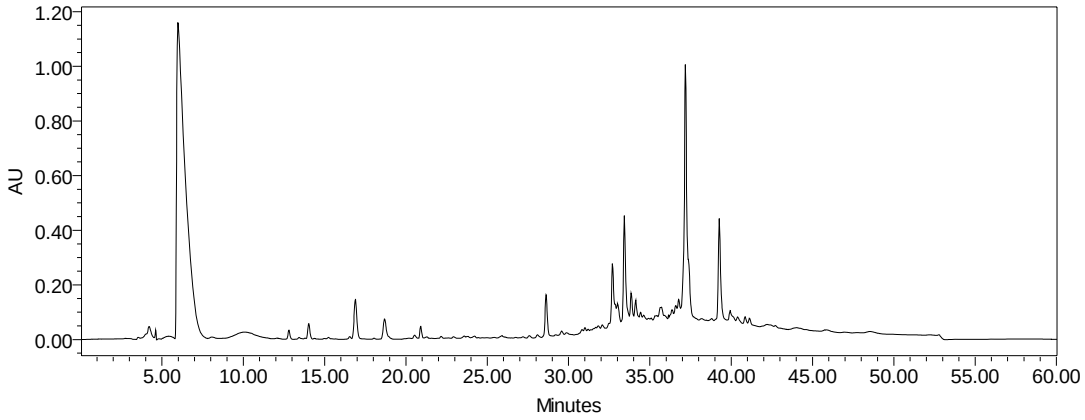

Figure A34 HPLC chromatogram of the roots (location A) of *Peucedanum caffrum*.

Table A21 Data for the HPLC chromatogram of *Peucedanum caffrum* (roots A).

Retention time	% Area	Absorbance maxima
4.210	0.49	207.4; 278.0; 360.4
4.612	0.18	260.3; 337.3
5.989	57.16	265.0
12.804	0.35	229.7; 289.8; 389.6
14.024	0.68	255.6; 323.0; 389.6
16.891	2.26	222.7; 283.9
18.686	1.10	228.5; 308.8
20.907	0.44	261.5
28.618	1.88	237.9; 323.0
32.075	0.14	237.9; 312.3
32.563	0.17	236.7; 312.3
32.702	3.42	232.0; 312.3
33.014	1.01	234.4; 312.3
33.430	5.01	225.0; 315.9
33.849	1.08	234.4; 312.3
34.129	0.73	234.4; 312.3
34.429	0.13	235.6; 312.3
35.326	0.20	236.7; 312.3
35.661	0.63	235.6; 312.3
36.263	0.12	236.7; 312.3
36.366	0.39	235.6; 311.1
36.593	0.62	235.6; 311.1
36.771	0.85	235.6; 313.5
37.188	15.39	228.5; 308.8
39.266	4.78	230.9; 311.1
39.937	0.28	236.7; 309.9
40.857	0.29	312.3
41.123	0.26	312.3

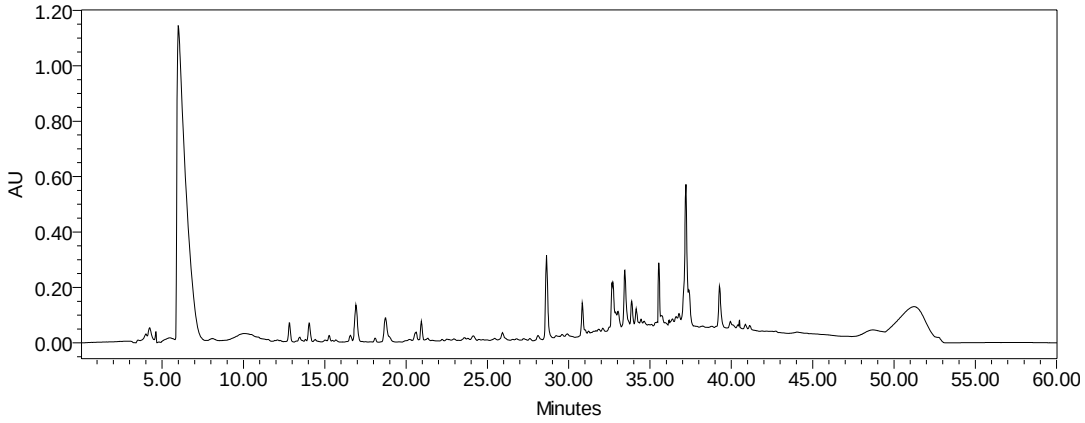

Figure A35 HPLC chromatogram of the roots (location B) of *Peucedanum caffrum*.

Table A22 Data for the HPLC chromatogram of *Peucedanum caffrum* (roots B).

Retention time	**% Area**	**Absorbance maxima (flavonoid tentatively identified)**
4.231	0.62	206.3; 349.2; 369.2
4.614	0.27	260.3; 336.1; 363.4
5.999	60.63	265.0
12.829	0.80	216.8; 289.8; 313.5
14.045	0.85	255.6; 324.2 **(isoflavone)**
15.271	0.31	221.5; 260.3; 292.2
16.575	0.35	236.7; 325.4
16.919	2.28	221.5; 283.9
18.732	1.35	226.2; 308.8
20.615	0.46	220.3; 288.6; 323.0
20.939	0.76	261.5; 390.8
25.928	0.26	240.3; 325.4
28.636	4.15	236.7; 323.0
30.839	1.13	225.0; 248.5; 267.4; 312.3
32.682	3.14	232.0; 267.4; 313.5
33.031	0.81	234.4; 312.3
33.450	3.00	232.0; 313.5
33.872	1.12	234.4; 312.3
34.152	0.88	235.6; 312.3
34.447	0.74	236.7; 312.3
35.544	1.75	236.7; 311.1
36.168	0.11	235.6; 311.1
36.604	0.84	236.7; 311.1
36.783	0.62	236.7; 312.3
37.202	9.58	229.7; 311.1
39.277	2.02	234.4; 311.1
39.943	0.44	309.9
40.499	0.30	311.1
40.864	0.43	312.3

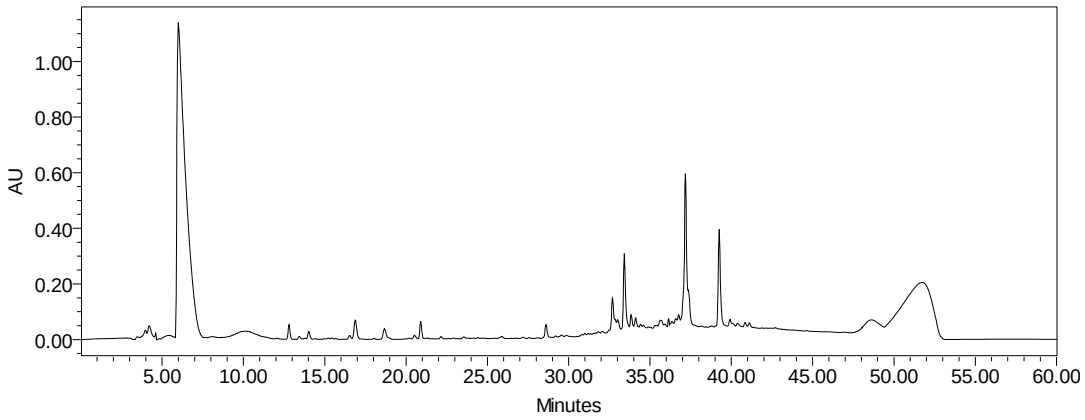

Figure A36 HPLC chromatogram of the roots (location C) of *Peucedanum caffrum*.

Table A23 Data for the HPLC chromatogram of *Peucedanum caffrum* (roots C).

Retention time	% Area	Absorbance maxima
3.975	0.31	278.0; 333.7; 345.6
4.207	0.79	207.4; 278.0; 359.4
4.607	0.08	257.9; 333.7; 363.4
6.000	67.72	265.0
12.805	0.69	229.7; 289.8; 313.5
14.021	0.38	254.4; 325.4
16.881	1.25	222.7; 283.9
18.672	0.65	229.7; 308.8; 388.4
20.900	0.80	261.5; 392.0
28.605	0.68	240.3; 323.0
32.694	2.05	233.2; 312.3
33.011	0.57	237.9; 312.3
33.420	4.13	229.7; 315.9
33.841	0.77	236.7; 312.3
34.120	0.65	236.7; 312.3
34.422	0.57	239.1; 312.3
35.637	0.57	239.1; 312.3
36.144	0.19	235.6; 311.1
36.586	0.22	311.1; 392.0
36.763	0.48	237.9; 312.3
37.179	10.59	229.7; 308.8; 392.0
39.254	5.17	230.9; 311.1; 392.0
39.922	0.24	309.9
40.845	0.22	312.3
41.110	0.21	312.3; 399.2

A11. *Schkuhria pinnata* (Lam.) Kuntze ex Thell. (Asteraceae)

A11.1 Botanical description

Schkuhria pinnata is an annual herb. The leaves are pinnate in shape and occur in an alternate arrangement. The ends of the stems have small yellow flowers (Figure A37) which are described as "ray florets" (von Koenen, 2001).

Figure A37 Leaves and flower heads of *Schkuhria pinnata* (S.v.Vuuren).

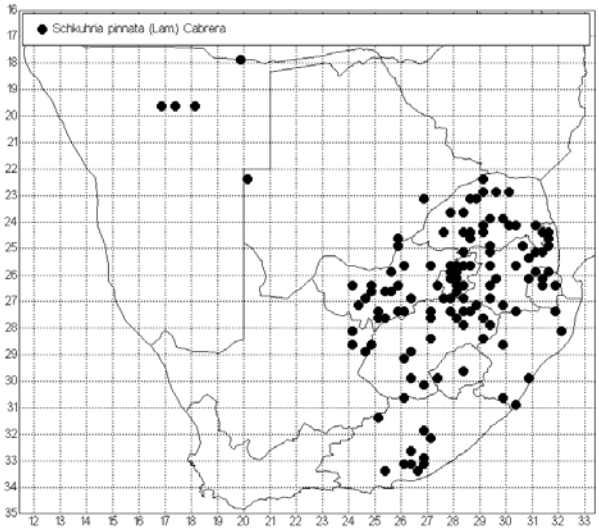

Figure A38 Distribution map of *Schkuhria pinnata* (SANBI).

A11.2 Geographical distribution

Schkuhria pinnata is found in Namibia, Botswana, Swaziland and Lesotho. Within South Africa it is widely distributed in the northern, central and eastern regions of the country. The provinces in which it can be found are North West, Northern Province, Mpumalanga, Gauteng, Free State, KwaZulu-Natal and the Eastern Cape (Figure A38).

A11.3 Traditional medicinal uses for respiratory ailments

The leaves are crushed finely and soaked in water and this is taken for upper and lower respiratory tract infections and influenza (Watt and Breyer-Brandwijk, 1962; von Koenen, 2001).

A11.4 HPLC profiles

Figure A39 is the HPLC chromatogram for the leaf extract of *Schkuhria pinnata*. Data for the chromatogram comprising retention time, % area and the absorbance maxima of the corresponding UV spectra is given in Table A24. No flavonoids were tentatively identified for this plant species.

A11.5 Literature review

A11.5.1 Antimicrobial activity

A summary of the antimicrobial studies that have been carried out on *Schkuhria pinnata* is given in Table A25.

A11.5.2 Chemistry

- A sesquiterpene lactone, eucannabinolide, was isolated from the stems of *Schkuhria pinnata* and was shown to have *in vitro* anticancer activity (Fouche *et al.*, 2008).
- Pacciaroni *et al.* (1995) isolated five new heliangolides and other sesquiterpene lactones that are common to the *Schkuhria* genus from the aerial parts of *Schkuhria pinnata* var. *abrotanoides*. The sesquiterpene lactones that were isolated were pectolinarigenin, the heliangolide eucannabinolide, schkuhrin II, chromolaenolide, santhemoidin A, diacetate heliangolide and schkuhripinnatolide.

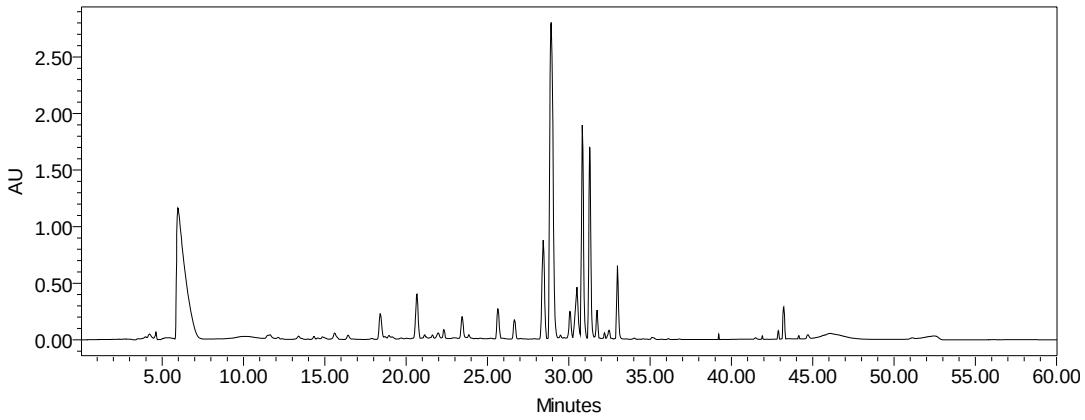

Figure A39 HPLC chromatogram of the leaves of *Schkuhria pinnata*.

Table A24 Data for the HPLC chromatogram of *Schkuhria pinnata* (leaves).

Retention time	% Area	Absorbance maxima
4.616	0.20	262.6; 343.2
5.973	25.21	265.0
15.606	0.39	234.4
18.413	1.42	207.4; 257.9; 351.6
20.662	2.52	None
22.322	0.33	None
23.442	1.11	325.4
25.643	1.58	325.4
26.660	1.00	None
28.430	5.89	None
28.916	25.14	222.7
30.076	1.29	273.3; 336.1
30.500	3.43	342.0
30.841	10.28	210.9
31.293	8.83	210.9
31.737	1.12	None
32.197	0.17	237.9; 308.8
32.477	0.39	233.2
32.996	3.07	216.8; 275.6; 330.1
39.221	0.08	265.0; 325.4; 369.2
41.901	0.08	245.0; 270.9; 326.6
42.885	0.25	268.5
43.215	1.32	234.4; 268.5; 308.8
44.145	0.08	268.5
46.066	4.81	265.0

Table A25 Summary of antimicrobial studies conducted on *Schkuhria pinnata*.

Reference	Plant part	Type of extract	Antimicrobial activity	Results
Muthaura *et al.* (2007)	Whole plant	Water and methanol	Antimalarial	The methanol extract showed high activity against the chloroquine susceptible strain of *Plasmodium falciparum* having an IC_{50} value of 1.30 µg/ml. The water extract had higher chemosuppression of the parasite infection (64.22%) than the methanol extract in an *in vivo* test where mice were infected with *Plasmodium berghei.*
Luseba *et al.* (2007)	Shoots	Dichloromethane and 90% methanol	Antibacterial	The dichloromethane extracts of the shoots of *S. pinnata* obtained MIC values of 0.31, 0.63 and 1.25 mg/ml against *S. aureus* (ATCC 29213), *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853) respectively. The 90% methanol extract obtained MIC values of 0.31, 0.31 and 1.25 mg/ml respectively against the same pathogens.
Muñoz *et al.* (2000)	Whole plant	Ethanol	Antimalarial	*In vivo* tests showed that the extract had moderate activity against *Plasmodium petteri* where the percentage inhibition of the parasite was 62% at a dose of 1000 mg/kg of extract that was administered to the infected mice.
Abdel-Malek *et al.* (1996)	Leaves, flowers, stems	Aqueous, organic and alcoholic	Anti-HIV activity	The therapeutic index (TI) was calculated as the ratio of the concentration of the extract required to protect 50% of the T-lymphoblastoid cells from the effects of the HIV virus *vs.* the concentration of the extract that is toxic to the viability of the cells. The aqueous concentration of the leaves, flowers and stems of *S. pinnata* obtained a TI of 8.

- Eight sesquiterpene lactones were isolated from the aerial parts of *Schkuhria pinnata* by Ganzer and Jakupovic (1990) and they comprised of two germacranolides, three

heliangolide derivatives named schkuhripinnatolides A C, two melampolides and a guaianolide.

- Bohlmann and Zdero (1981) isolated the diacetate heliangolide from the aerial parts of *Schkuhria pinnata*.

A11.5.3 Toxicity

- The cytotoxicity on Vero E6 cells was low whereby methanol and water extracts of *Schkuhria pinnata* obtained CC_{50} values (the concentration of plant extract that causes 50% of cell lysis and death) of 161.5 µg/ml and 22.51 µg/ml respectively. No toxic effects were observed in mice that were given an oral dose of the plant extract (Muthaura *et al.*, 2007).
- No mutagenic effects were recorded for the dichloromethane and 90% methanol extracts of *Schkuhria pinnata* in the bacterial Ames test (Luseba *et al.*, 2007) using *Salmonella typhimurium* (strain TA98).

A12. *Terminalia sericea* Burchell ex DC. (Combretaceae)

A12.1 Botanical description

Terminalia sericea grows to be a small to medium sized tree having an upright trunk and the branches spreading out widely (Figure A40). The colour of the bark is grey to pale brown and it has a rough cracked surface (van Wyk *et al.*, 2000). The leaves have an obovate to lanceolate shape i.e. they are longish getting broader towards the apex (von Koenen, 2001). They have characteristic silver-hairs and they are grouped together at the tips of the branches. The leaves either have short stalks or are sessile i.e. directly attached to the branch (van Wyk *et al.*, 2000; von Koenen, 2001). The flowers are cream coloured and have a bad smell. The fruit is comprised of thin membranes (wings) that enclose a thick seed like structure. (van Wyk *et al.*, 2000).

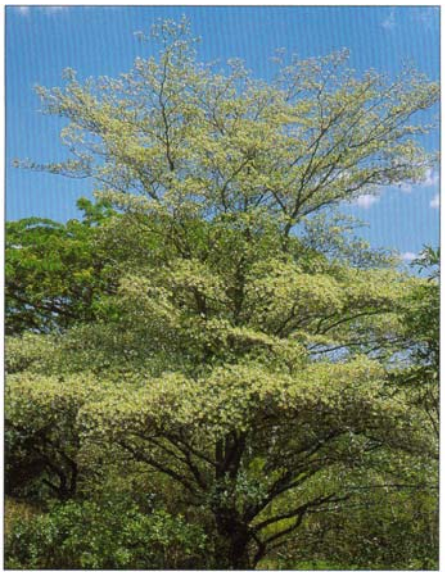

Figure A40 *Terminalia sericea* tree (van Wyk *et al.*, 2000).

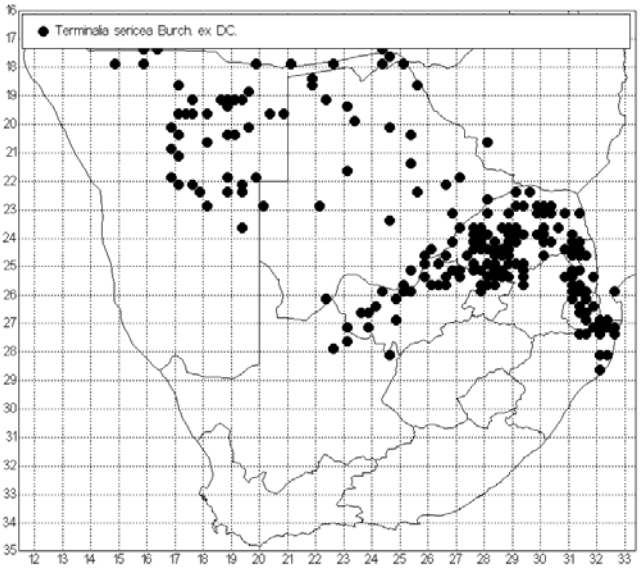

Figure A41 Distribution map of *Terminalia sericea* (SANBI).

A12.2 Geographical distribution

In South Africa, *Terminalia sericea* is usually found in the sandy savanna areas in the northern parts of South Africa (van Wyk *et al.*, 2000). The distribution of *Terminalia sericea* spans across the North West Province, Northern Province, parts of Mpumalanga, Swaziland and KwaZulu-Natal (Figure A41). Distribution within the rest of Africa includes Namibia, Botswana, Zimbabwe, Angola, Mozambique, Zambia, Malawi and Tanzania (Neuwinger, 1994).

A12.3 Traditional medicinal uses for respiratory ailments

The outer layers of the roots are prepared as hot infusions to treat pneumonia (Hutchings *et al.*, 1996; van Wyk *et al.*, 2000; von Koenen, 2001). In Ovamboland in Namibia, the roots are used to treat coughs (von Koenen, 2001). In Zimbabwe and Botswana the roots are used for sore throats (Hutchings *et al.*, 1996).

A12.4 HPLC profiles

Figure A42 is the HPLC chromatogram of the root extract of *Terminalia sericea*. Data for the chromatogram comprising retention time, % area and the absorbance maxima of the corresponding UV spectra is given in Table A26. No flavonoids were tentatively identified for this plant species.

A12.5 Literature review

A12.5.1 Antimicrobial activity

A summary of the antimicrobial studies that have been carried out on *Terminalia sericea* is given in Table A27.

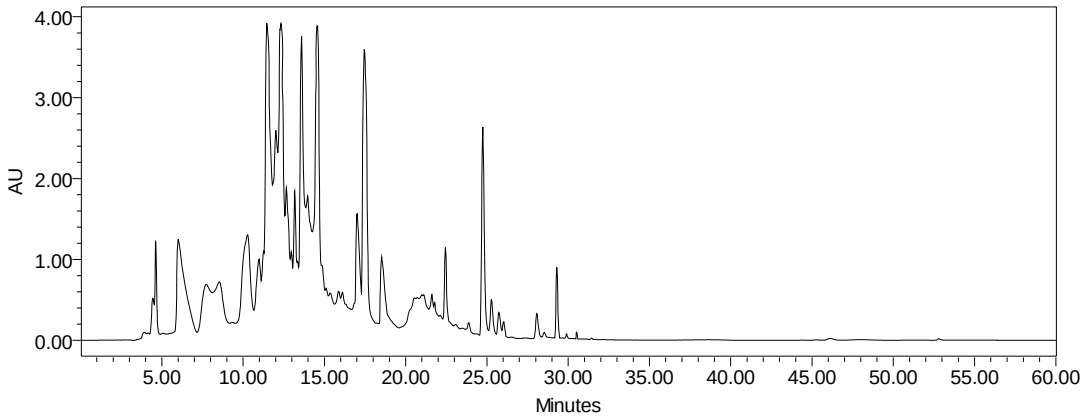

Figure A42 HPLC chromatogram of the roots of *Terminalia sericea*.

Table A26 Data for the HPLC chromatogram of *Terminalia sericea* (roots).

Retention time	% Area	Absorbance maxima
4.623	1.19	377.6
6.012	5.44	262.6; 380.0
7.729	2.91	212.1; 257.9; 377.6
8.535	2.78	212.1; 257.9; 377.6
10.275	4.68	212.1; 259.1; 377.6
10.979	1.82	205.1; 256.7; 378.8
11.457	11.95	239.1; 255.6; 365.6
12.016	5.50	219.1; 257.9; 377.6
12.326	9.53	255.6; 289.8; 360.4
12.663	3.31	216.8; 256.7; 377.6
12.962	0.87	212.1; 259.1; 377.6
13.177	2.69	216.8; 365.6
13.592	7.28	250.9; 378.8
13.966	3.79	215.6; 256.7; 380.0
14.573	9.79	239.1; 253.2; 364.4
15.104	0.36	206.3; 256.7; 374.0
15.353	0.35	205.1; 257.9; 372.8
15.874	0.31	202.8; 255.6; 19.377.6
16.109	0.22	205.1; 257.9; 377.6
17.012	2.58	253.2; 360.4
17.456	8.08	233.2; 289.8
18.527	2.01	253.2; 365.6
20.539	1.38	202.8; 273.3
20.993	1.26	201.6; 269.7
21.611	0.48	212.1; 287.4; 384.8
21.778	0.19	254.4; 359.4
22.445	1.10	218.0; 254.4; 360.4

Table A26 continued.

Retention time	% Area	Absorbance maxima
23.878	0.13	219.1; 304.0
24.747	4.03	218.0; 255.6; 360.4
25.279	0.78	220.3; 255.6; 359.4
25.739	0.49	255.6; 358.6
26.026	0.26	219.1; 273.3
28.070	0.48	221.5; 255.6; 358.5
28.526	0.09	255.6; 357.5
29.300	0.94	None
29.902	0.04	222.7; 274.4
30.522	0.05	254.4

A12.5.2 Chemistry

- Anolignan B was isolated from the ethyl acetate root extract (Eldeen *et al.*, 2006). It showed anti-inflammatory activity and antibacterial activity against both Gram-positive and Gram-negative bacteria.
- Terminoic acid which was isolated from *Terminalia sericea* obtained MIC value of 0.33 mg/ml against *Staphylococcus aureus* (Kruger, 2004 in Eloff, *et al*., 2008).
- Resveratrol-3-*O*-β-D-rutinoside, a hydroxystilbene glycoside has been isolated from *Terminalia sericea* (Bombardelli *et al.*, 1975 in Moshi and Mbwambo, 2005).
- The polysaccharides from the gum exudates contain galacturonic, glucuronic and 4-0-methylglucuronic acids and galactose, arabinose, rhamnose and xylose (Anderson and Bell, 1974).
- Triterpenoids, sericic acid and sericoside were isolated from the roots of *Terminalia sericea* (Bombardelli *et al.*, 1974).

Table A27 Summary of antimicrobial studies conducted on *Terminalia sericea*.

Reference	Plant part	Type of extract	Antimicrobial activity	Results
Tshikalange *et al.* (2008)	Roots	Chloroform, ethyl acetate, water and 70% acetone	In vitro anti-HIV-1	Recorded as having the highest inhibition of α-glucosidase (IC_{50} = 92 µg/ml) and also showed good inhibition of HIV-1 reverse transcriptase (IC_{50} = 43 µg/ml).
Eldeen and van Staden (2007)	Bark and root	Dichloromethane, ethyl acetate and ethanol	Antimycobacterial	Activity against *M.aurum* A+ with MIC values ranging between 1.56-3.12 mg/ml.
Steenkamp *et al.* (2007a)	Roots	Methanol	Antibacterial	MIC values of 2.50 and 5.00 mg/ml against *S. epidermidis* and *S. aureus*.
		Water	Antibacterial	MIC values of 1.00 and 2.00 mg/ml against *S. epidermidis* and *S. aureus*.
Steenkamp *et al*. (2007b)	Roots	Methanol and water	Antifungal	MIC values of 2.50 and 2.00 mg/ml for the methanol and water extracts respectively against *C. albicans* (ATCC 10231).
Masoko *et al.* (2005)	Leaves	Acetone, hexane, dichloromethane and methanol	Antifungal	Average MIC values ranged between 0.46 – 0.49 mg/ml against *C. albicans*, *C. neoformans*, *A.fumigatus*, *M. canis* and *S. schenkii*.
Tshikalange *et al.* (2005)	Roots	Aqueous	Antibacterial	MIC values of 20.0 mg/ml against *B. cereus*, *B. subtilis* and *S. aureus* and an MIC value of 1.0 mg/ml against *B. pumilus*.
Moshi and Mbwambo (2005)	Roots	Aqueous, ethanol, ethylacetate, butanol, dichloromethane: methanol, methanol, dichloromethane	Antibacterial	Inhibition of *S. aureus*, *E. coli*, *B. anthracis*, and *P. aeruginosa* by the disc diffusion method.
			Antifungal	Inhibition of *C. albicans* and *A. niger* by the disc diffusion method.
Eldeen *et al.* (2005)	Bark and root	Ethyl acetate, ethanol and aqueous	Antibacterial	MIC values obtained against *B. subtilis*, *S. aureus*, *E. coli* and *K. pneumoniae*. The MIC values ranged between 0.390 – 3.125 mg/ml with the ethyl acetate root extract obtaining the lowest MIC value against *B. subtilis*.

Table A27 continued.

Reference	Plant part	Type of extract	Antimicrobial activity	Results
Steenkamp *et al.* (2004)	Bark	Water and methanol	Antibacterial	MIC value of 1 mg/ml obtained against *S. aureus* and *S. pyogenes*
Fyhrquist *et al.* (2002)	Roots	Acetone, ethanol, methanol and water	Antibacterial and antifungal	Inhibition of the growth of *S. aureus, E. coli, Enterobacter aerogenes, S. epidermidis, B. subtilis, Micrococcus luteus, Sarcina* sp. and *C. albicans* using the hole-plate agar diffusion method.
	Roots	Methanol	Antibacterial	MIC values of 1.5 mg/ml, 5.3 mg/ml, 1.8 mg/ml, 1.0 mg/ml and 6.1 mg/ml were obtained against *E. aerogenes, S. aureus, S. epidermidis, M. luteus* and *Sarcina* spp. respectively by the cylinder agar diffusion method.
Eloff (1999)	Leaves	Acetone	Antibacterial	MIC values of 3.0 mg/ml, 1.2 mg/ml, 6.0 mg/ml and 0.4 mg/ml were obtained against *S. aureus, E. coli, P. aeruginosa* and *E. faecalis* respectively using the microplate serial dilution technique

A12.5.3 Toxicity

- In the monkey kidney cell cytotoxicity test, *Terminalia sericea* extracts showed considerable toxicity whereby it obtained an ID_{50} value (dose that inhibits 50% of cell growth after incubation period) of 24 µg/ml (Tshikalange *et al.*, 2005).
- The intermediate and polar extracts, with the exception of the dichloromethane and the petroleum ether extracts of the roots of *Terminalia sericea* showed toxicity against brine shrimps (Moshi and Mbwambo, 2005). The LC_{50} values ranged between 5.4 – 17.4 µg/ml.

A13. *Vitex rehmannii* Guerke (Verbenaceae)

A13.1 Botanical description

Vitex rehmannii is a tree that grows to a height of 3-4 meters high and it has many branches. The bark is dark grey-brown in colour and is roughly textured with cracks on the surface. The leaves have a palmately compound arrangement i.e. the leaves are grouped together and spread out from the same point (Figure A43). The leaves are a yellow-green colour and their shape is described as lanceolate or elliptic i.e. they are long, narrow leaves that are broader towards the base and which comes to a point at the two ends. The flowers occur in clusters and are located in the centre of a group of leaves. They are white to lilac in colour (Bredenkamp and Botha, 1996).

Figure A43 Leaves and flowers of *Vitex rehmannii* (A. Viljoen).

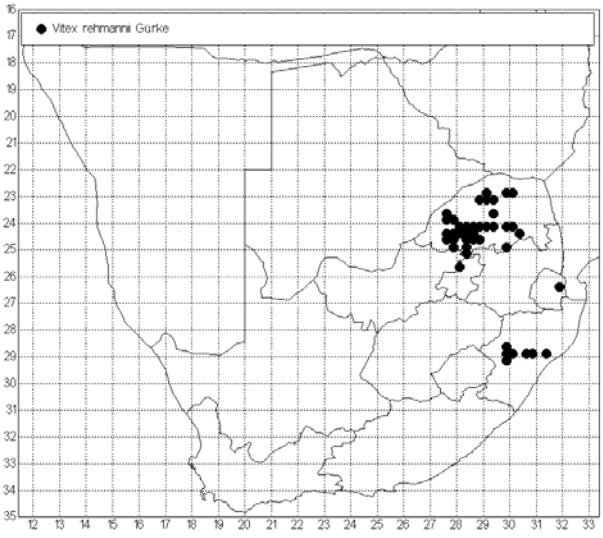

Figure A44 Distribution map of *Vitex rehmannii* (SANBI).

A13.2 Geographical distribution

The distribution (Figure A44) of *Vitex rehmannii* consists of North-West province, Gauteng, Northern Province (particularly common in the Waterberg area), Mpumalanga, Swaziland and KwaZulu-Natal (Bredenkamp and Botha, 1996).

A13.3 Traditional medicinal uses

Traditional medicinal uses relating specifically to respiratory ailments is reported by Hutchings *et al*. (1996) where unstipulated parts of the plant are used to treat pleurisy which is an inflammation of the tissue lining the lungs and the pleural cavity. Other uses include the treatment of stomach aches with enemas of leaf infusions (Watt and Breyer-Brandwijk, 1962; Hutchings *et al.*, 1996).

A13.4 HPLC profiles

Figure A45 is the HPLC chromatogram for the leaf extract of *Vitex rehmannii*. Data for the chromatogram comprising retention time, % area and the absorbance maxima of the corresponding UV spectra is given in Table A28. A flavonol, two isoflavones and a flavone were tentatively identified for the leaf extract of *Vitex rehmannii* (Table A28).

A13.5. Literature review

A13.5.1 Antimicrobial activity

A summary of the antimicrobial studies that have been carried on *Vitex rehmannii* is given in Table A29.

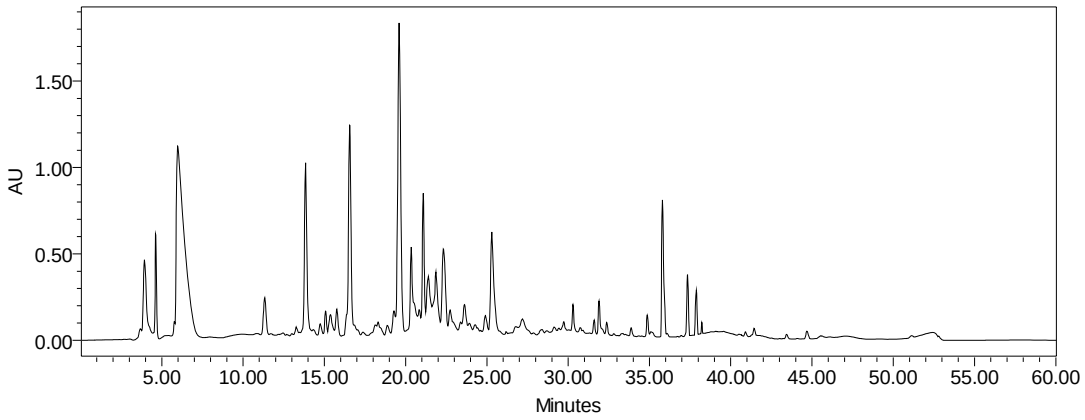

Figure A45 HPLC chromatogram of the leaves of *Vitex rehmannii*.

Table A28 Data for the HPLC chromatogram of *Vitex rehmannii* (leaves).

Retention time	% Area	Absorbance maxima (flavonoid tentatively identified)
3.937	3.12	201.6
4.625	2.37	229.7
5.977	23.84	265.0
11.331	1.66	206.3; 259.1; 293.4
13.843	6.60	255.6
14.744	0.47	259.1
15.075	0.78	246.1
15.376	1.23	270.9; 336.1
15.766	1.10	202.8; 268.5; 348.0
16.558	8.85	257.9; 326.6
19.283	0.91	203.9; 255.6; 352.8 **(flavonol)**
19.604	15.57	247.3; 329.0
20.346	2.11	228.5; 312.3
21.082	3.70	219.1; 242.6; 327.8
21.397	2.51	333.7
21.865	2.92	247.3; 330.1
22.325	4.09	247.3; 326.6 **(isoflavone)**
22.737	0.49	246.1; 324.2 **(isoflavone)**
23.619	0.81	232.0; 282.7; 317.1
24.905	0.71	242.6
25.303	5.32	243.8; 308.8
30.295	0.70	241.4; 273.3; 343.2
31.600	0.40	269.7; 336.1 **(flavone)**
31.900	0.85	242.6; 273.3; 343.2
32.372	0.34	255.6; 348.0
34.868	0.55	237.9; 272.1; 336.1
35.798	4.81	368.0
37.343	1.74	221.5; 326.6
37.881	1.29	248.5; 272.1; 324.2
38.223	0.18	246.1; 324.2

Table A29 Summary of antimicrobial studies conducted on *Vitex rehmannii*.

Reference	Plant part	Type of extract	Antimicrobial activity	Results
Nyiligira *et al.* (2008)	Aerial parts	Acetone	Antibacterial	MIC values ranged between 0.02 – 4.00 mg/ml against bacteria *S. aureus, B. cereus, E. faecalis, E. coli* and *S. typhimurium.*
			Antifungal	MIC value of 0.63 mg/ml was obtained against *C. neoformans.*
			Antimalarial	An IC_{50} value of 9.16 µg/ml was obtained against the chloroquine resistant Gambian FCR-3 strain of *Plasmodium falciparum*.
Nyiligira *et al.* (2004b)	Aerial parts	Essential oil	Antibacterial	The essential oil component of the leaves obtained MIC values of 16 mg/ml and 8 mg/ml against *S. aureus* and *B. cereus* respectively.

A13.5.2 Chemistry

- Two compounds have been isolated from the leaves of *Vitex rehmannii viz.* a labdane-type diterpene, 12S,16*S*/R-dihydroxy-*ent*-labda-7,13-dien-15,16-olide and cirsimaritin, a flavonoid (Nyiligira, 2004a; Nyiligira *et al.*, 2008). The labdane-type diterpene was responsible for the antimicrobial activity against *Staphylococcus aureus*.
- The major compounds of the essential oil of *Vitex rehmannii* comprise spathulenol (10%), caryophyllene oxide (9.8%), elema-1,11-dien-15-ol (7.2%) and caryophylladienol II (6.5%) (Nyiligira *et al.*, 2004b).
- Chemical constituents isolated from the air-dried leaves and stems of *Vitex rehmannii* comprise of agnuside (1.0%), aucubin (0.1%) and ecdysterone (0.005%) (Rimpler, 1972).

A13.5.3 Toxicity

- The aerial parts of *Vitex rehmannii* displayed toxicity (Nyiligira *et al.*, 2008) in the MTT assay (tetrazolium cellular viability assay) on transformed human kidney epithelium cells (IC_{50}=7.67 µg/ml and safety index = 0.84). The labdane-type diterpene, 12S,16*S*/R-dihydroxy-*ent*-labda-7,13-dien-15,16-olide, which was isolated from the leaves showed high toxicity (Nyiligira *et al.*, 2008) in the MTT assay (IC_{50}=1.27 µg/ml and safety index=0.53).

A14. *Zanthoxylum davyi* (Verdoorn) Waterm.(Rutaceae)

A14.1 Botanical description

Zanthoxylum davyi grows to be a medium to tall tree with height ranging between 8-30 meters. The branches and the main trunk typically have knobs with spiked tips (Figure A46). This feature is characteristic of the *Zanthoxylum* genus. The leaves and the unripe fruit have a characteristic lemon smell (Tarus *et al.*, 2006). The leaves have an alternate arrangement and their shape is elliptic i.e. it is broad in the middle region and narrowing at the bottom and top ends (Schmidt *et al.*, 2002).

Figure A46 Leaves and bark of *Zanthoxylum davyi* (Schmidt *et al.*, 2002).

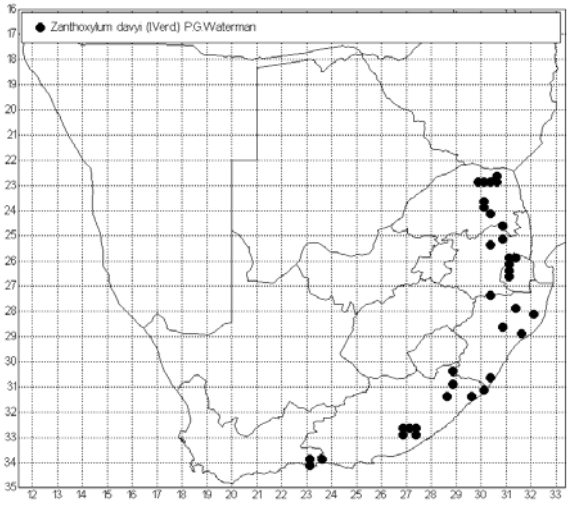

Figure A47 Distribution map of *Zanthoxylum davyi* (SANBI).

A14.2 Geographical distribution

The forests of KwaZulu-Natal and Transkei are the most common distribution of *Zanthoxylum davyi*. The northerly distribution spans through Mpumalanga, Swaziland and Zimbabawe.

(Tarus *et al.*, 2006). There is also a sparse distribution in the Northern Province and the Eastern and Western Cape Province (Figure A47).

A14.3 Traditional medicinal uses for respiratory ailments

The Zulu chew the cooked powdered bark to treat sever coughs and colds. The Vhavenda use the leaves for chest pains and the roots for sore throats. They also use the bark for chronic coughs (Hutchings *et al.*, 1996). Infusions are made of the leaves so as to treat colds and flu and generalised complaints relating to the chest (Schmidt *et al.*, 2002).

A14.4 HPLC profiles

The HPLC chromatograms for the leaf, root, bark and thorn extracts are presented in Figures A48 to A51. Data for the chromatograms comprising retention time, % area and the absorbance maxima of the corresponding UV spectra is given in Tables A30 to A33. No flavonoids were tentatively identified for this plant species.

A14.5 Literature review

A14.5.1 Antimicrobial activity

A summary of the antimicrobial studies that have been carried on *Zanthoxylum davyi* is given in Table A34.

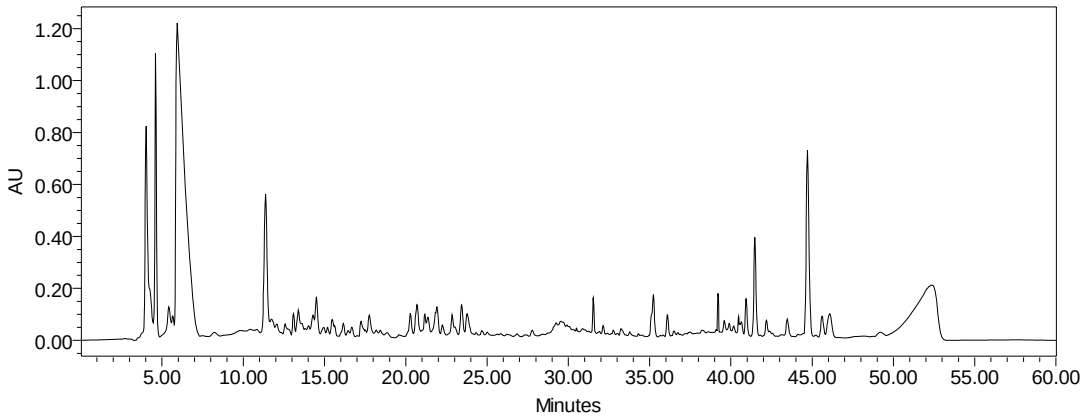

Figure A48 HPLC chromatogram of the leaves of *Zanthoxylum davyi*.

Table A30 Data for the HPLC chromatogram of *Zanthoxylum davyi* (leaves).

Retention time	% Area	Absorbance maxima
4.020	9.10	222.7; 273.3
4.607	6.66	221.5; 274.4
5.421	0.66	220.3; 274.4
5.942	38.04	265.0
11.371	5.70	206.3; 260.3; 293.4
13.088	0.59	221.5; 267.4
13.388	1.57	288.6;
14.500	2.41	232.0
15.471	0.72	221.5; 296.9; 320.6
17.246	0.89	201.6; 228.4; 279.2
17.754	2.05	232.0
20.279	1.08	205.1; 278.0
20.691	1.46	228.5; 281.5
21.167	1.46	221.5; 325.4
21.913	1.49	225.0; 283.9
22.851	0.75	229.7; 313.5
23.434	1.13	239.1; 311.1
23.769	1.78	236.7; 300.5
31.535	4.44	223.8; 280.3; 318.3
35.231	1.64	236.7; 279.2; 326.6; 399.2
36.092	0.62	236.7; 279.2; 324.2
39.212	0.62	248.5; 279.2; 326.6
40.472	0.73	246.1; 273.3; 327.8
40.933	1.04	246.1; 275.6
41.472	3.18	242.6; 273.3; 324.2
42.185	0.71	273.3
43.468	0.79	248.5; 273.3; 324.2
44.708	7.98	267.4; 333.7
45.608	0.72	272.1; 315.9

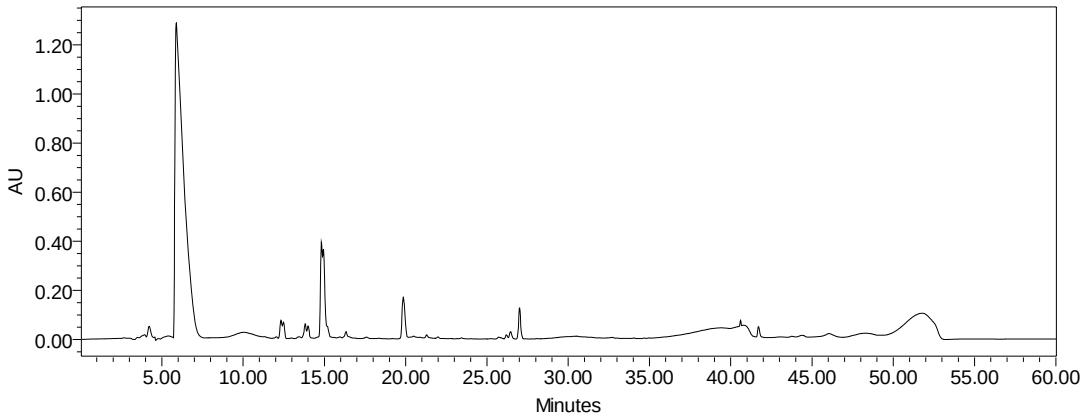

Figure A49 HPLC chromatogram of the roots of *Zanthoxylum davyi*.

Table A31 Data for the HPLC chromatogram of *Zanthoxylum davyi* (roots).

Retention time	% Area	Absorbance maxima
3.494	0.13	205.1; 289.8; 304.0; 325.4
3.937	0.54	205.1; 279.2;301.6;314.7;338.5
4.222	1.20	207.4;301.6; 345.6; 365.6
4.783	0.10	257.9; 272.1; 301.6; 332.5
5.393	0.71	205.1; 279.2; 304.0; 325.4
5.898	64.29	265.0
12.045	0.06	202.8; 278.0; 338.5
12.333	0.84	202.8; 278.0; 369.2
12.483	0.74	206.3; 273.3; 325.4
13.450	0.14	205.1; 229.7; 280.3; 365.6
13.820	0.74	202.8; 279.2; 365.6
13.999	0.56	206.3; 229.7;340.9
14.632	0.04	202.8; 229.7; 279.2; 365.6
14.821	4.04	203.9; 278.0
14.940	5.10	202.8; 279.2
16.334	0.27	209.8; 229.7; 336.1
17.595	0.06	202.8; 230.9; 280.3; 365.6
19.859	2.79	202.8; 278.0
20.495	0.04	229.7; 278.0; 336.1
21.294	0.13	230.9; 279.2
21.991	0.07	235.6; 279.2; 338.5
23.442	0.04	243.8
25.723	0.15	240.3; 325.4
26.198	0.20	236.7; 285.1; 325.4
26.453	0.42	212.1; 236.7; 325.4
27.008	1.52	234.4; 327.8
40.604	0.13	246.1; 325.4
41.707	0.49	311.1
46.050	0.27	266.2; 325.4
51.832	14.18	273.3; 325.4

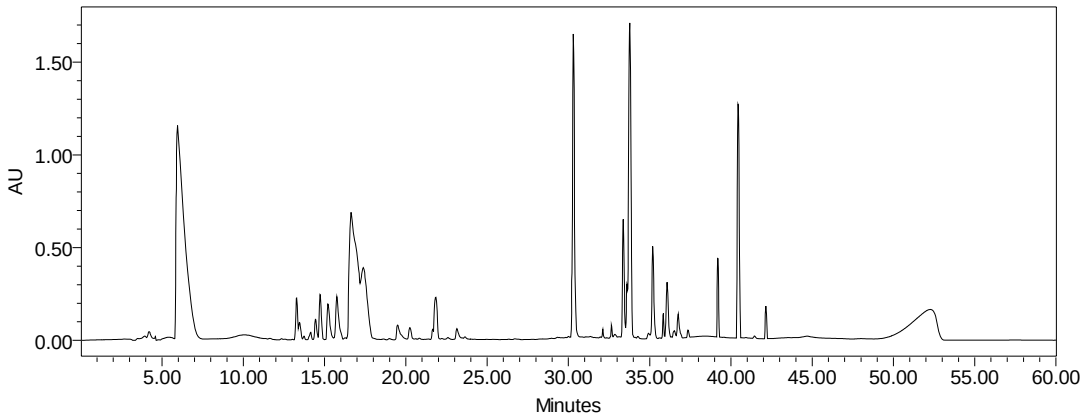

Figure A50 HPLC chromatogram of the bark of *Zanthoxylum davyi*.

Table A32 Data for the HPLC chromatogram of *Zanthoxylum davyi* (bark).

Retention time	% Area	Absorbance maxima
5.957	26.24	265.0
13.282	1.19	219.1; 325.4
13.458	0.54	285.1; 326.6
14.444	0.65	223.8; 268.5; 300.5
14.727	1.49	203.9; 229.7; 281.5
15.212	1.54	222.7; 280.3; 305.2
15.755	2.03	205.1; 228.5; 282.7
16.630	15.00	222.7; 270.9; 304.0
17.382	6.95	205.1; 274.4
19.494	0.81	205.1; 232.0; 282.7
20.247	0.42	205.1; 230.9; 278.0
21.842	2.12	205.1; 232.0; 282.7; 337.3
23.143	0.63	240.3; 286.3
30.310	9.12	248.5; 331.3
32.121	0.12	241.4; 285.1
32.654	0.21	239.1; 281.5
33.374	3.08	237.9; 288.6; 331.3
33.611	1.10	240.3; 286.3; 326.6
33.776	11.37	236.7; 186.3; 326.6
35.193	2.59	259.1; 326.6; 364.4
35.837	0.44	239.1; 282.7; 366.8
36.075	1.79	235.6; 272.1; 366.8
36.512	0.20	278.0
36.753	0.65	241.4; 274.4; 326.6
37.362	0.18	259.1; 326.6
39.195	1.68	278.0; 326.6
40.445	7.12	263.8; 326.6
42.150	0.74	278.0; 326.6

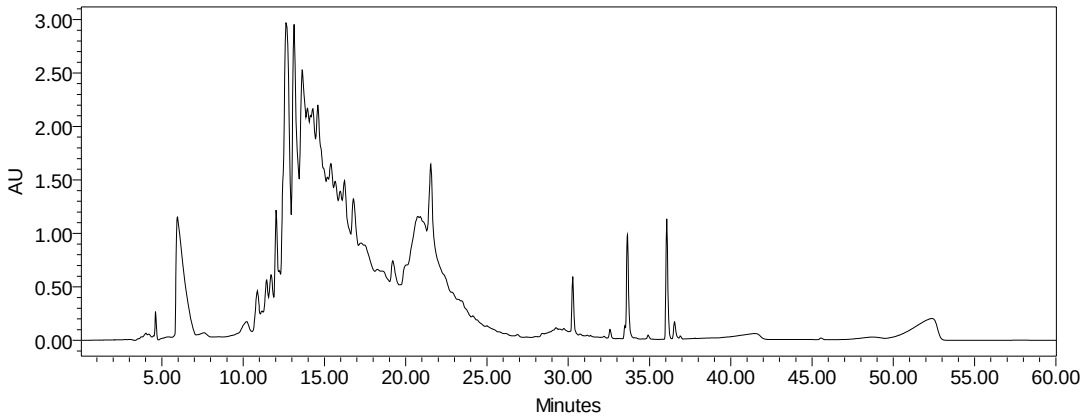

Figure A51 HPLC chromatogram of the thorns of *Zanthoxylum davyi*.

Table A33 Data for the HPLC chromatogram of *Zanthoxylum davyi* (thorns).

Retention time	% Area	Absorbance maxima
4.613	0.30	278.0
5.962	7.66	265.0
10.875	1.31	203.9; 278.0
11.441	0.98	202.8; 278.0
11.722	1.14	202.8; 278.0
12.031	2.63	201.6; 278.0
12.640	13.55	228.5; 278.0
13.128	10.19	228.5; 278.0
13.636	9.03	220.3; 273.3
13.953	4.07	209.8; 274.4
14.280	7.39	209.8; 278.0
14.596	10.10	209.8; 280.3
15.185	1.62	203.9; 279.2
15.396	3.33	203.9; 279.2
15.657	2.51	203.9; 280.3
15.971	1.90	203.9; 279.2
16.230	2.87	203.9; 280.3
16.784	1.47	203.9; 280.3
19.202	0.63	202.8; 280.3
20.048	0.57	202.8; 280.3
20.747	6.85	203.9; 280.3
21.541	4.77	203.9; 279.2; 348.0; 377.6
30.274	0.84	248.5; 319.4
32.569	0.13	237.9; 331.3
33.484	0.14	246.1; 294.5; 358.5; 377.6
33.640	1.77	275.6; 325.4
34.914	0.07	256.7; 332.5; 380.0
36.060	1.89	230.9; 283.9
36.539	0.28	276.8; 384.8

Table A34 Summary of antimicrobial studies conducted on *Zanthoxylum davyi*.

Reference	Plant part	Type of extract	Antimicrobial activity	Results
Tshikalange *et al.* (2008)	Roots	70% acetone	*In vitro* anti-HIV-1	The acetone root extracts (concentration=15 µg/ml) inhibited HIV viral proteins, NF-κB and Tat, at % inhibition values of 54.0 and 50.0% respectively.
Steenkamp *et al.* (2007a)	Bark	Methanol and water	Antibacterial	The methanol extract obtained an MIC value of 1 mg/ml against the *S. epidermidis* and *S. aureus*.
Steenkamp *et al.* (2007b)	Bark	Methanol and water	Antifungal	The methanol extract obtained an MIC value of 0.50 mg/ml and the water extract obtained an MIC value of 3.25 mg/ml against *C. albicans* (ATCC 10231).

A14.5.2 Chemistry

Five benzo[c]phenanthridine alkaloids, chelerythrine, dihydrochelerythrine, bocconoline, 6-hydroxydihydrochelerythrine and 6-methoxy-7-demethyldihydrochelerythrine were isolated from the stem bark of *Zanthoxylum davyi*. Two other compounds that were also isolated were 4-methoxy-1-methyl-2(1H)-quinolinone and *meso*-sesamin (Tarus *et al.,* 2006).

A14.5.3 Toxicity

No studies were found reporting on the toxicity of *Zanthoxylum davyi*.

A15. *Ziziphus mucronata* Willd. subsp. *mucronata* (Rhamnaceae)

A15.1 Botanical description

Ziziphus mucronata is a tree growing to a height of about 5-10 m and it has a wide spreading crown (van Wyk *et al.*, 2000). The bark is grey-brown in colour and has many cracks that occur in a lengthwise pattern (van Wyk *et al.*, 2000; Venter and Venter, 2002). The leaves are green and shiny and have an alternate arrangement i.e. the leaves are arranged alternately along the stem (Figure A52). The upper part of the leaf is crenate i.e. it has rounded blunt teeth. A pair of thorns appears at the base of the leaf and one thorn is straight and the other is curved. The yellow-green flowers grow in a group at the point where the leaf joins the stem. This plant has fruit that are reddish-brown berries (van Wyk *et al.*, 2000; von Koenen, 2001; Venter and Venter, 2002).

Figure A52 Leaves and bark of *Ziziphus mucronata* (S.v.Vuuren).

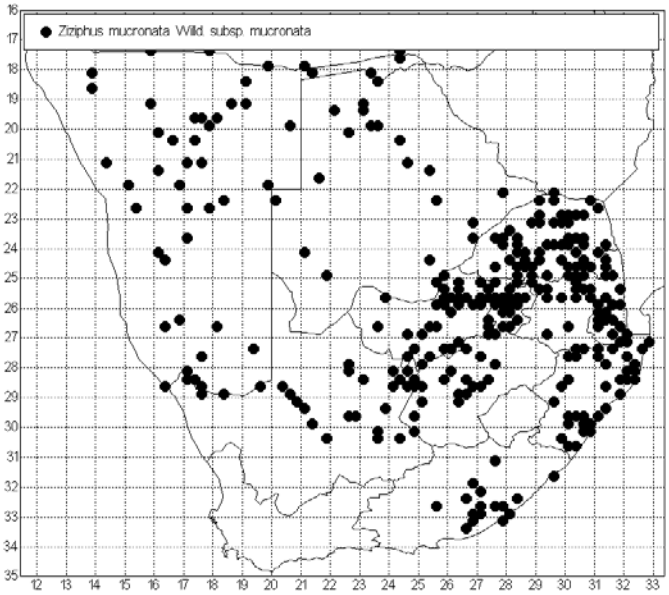

Figure A53 Distribution map of *Ziziphus mucronata* (SANBI).

A15.2 Geographical distribution

van Wyk *et al.* (2000) reports that *Ziziphus mucronata* is one of the most widely distributed of all the South African trees. The southern African distribution includes Namibia, Botswana and Swaziland. The provinces within South Africa where it is found are Northern Cape, Eastern Cape, KwaZulu-Natal, Free State, Gauteng, Mpumalanga, North West Province and Northern Province (Figure A53).

A15.3 Traditional medicinal uses for respiratory ailments

The roots, bark or leaves are usually used, either separately or in combination (van Wyk *et al.*, 2000). Chronic coughs and chest complaints are treated with bark infusions which are sometimes used together with the leaves or roots (Watt and Breyer-Brandwijk, 1962; Hutchings *et al.*, 1996; van Wyk *et al.*, 2000; von Koenen, 2001). Hutchings *et al.* (1996) reports this use to be specific to the Zulu. The pulp of the roots is also used for swollen glands in tuberculosis (Watt and Breyer-Brandwijk, 1962; von Koenen, 2001). In West Africa colds are treated with leaves, fruits and stems (Hutchings *et al.*, 1996).

A15.4 HPLC profiles

The HPLC chromatograms for the leaf extract and the root extract are presented in Figures A54 and A55 respectively. Data for the chromatograms comprising retention time, % area and the absorbance maxima of the corresponding UV spectra is given in Tables A35 and A36. A flavonol was tentatively identified for the leaf extract of this plant species (Table A35).

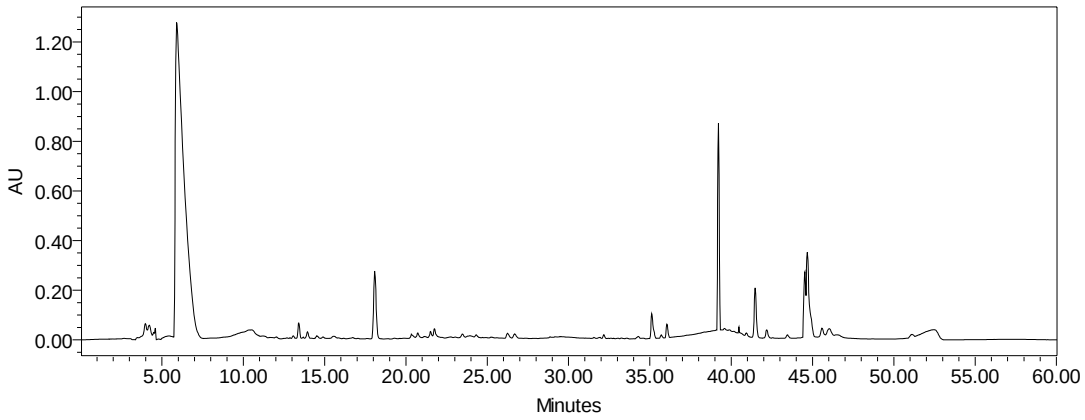

Figure A54 HPLC chromatogram of the leaves of *Ziziphus mucronata*.

Table A35 Data for the HPLC chromatogram of *Ziziphus mucronata* (leaves).

Retention time	**% Area**	**Absorbance maxima (flavonoid tentatively identified)**
3.977	0.78	202.8; 278.0; 380.0
4.218	0.92	206.3; 261.5; 334.9
4.587	0.53	261.5; 363.4
5.907	66.07	265.0
13.067	0.11	227.4; 278.0; 377.6
13.411	0.64	202.8; 278.0; 393.2
13.947	0.29	234.4; 393.2
18.083	4.12	255.6; 354.0 **(flavonol)**
20.344	0.22	207.4; 233.2; 278.0; 323.0
20.725	0.21	232.0; 266.2; 342.0
21.515	0.24	233.2; 278.0
21.753	0.35	255.6; 348.0
26.251	0.31	237.9; 312.3
26.686	0.35	240.3; 317.1
32.168	1.42	313.5
35.115	1.32	237.9; 279.2; 321.8
35.699	0.13	278.0
36.047	0.58	241.4; 308.8
39.213	7.74	278.0; 326.6
40.477	0.17	246.1; 274.4
40.930	0.13	278.0
41.473	2.51	246.1; 273.3; 325.4
42.184	0.40	273.3
43.456	0.17	274.4
44.525	3.02	269.7; 332.5
44.680	6.14	268.5; 337.3
45.583	0.52	268.5; 332.5
46.027	0.63	268.5; 332.5

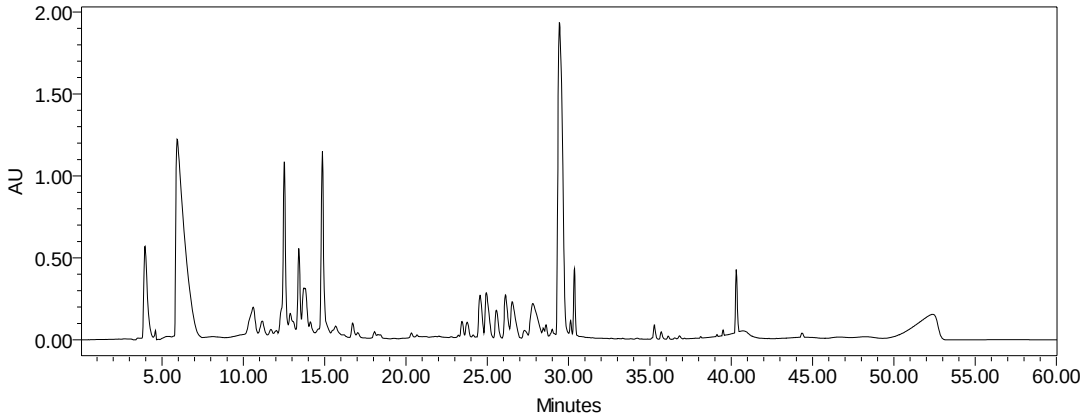

Figure A55 HPLC chromatogram of the roots of *Ziziphus mucronata*.

Table A36 Data for the HPLC chromatogram of *Ziziphus mucronata* (roots).

Retention time	% Area	Absorbance maxima
3.958	5.53	202.8
4.596	0.23	259.1; 337.3; 363.4
5.938	26.59	265.0
10.606	2.15	206.3; 269.7
12.520	7.03	207.4; 270.9; 324.2
12.886	1.00	207.4; 270.9; 324.2
13.414	2.83	203.9; 278.0
13.730	2.68	202.8; 280.3
14.856	6.42	203.9; 278.0
16.724	0.48	206.3; 278.0
23.444	0.58	232.0; 275.6
23.761	0.69	233.2; 275.6
24.554	2.27	280.3
24.943	2.74	280.3
25.559	1.38	226.2; 278.0
26.118	2.29	278.0
27.803	3.47	227.4; 276.8
28.613	0.59	239.1; 275.6
29.437	23.86	268.5; 318.3
30.125	0.39	236.7; 278.0; 312.3
30.357	1.78	269.7; 318.3
35.272	0.41	273.3
35.697	0.33	262.6; 294.5; 366.8
39.499	0.11	261.5; 294.5
40.312	1.81	249.7; 273.3

A15.5 Literature review

A15.5.1 Antimicrobial activity

A summary of the antimicrobial studies that have been carried out on *Ziziphus mucronata* is given in Table A37.

Table A37 Summary of antimicrobial studies conducted on *Ziziphus mucronata*.

Reference	Plant part	Type of extract	Antimicrobial activity	Results
Luseba *et al.* (2007)	Shoots	Dichloromethane and 90% methanol	Antibacterial	The dichloromethane extract obtained an MIC value of 2.5 mg/ml against *S. aureus* (ATCC 29213), *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853). The methanol extract obtained an MIC value of 1.25 mg/ml against *P. aeruginosa* (ATCC 27853).
McGaw *et al.* (2007)	Bark and leaves	Hexane, methanol and water	Antibacterial	The MIC values were determined against *E. coli, S. aureus, E. faecalis* and *P. aeruginosa.* The methanol leaf extract obtained an MIC value of 0.2 mg/ml against *S. aureus*. The MIC values for the remainder of the extracts ranged between 1.6 and 12.5 mg/ml.
Bessong *et al.* (2005)	Leaves	Aqueous and methanol	Anti-HIV activity	The inhibitory activity of the plants extracts was measured against HIV-1 reverse transcriptase (RT). The aqueous and methanol extracts of the leaves obtained IC_{50} values of 77.5 and 81.5 µg/ml respectively, against the RNA-dependant-DNA polymerase activity of RT. Also, the aqueous and methanol extracts obtained IC_{50} values of >100 and 75.0 µg/ml respectively, against the ribonuclease H activity of RT.

Table A37 continued.

Reference	Plant part	Type of extract	Antimicrobial activity	Results
Adamu *et al.* (2005)	Root, leaves and bark	Aqueous	Antibacterial	In the agar well diffusion method, the aqueous extract of the roots/leaves showed weak inhibition of *Proteus mirabilis* and *S. aureus* whereas the aqueous extract of the root/bark showed strong inhibition of *Proteus mirabilis, P. aeruginosa, S. aureus* and *E. coli* where inhibition zones against these bacteria were > 15 mm.
Clarkson *et al.* (2004)	Leaves	Dichloromethane	Antiplasmodial	An IC_{50} value of 12.0 µg/ml was recorded against a chloroquine-sensitive strain (D10) of *Plasmodium falciparum*.
Prozesky *et al.* (2001)	Stem bark	Acetone	Antiplasmodial	The acetone extract of the stem bark obtained an IC_{50} value of 4.13 µg/ml against a chloroquine resistant strain (PfUP1) of *Plasmodium falciparum*.
Mølgaard *et al.* (2001)	Root bark	Aqueous	Anthelmintic	All the cestodes of *Hymenolepis diminuta* were killed at a concentration of 1.6 mg/ml after one hour of exposure to the extract.
Kudi and Myint (1999)	Leaf	80% ethanol	Antiviral	The leaf extract caused 75% inhibition of poliovirus and astrovirus at a concentration of 2 mg/ml.
Rabe and van Staden (1997)	Leaf	Methanol	Antibacterial	Antibacterial activity was determined by the disc diffusion method and was recorded as the ratio of the zone of inhibition obtained by the extract to the zone of inhibition obtained by the control, neomycin. The methanol leaf extract obtained values of 0.30, 0.13 and 0.14 against *S. aureus*, *S. epidermis* and *B. subtilis* respectively.

A15.5.2 Chemistry

- Anthocyanins were extracted from *Ziziphus mucronata* and were shown to have antisickiling activity (Mpiana *et al.*, 2008).

- Two compounds were isolated from the leaves of *Ziziphus mucronata viz.* 2,3-dihydroxyl-up-20-en-28-oic and zizyberanalic acid. The first compound showed good antibacterial activity against *Staphylococcus aureus* (Moloto, 2004 in McGaw and Eloff, 2008a).
- Mucronine J, a cyclopeptide alkaloid, was isolated from the dichloromethane extract of the root bark of *Ziziphus mucronata* (Auvin *et al.*, 1996).
- Mucronine D, which was previously isolated from the bark of *Ziziphus mucronata* by Tschesche *et al.* (1972), was also isolated from the roots together with two additional cyclopeptide alkaloids by Barboni *et al.* (1994).
- Peptide alkaloids, abyssenines A, B and C, were isolated from the bark of *Ziziphus mucronata*. Leaves were shown to contain the alkaloids, mucronine G and H, abyssenine C and the isoquinoline alkaloid (-)*N*-methylcoclaurine (Tschesche *et al.*, 1974a).
- The peptide alkaloids, mucronines E, F, G and H were isolated from *Ziziphus mucronata* by Tschesche *et al.* (1974b) and mucronines A, B and C was isolated by Fehlhaber *et al.* (1972).

A15.5.3 Toxicity

- The 90% methanol extracts of the leaves of *Ziziphus mucronata* showed genotoxic effects in the Ames test (Elgorashi *et al.*, 2003).
- The dichloromethane and the methanol extracts of the leaves of *Ziziphus mucronata* showed genotoxic effects on human white blood cells in the micronucleus and alkaline comet assay tests which are tests to measure DNA damage (Taylor *et al.*, 2003).

- In the brine shrimp lethality test, the hexane leaf extract obtained an LC_{50} value (the concentration of extract that results in the death of 50% of the larvae) of 0.9 mg/ml (McGaw *et al.*, 2007).