# Evaluating the predictive value of a database of antimicrobial activities of leaf extracts of 537 southern African tree species against six important bacterial and fungal pathogens

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# Declaration

The research presented in this report was carried out in the Phytomedicine Programme, Department Paraclinical Sciences, Faculty Veterinary Science, University of Pretoria under the supervision of Prof J.N. Eloff.

I, the undersigned Elisabeth Pauw, declare that this thesis submitted to the University of Pretoria for the degree Philosophiae Doctor, is a result of my own investigations except when the work of others is acknowledge and has not been submitted to any other institution.

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Elisabeth Pauw

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Prof J.N. Eloff (promotor)

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## Abstract

Infectious diseases are the world's leading cause of premature deaths in humans and animals. The resistance to antibiotics and the emergence of new infectious diseases has increased the need for additional effective antimicrobial products. Despite numerous publications investigating antimicrobial activity of plant extracts it appears that no effective single product antimicrobial has yet been developed from plants. In many cases, however crude plant extracts have excellent activity and may provide useful products.

Plants are frequently selected based on traditional use. Traditional healers usually use aqueous extracts of plants which in our experience generally have very low activities and it may be one of the reasons why no new products were developed from plants. Another approach to select plants for research is to use the taxonomic approach based on the premises that: (1) there is a correlation between active chemical compounds and antimicrobial activity; and (2) species in a family or order may have similar activities if the chemical precursors are inherited from a common ancestor. Future screening programmes could then concentrate on close relatives of species within these promising families and orders.

The main aim of this study was to randomly screen leaf extracts of several hundred southern African tree species against important microbial pathogens to determine which taxa have the highest activity and may yield useful products to treat infections in human and animal health markets. A wide selection of plant species improved the possibility of finding promising extracts and has the advantage that active compounds may be discovered from plants that are not used traditionally. To ensure sustainable use only leaves of trees were examined. A spin off of this study would also indicate the susceptibility of different organisms, correlate the antimicrobial activities of the different organisms and determine what minimum inhibitory concentration (MIC) represents a good activity based on investigating many extracts against many microbes.

The antimicrobial activity was determined by using a sensitive serial dilution microplate method. Acetone extracts were tested against two Gram-positive bacteria, two Gram-negative bacteria and two fungi, i.e. *Staphylococcus aureus, Enterococcus faecalis*, *Escherichia coli, Pseudomonas aeruginosa, Candida albicans* and *Cryptococcus neoformans*.

Small and mostly insignificant differences were found between the susceptibility of the microbial pathogens to the extracts. *E. faecalis* was the most sensitive bacterium and *C. neoformans* the most sensitive fungal organism. The strongest correlations in activities among the pathogens were between *C. albicans* and *C. neoformans*, and among the pathogen classes between Gram-positive and Gram-negative bacteria.

The tree extracts analysed in the present study had a wide range of activities against the different pathogens. Twenty six per cent of the extracts inhibited the pathogens at MIC levels of 0.16 mg/ml. This clearly shows that 0.16 mg/ml is not low enough to discriminate between promising species. Some of the extracts inhibited the growth of more than one pathogen while other extracts had selective activities and could be the most promising to follow up.

The study identified families and orders with either statistically significantly higher or lower antimicrobial activities. Among the large families, Combretaceae and Fabaceae had high mean activities against all test pathogens. The families Anacardiaceae and Moraceae had high activities against both Gram-positive and Gram-negative bacteria whereas the families Proteaceae and Meliaceae had higher antifungal activities. Among the large orders, Fabales had relatively high activities against all the pathogen classes. Considering that plants in related taxa often contain similar compounds and therefore similar activities, future studies could analyse more representative species in the promising taxa.

Many tree species, genera, families and orders, including well-known and lesser known medicinal taxa in southern Africa, were identified with promising activities. To evaluate the potential use of these results, additional cytotoxicity, phytochemical and pharmacological studies should be carried out. The study, although still exploratory, underlined the potential of southern African tree extracts as sources of antimicrobial products. Application of these results within the Phytomedicine Programme has led to patents and products that were as good as commercial products in animal and field trials. We hope that our results will provided a starting point for discovering new products with useful activities.

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# List of abbreviations

| | |
|---|---|
| AIDS | Acquired immune deficiency syndrome |
| ANOVA | Analysis of variance |
| APG | Angiosperm Phylogeny Group |
| Ca | *Candida albicans* |
| CFU | Colony-forming units |
| Cn | *Cryptococcus neoformans* |
| Ec | *Escherichia coli* |
| Ef | *Enterococcus faecalis* |
| DF | Degrees of freedom |
| INT | *p*-iodonitrotetrazolium violet |
| LSM | Least square means |
| MIC | Mimimum inhibitory concentration |
| MRSA | Methicillin-resistant *Staphylococcus aureus* |
| NAPRALERT | Natural Products Alert |
| NCCLS | National Committee for Clinical Laboratory Standards |
| Pa | *Pseudomonas aeruginosa* |
| PMDB | Phytomedicine Tree Data Base |
| PRECIS | The National Herbarium Pretoria (PRE) Computerized Information System |
| PRU | HGWJ Schweickerdt Herbarium |
| Sa | *Staphylococcus aureus* |
| SANBI | South African National Biodiversity Institute |
| SAS | Statistical Analysis Software |
| SD | Standard deviation |
| UP | University of Pretoria |
| WHO | World Health Organisation |

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# Chapter 1

# Introduction and objectives

## 1.1 Introduction to the study

Plants produce a diverse range of bioactive molecules with a wide spectrum of activities, making them a rich source of different types of compounds that could be used as medicine (Vlietinck and Vanden Berghe, 1991; Ripa *et al.,* 2009). Throughout the world, plants are used to treat many illnesses, particularly infectious diseases, and were once used as the primary medicine all over the world (Van Wyk *et al*., 1997; Khafagi and Dewedar, 2000). Infections are the world's leading cause of premature deaths, killing almost 50 000 people every day (Ahmad and Beg, 2001).

Pharmacological industries produced a large amount of commercial antibiotics in the last three decades but the extensive use of antibacterial and antifungal agents resulted in a significant upsurge in resistance to these drugs (Neu, 1992; Cowan, 1999; Levy and Marshall, 2004). Consequently, the treatment of bacterial and fungal pathogens that are drug-resistant is even more complicated in acquired immune deficiency syndrome (AIDS) patients (Diamond, 1991). These developments have increased the need to search for new antibacterial products with improved activity (Eloff *et al*., 2005).

In recent years, there has been growing world-wide interest in natural and traditional medicines as an alternative form to treat infectious diseases (Van Wyk *et al*., 1997). This is partially based on the widely held, but not necessarily correct assumption, that there is a lower incidence of adverse reactions to natural medicine. The World Health Organisation (WHO) estimated that about 80% of the rural population of the developing countries rely exclusively on plants to meet their health care needs (Farnsworth *et al*., 1985). Nevertheless, of all the 250 000 species of higher plants known on Earth, only a fraction has been scrutinised for all aspects of their potential therapeutic medicinal value (Farnsworth, 1984). Furthermore, natural products and their derivatives (including from microorganisms) represent more than 50% of all drugs in clinical use in the world (Van Wyk and Wink, 2004). The importance of natural products in drug discovery has been discussed in many scientific papers (Farnsworth *et al.,* 1995; Cordell, 2000).

Southern Africa has a rich floral diversity comprising in the order of 10% of the world's plant diversity on less than 2.5% of Earth's land surface (Germishuizen and Meyer, 2003). This diversity represents a very valuable resource for commercial development as well as basic scientific studies (Van Wyk, 2008). In South Africa in particular, many rural ethnic groups rely on

traditional indigenous plant knowledge to treat various diseases in both humans and livestock (Rabe and Van Staden, 1997; Grierson and Afolayan, 1999; Masika and Afolyan, 2002; McGaw and Eloff, 2008). As much as 15% of the 24 000 taxa recorded in southern Africa are used in traditional medicines (Van Wyk and Gericke, 2000; Arnold *et al.*, 2002) and an estimated 500 plant species are traded in informal medicinal plant markets (McGaw *et al*., 2005). Traditional medicine remains more affordable than the expensive Western medicine and is also easily accessible by the poorer communities.

Notwithstanding the large southern African plant biodiversity and history of traditional plant use (Van Wyk, 2002), relatively limited scientific work has been done on the medicinal plants of this region (George *et al*., 2001; Van Vuuren, 2008). Recently, more information on the biological activity and chemistry of plants used in traditional medicine in southern Africa have been documented (Van Vuuren, 2008) and several studies provided scientific support for the use of various African plants for treating infections and diseases (Fennel *et al*., 2004a). Among the best known South African medicinal plant species that have contributed to herbal medicines used world-wide are *Aloe ferox* (aloe)*, Agathosoma betulina* (buchu)*, Harpagophytum procumbens* (devil's claw)*, Sutherlandia frutescens* (cancer bush), *Hoodia gordonii* (ghaap) and *Aspalathus betulina* (rooibos tea) (George *et al*., 2001; Van Wyk, 2002; Gurib-Fakim, 2006). Many more medicinal plants have been identified in the first African herbal Pharmacopoeia (Brendler *et al*., 2010).

Contrary to expectations, thousands of publications on antimicrobial activity of plant extracts have not led to the development of any new commercial antimicrobial compounds of significant importance world-wide (Gertch, 2009). Many large-scale screening programmes of the past failed to produce worthwhile plant-derived antimicrobial pharmaceutical products (Lewis and Ausubel, 2006; Eloff and McGaw, in press). Consequently, the major pharmaceutical companies have lost interest in screening higher plants for their biological potential (Cordell and Colvard, 2005).

According to Eloff and McGaw (in press) reasons for the failure of these screening programmes could be that inaccurate and misleading methods such as agar diffusion were used by many inexperienced researchers. Many scientists following traditional healers, used an inefficient extractant such as water while it has been shown that the antimicrobial compounds are usually intermediate polarity compounds that are not extracted by water (Kotze and Eloff, 2002; Eloff *et al*., 2005). Furthermore, several publications considered minimum inhibitory concentrations (MIC's) higher than 5 mg/ml as active (Eloff, 2004; Rios and Recio, 2005; Cos *et al*., 2006; Gertch, 2009).

Another possible reason for inefficient screening programmes is that the majority of scientists selected plant species for research studies based on traditional knowledge. Traditional healers typically use aqueous extracts of plants which, as stated above, generally have very low activities (Rabe and van Staden, 1997; Kotze and Eloff, 2002). Water does not extract the antimicrobial compounds that usually have an intermediate or non-polar character (Kotze and Eloff, 2002). The activity of aqueous extracts used by traditional healers may be indirect by stimulating the immune system of the host rather than killing the pathogens. Aqueous extracts of plant species used in ethnomedicine may therefore not have high direct antimicrobial activity and scientists may therefore have focused on the wrong species.

Moreover, many scientists focused on the isolation of compounds not recognising that phytomedicines contain a mixture of compounds that often acts synergistically (van Wyk and Wink, 2004). Experience in the Phytomedicine Programme showed that antimicrobial compounds isolated from extracts never had the expected activity based on the activity of crude extracts and fractions (Eloff *et al*., 2008). This is probably because plant metabolites may work in combination with other compounds to regulate microbial infections and may therefore not be effective alone (Lewis and Ausubel, 2006).

For these reasons coupled with the large number of plant species that have not yet been examined for their antimicrobial activities and the urgent need to discover new antimicrobial agents, a randomly wide-screening of southern African plant species to identify promising antimicrobial plant extracts as leads for further in-depth research is justified. Such an approach has not been followed in southern Africa before, except partially in the work of Noristan Pharmaceuticals (Fourie *et al*., 1992). The methods they used to determine antimicrobial activity are now outdated, thereby rendering comparisons impossible.

A wide-screening approach offered the potential to discover plants with antimicrobial activities that are available in rural areas but which are not used traditionally. It also reduced the administrative complications of investigating plants based on its use in traditional medicine due to the new legislation to prevent biopiracy in South Africa (Eloff and McGaw, in press). Promising tree species may be tested further in depth for potential as phytomedicines in the herbal medicine industry. Although not the main aim of this study, the probability of isolating compounds with the potential to be used as new drugs for the treatment of infectious diseases would be improved.

There is often a correlation between the presence of secondary compounds in taxonomically closely related taxa (Wink, 2003; Heinrich *et al*., 2004). To improve the selection process for future studies, the study also aimed to ascertain which genera, families and orders have the highest antimicrobial activities that may yield useful products for the herbal medicine of animal

health markets. Given that a related taxon may contain similar or related pharmacological compounds and therefore similar bioactivity, the correlation between taxonomy and antimicrobial activity was investigated. Further studies could then focus on related taxa in promising families and orders to facilitate and improve the selection process.

## 1.2 Literature review

A substantial amount of relevant research is available in the literature and this review will investigate several major themes relating to the study. The first section presents an assessment of infectious diseases and the development of antimicrobials from plants, followed by a brief summary of the sources of antimicrobial activity in plants. Thereafter the use of plant extracts versus isolated compounds as phytomedicine is reviewed as well as the inconsistencies in the *in vitro* determination of antimicrobial activity. Previous antibacterial and antimicrobial screening of plant extracts and the different approaches for selecting plants for antimicrobial screening are discussed. Finally, the correlation between phylogenetic relationships and pharmacological activity is examined.

### 1.2.1 Infectious diseases and the development of antimicrobials from plants

Bacterial infections are the main cause of deaths in the developing countries (Iwu *et al*., 1999). The incidence of resistance to antibiotics intensified in bacteria driven by the unwise and excessive use of antimicrobials especially in developing countries where antibiotics are easy accessible without prescription (Levy and Marchall, 2004). This increased the need to find alternative, safe and effective antibacterial agents to treat infections. Furthermore, the highly efficient permeability barrier of Gram-negative bacteria has been largely responsible that no new classes of broad-spectrum compounds that are equally active against Gram-positive and Gram-negative bacteria were produced. The last class of broad-spectrum compounds that were discovered by the pharmaceutical industry was fluoroquinolones, some 40 years ago (Lewis and Ausubel, 2006).

Similarly, infections caused by fungal pathogens have increased in South Africa as well as world-wide during the last few decades, particularly under immune-compromised patients (Rex *et al*., 1997; McCarthy *et al*., 2006). In animals, systemic fungal infections often pose problems for veterinarians as drugs are generally unavailable (Hector, 2005). Drugs used in the treatment of fungal infections are deficient due to toxic side effects and furthermore the organisms developed resistance towards these drugs (Enoch *et al*., 2006; Pitman *et al*., 2011). This increased the need to also find alternative, safe, effective broad spectrum antifungal agents.

It is estimated that more than 75% of the antibacterials in clinical use are of natural origin, mostly from fungal sources (Newman *et al*., 2003). However, there is considerable potential to develop ant-infective agents from plants, especially against infections that are presently difficult to treat (Iwu *et al*., 1999). These plant-based materials might inhibit bacteria through mechanisms other than conventionally used antibiotics and therefore they may be valuable in the treatment of microbes that have developed a resistance to antibiotics in use (Eloff, 1998b; Lewis and Ausubel, 2006). According to Lewis and Ausubel (2006), it may be possible to develop different types of antibacterials from plants such as traditional antibiotics, multi drug resistant inhibitors and compounds that target bacterial virulence.

### 1.2.2 Sources of antimicrobial activity in plants

Because plants are sessile and unable to avoid or fight their natural enemies they have evolved chemical defence mechanisms to protect themselves against herbivores, worms, insects and microorganisms like bacteria, fungi and viruses (Moerman and Estabrook, 2003; Wink, 2003). The success of plant defence mechanisms in combating pathogen infections is evident in the scarcity of disease in wild plants (Lewis and Ausubel, 2006). The active ingredients in plants are chemical compounds that act directly or indirectly to treat or prevent diseases (Van Wyk *et al*., 1997, Verpoorte, 1998). These chemicals, mostly secondary metabolites, interact with the physiology state of the attacking organism (Moerman and Estabrook, 2003).

Secondary metabolites are produced by all higher plants but are not directly involved in basic essential processes for example photosynthesis or respiration (Theis and Lerdau, 2003). It was first believed that secondary metabolites were produced as waste products with no apparent function, but later it was recognised that these secondary metabolites also act as chemical defence against pests and diseases (Verpoorte, 1998) and to attract pollinating or seed dispersal animals (Wink, 2003).

Plants have an almost limitless ability to synthesise secondary metabolites (Van Wyk and Wink, 2004). Verpoorte (1998) estimated that more than 100 000 secondary metabolites have been isolated from natural products and taken up in the NAPRALERT database and he extrapolated that at least a million more compounds could be isolated from plant species. In a plant taxon, the secondary metabolites form a complex pattern and a few major compounds usually dominate within a specific taxon (Wink 2003). Among the variety of secondary metabolites, three main classes can be distinguished: phenolics, alkaloids and terpenes, which all have antimicrobial activities (Cowan, 1999; Cos *et al*., 2006).

Cox (1994) regarded the pharmacological activity of most plants as occurring by chance, but according to Verpoorte (1998) secondary metabolites formed as a consequence of natural

selection. Jones and Firn (1991) hypothesise that even though a compound is active against an organism in one plant species and the same compound is found in related plant species it does not necessary indicate that the particular compound specifically evolved as a defence mechanism. They proposed further that plants with the greatest chemical diversity may have an increased probability of producing active compounds and that plants will retain inactive compounds since they increase the probability of producing different active compounds. Similarly, Douwes *et al*. (2008) recommended that plant taxa with high chemical diversity would provide the greatest potential for drug development.

The patterns among the secondary metabolites may change which will have an influence the bioactivity of the plant. Factors such as genotypes, the physiological growth cycle of the plant, different development stages and environmental conditions like the soil structure, daily and seasonal changes and phenotypic differences between younger and older parts of the plants, may cause variations in secondary metabolites (Verpoorte, 1998, Wink, 2003). In addition, infected plants may contain a different combination of compounds compared to a healthy plant (Wink, 2003).

There is substantial evidence that there is a correlation between the taxonomy and biological activity of plant families. Some angiosperm families such as the Apocynaceae, Euphorbiaceae, Menispermaceae and Solanaceae contain a high concentration of biologically active compounds (Balick, 1990). Other families are known to be rich in alkaloids such as Rubiaceae, Fabaceae, Boraginaceae, Apocynaceae, Asteraceae, Rutaceae, Papaveraceae, Amaryllidaceae, Berberidaceae, Ranunculaceae and Solanaceae (Van Wyk and Wink, 2004).

### 1.2.3 The use of plant extracts versus isolated compounds as phytomedicine

The pharmacological activity of medicinal plants is very complex and a series of closely related compounds is apparently responsible for the chemical defences. This combination of different compounds can give the plant protection against several organisms in which case no single active compound can be isolated (Verpoorte, 1998). This mixture of certain compounds in phytomedicines often has an additive or synergistic effect (Van Wyk and Wink, 2004). Synergy in biological systems occurs when the effect of two or more compounds applied together is greater than the sum of the effects when identical amounts of each compound are used (Houghton and Raman, 1998) and that may explain why the activity of an extract is frequently greater than that of pure isolated compounds (Williamson, 2001).

Furthermore, phytomedicines may contain substances which are not active themselves but they enhance the activity of the antimicrobials (Gilbert and Alves, 2003; Kalemba and Kunicka, 2003). This synergy may provide the plant with protection from degradation by enzymes of an

active substance; it may increase the efficacy of the crude drug by providing signals to the cells of the host; it may overcome multi-drug resistance systems; and it may also aid transport across cell and membrane walls (Gilbert and Alves, 2003). In addition, there may also be plant substances present that stimulate the immune system of animal hosts (Cowan, 1999; Gilbert and Alves, 2003).

### 1.2.4 Inconsistencies in the *in vitro* determination of antimicrobial activity

Antimicrobial activity identified by *in vitro* tests provides a basic understanding of a plant's efficacy. Although numerous papers and studies on antimicrobial agents have been published over the years, one of the main problems is the lack of uniformity in the criteria selected to study the activity (Rios and Recio, 2005; Manou *et al*., 1998).

First of all, several different methods are used to determine antimicrobial activity *in vitro* and the results obtained between the different methods may vary. In earlier work, many researchers used the agar diffusion technique which measures the size of the zone inhibiting the test pathogen (diameter in mm). However, several factors may have an influence on the inhibition zone. This method is inadequate if the extracts are poorly soluble such as non-polar extracts (Rios *et al*., 1988). This is because if there are antimicrobial compounds with different polarities in the extract, the non-polar compounds will diffuse very slowly in the aqueous agar medium (Eloff and McGaw, in press). The other two methods commonly used are agar and broth dilution methods, both quantitative dilution methods (Rios *et al*., 1988; Kalemba and Kunicka, 2003; Van Vuuren, 2008). In these assays, the activity of the plant extracts is measured as the mimimum inhibitory concentration (mg/ml) which is defined as the lowest concentration inhibiting microbial growth (Wiegand *et al*., 2008). According to Rios *et al*. (1988), the liquid dilution method is the most accurate technique to establish the real potency.

As mentioned before, results obtained between the different methods may vary. A study by Klančnik *et al*. (2010) on essential oils found that the MIC values obtained by the agar dilution method (MIC in mg/ml) were generally lower than those obtained by the agar diffusion method (diameter in mm). In another study there was a very low correlation between the antimicrobial actvity using the serial broth dilution methods and agar diffusion (Eloff and McGaw, in press). Among the dilution methods, a good correlation was found in the sensitivity for antibacterial activity of essential oils measured against Gram-positive bacteria between the agar and broth dilution method. However, agar dilution methods was less sensitive than serial broth microdilution methods for Gram-negative bacteria, where a lower concentration of plant extract was sufficient for growth inhibition by the broth dilution method (Klančnik *et al*., 2010). Samie *et al*. (2005) reported that the disc diffusion and microdilution methods gave conflicting results. In their study, extracts of *Ximenia caffra* presented no inhibition zone while with the microdilution

method, MIC's of 6 mg/ml was recorded. However, 6 mg/ml could be considered as a very low activity.

In addition to the different methods used, the results of antibacterial activities are also influenced by different ways of defining MIC's (Burt, 2004). A common problem when reporting activity of plant extracts is that positive activity is claimed for excessively high concentrations of plant extract. This was highlighted by Ríos and Recio (2005) who reported that some papers claim activity with concentrations higher than 1 mg/ml (1000 µg/ml) for extracts. Salvat *et al*. (2001) reported extracts as active at MIC's of 0.5 mg/ml (500 µg/ml) or lower and Buwa and Van Staden (2006) reported MIC values of 0.39 mg/ml as high antibacterial activities and MIC's of 0.78 mg/ml as good antibacterial activities against several bacterial and fungal pathogens. Similarly, Gertch (2009) found that in a large number of ethnopharmacological papers use concentrations higher than 0.2 mg/ml (200 µg/ml) as noteworthy activity.

Several recent authors recommended that plant extracts should have an inhibitory concentration of below 0.1 mg/ml (100 µg/ml) for extracts and 25 µM for pure compounds to be considered worthwhile as a promising lead (Eloff, 2004; Rios and Recio, 2005; Cos *et al*., 2006; Gertsch, 2009). This concentration is now widely accepted as a benchmark for activity. However, the concentration has not been scientifically determined and it remains under discussion.

Taking all these factors into consideration, it is therefore difficult to compare antimicrobial activity of plant extracts in different studies because methods that are not equally sensitive were used.

### 1.2.5 Antibacterial and antifungal screening

A large number of plant extracts were screened over the last few decades against several bacteria in an attempt to discover plant extracts or compounds that would be effective in the treatment of bacterial infections. The plant extracts and compounds exhibited activities against a large number of bacterial species, including sensitive and resistant strains. A review by Gibbons (2004) identified 116 single chemical compounds isolated from plants with MIC's lower than 64 µg/ml against different *Staphylococci*, some had activities lower than 1 µg/ml.

Several studies focused on the antibacterial activities of plants from Africa. One such study by Vlietinck *et al*. (1995) investigated 100 plant species from Rwanda, using both agar diffusion and dilution assays. They found that 45% of the plant extracts were active against *S. aureus*, 2% against *E. coli*, 16% against *P. aeruginosa* and 7% against *C. albicans*. In another study, Fankam *et al*. (2011) evaluated the antibacterial activity of extracts of 11 plants from Cameroon

against Gram-negative bacteria, including multi-drug resistant strains, and found MIC values ranging from 32 to 1024 µg/ml.

More specifically in southern African, ethnopharmacological developments were reviewed by Light *et al.* (2005) and Van Vuuren (2008). In other published studies, a number of southern African plant species were identified with promising antibacterial activities and numerous antibacterial compounds were isolated (Rabe and Van Staden, 1997; Grierson and Afolayan, 1999; Fyhrquist, *et al*., 2002; Masika and Afolayan, 2002; Fennel *et al*., 2004a; Eloff *et al*., 2005; Buwa and Van Staden, 2006; Eloff and McGaw, 2006, 2008; Mathabe *et al*., 2006).

Some studies focused specifically on plants used in ethnoveterinary medicine. One such study by Masika and Afolayan (2002), recorded MIC's of 0.1 mg/ml and higher against Gram-positive bacteria for extracts of three plant species used in veterinary medicine. In another study McGaw *et al.,* 2007 reported activities less than 0.1 mg/ml for a third of the 17 ethnoveterinary plants screened against a panel of nosocomial bacteria.

Although most studies focused on antibacterial screening there was an increase in the last decade in the number of published papers investigating the antifungal activities of plant extracts in the two leading journals in this field (Sortino *et al.,* 2012). Their review provided the structures of 89 antifungal compounds that have been isolated. Abad *et al*. (2007) found that the antifungal studies of plants were performed mainly on members of the Asteraceae and Liliaceae, but also on several plants which belonged to the families Fabaceae (Leguminosaceae), Rutaceae, Myrtaceae, Lamiaceae, Combretaceae, Zingiberaceae, Amaryllidaecae and Euphorbiaceae. Furthermore, Sortino *et al.* (2012) examined the antifungal studies registered in the NAPRALERT database and found that Solanaceae, Rutaceae, Brassicaceae and Lamiaceae had the largest percentage of active species among the families investigated.

In southern Africa, the number of papers reporting on antifungal research also increased and several plant extracts were reported to have promising antifungal activities (Masika and Afolayan, 2002, Motsei *et al*., 2003, Buwa and Van Staden, 2006, Samie *et al*., 2010). Numerous studies focused on species of the family Combretaceae and confirmed the ethnomedicinal use of several members of this family (Fyhrquist, *et al*., 2004, Masoko *et al*., 2005).

The majority of these antibacterial and antifungal studies were based on plants used in traditional medicine including veterinary medicine. Although these studies provided promising results, it is not enough considering the large amount of African plants which have not yet been screened (Karou *et al*., 2007). In the southern African context this statement was confirmed by

McGaw and Eloff (2008) who indicated that more plants need to be evaluated for ethnoveterinary use given the rich biodiversity and the widespread traditional use of plants as medicine.

Considering the tens of thousands of plant species throughout the world, researchers adopted several approaches to select plants for antimicrobial screening and those approaches may be separated into random and targeted approaches (Farnsworth and Bingel, 1977; Balick, 1990; Cox, 1990; Khafagi and Dewedar, 2000).

The random approach involves the collection of as many different plant species as possible found in a study area. No consideration is taken of taxonomical relationships, ethnobotanical uses and/or other qualities (Balick, 1990; Khafagi and Dewedar, 2000). This approach has led to the discovery of a number of useful drugs (George *et al*., 2001).

A narrower approach is to target certain plant species based on different criteria. The most common targeted approach is ethnodirected and is based on traditional medicinal uses of a plant. Plants used traditionally as medicine for specific diseases are identified and tested to validate their use scientifically. Ethnomedicinal leads have earlier resulted in the discovery of several important drugs (Houghton and Raman, 1998). In other targeted approaches, such as the phytochemical approach the aim is to collect and screen all the species known to be rich in specific bioactive compounds. while the chemotaxonomic and phylogenetic targeted approaches are based on the collection of a number of close relatives or of all the species in the same plant family that are known to contain useful compounds (Cotton, 1996) because similar activities are expected of related plants due to similarities in secondary metabolites (Douwes *et al*., 2008). However, according Pezzuto (1997) the approach to use chemotaxonomic and phytochemical relationships in the selection process is not directed towards the discovery of new therapeutic compounds.

In another targeted approach, described as a plant-ecological approach, the focus is on plants with particular characteristics, for example specific growth form, leaf size, plants in a particular habitat, etc. Many of the plants used traditionally today were likely selected based on early plant-ecological observations. One such example is the preparation of insecticides from plants without insect infestations (Verpoorte, 1998).

The question is which approach must be followed to discover new leads for drug development? All these screening approaches have specific drawbacks that may result in certain plants or groups of plants being excluded as leads. Many screening programmes in the 1960's randomly screened plants for anti-cancer activities which had, according to Cox and Balick (1994), an

extremely low success rate and is less productive than the ethnodirected approach (Balick, 1994).

In recent years, the ethnodirected approach was applied by a number of researchers in their bioprospecting endeavours (Cox and Balick, 1994; Farnsworth *et al*., 1985; Farnsworth, 1990; Light *et al*., 2005). All these studies showed that ethnodirected selections have a far higher chance of success than random selection procedures. Khafagi and Dewedar (2000) also found that 83% of plants collected by the ethnodirected approach showed antimicrobial activity, compared to only 42% of plants collected by the random method. Unfortunately they did not compare water extracts used traditionally and they used a 20 mg/ml agar diffusion assay that makes it very difficult to compare with MIC's. Similarly, Svetaz *et al*. (2010) investigated the antifungal activity of 327 plant species from seven Latin American countries and found a higher percentage of plants with antifungal activities among extracts of plants used to treat fungal infections compared to plants not used medicinally. In addition, the number of hits was higher against dermatophytic infections that were easily diagnosed by traditional healers compared to other fungus infections that were less easy to diagnose. In South Africa, results of a study by Rabe and Van Staden (1997) also supported the concept that the etnobotanical approach in screening plants for bioactivity is successful.

However, according to George *et al*. (2001) the search for drugs by means of ethnobotanical leads is cost and time effective but that random screening also deliver leads and cannot be ignored. The ethnomedicinal approach may not necessarily always yield plants with high antimicrobial activities and the strict pursuit of ethnomedicinal leads only might have led to important drugs being missed, for example camptothecin and homoharringtonine (Cragg *et al*., 1994). Studies conducted in the Phytomedicine Programme at the University of Pretoria (UP) established that in many cases species with high antibacterial activities were not used by rural communities, even though the plant species occur in their area.

In a study by the National Cancer Institute in the USA the number of hits obtained by ethnobotanical leads was compared to the number of hits with plants collected randomly. They found that initially it seemed that ethnobotanical leads were more effective, but after dereplication, no differences were found (Balick, 1994). Similarly, Eloff and McGaw (in press) found no significant differences in antibacterial activity between the trees randomly selected and the trees used traditionally. However, the study compared trees that were used for general medicinal purposes and a different set of results could have been expected if trees only used against microbial infections were compared.

Because water is usually the only extractant available to rural inhabitants, and water does not extract, it is not surprising that ethnobotanical leads may not necessarily always yield high

antimicrobial activities. In more than 90% of cases the antibacterial compounds were relatively non-polar compounds and practically no antibacterial activity was found in the water extracts (Kotze and Eloff, 2002). In a study by Rabe and Van Staden (1997), only 15% of the 27 water extracts of ethnomedicinal plants of southern Africa were found to have active antimicrobial activities. Similarly, Clarkson *et al.* (2004) reported that most of the water extracts did not have any antiplasmodial activity whereas extractants such as DCM and MeOH had the greatest activity.

To conclude, the urgent need to find novel agents against diseases requires as many sources as possible from which to search (Cragg *et al*., 1994). A random or wide screening approach has the advantage that active compounds may be discovered from plants with unfavourable characteristics, like toxic plants, that are not used traditionally (Khafagi and Dewedar, 2000).

### 1.2.6 Phylogenetic relationships and biological activity

A correlation often exists between the occurrences of certain secondary compounds in taxonomically related taxa. Species in the same plant order, family and genera may have inherited chemical compounds for defence against other organisms from common ancestors (Wink, 2003; Heinrich *et al*., 2004). Therefore, the systematic position of a plant used traditionally as a medicine allows some interpretations to be made about the biologically active compounds possibly present in the species (Heinrich *et al*., 2004).

The incidence of certain types of secondary metabolites is usually restricted to a few families or genera (Verpoorte, 1998) and close relatives may display similar pharmacological activities. Many secondary compounds are used as taxonomic markers because of their restricted distribution (Bennet and Wallsgrove, 1994). In a monophyletic clade almost all members share the same chemical characteristics (Wink, 2003). Similar bioactivities are therefore expected of related plants due to similarities in secondary metabolites (Douwes *et al*., 2008). This was evident in a collaborative study by the United States National Cancer Institute and Department of Agriculture which collected a broad taxonomic range of plants at random and tested it for anti-tumour activity. It was found that certain families such as Apocynaceae and Rutaceae were better sources of active extracts while other families provided few active extracts (Cragg *et al*., 1994). This knowledge can be applied to screen related species of a plant with promising activity (Verpoorte, 1998)

Similarly, some plant families and orders are regarded by ethnobotanists as superior sources of plant medicine. It could therefore be that plants in related taxa share favourable traits or similar types of compounds. For example, the Combretaceae is a relatively small family but is used quite extensively by traditional healers in KwaZulu-Natal (Eloff, 1998a). The preference of some

families by traditional healers was also confirmed in a study by Moerman and Estabrook (2003) who reported that indigenous people in North America selected particular families as medicinal plants regardless of family size. Families such as Asteraceae, Rosaceae and Solanaceae were used extensively. The study also identified large families that are ignored as medicinal plants, most notably the family Poaceae. Interesting, the family Rubiaceae was a minor source of medicinal plants in North America even though important drugs were developed from this family.

Locally, a study by Douwes (2005) on southern African plant species identified families that hold significantly more ethnomedicinal taxa. Families such as Euphorbiaceae, Rubiaceae, Malvaceae, Anacardiaceae, Fabaceae and Asteraceae were identified. In addition, Douwes *et al.* (2008) identified seven “hot” plant orders such as Malpigiales, Fabales, Gentianales, Asterales, Solanales, Malvales and Sapindales that have significantly more taxa used ethnomedicinally. Furthermore, the study also identified that the orders Rosales, Proteales, Poales, Asparagales and Caryphyllales were used less than predicted. Highly diverse bioactives were found in the plant families from the “hot” plant orders and showed that these taxa are selected traditionally on the basis of bioactivity, which is reflected in chemical diversity.

However, the occurrence of certain secondary metabolites is not always restricted and may occur in several unrelated groups (Wink, 2003). This may be due to the independent development of the responsible gene (convergence) or alternatively it could be due to genes that are switched on and off during specific environmental stress factors (Wink, 2003). Theis and Lerdau (2003), using terpenes as a case study, postulated that the evolution of metabolic functions can occur as a result of change at any level of biological organisation. Factors that are involved in functional shifts are spatial distribution of terpenes within plants, changes in genetic architecture and changes in gene regulation that changes terpene quantities

## 1.3 Aim and objectives

### 1.3.1 Aim

The main aim of the study was to facilitate the discovery of plant extracts with high activities that may yield products that can be used to combat microbial infections in animals and humans. We designed the study to firstly randomly screen extracts of a large number of southern African tree species for their antimicrobial activity against different pathogens and secondly to determine whether antimicrobial activity is associated with plant taxonomy. The rationale for the latter aim was that taxa with general high activity could offer more promising leads. If correlations were to be found, it could lead to a better guided approach in selecting tree species from promising families for continuing studies rather than based on ethnopharmacology. This information could

also be useful for plants growing outside South Africa. A number of closely related species, assumed to contain related active biochemical substances could then be screened. This would accelerate the discovery of plants with bioactive compounds. The focus was to sample leaves of representatives of all southern African tree genera and families and to use a standardised method for analysing the antimicrobial activities.

The final purpose of this study was to develop antimicrobial extracts rather than isolating single compounds since plant extracts have the advantage of synergistic effects and would be cheaper and more freely available. In cases where a very active extract is discovered, we will isolate and characterise the active compounds for quality control.The study was part of a preliminary extensive screening programme of the Phytomedicine Programme, Department Paraclinical Sciences, University of Pretoria. Preliminary data obtained in this study provided material for several MSc and PhD projects for students in the Phytomedicine Programme. In one case a patent application is in preparation.

### 1.3.2 Objectives

To achieve the aim, the following objectives, listed below, were addressed:

1. To screen leaf extracts of several hundred tree species for antimicrobial activity against six important pathogens.
2. To determine a standard to establish what concentration of extract can be considered to have significant antimicrobial activity based on the antimicrobial activities of a large number of tree leaf extracts.
3. To identify tree species and genera with high antibacterial and/or antifungal activities against six important pathogens.
4. To evaluate the overall susceptibility of the six different pathogens used in the study to acetone leaf extracts of several hundred southern African tree species.
5. To determine if there are correlations between the activities of tree leaf extracts against different pathogens
6. To identify tree families with the best likelihoods of delivering extracts with high antimicrobial activity against Gram-positive bacteria, Gram-negative bacteria and fungi.
7. To identify tree orders with the best likelihoods of delivering extracts with high antimicrobial activity against Gram-positive bacteria, Gram-negative bacteria and fungi.
8. To analyse and interpret intra-taxa variation in the context of a wide screening approach.

## 1.4 Scope and limitations of the present study

This study covered trees from the floristic area of southern Africa which has an enormous biodiversity and land area. It was practically impossible to screen all 24 000 southern African plant species (Germishuizen and Meyer, 2003; Light *et al*., 2005). In order to reduce this project to be more manageable, the study focused on tree species because the identification of trees is easier and can be done with a higher degree of certainty.

In addition, the project also had the following limiting factors in order to be more practicable:

### 1.4.1 Plant sample material

Different parts of a plant could yield different antimicrobial activities (Benli *et al*., 2007). However, if leaves, roots, bark and flowers were sampled, it would complicate the project and quadruple the number of analyses. Collection of flowers would have been impractical due to flowering seasons that vary significantly among species. Moreover, because of the destructive nature of harvesting roots and bark, it would have been difficult to get permission from nature conservation and landowners and would have ruled out the possibility of collecting plants in botanical gardens. It is also easier to recollect leaves from the same plant for follow up work. The most important reason for selecting leaves is because leaves represent a renewable and abundant resource to develop useful antimicrobial extracts. However, in some cases the leaves may not be the plant part with the most promising activities and promising antimicrobial results found by analysing the leaf extracts may not be applicable to other plant parts.

### 1.4.2 Sample size

Given that the population of tree species in the southern African region approximates 2 100 (Van Wyk *et al.,* 2011), time and financial constraints limited the scope of this project to a sample thereof. To reach a wide representation of the tree population, the project was structured to sample at least one tree species per genus. In larger genera, more than one species per genus were sampled. The 537 species included in this study therefore approximate 25 per cent of the tree species occurring in southern Africa. Statistical analysis of the data to determine taxonomic relationships had to exclude those minor families and orders of which the sample sizes were too small.

### 1.4.3 Variability in bioactivity

The bioactivity of a plant can be affected by different factors. Firstly, the patterns of secondary metabolites may change between different development stages and between different tissues and organs which may influence the bioactivity of the plant. Furthermore, infections or wounding

of a plant can also activate the synthesis of different compounds which could have an effect on the activity of the plant extract (Verpoorte, 1998; Wink, 2003). Secondly, the bioactivity of plants may vary due to external environmental conditions such as daily and seasonal changes and soil structure (Benli *et al.,* 2007; Würger, 2011). Consequently, the same chemotype will produce a different chemical profile depending on the environment (Houghton and Raman, 1998). Finally, due to genetic variability, intra-specific differences in antimicrobial activities may occur between individuals and populations (Verpoorte, 1998).

In was not possible to account for all these variants at this wide level of screening in this study. However, to counteract for some of the variation, we collected leaves mainly during the active growing season, we limited the geographical area to a few botanical gardens and we sampled from mature and disease-free trees only.

### 1.4.4 Solvent used in extraction process

To reduce the number of assays, only one extractant was used. However, there is not a single solvent that are able to extract all compounds from a plant. In this study, acetone was used as an extractant mainly due to its ability to extract compounds of a wide range of polarities, its non-toxicity to bioassay systems and its ease of removal from extracts (Eloff, 1998a). By using more extractants, different quantities and types of compounds could have been extracted (Verpoorte, 1998).

### 1.4.5 Panel of microbes

In this study, the panel of microbial organisms was limited to six pathogenic species representing Gram-positive bacteria, Gram-negative bacteria and fungi and should be expanded in future work and also include resistant bacteria and fungi. The four bacteria used (*Staphylococcus aureus, Enterococcus faecalis, Escherichia coli* and *Pseudomonas aeruginosa*) were the four isolates recommended by the National Committee for Clinical Laboratory Standards (National Committee for Clinical Laboratory Standards (NCCLS), 1992) to evaluate antibiotics. Two of the most important disease-causing fungi in animals, *Candida albicans* and *Cryptococcus neoformans* were used (Fan-Harvard *et al*., 1991).

### 1.4.6 Cytotoxicity

Antimicrobial activity reported in this study did not discriminate on the basis of specific cell toxicity and could be related to a general toxicity. Therefore, promising extracts *in vitro* may be cytotoxic. For a plant extract and/or compound to be developed as a potential drug it has to be efficient as well as non-toxic and future studies should include cytotoxicity studies in parallel.

About 30% of the failures in the development of drugs have been associated with toxicity and safety issues (Kola and Landis, 2004).

Extracts with selective toxicity against one or only a few pathogens may possibly not be toxic against animal cells, whereas extracts with antimicrobial activity against many pathogens may contain a general metabolic toxin that could also be toxic to human cells.

## 1.5 Organisation of the study

The dissertation is organised into ten chapters. Chapter 2 outlines the study area and taxonomy applied in the study. Chapter 3 provides a detailed description of the data base including the taxa collected (tree species, genera, families and orders). Chapter 4 outlines the general procedures that were followed to determine the antimicrobial activities of the tree extracts.

In Chapter 5, the first aim was to determine the MIC's that should be considered as significant based on a large number of assays and secondly, the aim was to identify species with promising activities based on specific criteria. The susceptibility of the different pathogens used in the study, and the correlation between antimicrobial activities of the different pathogens is investigated in Chapter 6.

The antimicrobial activities of the families are compared in Chapter 7 to enable us to determine if species within some of the families have a higher combined antibacterial activity. In the same way, the mean antimicrobial activities of the orders are compared in Chapter 8. Chapter 9 investigated several families and orders to provide an insight into the intra-family and intra-order variation in the context of wide screening.

Finally, a summary of the results and discussion on some possible future research is presented in Chapter 10.

# CHAPTER 2

# Study area, material and taxonomy

## 2.1 Study area and study material

The study covered trees from the floristic area of southern Africa, south of the Kunene, Okavango and Zambezi rivers. The area includes the countries of South Africa, Lesotho, Swaziland, Namibia, Botswana, Zimbabwe and a part of Mozambique. This is a large area, covering several climatic zones and a wide range of vegetation types (Light *et al*., 2005; Van Wyk *et al*., 2011). An estimated 24 000 higher plant taxa of 368 families are recorded in the Flora of Southern Africa comprising more than 10% of the world's vascular plant flora (Germishuizen and Meyer, 2003). According to Goldblatt (1978) about 80% of these plants are endemic to this region.

It was practically impossible to screen all the approximately 24 000 southern African plant species (Germishuizen and Meyer, 2003). In order to reduce this project to more feasible dimensions the study focused on extracts of tree species because identification of trees is easier and can be done with a higher degree of certainty. We sampled leaf material only because leaves are a renewable resource and it is also easier to recollect leaves from the same plant for follow-up work. Furthermore, if roots, bark or flowers were to be sampled in addition to leaves, it would have complicated the project and quadrupled the number of analyses. Collection of flowers would have been impractical due to the flowering seasons that vary significantly among species. Moreover, because of the destructive nature of harvesting roots and bark, it would have been difficult to get permission from nature conservation and landowners and would probably have ruled out the possibility of collecting plants in botanical gardens.

A total of about 448 genera and 2 100 tree species are found in southern Africa (Van Wyk *et al.,* 2011). In practice the distinction between a tree and a shrub is not always very clear and the 2 100 species include trees with a single main stem, multi-stemmed trees, marginal trees which are closer to shrubs as well as robust, woody climbers (Van Wyk *et al*., 2011).

## 2.2 Plant taxonomy and classification

### 2.2.1 Background

Plant classification systems group plants into organised categories to show relationships between plants and evolves continuously as new knowledge becomes available. Different opinions exist of how plants should be categorised and different plant classification systems, each with its own arrangement of the genera, species and families, are used internationally. Traditional plant classifications systems were based on morphological and biochemical characteristics of the plants. Some systems that were frequently used include: Cronquist system, Thorne system and Takhtajan system. The advent of genetic evidence led to new classification systems which grouped plants based on relationships determined mostly by DNA analyses.

Higher-level classifications have always been problematic and are still uncertain. A recent publication by Chase and Reveal (2009), which was published in association with the Angiosperm Phylogeny Group III classification (APG III), placed all land plants in the class Equisitopsida with 16 major subclasses. Based on this classification system, the trees in southern Africa are grouped into five subclasses: Polypodiidae (tree ferns), Magnoliidae (angiosperms), Cycadidae, Gnetidae and Pinidae (gymnosperms). The Magnoliidae (angiosperms) is the largest group and very diverse morphologically and ecologically (Soltis and Soltis 2004).

In recent years angiosperm taxonomy underwent significant changes. The APG classification was first published in 1998 followed up by two revised and updated versions. The latest update was published in 2009 and is currently widely accepted. The APG III classification retains the Linnean system of orders and families. The system formally recognises orders and families, but not groupings above order level, for which the informal term “clade” is used (APG, 1998). One of the criteria for formal recognition in the APG classification is that a group has to be monophyletic, i.e. include all the descendants of a common ancestor. The traditional division of angiosperms into monocotyledon and dicotyledon plants were changed with the APG system. Although the monocotyledon plants were recognised as a separate clade, the dicotyledon plants were not. Three main groups (unranked clades) described by the APG III system are the magnoliids, monocots and eudicots (APG III, 2009).

Several orders are recognised in each unranked clade and increased from 40 to 45 and 59 respectively in the 1998, 2003 and 2009 publications. Well-supported monophyletic families that were not placed in an order in the first publication were grouped into new orders in the APG II and III systems. Newly presented orders that are of importance in this study are Canellales,

Gunnerales, Crossosomatales and Celastrales (APG II), as well as Bruniales, Buxales, Escalloniales, Vitales and Zygophyllales (APG III).

The first APG classification system recognised 462 families which were reduced to 457 families in the APG II because several families were restructured and placed into orders and some families, in the orders Asparagales, Lamiales and Malpighiales, were recircumscribed (APG II, 2003). In addition, the family traditionally known as Euphorbiaceae was split into several smaller families because it was not a monophyletic group. Five different families based on this split are now recognised by the APG III system, i.e. Euphorbiaceae, Pandanaceae, Phyllanthaceae, Picrodendraceae and Putranjivaceae (APG II, 2003; APG III, 2009).

Although some new families were recognised in the APG III, several changes resulted in fewer families (413 compared to 462) in total. Unplaced families were substantially reduced although some families were still unplaced, e.g. Boraginaceae and Icacinaceae. Some families were not recognised any longer, e.g. Heteropyxidaceae, Maesaceae, Myrsinaceae, Oliniaceae and Rhynchocalycaceae. Some other recent changes under the APG III system of importance for this study are the placement of the genus *Chloristylis* (=*Itea*) under the family Iteaceae (order Saxifragales). It was formely placed under the family Escalloniaceae and, the family Bruniaceae was placed in its own order, Bruniales.

From the above instances, it is clear that the classification of plants is still uncertain and the placement of genera and species within families and orders is continuously changing. Many of these adjustments were made during the course of the study. It is therefore necessary to state what taxonomic treatment, especially at suprageneric and suprafamilial levels, was applied since one of the aims of this study was to investigate to what degree antimicrobial activities are associated with taxonomic relations between different trees. A brief overview of the taxonomy applied in this study is provided in sections 2.2.2 (orders) and 2.2.3 (families).

### 2.2.2 Taxonomical arrangement and circumscription of the southern African tree orders

The order level classification of the subclasses Polypodiidae, Cycadidae, Gnetidae and Pinidae, traditionally known as tree ferns and gymnosperms, followed the SANBI (South African National Biodiversity Institute) Internet Taxonomy System of the Trees of southern Africa (SANBI, 2008). All the orders in these four subclasses are listed in Table 2.1. The order level classification of the subclass Magnoliidae (angiosperms) followed the APG III (2009) classification system and is summarised in Table 2.2. Grouping of the trees of southern Africa at suprafamilial (order) level resulted in a total of 44 orders.

**Table 2.1:** The taxonomic arrangement of the southern African tree orders of the subclasses Polypodiidae, Cycadidae, Gnetidae and Pinidae (tree ferns and gymnosperms). The orders are arranged alphabetically ([1] = order not represented in study; [2] = family not represented in study).

| Order | Subclass / Traditional group | Family |
|---|---|---|
| Coniferales | Pinidae / gymnosperm | Cupressaceae |
| Cyatheales | Polypodiidae / tree fern | Cyatheaceae |
| Cycadales | Cycadidae / gymnosperm | Cycadaceae, Zamiaceae |
| Pinales | Pinidae / gymnosperm | Podocarpaceae |
| [1]Welwitschiales | Gnetidae / gymnosperm | [2]Welwitschiaceae |

**Table 2.2:** The taxonomic arrangement of the southern African tree orders of the subclass Magnoliidae (angiosperms). The orders are arranged alphabetically ([1] = order not represented in study; [2] = family not represented in study).

| Order | Family |
|---|---|
| Apiales Nakai | Apiaceae, Araliaceae, Pittosporaceae |
| Aquifoliales Senft | Aquifoliaceae |
| Arecales Bromhead | Arecaceae |
| Asparagales Link | Asphodelaceae, Dracaenaceae |
| Asterales Link | Asteraceae |
| Brassicales Bromhead | Capparaceae, Moringaceae, [2]Salvadoraceae |
| Bruniales Dumort. | Bruniaceae |
| Buxales Takht. ex Reveal | Buxaceae |
| Canellales Cronquist | Canellaceae |
| Caryophyllales Juss. ex Bercht. & J.Presl | [2]Chenopodiaceae, [2]Mesembryanthemacea, [2]Nyctaginaceae, Portulacaceae, [2]Tamaricaceae |
| Celastrales Link | Celastraceae |
| Cornales Link. | [2]Cornaceae, Curtisiaceae |
| Crossosomatales Takht. ex Reveal | Aphloiaceae |
| Ericales Bercht. & J.Presl | Ebenaceae, Ericaceae, Lecythidaceae, Maesaceae, Myrsinaceae, Sapotaceae |
| Fabales Bromhead | Fabaceae, Polygalaceae |

| Order | Family |
|---|---|
| Fagales Engl. | Myricaceae |
| Gentianales Juss. ex Bercht. & J.Presl | Apocynaceae, [2]Asclepiadaceae, Gentianaceae, Rubiaceae, Strychnaceae |
| Geraniales Juss. ex Bercht. & J.Presl | Greyiaceae, Melianthaceae |
| [1]Huerteales Doweld | [2]Gerrardinaceae |
| Lamiales Bromhead | Acanthaceae, [2]Avicenniaceae, Bignoniaceae, Buddlejaceae, Lamiaceae, Oleaceae, [2]Pedaliaceae, Scrophulariaceae, Verbenaceae |
| Laurales Juss. ex Bercht. & J.Presl | Hernandiaceae, Lauraceae, [2]Monimiaceae |
| Magnoliales Juss. ex Bercht. & J.Presl | Annonaceae |
| Malpighiales Juss. ex Bercht. & J.Presl | Chrysobalanaceae, Clusiaceae, Dichapetalaceae, Erythroxylaceae, Euphorbiaceae, Flacourtiaceae, [2]Hypericaceae, Kiggelariaceae, [2]Linaceae, Malpighiaceae, Ochnaceae, [2]Passifloraceae, Phyllanthaceae, Picrodendraceae, Putranjivaceae, Rhizophoraceae, Salicaceae, [2]Turneraceae, Violaceae |
| Malvales Juss. ex Bercht. & J.Presl | Bombaceae, [2]Brownlowiaceae, [2]Dipterocarpaceae, Helicteraceae, Malvaceae, Pentapetaceae, Sparrmanniaceae, Sterculiaceae, Thymelaeaceae |
| Myrtales Juss. ex Bercht. & J.Presl | Combretaceae, Heteropyxidaceae, Lythraceae, Melastomataceae, Myrtaceae, Oliniaceae, [2]Rhynchocalycaceae, [2]Sonneratiaceae |
| Oxalidales Bercht. & J.Presl | [2]Connaraceae, Cunoniaceae |
| Pandanales R.Br. ex Bercht. & J.Presl | Pandanaceae |
| [1]Piperales Bercht. & J.Presl | [2]Piperaceae |
| [1]Poales Small | [2]Poaceae |
| Proteales Juss. ex Bercht. & J.Presl | Proteaceae |
| [1]Ranunculales Juss. ex Bercht. & J.Presl | [2]Menispermaceae |
| Rosales Bercht. & J.Presl | Cecropiaceae, Celtidaceae, Moraceae, Rhamnaceae, Rosaceae, Urticaceae |
| Santalales R.Br. ex Bercht. & J.Presl | Olacaceae, [2]Opiliaceae, [2]Santalaceae |
| Sapindales Juss. ex Bercht. & J.Presl | Anacardiaceae, Burseraceae, Kirkiaceae, Meliaceae, Ptaeroxylaceae, Rutaceae, Sapindaceae |
| Saxifragales Bercht. & J.Presl | Crassulaceae, Hamamelidaceae, Iteaceae |
| [1]Solanales Juss. ex Bercht. & J.Presl | [2]Montiniaceae, [2]Solanaceae |
| Vitales Juss. ex Bercht. & J.Presl | Vitaceae |
| Zingiberales Griseb. | Musaceae, Strelitziaceae |
| Zygophyllales Link | Balanitaceae, [2]Zygophyllaceae |

### 2.2.3 Taxonomical arrangement and circumscription of the southern African tree families

For the purpose of the study, the genera were grouped into families following Van Wyk *et al*. (2011), who adapted the APG III system for southern African plant taxonomic practices. An exception was made in the case of the families Caesalpiniaceae, Fabaceae (in a narrow sense) and Mimosaceae which were grouped into one family (Fabaceae); with Caesalpinioideae, Faboideae (Papilionoideae) and Mimosoideae recognised as subfamilies (APG III, 2009). Based on this classification system, the trees of southern Africa are grouped into approximately 133 families (Van Wyk *et al*., 2011). The traditionally known tree ferns are represented by one family (Cyatheaceae), the gymnosperms by four families (Cupressaceae, Podocarpaceae, Welwitschiaceae and Zamiaceae), while the remainder of the families are all angiosperms.

A condensed list of the circuscription of the families and genera of the trees of southern Africa, as applied in this study, is shown in Table 2.3. A detailed version is tabled in Appendix A, Table A.1, in which differences with the APG III classification system are noted and alternative placings of families, as described by Van Wyk *et al.* (2011), are given.

The most important differences with the APG III classification are summarised below:

(1) The family Salicaceae is used in a narrow sense and contains only one species, *Salix mucronata*. In the APG system, the Salicaceae is expanded to include several genera previously placed under Flacourtiaceae.

(2) The family Flacourtiaceae is retained whereas the APG system places some of the genera in other families (mainly Salicaceae).

(3) The circumscription of the family Scrophulariaceae is as follows:

- *Anastrabe, Bowkeria, Halleria* and *Ixianthes* are placed in Scrophulariaceae and in a broad sense under Stilbaceae. In the APG III system, these genera are placed under Stilbaceae.
- *Buddleja, Gomphostigma* and *Manuleopsis* are placed in their own family, Buddlejaceae (in a narrow sense) and in Scrophulariaceae (in a broad sense). In the APG III system these genera are all placed in Scrophulariaceae.

(4) Families Bombaceae, Brownlowiaceae, Helicteraceae, Pentapetaceae, Sparrmanniaceae and Sterculiaceae are retained as separate families and are not included in Malvaceae. The family Malvaceae is used in the narrow sense and include only a few genera. The circumscription of the Malvaceae in APG III system is broader and includes previous families such as Bombaceae, Sterculiaceae and Tiliaceae.

**Table 2.3:** Arrangement of families representing the tree species of southern Africa. The families are arranged alphabetically (bold font - no extracts from the entire genus and/or family were analysed; [1] = tree species within the genus and/or family are found outside South African borders only; [2] = classified in different family under APG III; [3] = non-indigenous tree species).

| Family | Genus |
|---|---|
| Acanthaceae Juss. | *Anisotes, Brillantaisia, Duvernoia, Justicia, Mackaya, Metarungia, Sclerochiton* |
| Anacardiaceae R.Br. | *Harpephyllum, Heeria, Lannea, Laurophyllus, Loxostylis, Ozoroa, Protorhus, Searsia, Sclerocarya, Smodingium* |
| Annonaceae Juss. | *Annona, Artabotrys, Cleistochlamys, Friesodielsia, Hexalobus, Monanthotaxis, Monodora, Sphaerocoryne, Uvaria, Xylopia* |
| Aphloiaceae Takht. | *Aphloia* |
| Apiaceae Lindl. | *Heteromorpha, Polemannia, Polemanniopsis, Steganotaenia* |
| Apocynaceae Juss. | *Acokanthera, Adenium, Anchylobotrys, Baissea, Callichilia, Carissa, Diplorhynchus, Furtumia, Gonioma, Holarrhena, Landolphia, Mascarenhasia, Mondia, Oncinotus, Pachypodium, Pleiocarpa, Pleioceras, Rauvolfia, Strophantus, Tabernaemontana, Voacanga, Wrightia, [3]Pachypodium* |
| Aquifoliaceae Bercht. & J.Presl | *Ilex* |
| Araliaceae Juss. | *Cussonia, Polyscias, Schefflera, Seemannaralia* |
| Arecaceae Bercht. & J.Presl | *Borassus, Hyphaene, Jubaeopsis, Phoenix, Raphia* |
| [1]Asclepiadaceae R.Br. | *[1]Fockea* |
| [2]Asphodelaceae Juss | *Aloe* |
| Asteraceae Bercht. & J.Presl | *Berkheya, Brachylaena, Chrysanthemoides, Didelta, Distephanus, Euryops, Lopholaena, Metalasia, Microglossa, Oldenburgia, Osmitopsis, Othonna, Psiadia, Senecio, Solanecio, Tarchonanthus, Vernonia, Zoutpansbergia* |
| [2]Avicenniaceae End.ex Schnizl | *Avicennia* |
| Balanitaceae Endl | *Balanites* |
| Bignoniaceae Juss. | *Catophractes, Dolichandrone, Fernandoa, Kigelia, Markhamia, Podranea, Rhigozum, Stereospermum, Tecomaria (=Tecoma)* |
| [2]Bombaceae Kunth | *Adansonia* |
| Boraginaceae Juss. | *Cordia, Ehretia* |
| Brownlowiaceae | *[1]Carpodiptera* |
| Bruniaceae R.Br. ex DC. | *Berzelia, Raspalia* |
| [2]Buddlejaceae Wilhelm | *Buddleja, Gomphostigma, Nuxia* |
| Burseraceae Kunth | *Commiphora* |

| Family | Genus |
|---|---|
| Buxaceae Dumort. | *Buxus* |
| Canellaceae Mart. | *Warburgia* |
| Capparaceae Juss. | *Bachmannia*, *Boscia*, *Cadaba*, *Capparis*, *Cladostemon*, *Maerua*, *Thilachium* |
| [2]Cecropiaceae C.Berg | *Myrianthus* |
| Celastraceae R.Br. | *Allocassine*, *Brexia*, *Cassine*, *Catha*, *Elaeodendron*, *Gloveria*, *Gymnosporia*, *Hippobromus*, *Lauridia*, *Lydenburgia*, *Maurocenia*, *Maytenus*, *Mystroxylon*, *Pleurostylia*, *Pseudosalacia*, *Pterocelastrus*, *Putterlickia*, *Robsonodendron*, *Salacia* |
| [2]Celtidaceae Engl. | *Celtis*, *Chaetacme*, *Trema* |
| [2]Chenopodiaceae Vent | *Salsola* |
| Chrysobalanaceae R.Br. | *Maranthes*, *Parinari* |
| [2]Clusiaceae Lindl. | *Garcinia*, *Harungana*, *Hypericum*, *Psorospermum* |
| Combretaceae R.Br. | *Combretum*, *Pteleopsis*, *Terminalia*, *Lumnitzera*, [3]*Quisqualis*, [3]*Bucida* |
| Connaraceae R.Br. | *Cnestis*, *Rourea* |
| [1]Cornaceae Takht. | [1]*Afrocrania*, [1]*Alangium* |
| Crassulaceae J.St.-Hil. | *Crassula*, *Tylecodon* |
| Cunoniaceae R.Br. | *Cunonia*, *Platylophus* |
| Cupressaceae Bartlett | *Widdringtonia*, *Juniperus* |
| Curtisiaceae Takht | *Curtisia* |
| Cyatheaceae | *Cyathea* |
| Cycadaceae Persoon | *Cycas* |
| Dichapetalaceae Baill. | *Tapura* |
| Dipterocarpaceae Blume | *Monotes* |
| Dracaenaceae R.A. Salisbury | *Dracaena* |
| Ebenaceae Gürke | *Diospyros*, *Euclea* |
| Ericaceae Juss. | *Erica*, *Vaccinium* |
| Erythroxylaceae Kunth | *Erythroxylum*, *Nectaropetalum* |
| Euphorbiaceae Juss. | *Acalypha*, *Alchornea*, *Argomuellera*, *Cavacoa*, *Croton*, *Erythrococca*, *Euphorbia*, *Excoecaria*, *Macaranga*, *Maprounea*, *Micrococca*, *Necepsia*, *Sapium*, *Schinziophyton*, *Sclerocroton*, *Shirakiopsis*, *Spirostachys*, *Suregada*, *Synadenium*, *Tannodia*, [3]*Jatropha* |

| Family | Genus |
|---|---|
| Fabaceae Lindl. (in broad sense) | Subclass Caesalpinioideae: *Adenolobus*, *Afzelia*, *Baikiaea*, *Bauhinia*, *Brachystegia*, *Burkea*, *Caesalpinia*, *Cassia*, *Colophospermum*, *Dialum*, *Erythrophleum*, *Guibourtia*, *Haematoxylon*, *Hymenaea*, *Julbernardia*, *Parkinsonia*, *Peltophorum*, *Piliostigma*, *Pterolobium*, *Schotia*, *Senna*, *Umtiza*, [3]*Tamarindus*<br>Subclass Mimosoideae: *Acacia*, *Adenopodia*, *Albizia*, *Amblygonocarpus*, *Dichrostachys*, *Elephantorrhiza*, *Entada*, *Faidherbia*, *Newtonia*, *Xylia*, [3]*Leucaenia*<br>Subclass Papilionoideae: *Aeschynomene*, *Baphia*, [1]*Baphiopsis*, *Bolusanthus*, *Calpurnia*, *Cordyla*, *Craibia*, *Crotalaria*, *Cyclopia*, *Crotalaria*, *Dalbergia*, *Erythrina*, *Flemingia*, *Hypocalyptus*, *Indigofera*, *Millettia*, *Mundulea*, *Ormocarpum*, *Otholobium*, *Philenoptera*, *Podalyria*, *Psoralea*, *Pterocarpus*, *Rhynchosia*, *Sesbania*, *Sophora*, *Stirtonanthus*, *Swartzia*, *Tephrosia*, *Virgilia*, *Wiborgia*, *Xanthocercis*, *Xeroderris* |
| [2]Flacourtiaceae (in narrow sense) | *Casearia*, *Dovyalis*, *Flacourtia*, *Homalium*, *Oncoba*, *Pseudoscolopia*, *Scolopia*, *Trimeria* |
| Gentianaceae Juss. | *Anthocleista* |
| [1]Gerrardinaceae Alford | [1]*Gerrardina* |
| [2]Greyiaceae (Gürke) Hutch | *Greyia* |
| Hamamelidaceae R.Br. | *Trichocladus* |
| [2]Helicteraceae J. Agardh | [1]*Triplochiton* |
| Hernandiaceae Blume | *Gyrocarpus* |
| [2]Heteropyxidaceae Engler & Gilg | *Heteropyxis* |
| Icacinaceae Miers | *Apodytes*, *Cassinopsis* |
| Iteaceae J.Agardh | *Choristylis* |
| [2]Kiggelariaceae Link | *Kiggelaria*, *Rawsonia*, *Xylotheca* |
| Kirkiaceae Takht. | *Kirkia* |
| Lamiaceae Martinov | *Achyrospermum*, *Clerodendrum*, *Hemizygia*, *Karomia*, *Plectranthus*, *Premna*, *Rotheca*, *Syncolostemon*, *Tetradenia*, *Tinnea*, *Vitex* |
| Lauraceae Juss. | *Cryptocarya*, *Dahlgrenodendron*, *Ocotea* |
| Lecythidaceae A.Rich. | *Barringtonia* |
| Linaceae DC. ex Perleb | *Hugonia* |
| Lythraceae J.St.-Hil. | *Galpinia* |
| [2]Maesaceae Anderb.,B. Stähl & Källersjö | *Maesa* |
| Malpighiaceae Juss. | *Acridocarpus*, *Triaspis* |
| Malvaceae Juss. | *Abutilon*, *Azanza*, *Hibiscus*, [3]*Pavonia*, *Thespesia* |
| Melastomataceae Juss. | *Dissotis*, *Memecylon*, *Warneckea* |
| Meliaceae Juss. | *Ekebergia*, *Entandrophragma*, *Khaya*, *Lovoa*, *Nymania*, *Pseudobersama*, *Trichilia*, |

| Family | Genus |
|---|---|
| | *Turraea, Xylocarpus* |
| Melianthaceae Horan. | *Bersama* |
| Menispermaceae Juss. | *Cocculus, Tiliacora, Tinospora* |
| [2]Mesembryanthemaceae Fenzl | *Stoeberia, Mestoklema* |
| Monimiaceae Juss. | *Xymalos* |
| Montiniaceae Nakai | *Montinia* |
| Moraceae Gaudich. | *Ficus, Maclura, Morus, Trilepisium* |
| Moringaceae Martinov | *Moringa* |
| Musaceae Juss. | *Ensete* |
| Myricaceae A.Rich. ex Kunth | *Morella* |
| [2]Myrsinaceae R.Br. | *Embelia, Myrsine, Rapanea* |
| Myrtaceae Juss. | *Eugenia, Metrosideros, Syzygium* |
| Nyctaginaceae Juss. | *Phaeoptilum, Pisonia* |
| Ochnaceae DC. | *Brackenridgea, Ochna* |
| Olacaceae R.Br. | *Olax, Strombosia, Ximenia* |
| Oleaceae Hoffmanns. & Link | *Chionanthus, Olea, Schrebera, [3]Jasminum* |
| [2]Oliniaceae Arn.ex Sond. | *Olinia* |
| Opiliaceae Valeton | *Opilia* |
| Pandanaceae R.Br. | *Pandanus* |
| Passifloraceae Juss. ex Roussel | *Paropsia, Adenia* |
| Pedaliaceae R.Br. | *Sesamothamnus* |
| [2]Pentapetaceae Bercht & J. Presl. | *Dombeya* |
| Phyllanthaceae Martinov | *Antidesma, Bridelia, Cleistanthus, Flueggea, Heywoodia, Hymenocardia, Lachnostylis, Margaritaria, Phyllanthus, Pseudolachnostylis, Uapaca* |
| Picrodendraceae Small | *Androstachys, Hyaenanche* |
| Piperaceae C.A. Agardh | *Piper* |
| Pittosporaceae R.Br. | *Pittosporum* |
| Poaceae Barnhart | *Thamnocalamus, Oreobambos, Oxytenanthera* |
| Podocarpaceae Endl. | *Podocarpus* |
| Polygalaceae Hoffmanns. & Link | *Carpolobia, Nylandtia, Polygala, Securidaca* |

| Family | Genus |
|---|---|
| Portulacaceae Juss. | *Ceraria*, *Portulacaria* |
| Proteaceae Juss. | *Brabejum*, *Faurea*, *Leucadendron*, *Leucospermum*, *Mimetes*, *Paranomus*, *Protea*, [3]*Grevillea* |
| [2]Ptaeroxylaceae Sonder | *Ptaeroxylon* |
| Putranjivaceae Meisn. | *Drypetes* |
| Rhamnaceae Juss. | *Berchemia*, ***Colubrina***, ***Helinus***, ***Noltea***, ***Lasiodiscus***, *Phylica*, *Rhamnus*, ***Scutia***, *Ziziphus* |
| Rhizophoraceae Pers. | ***Bruguiera***, *Cassipourea*, ***Ceriops***, ***Rhizophora*** |
| [2]Rhynchocalycaceae L.A.S.Johnson | ***Rhynchocalyx*** |
| Rosaceae Juss. | ***Cliffortia***, *Leucosidea*, *Prunus* |
| Rubiaceae Juss. | ***Afrocanthium***, ***Aidia***, ***Anthospermum***, *Alberta*, *Breonadia*, *Burchellia*, ***Burttdavya***, *Canthium (=Plectroniella)*, ***Carphalea***, *Catunaregam*, *Cephalanthus*, ***Chassalia***, ***Coddia***, *Coptosperma*, ***Craterispermum***, *Cremaspora*, ***Crossopteryx***, ***Didymosalpinx***, *Feretia*, *Gardenia*, ***Guettarda***, ***Heinsenia***, *Hymenodictyon*, *Hyperacanthus*, *Ixora*, *Keetia*, *Kraussia*, *Lagynias*, ***Lasianthus***, ***Leptactina***, ***Mitriostigma***, ***Multidentia***, *Mussaenda*, *Oxyanthus*, ***Pachystigma***, ***Pauridiantha***, *Pavetta*, ***Polysphaeria***, *Psychotria*, *Psydrax*, *Pyrostria*, *Rothmannia*, ***Rytigynia***, ***Rutidea***, ***Tapiphyllum***, *Tarenna*, *Tricalysia (=Empogona)*, ***Sericanthe***, *Vangueria*, ***Vangueriopsis***, [3]*Coffea* |
| Rutaceae Juss. | *Calodendrum*, ***Citropsis***, *Clausena*, *Coleonema*, ***Empleurum***, ***Fagaropsis***, *Oricia*, *Teclea*, *Toddalia*, *Toddaliopsis*, *Vepris*, *Zanthoxylum* |
| Salicaceae Mirb. | *Salix* |
| Salvadoraceae Lindl. | *Azima*, *Salvadora* |
| *Santalaceae* R.Br. | *Osyris* |
| Sapindaceae Juss. | *Allophylus*, *Aporrhiza*, *Atalaya*, *Blighia*, *Deinbollia*, *Dodonaea*, *Erythrophysa*, ***Filicium***, ***Glenniea***, *Haplocoelum*, *Hippobromus*, ***Lecaniodiscus***, ***Lepisanthes***, ***Macphersonia***, *Pancovia*, *Pappea*, *Smelophyllum*, *Stadmannia*, *Zanha* |
| Sapotaceae Juss. | *Chrysophyllum*, *Englerophytum*, *Inhambanella*, *Manilkara*, *Mimusops*, ***Pouteria***, *Sideroxylon Synsepalum*, *Vitellariopsis* |
| Scrophulariaceae Juss. | *Anastrabe*, ***Antherothamnus***, *Bowkeria*, ***Manuleopsis***, *Freylina*, *Halleria*, ***Ixianthes*** |
| Solanaceae Juss. | *Solanum* |
| [1]Sonneratiaceae Engl. & Gilg | [1]*Sonneratia* |
| [2]Sparrmanniaceae J. Agardh | ***Glyphaea***, *Grewia*, *Sparrmannia* |
| [2]Sterculiaceae Vent. | *Cola*, ***Heritiera***, *Sterculia* |
| Strelitziaceae Hutch. | *Strelitzia* |
| [2]Strychnaceae Link. | *Strychnos* |

| Family | Genus |
|---|---|
| Tamaricaceae Link | *Tamarix* |
| Thymelaeaceae Juss. | *Dais, Englerodaphne, Passerina, Peddiea, Synaptolepis* |
| [1]Turneraceae DC. | *[1]Turnera* |
| Urticaceae Juss. | *Obetia, Pouzolzia, Urera* |
| Verbenaceae J.St.-Hil. (in narrow sense) | *Lippia, [3]Lantana* |
| Violaceae Batsch | *Rinorea* |
| Vitaceae Juss. | *Cissus, Cyphostemma, Rhoicissus* |
| [1]Welwitschiaceae | *[1]Welwitschia* |
| Zamiaceae Horaninow | *Encephalartos* |
| [1]Zygophyllaceae R.Br. | *[1]Neoluederitzia* |

### 2.2.4 Taxonomical arrangement and circumscription of southern African tree genera and species

The trees of southern Africa comprise a total of 2 100 tree species arranged in 530 genera. Taxa names at genus and species level followed the PRECIS (National Herbarium Pretoria (PRE) Computerised Information System) database (SANBI, 2005). If applicable, the species were subdivided into subspecies (subsp.). Other infraspecific taxa, e.g. varieties (var.) and forms (f) were not recognised.

## 2.3 Size scale of southern African tree orders and families

Plant classification results in an unequal distribution of representative species per family and order. Consequently some plant families and orders are very large containing several genera and species, while others contain only one or a few species. Two of the largest plant families are Asteraceae with an estimated 1 620 genera globally and Fabaceae with 745 genera, each genus encompassing several thousands of species (Brummit, 1992; SysTax, online data system). In contrast, the family Aphloiaceae comprises of only one species (Brummit, 1992; Stevens, 2001 onwards).

In this study, the boundaries of the study moderated the size of the families and orders. For example, we focused only on tree species as opposed to all higher plant species and the study

area was limited to southern Africa. Therefore, we determined the sizes of southern African tree families and orders measured by the number of genera.

Based on Van Wyk *et al*. (2011) and Coates Palgrave (2005), the orders encompassing tree species in southern Africa contained from 1 to 77 genera per order (Coates Palgrave, 2005; Van Wyk *et al*., 2011). The largest tree orders were Gentianales (77 genera), Fabales (76 genera), Malpigiales (74 genera) and Sapindales (56 genera).

Within families, the number of southern African tree genera varied from 1 to 72 per family. The largest tree families in the southern African region were Fabaceae and Rubiaceae with respectively 72 and 53 tree genera. Other large families were Euphorbiaceae (25 genera), Apocynaceae (22 genera), Celastraceae and Sapindaceae (19 genera each).

It is surprisingly that the largest order was composed of only five more genera compared to the largest family. Since orders are more inclusive, we expected a larger difference. The largest family, Fabaceae enclosed 72 genera while the largest order, Gentianales consisted of 77 genera. The reason is that very large families were often grouped with very small families in an order whereby the differences are smoothed out. For example the order Fabales encompass two families of which Fabaceae contained 72 tree genera but Polygalaceae contained only four tree genera in the southern African region. Another example is the order Gentianales encompassing 77 genera, grouped into four families. The two smallest families in this order (Strychnaceae and Gentianaceae) each contained a single tree genus while the largest family in the order (Rubiaceae) enclosed 53 tree genera.

The frequency distribution of 40 southern African tree orders and 100 southern African tree families, across size classes and based on the number of genera, are shown in Figures 2.1 and 2.2 respectively. Twelve of the forty orders enclosed only a single tree genus while ten orders enclosed between two and three genera (Figure 2.1). Overall, only five of the forty orders contained thirty or more genera. There were also more small families compared to large families (Figure 2.2). Among the 100 families, the majority (69) contained three or fewer tree genera. Twenty three of the families contained between four and twelve tree genera and only eight of the families contained more than thirteen tree genera.

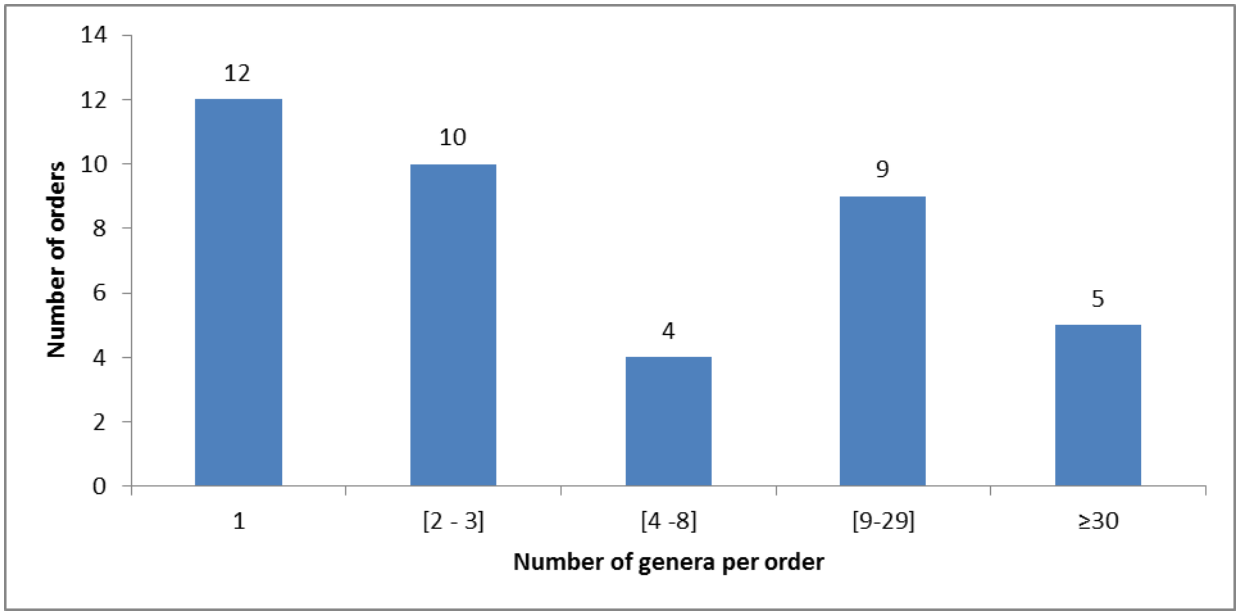


**Fig. 2.1:** Frequency distribution of southern African tree orders across size classes based on number of genera. The genera were grouped into size classes: [1]; [2 to 3]; [4 to 8]; [9 to 29] and [≥30].

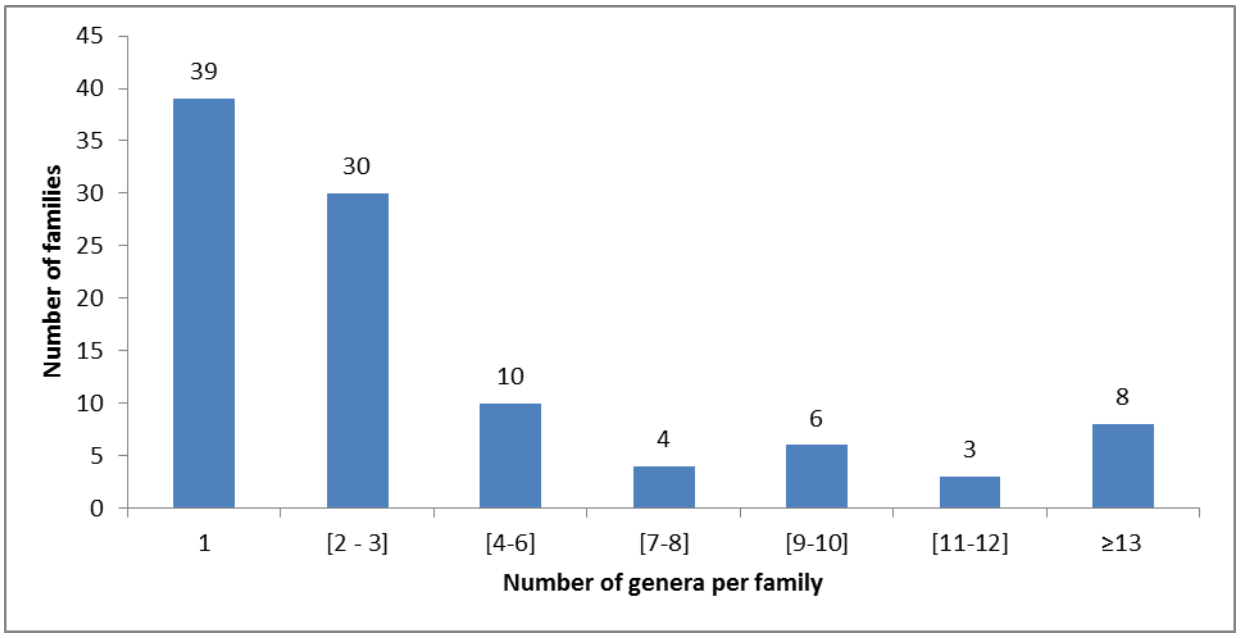


**Fig. 2.2:** Frequency distribution of southern African tree families across size classes based on number of genera. The genera were grouped into size classes: [1]; [2 to 3]; [4 to 6]; [7 to 8]; [9 to 10] and [≥13].

## 2.4 Conclusions

Many adjustments in the classification of plants occurred during recent years. For the purpose of the study, the families were grouped by mainly following Van Wyk *et al*. (2011) which is based largely on the APG III classification system, but conformed to South African plant taxonomy practices. The order level classification mostly followed APG III (2009). Based on these classification systems, approximately 2 100 tree species, belonging to 530 genera, 133 families and 44 orders occur in southern Africa. The largest number of tree species is grouped into the subclass Magnoliidae (angiosperms).

Classification systems assemble families and orders containing unequal numbers of species. In this study, the size differences were furthermore influenced by the boundaries of the study. We found large differences in sizes between the families representing the trees of southern Africa. Similar size differences occurred between the orders. The largest southern African tree orders were Gentianales, Fabales, Malpigiales and Sapindales. while the largest tree families were Fabaceae, Rubiaceae, Euphorbiaceae, Apocynaceae, Celastraceae and Sapindaceae. In addition to size differences, we found that the range of family and order sizes was extremely skewed with far more small than large families and orders.

The next chapter discusses the collection of the tree species and the organisation of the data.

# CHAPTER 3

# Description of the data base

## 3.1 Plant collection

Tree leaf samples of southern African trees were nearly exclusively collected in national botanical gardens of the South African National Biodiversity Institute (SANBI) and regional botanical gardens across South Africa. These gardens were selected based on easy access to facilitate the recollection of leaves from the same tree for future collections to expand the work. Several collection trips were undertaken between May 2004 and March 2009 to sample the leaf material.

The national and regional botanical gardens where most of the tree leaf samples were collected are listed below:

- Pretoria National Botanical Garden, SANBI, Pretoria
- Lowveld National Botanical Garden, SANBI, Nelspruit
- KwaZulu-Natal National Botanical Garden, SANBI, Durban
- Kirstenbosch National Botanical Garden, SANBI, Cape Town
- Manie van der Schijff Botanical Garden, University of Pretoria, Pretoria

We sampled leaf material only because it was easier to access and it is also easier to recollect leaves from the same plant for follow up work. If a product is to be developed from the plant material, leaves of trees will be a rational and sustainable resource. The identification of the leaf material was verified by the respective botanical garden herbariums. In the botanical gardens most trees were labeled and voucher specimens of the trees along with collection data and origin of collection are kept in the respective national botanical garden herbarium. GPS-coordinates were recorded for unlabeled trees. In all cases voucher specimens are also stored in the HGWJ Schweickerdt Herbarium of the University of Pretoria.

The collection of samples that provided the leaf material for the study has been labeled the Phytomedicine Tree Database (PMDB), Paraclinical Sciences, Faculty of Veterinary Sciences, University of Pretoria.

## 3.2 Tree species, genera, families and orders collected for the database

As outlined in Chapter 2, we considered approximately 44 orders, 133 families, 530 genera and 2 100 tree species found in the southern African region (Van Wyk *et al.,* 2011). This includes a few marginal (shrub-like) tree species as well as woody climbers.

To screen representative samples of all 2 100 species was not practical due to time and financial restrictions as well as the limited number of trees present in botanical gardens. The target was therefore to collect good representative members of at least one species per genus in a family. However, among the larger genera, we sampled more than one species. This approach reduced the total number of species to a more manageable size. For practical reasons, we sampled only in one southern African country namely South Africa.

In total, samples of 537 species spanning 350 genera, 101 families and 38 orders were collected. A detailed list of the genera, families and orders collected is available from the Phytomedicine Programme, Department Paraclinical Sciences, University of Pretoria.

The collection of samples of some representatives proved to be challenging and in some cases, due to the unavailability of certain tree species during the collection period, several tree species were not collected. Representatives of about 32% of the genera were not sampled because they are found entirely outside the borders of South Africa (e.g. *Borassus, Crossopteryx, Guettarda, Ixianthes, Mondia, Raspalia* and *Rytigynia*) and trees were not accessible in South African botanical gardens. In general, samples of small, immature trees, trees with small, scale-like leaves (e.g. *Phaeoptilum, Salsola* and *Tamarix*) or succulent leaves (e.g. *Sesamothamnus, Stoeberia* and *Tylecodon*) were not sampled as the trees would have been severely damaged. It would in any case be difficult to use these plants sustainably if results were very positive. Sampling of creeping or vine-like growth forms (e.g. *Cocculus, Landolphia, Tiliacora, Tinospora, Adenia* and *Salacia*), mangroves (e.g. *Avicennia, Bruguiera, Ceriops, Lumnitzera* and *Rhizophora*) and bamboos (e.g. *Thamnocalamus* and *Oxytenanthera*) were not collected as part of this study due to the inaccessibility due to their growth forms and/or habitats.

Consequently, a few families were excluded from the Phytomedicine Tree Database but in most cases these families were relatively small, containing only one or two tree genera, each containing few species (Table 3.1). Families such as Avicenniaceae and Rhizophoraceae (both enclose mangroves), Poaceae (bamboo plants) and Menispermaceae (creepers) were not collected. The families Chenopodiaceae, Mesembryanthemaceae and Tamaricaceae contain mainly tree species distributed in the arid regions of South Africa, with small, scale-like or succulent leaves which were not feasible to be sampled. Representatives of at least 15 of the families that were not represented are found entirely outside the borders of South Africa. One of these families, Welwitschiaceae, contains a single protected endemic species.

**Table 3.1:** List of the incomplete tree families and genera in the Phytomedicine Tree Database (Bold font = families and genera that occur entirely outside the borders of South Africa; [1] = entire families that were not represented in the database).

| Family | Tree genera not represented |
|---|---|
| Acanthaceae | *Anisotes*, ***Brillantaisia***, *Justicia* |
| Anacardiaceae | *Laurophyllus* |
| Annonaceae | ***Cleistochlamys***, *Sphaerocoryne* |
| Apiaceae | *Polemannia, Polemanniopsis* |
| Apocynaceae | *Adenium, Anchylobotrys*, ***Baissea***, *Callichilia, Landolphia, Mondia*, ***Pleioceras*** |
| Araliaceae | *Seemannaralia* |
| Arecaceae | *Borassus* |
| [1]Asclepiadaceae | ***Fockea*** |
| Asteraceae | *Berkheya, Didelta, Distephanus, Lopholaena*, ***Microglossa***, *Osmitopsis, Othonna, Psiadia, Senecio, Vernonia, Zoutpansbergia* |
| [1]Avicenniaceae | *Avicennia* |
| Bignoniaceae | *Catophractes*, ***Fernandoa*** |
| [1]Brownlowiaceae | *Carpodiptera* |
| Bruniaceae | *Raspalia* |
| Capparaceae | *Bachmannia, Cadaba* |
| Celastraceae | *Allocassine*, ***Brexia***, *Gloveria, Hippobromus, Lauridia, Robsonodendron, Salacia* |
| [1]Chenopodiaceae | *Salsola* |
| Chrysobalanaceae | *Parinari* |
| Clusiaceae | ***Psorospermum*** |
| Combretaceae | *Lumnitzera* |
| [1]Connaraceae | *Cnestis*, ***Rourea*** |
| [1]Cornaceae | ***Afrocrania***, ***Alangium*** |
| Crassulaceae | *Tylecodon* |
| Cunoniaceae | *Platylophus* |
| [1]Dipterocarpaceae | *Monotes* |
| [1]Dracaenaceae | *Dracaena* |
| Ericaceae | *Vaccinium* |

| Family | Tree genera not represented |
|---|---|
| Euphorbiaceae | *Maprounea*, *Micrococca*, *Sapium*, *Shirakiopsis* |
| Fabaceae | *Adenolobus* , *Dialum*, *Guibourtia*, *Haematoxylon*, *Julbernardia*, *Parkinsonia*, *Piliostigma*, *Pterolobium*, *Adenopodia*, *Amblygonocarpus*, *Aeschynomene*, *Cyclopia*, *Flemingia*, *Hypocalyptus*, *Otholobium*, *Rhynchosia*, *Sesbania*, *Sophora*, *Stirtonanthus*, *Swartzia*, *Tephrosia*, *Wiborgia* |
| Flacourtiaceae | *Casearia*, *Scolopia* |
| [1]Gerrardinaceae | *Gerrardina* |
| [1]Helicteraceae | *Triplochiton* |
| Lamiaceae | *Achyrospermum*, *Hemizygia*, *Plectranthus*, *Premna*, *Rotheca*, *Tetradenia* |
| Lauraceae | *Dahlgrenodendron* |
| [1]Linaceae | *Hugonia* |
| Malpighiaceae | *Triaspis* |
| Meliaceae | *Nymania*, *Xylocarpus* |
| [1]Menispermaceae | *Cocculus*, *Tiliacora*, *Tinospora* |
| [1]Mesembryanthemaceae | *Stoeberia*, *Mestoklema* |
| [1]Monimiaceae | *Xymalos* |
| [1]Montiniaceae | *Montinia* |
| Myrsinaceae | *Embelia* |
| [1]Nyctaginaceae | *Phaeoptilum*, *Pisonia* |
| Ochnaceae | *Brackenridgea* |
| Olacaceae | *Olax* |
| [1]Opiliaceae | *Opilia* |
| [1]Passifloraceae | *Paropsia*, *Adenia* |
| [1]Pedaliaceae | *Sesamothamnus* |
| [1]Piperaceae | *Piper* |
| [1]Poaceae | *Thamnocalamus*, *Oreobambos*, *Oxytenanthera* |
| Polygalaceae | *Carpolobia*, *Nylandtia* |
| Portulacaceae | *Ceraria* |
| Rhamnaceae | *Colubrina*, *Helinus*, *Noltea*, *Lasiodiscus*, *Scutia* |
| Rhizophoraceae | *Bruguiera*, *Ceriops*, *Rhizophora* |
| [1]Rhynchocalycaceae | *Rhynchocalyx* |

| Family | Tree genera not represented |
|---|---|
| Rosaceae | *Cliffortia* |
| Rubiaceae | *Afrocanthium*, ***Aidia***, ***Anthospermum***, ***Burttdavya***, ***Carphalea***, ***Chassalia***, *Coddia*, ***Craterispermum***, *Crossopteryx*, ***Didymosalpinx***, *Guettarda*, *Heinsenia*, ***Lasianthus***, *Leptactina*, *Mitriostigma*, ***Multidentia***, *Pachystigma*, ***Pauridiantha***, ***Polysphaeria***, *Rytigynia*, ***Rutidea***, ***Tapiphyllum***, *Tarenna*, *Tricalysia*, ***Sericanthe***, ***Vangueriopsis*** |
| Rutaceae | ***Citropsis***, *Empleurum*, ***Fagaropsis*** |
| [1]Salvadoraceae | *Azima*, *Salvadora* |
| [1]Santalaceae | *Osyris* |
| Sapindaceae | ***Filicium***, ***Glenniea***, *Lecaniodiscus*, ***Lepisanthes***, ***Macphersonia*** |
| Sapotaceae | ***Pouteria*** |
| Scrophulariaceae | *Antherothamnus*, ***Manuleopsis***, *Ixianthes* |
| [1]Solanaceae | *Solanum* |
| [1]Sonneratiaceae | ***Sonneratia*** |
| Sparrmanniaceae | ***Glyphaea*** |
| [1]Tamaricaceae | *Tamarix* |
| [1]Turneraceae | ***Turnera*** |
| Vitaceae | *Cissus*, ***Cyphostemma*** |
| [1] Welwitschiaceae | ***Welwitschia*** |
| [1]Zygophyllaceae | ***Neoluederitzia*** |

Plant orders of which representatives of entire families were not collected are shown in Table 3.2. The majority of these orders are relatively small and contain only a few families of which representative tree species, for reasons explained above, were not collected. Of these, entire orders that were not represented were Huerteales, Piperales, Poales, Ranunculales, Solanales and Welwitchiales. The order Caryophyllales, consisting of five southern African tree families, was poorly represented and only one representative species of the family Portulacaceae was collected. The other families in this order comprise mostly of single species with features that made sampling impractical (climbers, shrub-like plants, plants with small, scale-like and succulent leaves).

Although representatives of the families Boraginaceae and Icacinaceae were collected, they were excluded from the analysis at order level because both are not yet placed in an order in

the APG III system (APG III, 2009). Nonetheless, they were included in the analyses at the family, genus and species level.

**Table 3.2:** List of orders of which entire families were not represented in the Phytomedicine Tree Database ([1] = total number of southern African tree families per order; [2] = entire orders not represented in the database).

| Order | [1]Number of families | Tree families not represented in database |
|---|---|---|
| Brassicales | 3 | Salvadoraceae |
| Caryophyllales | 5 | Mesembryanthemaceae, Chenopodiaceae, Nyctaginaceae, Tamaricaceae |
| Gentianales | 5 | Asclepiadaceae |
| [2]Huertеales | 1 | Gerrardinaceae |
| Lamiales | 8 | Avicenniaceae, Pedaliaceae |
| Laurales | 3 | Monimiaceae |
| Malpighiales | 19 | Hypericaceae, Linaceae, Passifloraceae, Turneraceae |
| Malvales | 8 | Dipterocarpaceae, Brownlowiaceae |
| Myrtales | 8 | Rhynchocalycaceae, Sonneratiaceae |
| Oxalidales | 2 | Connaraceae |
| [2]Piperales | 1 | Piperaceae |
| [2]Poales | 1 | Poaceae |
| [2]Ranunculales | 1 | Menispermaceae |
| Santalales | 3 | Opiliaceae, Santalaceae |
| [2]Solanales | 2 | Montiniaceae, Solanaceae |
| [2]Welwitschiales | 1 | Welwitschiaceae |
| Zygophyllales | 2 | Zygophyllaceae |

### 3.3 Size scale of families and orders in the database

The number of representative species in the Phytomedicine Tree Database per family ranged from 1 to 55. The largest tree families were Fabaceae and Rubiaceae with 55 and 41 representative species respectively. Other large families were Euphorbiaceae, Apocynaceae, Celastraceae and Sapindaceae. The orders representing the tree species captured for the Phytomedicine Tree Database contained from 1 to 71 tree species per order. The largest orders were Malpighiales (71 tree species), Sapindales (64 tree species), Gentianales (64 tree species) and Fabales (57 tree species).

Frequency distributions, similar to those in Chapter 2.3, were compiled of the families and orders encompassing the tree species collected for the Phytomedicine Tree Database. As shown in Figure 3.1, the largest number of families (63 of 101) contained three or fewer representative species. Twenty four families contained between four and twelve species and only eleven families contained more than thirteen representative species. Among the orders represented in the Phytomedicine Tree Database, 11 of 38 orders enclosed only one representative species while six orders enclosed 30 or more representative tree species (Figure 3.2).

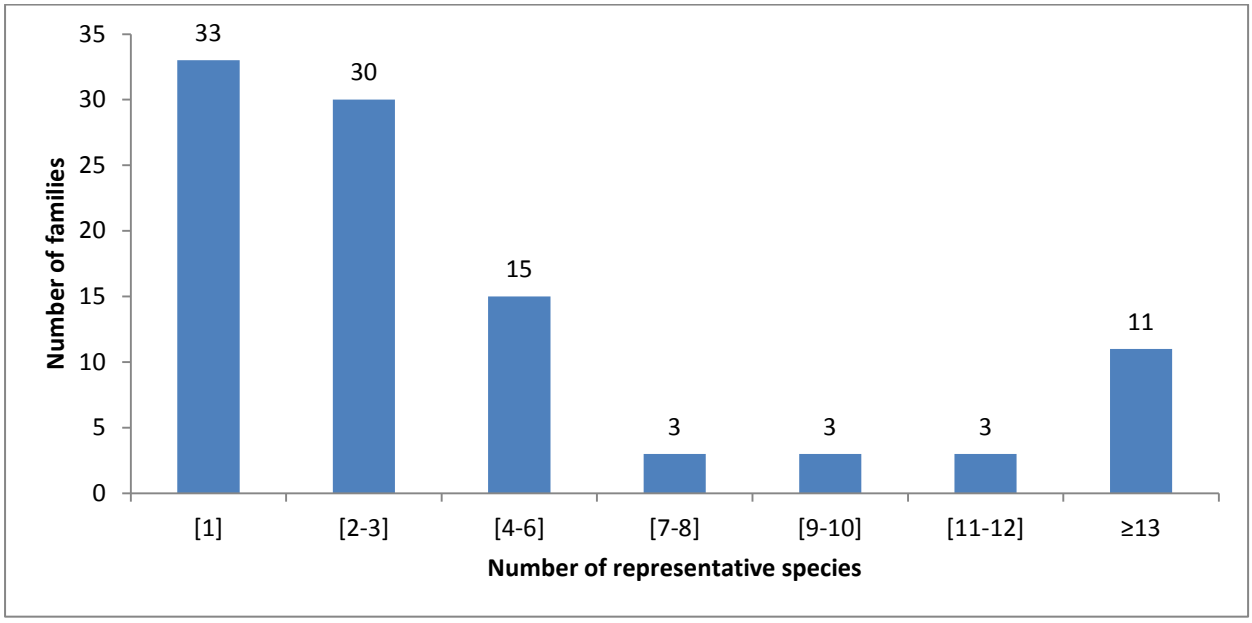


**Fig. 3.1:** Frequency distribution of families across size classes based on the number of tree species in the Phytomedicine Tree Database. The species were grouped into size classes: [1]; [2 to 3]; [4 to 6]; [7 to 8]; [9 to 10] and [≥13].

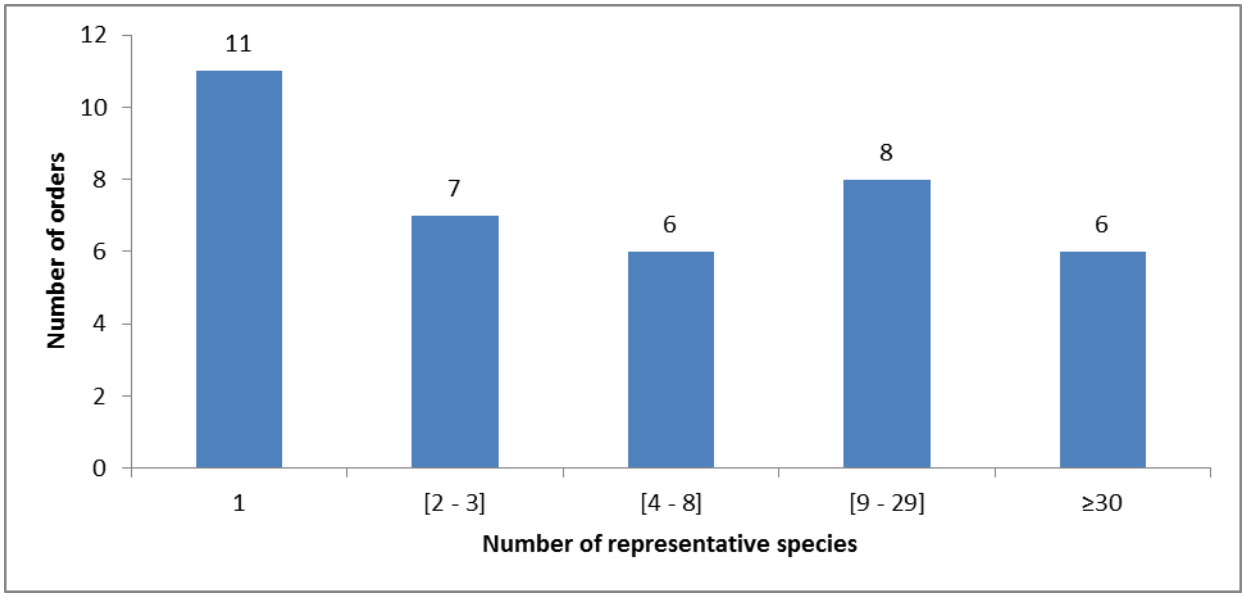


**Fig. 3.2:** Frequency distribution of orders across size classes based on the number of tree species in the Phytomedicine Tree Database. The species were grouped into size classes: [1]; [2 to 3]; [4 to 8]; [9 to 29] and [≥30].

## 3.4 Conclusions

The approximately 2 100 tree species of southern Africa belong to 530 genera, 133 families and 44 orders. Although the southern African region encompasses seven countries, we sampled only within South Africa for practical reasons. Our target was to collect good representative members of at least one species per genus in a family. Samples of genera occurring outside the borders of South Africa were only collected if the relevant representative tree species were found in one of the national botanical gardens. Therefore, a large proportion of tree genera (32%), distributed outside the South African borders, were not collected. Moreover, trees too small, unavailable or inaccessible to permit adequate sampling were excluded.

Nevertheless from the analysis presented here it does appear as if the Phytomedicine Tree Database contains a fair representation of the tree genera, families and orders occurring in southern Africa. After several collection trips to different botanical gardens within South Africa, a total of 717 samples of 537 species belonging to 350 genera, 101 families and 38 orders were collected. The families and orders, of which no representative species were collected, were generally small families and orders, mostly containing a single tree genus in the southern African region. In addition, a large number of species within these families and orders are found entirely outside South African borders.

The sizes of the families representing the tree species assimilated in the Phytomedicine Tree Database differed considerably. This was mainly due to classification systems that assemble unequal numbers of species in families (Chapter 2.3). The largest tree families were Fabaceae, Rubiaceae, Euphorbiaceae, Apocynaceae, Celastraceae and Sapindaceae while the largest orders were Malpighiales, Sapindales, Gentianales and Fabales. In addition to size differences, we found that the range of family and order sizes was extremely skewed with far more small than large families and orders. These differences will complicate comparisons and statistical analyses between families (and orders). Therefore, the families and orders were divided into groups according to size as set out in Chapter 7 and 8 respectively.

# CHAPTER 4

# General methods and data processing

## 4.1 Plant preparation

Fresh tree leaves were collected from labelled trees in the botanical gardens listed in Chapter 3.1. Harvested leaves were immediately stored in open mesh loosely woven bags normally used for selling oranges and vegetables. This approach ensured air flow for quick drying and minimised chemical changes by microbial attack after collection. The leaf material was examined and any leaves attacked by insects or microbes were removed.

The leaves were dried indoors at room temperature under good ventilation conditions and, when completely dried, ground to a fine powder using a Jankel and Künkel Model A10 mill. After extraction, the excess powder was stored in tightly closed glass containers in the dark at room temperature for future studies. Dried material was used because there are fewer problems associated with large scale extraction of dried plant material compared to fresh plant material (Eloff, 1998a) and dried material may retain its biological activity for many decades (Eloff, 1999).

## 4.2 Extraction method

There is no one solvent that is able to extract all the compounds from a plant and by using different solvents, different quantities and types of compounds can be extracted (Verpoorte, 1998). Eloff (1998c) found that acetone was the best choice as an extractant mainly due to its ability to extract compounds of a wide range of polarities (Kotze and Eloff, 2002), its non-toxicity to bioassay systems (Eloff *et al*., 2007) and its ease of removal from extracts. Therefore, acetone (technical grade, Merck) was used as an extractant in the assays using a ratio of 1:10 of leaf material to extractant.

The extraction procedure developed and described by Eloff (1998c) was used. Three gram (3 g) of all tree leaf samples was extracted with 30 ml acetone. The mixture was shaken at high speed for 10 minutes in a Labotec model 20.2 shaking machine. The extracts were then centrifuged at 6000 rpm for 10 minutes. After centrifuging, the supernatants were filtered through Whatman No 1 filter paper and transferred into pre-weighed labelled glass vials. The solvent was removed under a stream of air at room temperature for quantitative determination.

## 4.3 Microbial test organisms

The panel of microbial organisms used in this study represented pathogenic species of different classes commonly associated with nosocomial infections. One of the reviewers of the initial project proposal, Prof Arnold Vlietinck of Antwerpen University, Belgium, recommended the microorganisms that were used in this study.

### 4.3.1 Bacterial test organisms

The four most important human bacterial pathogens (Sacho and Scoub, 1993) were selected as test organisms. The specific strains for antibacterial testing were recommended by the National Committee for Clinical Laboratory Standards (NCCLS, 1992). The bacteria were maintained in the Phytomedicine Laboratory at Onderstepoort, University of Pretoria. The bacterial strains consisted of two Gram-positive strains, *Staphylococcus aureus* and *Enterococcus faecalis,* and two Gram-negative strains, *Escherichia coli* and *Pseudomonas aeruginosa* (Table 4.1). All the bacterial strains were subcultured from the original strains, stored at -70ºC and maintained on Mueller Hinton agar plates at 4º C. Three to five colonies of bacteria from a fresh 18-24 h agar plate culture were inoculated into 2 ml sterile distilled water with 0.02% Tween 80 (BDH). From this mixture, 1-10 µl were transferred to 10 ml MH broth to give a final concentration of approximately 5 x $10^5$ CFU/ml.

**Table 4.1:** Bacterial test organisms used in the study.

| Bacteria | Strain |
|---|---|
| *Enterococcus faecalis* | ATTC 29212, Gram-positive bacterium |
| *Staphylococcus aureus* | ATTC 29213, Gram-positive bacterium |
| *Escherichia coli* | ATTC 25922, Gram-negative bacterium |
| *Pseudomonas aeruginosa* | ATTC 27853, Gram-negative bacterium |

### 4.3.2 Fungal test organisms

Two of the most common and important disease-causing fungi in animals, *Candida albicans* and *Cryptococcus neoformans* (Fan-Harvard *et al*., 1991) were used (Table 4.2). The fungal organisms were maintained in the Microbiology Laboratory at Onderstepoort, University of Pretoria. The test organisms, both yeasts, were cultured from clinical cases of disease in animals at the Department of Veterinary Tropical Diseases, Faculty of Veterinary Sciences, University of Pretoria. All fungal strains were maintained on Sabouraud dextrose agar (Oxoid, Basingstoke, UK). Sabouraud Dextrose broth was used as liquid nutrient medium.

**Table 4.2:** Fungal test organisms used in the study ([1] = cultured from clinical cases of disease in animals by the Department of Veterinary Tropical Diseases, Faculty of Veterinary Sciences. None of the animals was treated prior to sampling).

| Fungal organism | Source |
|---|---|
| *Candida albicans* (yeast) | [1]Isolated from a Gouldian finch |
| *Cryptococcus neoformans* (yeast) | [1]Isolated from a cheetah |

## 4.4 Microdilution assays to determine minimum inhibitory concentrations (MIC)

The antimicrobial activity of antibiotics and extracts is commonly described in terms of its MIC, the lowest concentration of the extract that inhibits growth of the test organism. Between 707 and 717 crude extracts corresponding to about 537 species were screened. The choice of screening assays is very important for the selection of extracts that will be worth-while candidates for follow up studies.

### 4.4.1 Antibacterial microdilution assay

A sensitive serial dilution microplate method, developed by Eloff (1998b), was used to determine the MIC of plant extracts against four bacterial strains in triplicate. Extracts were tested over a two-fold serial dilution concentration range of 2.50 mg/ml to 0.02 mg/ml. This biological assay is used very widely internationally and was chosen because of its simplicity, reproducibility, sensitivity, and relatively low cost while being a rapid method at the same time.

The dried extracts were dissolved in acetone to a concentration of 10 mg/ml. The plant extracts (100 µl) was added to the first well of a 96 well microtitre plate and were serially diluted 1:1 with water. Overnight incubated bacterial cultures (100 µl) were added to each well. This 50% inoculum of microorganisms in the logarithmic growth phase means that contamination would not influence the results. Gentamicin (100 µl of 0.1 mg/ml) was used as positive control and acetone was used as solvent control. The plant extracts were tested in triplicate for each assay. The microplates were incubated overnight at 37°C in 100% relative humidity.

As an indicator of growth, 40 µl of 0.2 mg/ml INT (*p*-iodonitrotetrazolium violet, Sigma®) dissolved in hot water was added to the microplate wells and incubated at 37°C for 2 hours. Eloff (1998b) compared different tetrazolium salts and found that INT was the best indicator for the quantification of antimicrobial activity against common bacterial strains. The tetrazolium salt, which is colourless, acts as an electron acceptor which reduces biologically active organisms to a red coloured formazan product. The solution in the well either remains clear or its colour

intensity decreases when there is no bacterial growth. Measurement of MIC was done after 2 hours. The MIC in this study was expressed as the lowest concentration of the extract that led to a decrease in bacterial growth. The plates were compared visually for any change in colour intensity because the green colour of plant extracts and the precipitation of compounds of some extracts hinder reading of the data with a microplate reader (Eloff, 1998b).

### 4.4.2 Antifungal microdilution assay

For the antifungal assay, the sensitive serial dilution microplate method described by Eloff (1998b), as modified by Masoko *et al*. (2005), was used to test the activity of two fungal organisms. Two-fold serial dilutions of extracts were prepared as described in section 4.4.1. Actively growing fungal organisms were transferred from an agar plate by collecting conidia with a sterile cotton swab into a fresh Sabouraud Dextrose broth and 100 µl of this mixture was then added to each well as described in section 4.4.1. Amphotericin B was used as positive control and acetone was used as solvent control. As an indicator of growth, 40 µl of 0.2 mg/ml INT (*p*-iodonitrotetrazolium violet, Sigma®) dissolved in water was added to the microplate wells and the plates were incubated at 35°C for 24 hours. The determination of the MIC of the plant extracts was triplicated for each assay. Measurement of the MIC, the lowest concentration of the extract that inhibits fungal growth, was made after 24 hours.

## 4.5 Data processing

### 4.5.1 Mean minimum inhibitory concentrations (MIC)

The tree extracts were evaluated at concentrations ranging between 2.50 mg/ml and 0.02 mg/ml. For the purpose of the calculation of mean MIC's, a value of 0.02 mg/ml was assigned for the extracts with an MIC lower than 0.02 mg/ml and for the extracts with an MIC higher than 2.50 mg/ml, a value of 2.50 mg/ml was assigned.

The MIC's were determined in triplicate and expressed as mean ± standard deviation (SD) using Microsoft Excel 2010. Furthermore, average MIC's were calculated for species, families and orders against the six pathogens and against the three pathogen classes (Gram-positive bacteria, Gram-negative bacteria and fungi) analyses. All average values were expressed as geometrical mean values.

The geometric mean is the mean of a set of data on a logarithmic scale which normalises the averaged values so that no single value dominates the weighting. In other words it dampens the effect of very high or very low values. In the present study, if the average MIC is calculated against pathogen classes for the family and order analyses from the values obtained, high MIC values totally overshadows low MIC values. This case is similar to calculating the average of pH

values. For this reason, mean MIC values in this study were presented as geometric mean values which were more suitable compared to arithmetic mean values. In the succeeding chapters, the geometrical mean MIC's are referred to as mean MIC values.

For the calculation of geometric mean values, the following steps were followed: (1) convert each MIC value to $\log_{10}$; (2) calculate the average (mean) of the individual $\log_{10}$ values; and (3) calculate the antilog of the averaged $\log_{10}$ value to get the geometrical mean (Table 4.3).

**Table 4.3:** Examples to illustrate the calculation of the geometrical mean minimum inhibitory concentration (MIC, mg/ml).

| Family A | | Family B | |
|---|---|---|---|
| MIC value (mg/ml) | $\text{Log}_{10}$ MIC value | MIC value (mg/ml) | $\text{Log}_{10}$ MIC value |
| 0.16 | -1.83 | 0.16 | -1.83 |
| 2.50 | 0.92 | 0.32 | -1.14 |
| 0.63 | -0.46 | 0.63 | -0.46 |
| 0.02 | -3.91 | 0.16 | -1.83 |
| 0.63 | -0.46 | 0.63 | -0.46 |
| 0.08 | -2.53 | 1.25 | 0.22 |
| Arithmetic mean: 0.67 | Mean: -1.38 | Arithmetic mean: 0.53 | Mean: -0.92 |
| | $\text{Antilog}_{10}$: 0.25 | | $\text{Antilog}_{10}$: 0.40 |
| | Geometric mean: 0.25 | | Geometric mean: 0.40 |

### 4.5.2 Statistical analysis

After natural logarithmic transformation of the MIC values, the data were analysed statistically as described below. Differences were considered significant at $p < 0.05$.

The susceptibility of the six different pathogens used were compared by conducting a Friedman analysis of variance, using the statistical package Statistical Analysis Software (SAS), version 9.2, followed by a *post hoc* analysis.

The correlation between the antimicrobial activities, expressed as MIC (mg/ml), of the different pathogens and pathogen classes (Gram-positive bacteria, Gram-negative bacteria and fungi) was established by determining correlation coefficients (r) and coefficients of determination ($r^2$) using Microsoft Excel, Version 2010.

Analyses of variance (ANOVA) were performed to compare mean MIC of the different families and orders respectively against each of the respective pathogen classes (i.e. Gram-positive, Gram-negative and fungi). In cases where statistical significances were established, the practical significance of differences was challenged, in accordance with the recommendations of Cohen (1988). If differences were found ($p < 0.05$), a *post hoc* test (i.e. least square means (LSM)) was implemented. These analyses were carried out with the statistical software program SAS, version 9.2.

# CHAPTER 5

# Determination of significant antimicrobial activity and identification of promising southern African tree species

## 5.1 Introduction

The spread of resistant bacteria and fungi has increased the demand to find alternative medicines from plants (Eloff, 1998c). Southern Africa is exceptionally rich in plant diversity and a large number of plant species have not yet been examined for their antibacterial activities. For these reasons coupled with others discussed in the main introduction, the aim of the greater project was to collect and analyse several hundred leaf samples of southern African trees unguided by prior ethnopharmacological knowledge.

The aim of this Chapter was twofold. Firstly, the aim was to determine the MIC level that would represent significant antimicrobial activity. According to Gertch (2009), there is a tendency to attribute pharmacological effects to almost every plant extract and natural product. Several previous studies dosed excessively high extract concentrations or identified very high MIC's as active (Salvat *et al*., 2001; Buwa and Van Staden, 2006), but such standards are meaningless in ethnopharmacology (Ríos and Recio, 2005; Cos *et al*., 2006). When standards do not allow for sufficient differentiation, wrong conclusions could be made about pharmacological potential. For example, *in vitro* effects observed at extract concentrations higher than 0.2 mg/ml may not be significant and could trigger non-physiological effects and other common plant extracts could cause the same effect (Gertch, 2009). Consequently, Roersch (2012) pointed out that there is a need for a classification system for antimicrobial activities based on MIC values for extracts.

There is convergence among several authors that plant extracts should have an inhibitory concentration of 100 µg/ml or below (≤ 0.1 mg/ml) to be considered as active, but this remains an unconfirmed rule of thumb (Eloff, 2004; Rios and Recio, 2005; Cos *et al*., 2006; Gertsch, 2009). By investigating the antimicrobial activity of a large number of plant extracts in this study we should be able to propose MIC standards for evaluating plant extracts at a higher level of confidence.

Secondly, the aim was to identify species with promising activities based on specific criteria. Future studies may then focus on the identified promising species by examining different populations. It would also be useful to investigate related species within the same genus since it is well known that related plant species contain similar chemical compounds and therefore may have comparable biological activities. Promising species with high activity and low toxicity could then be used by traditional healers to control infections in animals and humans.

New legislation to prevent biopiracy in South Africa requires authorisation for research and use of traditional medicinal plants (Eloff and McGaw, in press). Our approach of wide-screening, unguided by traditional use, increased the possibility of finding promising tree species that were not used traditionally and which will be unconstrained by administrative obstacles. Such tree species could become important sustainable sources of antimicrobial extracts or compounds.

## 5.2 Materials and methods

The study area, material and taxonomical arrangements of the trees of southern Africa were described in Chapter 2. The collection of tree samples and the description of the tree species represented in the Phytomedicine Tree Database were discussed in Chapter 3 and the preparation of plant extracts, microbial pathogens, antimicrobial assays as well as the processing of the data were discussed in Chapter 4.

## 5.3 Results and discussion

MIC's (mg/ml) of between 704 and 717 southern African crude tree leaf extracts were tested against six pathogens and generated a total of 4 278 MIC observations. Each of these was determined in triplicate. Due to the large volume of data, the complete list of the species with corresponding MIC values may be obtained from the Phytomedicine Programme, Department Paraclinical Sciences, University of Pretoria. The crude tree extracts yielded a range of MIC's between 0.02 and 2.50 mg/ml (the lowest and highest concentrations at which extracts were tested). The level of antimicrobial activity of the extracts varied between tree species and between pathogens.

The results are structured around three major themes. In section 5.3.1, we examined the antimicrobial activities of all extracts to determine the MIC level that would represent significant antimicrobial activity. Thereafter, species with promising activities were short-listed based on a number of criteria (5.3.2). Finally, tree genera that contained a substantial number of promising species against each of the pathogens were identified (5.3.3).

### 5.3.1 Significant antimicrobial activities based on the MIC values of the extracts

In this section, we studied the overall distribution of activities across all the observations. We firstly determined the number of extracts inhibiting each of the pathogens at each concentration. We then determined the average number of extracts inhibiting the pathogens at each concentration by dividing the total number of active extracts by six and expressed that as a percentage of total number of extracts tested (Figure 5.1). In Figure 5.2 the area between concentrations of 0.16 mg/ml and 0.02 mg/ml is enlarged for extrapolation of the test results. We established that at MIC's of 1.25 mg/ml, on average 88% of the extracts inhibited the

pathogens and at concentrations of 0.31 mg/ml, more than half of the extracts (52%) on average inhibited the pathogens (Figure 5.1). At an MIC of 0.16 mg/ml, the percentage of extracts that inhibited the pathogens decreased to 26% on average and further to 9% at MIC's of 0.08 mg/ml. Similar results were found in a study by Shai *et al*. (2013) who evaluated extracts of 17 plant species against Gram-positive and Gram-negative bacteria. In their study, 9% of the extracts of medicinal plants were also active at MIC's of 0.08 mg/ml. We found that on average only 1% of the extracts inhibited the pathogens at a concentration of 0.02 mg/ml.

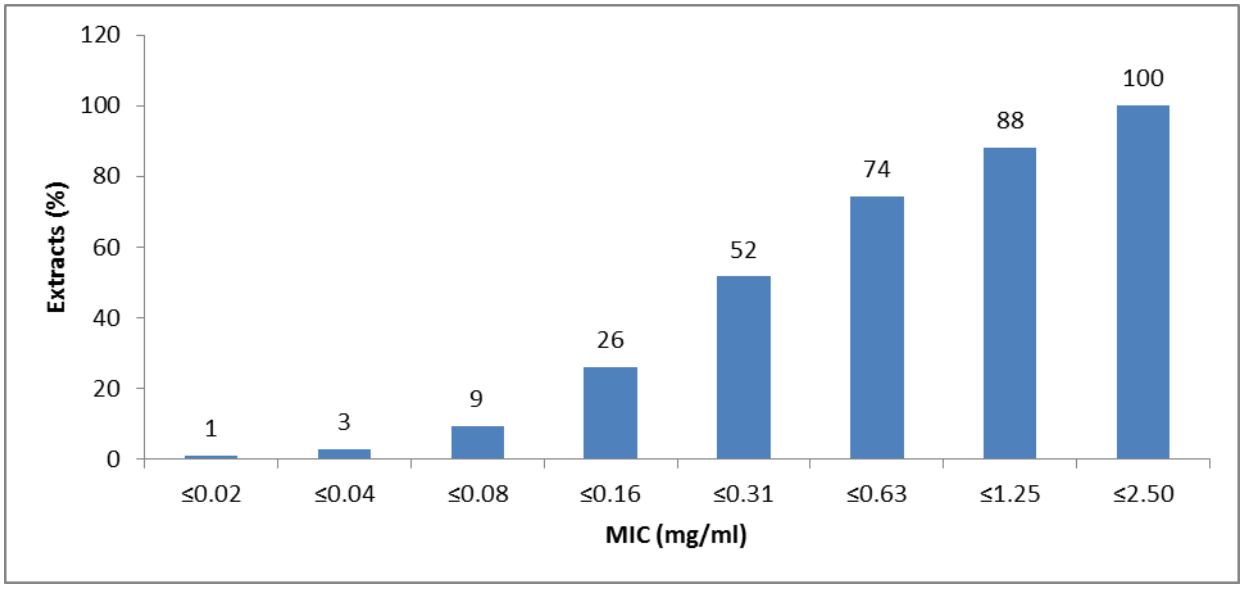


**Fig. 5.1:** The percentage of active extracts (n = 4278) calculated from the average number of extracts that was active against each of the pathogens at all the concentrations in the MIC spectrum.

As presented in the introduction of this chapter, several authors considered the activities of plant extracts with MIC's of 0.1 mg/ml and lower as significant (Eloff, 2004; Rios and Recio, 2005; Cos *et al*., 2006; Gertsch, 2009). Figure 5.2 shows that on average 13% of extracts were active against the six pathogens at an MIC of 0.1 mg/ml. The concentrations at which 10% of extracts were showing activity against all pathogens on average was 0.084. Judging from the foregoing results and discussion, the evidence from our wide-screening therefore seems to confirm that an MIC of 0.1 mg/ml or lower would be a reasonable and practical standard to determine significant antimicrobial activity in screening procedures. If a more stringent classification is needed (5% of extracts) then MIC's of roughly 0.06 mg/ml could be used as a standard.

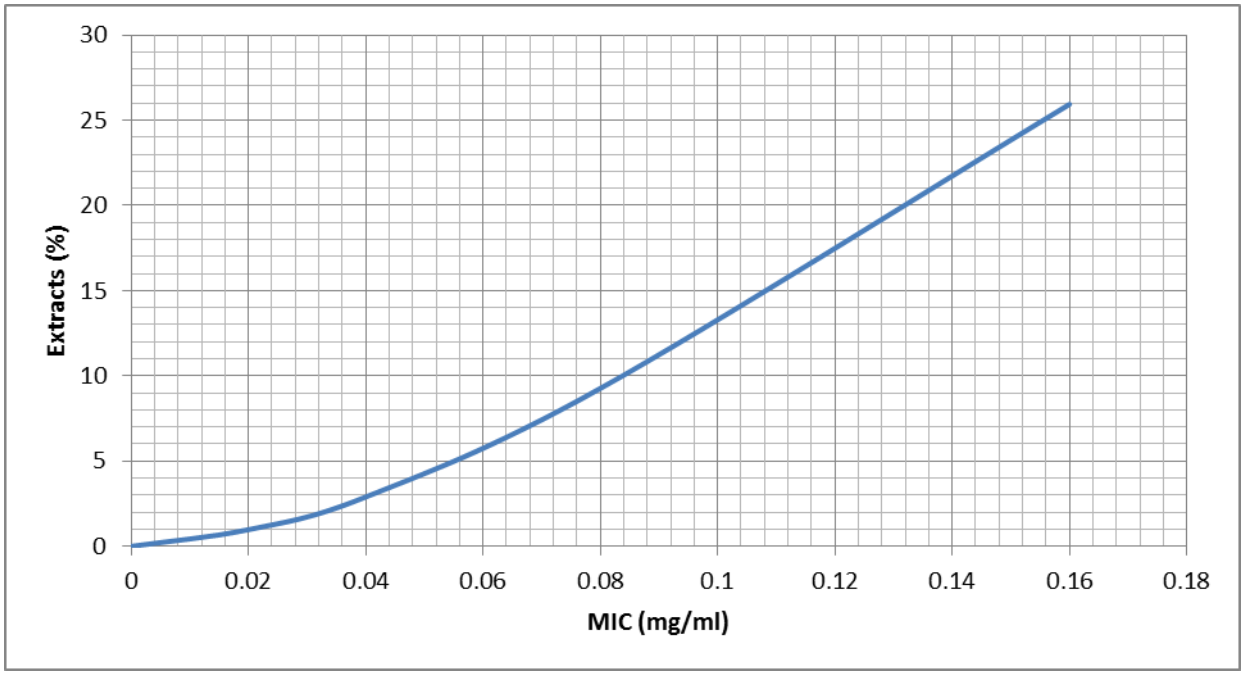


**Fig. 5.2:** Line diagram of the percentage of active extracts (n = 4278) calculated from the average number of extracts that was active at MIC's of 0.16 mg/ml and lower.

It is well-known that different pathogens have different sensitivities towards antimicrobial compounds and extracts. This will be discussed in Chapter 6. Here, we investigated if different benchmarks for activity testing should be considered for the different pathogens. Figure 5.3 shows the percentage of extracts that inhibited the individual pathogens at a range of concentrations. As an example of the different sensitivities, the number of extracts that inhibited the pathogens at MIC's of 0.08 mg/ml and lower varied between 5% (*E. coli*), 8% (*S. aureus*), 9% (both *E. faecalis* and *P. aeruginosa*), 10% (*C. albicans*) and 15% (*C. neoformans*). It is therefore necessary to refine the activity benchmark for each pathogen. In Figure 5.4 the area between concentrations of 0.16 mg/ml and 0.02 mg/ml is enlarged for extrapolation of the test results. The MIC benchmark to identify the top 10% extracts showing activity against all six pathogens should be an MIC of 0.06 mg/ml for *C. neoformans* and an MIC of 0.01 mg/ml for *E. coli*.

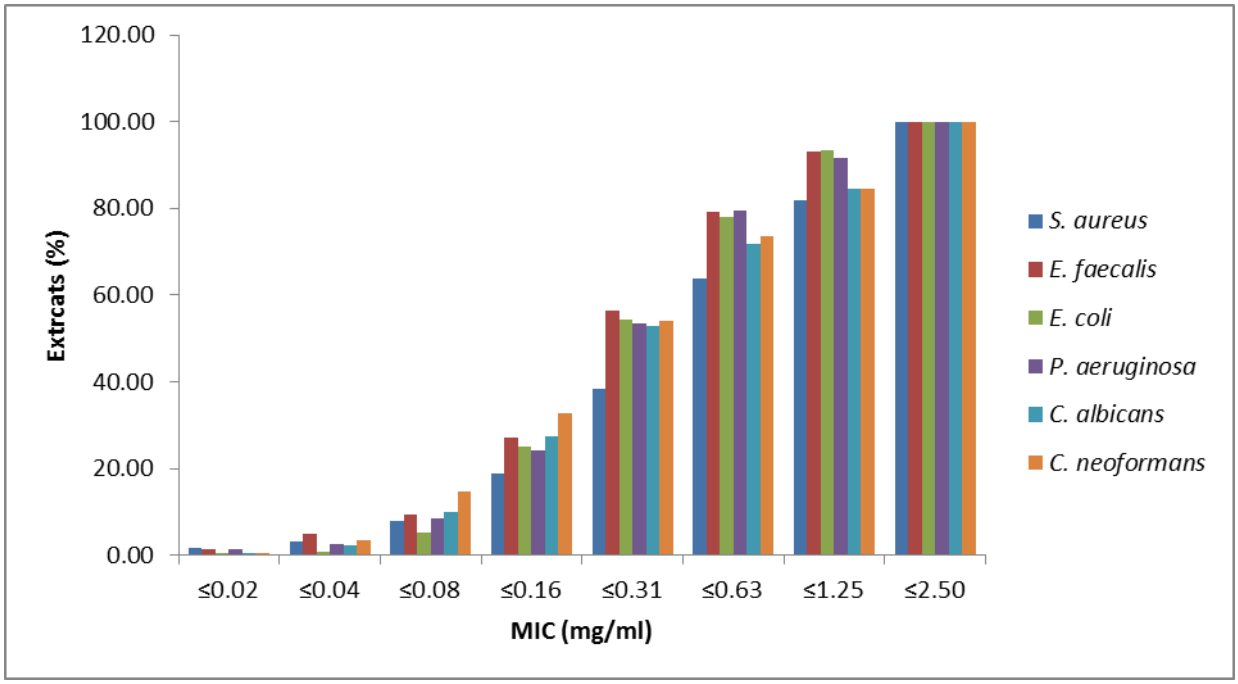


**Fig. 5.3:** The percentage of active extracts (n ≥ 704 and ≤ 717) against each of the six pathogens.

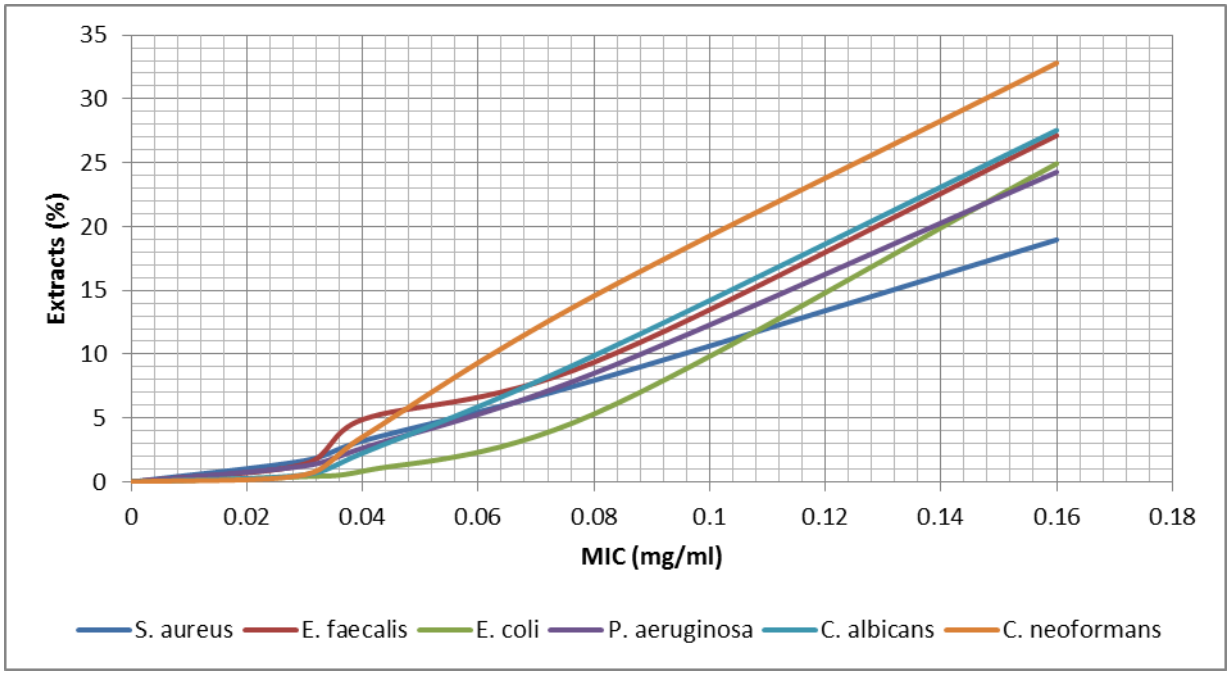


**Fig. 5.4:** Line diagram of the percentage of active extracts (n ≥ 704 and ≤ 717) against each of the six pathogens at MIC's of 0.16 mg/ml and lower.

### 5.3.2 Tree species with the most effective antimicrobial extracts

The main aim of this section was to select promising tree species for further studies. For practical reasons an MIC value of 0.16 mg/ml was used as the standard for anti-microbial activity even though this concentration is higher than the recommended MIC of 0.1 mg/ml for screening purposes. This was in part necessary because 0.1 mg/ml was not included in our serial dilutions. The two-fold level dilution series is a relatively coarse method used for our first level wide-screening and to prevent the omission of species with potential, a slightly higher MIC was therefore justified. According to Figure 5.1, an average of 26% of the extracts will qualify as having antimicrobial activity at an MIC of 0.16 mg/ml. In future, more detailed studies would include more assays and use stringent cut-off points to distinguish between tree species with interesting activities.

Consequently we considered an MIC of 0.04 mg/ml and lower as very high activity, an MIC > 0.04 mg/ml and ≤ 0.08 mg/ml as high activity and an MIC > 0.08 mg/ml and ≤ 0.16 mg/ml as interesting activity. Furthermore, we considered an MIC > 0.16 mg/ml as not interesting and an MIC ≥ 1.25 as very low activity.

Species with interesting, high or very high activity against one or more of the six pathogens were identified and a short-list was compiled based on three criteria: (1) tree extracts inhibiting five or six pathogens at an MIC of 0.16 mg/ml and lower (section 5.3.2.1); (2) tree extracts showing very high activity (MIC ≤ 0.04 mg/ml) against at least one of the six pathogens (5.3.2.2); and (3) tree extracts with MIC's ≤ 0.16 mg/ml against one of the pathogens and MIC's > 0.16 mg/ml against the rest of the pathogens (5.3.2.3).

#### 5.3.2.1 Extracts of tree species with interesting antimicrobial activity (MIC's ≤ 0.16 mg/ml) against five or six pathogens

A large number of extracts had MIC's of 0.16 mg/ml and lower against at least one of the pathogens. Among these, 29 extracts of 26 tree species inhibited at least five of the pathogens at MIC's of 0.16 mg/ml and lower (Table 5.1). Although these species are considered promising, they may contain general metabolic toxins that would limit their application other than for topical use. Several extracts showed general activity against all six pathogens and may not necessarily be useful as medicine because they might have a general metabolic toxin which could be harmful to the host cells as well. Those most active extracts (Phytomedicine Tree Database number in brackets) with MIC 0.16 mg/ml and below were: *Acacia sieberiana* (no. 338), *Bowkeria citrina* (no. 647), *Curtisia dentata* (no. 26), *Dodonaea viscosa* (no. 110), *Hypericum roeperianum* (no. 356), *Macaranga mellifera* (no 54), *Smodingium argutum* (no. 188), *Terminalia phanerophlebia* (no. 191) and two extracts of *Loxostylis alata* (nos. 614 and 736).

With the exception of *B. citrina* and *S. argutum*, the other seven species are all listed as medicinal plants, but not necessarily for antimicrobial activity, in southern Africa (Arnold *et al*. 2002).

**Table 5.1:** Acetone tree leaf extracts with interesting activities (MIC's ≤ 0.16 mg/ml) against five or six pathogens (MIC's ≤ 0.16 mg/ml in bold; No. = accession number in Phytomedicine Tree Database; SD = standard deviation; *Ef = E. faecalis; Sa = S. aureus; Ec = E. coli; Pa = P. aeruginosa; Ca = C. albicans; Cn = C. neoformans;* [1] = non-indigenous species).

| Tree species | No. | MIC (mg/ml) ± SD | | | | | |
|---|---|---|---|---|---|---|---|
| | | Gram-positive bacteria | | Gram-negative bacteria | | Fungi | |
| | | *Ef* | *Sa* | *Ec* | *Pa* | *Ca* | *Cn* |
| *Acacia sieberiana* | 338 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.08 ± 0.00 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.06 ± 0.02 |
| *Bowkeria citrina* | 647 | 0.16 ± 0.00 | 0.04 ± 0.00 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.02 ± 0.00 | 0.08 ± 0.00 |
| *Calpurnia aurea* | 202 | 0.02 ± 0.00 | 0.16 ± 0.00 | 0.08 ± 0.00 | 0.13 ± 0.05 | 0.63 ± 0.00 | 0.13 ± 0.05 |
| *Combretum mkuzense* | 18 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.08 ± 0.00 | 0.16 ± 0.00 | 0.63 ± 0.00 |
| *Curtisia dentata* | 26 | 0.03 ± 0.01 | 0.08 ± 0.00 | 0.08 ± 0.00 | 0.08 ± 0.00 | 0.04 ± 0.00 | 0.06 ± 0.02 |
| *Dodonaea viscosa* | 110 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.08 ± 0.00 | 0.08 ± 0.00 |
| *Elaeodendron croceum* | 11 | 0.02 ± 0.00 | 0.02 ± 0.00 | 0.04 ± 0.00 | 0.16 ± 0.00 | 0.31 ± 0.00 | 0.16 ± 0.00 |
| *Harpephyllum caffrum* | 324 | 0.08 ± 0.00 | 0.08 ± 0.00 | 0.16 ± 0.00 | 0.31 ± 0.00 | 0.04 ± 0.00 | 0.02 ± 0.00 |
| *H. caffrum* | 605 | 0.31 ± 0.00 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.16 ± 0.00 |
| *Heteropyxis natalensis* | 354 | 0.16 ± 0.00 | 0.04 ± 0.00 | 0.04 ± 0.00 | 0.16 ± 0.00 | 0.31 ± 0.00 | 0.04 ± 0.00 |
| *Hypericum roeperianum* | 356 | 0.05 ± 0.02 | 0.03 ± 0.01 | 0.08 ± 0.00 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.08 ± 0.00 |
| *Indigofera frutescens* | 178 | 0.06 ± 0.03 | 0.11 ± 0.06 | 0.06 ± 0.03 | 0.06 ± 0.03 | 0.79 ± 0.36 | 0.08 ± 0.00 |
| *Leucosidea sericea* | 609 | 0.02 ± 0.00 | 0.02 ± 0.00 | 0.16 ± 0.00 | 0.05 ± 0.02 | 0.63 ± 0.00 | 0.04 ± 0.00 |
| *Loxostylis alata* | 180 | 0.02 ± 0.00 | 0.06 ± 0.03 | 0.06 ± 0.03 | 0.03 ± 0.01 | 0.16 ± 0.00 | 0.04 ± 0.00 |
| *L. alata* | 614 | 0.08 ± 0.00 | 0.02 ± 0.00 | 0.03 ± 0.01 | 0.06 ± 0.03 | 0.31 ± 0.00 | 0.08 ± 0.00 |
| *L. alata* | 736 | 0.04 ± 0.00 | 0.08 ± 0.00 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.08 ± 0.00 | 0.08 ± 0.00 |
| Macaranga capensis | 53 | 0.03 ± 0.01 | 0.03 ± 0.01 | 0.08 ± 0.00 | 0.31 ± 0.00 | 0.08 ± 0.00 | 0.02 ± 0.00 |
| *M. mellifera* | 54 | 0.13 ± 0.05 | 0.16 ± 0.00 | 0.08 ± 0.00 | 0.10 ± 0.05 | 0.05 ± 0.02 | 0.02 ± 0.00 |
| *Maclura africana* | 302 | 0.04 ± 0.00 | 0.02 ± 0.00 | 0.31 ± 0.00 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.16 ± 0.00 |
| *Maesa lanceolata* | 615 | 0.04 ± 0.00 | 0.02 ± 0.00 | 0.02 ± 0.00 | 0.02 ± 0.00 | 0.63 ± 0.00 | 0.16 ± 0.00 |

| Tree species | No. | MIC (mg/ml) ± SD | | | | | |
|---|---|---|---|---|---|---|---|
| | | Gram-positive bacteria | | Gram-negative bacteria | | Fungi | |
| | | *Ef* | *Sa* | *Ec* | *Pa* | *Ca* | *Cn* |
| *Mystroxylon aethiopicum* | 10 | 0.04 ± 0.00 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.08 ± 0.00 | 0.20 ± 0.09 | 0.10 ± 0.05 |
| *Ochna pretoriensis* | 182 | 0.08 ± 0.00 | 0.11 ± 0.06 | 0.06 ± 0.03 | 0.11 ± 0.06 | 1.25 ± 0.00 | 0.13 ± 0.05 |
| *Polygala myrtifolia var. myrtifolia* | 622 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.05 ± 0.02 | 0.08 ± 0.00 | 0.31 ± 0.00 |
| *Searsia pyroides* | 570 | 0.04 ± 0.00 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.04 ± 0.00 | 0.31 ± 0.00 | 0.16 ± 0.00 |
| *Smodingium argutum* | 188 | 0.04 ± 0.00 | 0.06 ± 0.03 | 0.06 ± 0.03 | 0.06 ± 0.03 | 0.16 ± 0.00 | 0.10 ± 0.05 |
| *Terminalia phanerophlebia* | 191 | 0.02 ± 0.00 | 0.08 ± 0.00 | 0.06 ± 0.03 | 0.11 ± 0.06 | 0.04 ± 0.00 | 0.10 ± 0.05 |
| *Virgilia divaricate* | 192 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.22 ± 0.11 | 0.06 ± 0.03 | 0.04 ± 0.00 | 0.16 ± 0.00 |
| Xylia torreana | 159 | 0.16 ± 0.00 | 0.20 ± 0.09 | 0.16 ± 0.00 | 0.08 ± 0.00 | 0.16 ± 0.00 | 0.16 ± 0.00 |
| [1]*Leucaena leucocephala* | 342 | 0.31 ± 0.00 | 0.08 ± 0.00 | 0.08 ± 0.00 | 0.08 ± 0.00 | 0.16 ± 0.00 | 0.10 ± 0.05 |

### 5.3.2.2 Extracts of tree species with very high activity (MIC ≤ 0.04 mg/ml) against at least one of the six pathogens

Ninety-two crude tree extracts had MIC's of 0.04 mg/ml and lower against at least one of the six pathogens. Inhibitions at this concentration are considered as very high activities in this study. The activity of these crude extracts is already higher than that of the older registered antibiotics. Due to the size of the table it is presented in Appendix B, Table B.1. Among these, 34 extracts yielded MIC's of 0.02 mg/ml and lower and were considered to be the most promising (Table 5.2). These extracts were distributed among 31 species, 28 genera and 23 families. A few of the most promising species listed in Table 5.2 are accentuated below.

Two extracts of *Leucosidea sericea* (nos. 288 and 609) were very effective against *E. faecalis* and *S. aureus* with MIC's of 0.02 mg/ml each (Table 5.2). Extracts of *Elaeodendron croceum* (no. 11) and *Haplocoelum foliolosum* (no. 303) had very high activity against both of the Gram-positive bacteria while extracts of *Maesa lanceolata* (no. 615) had very high activities *against E. faecalis* (MIC, 0.04 mg/ml), *S. aureus*, *E. coli* and *P. aeruginosa* (MIC, 0.02 mg/ml each).

Extracts of two species of *Macaranga, M. mellifera* (no. 54) and *M. capensis* (no. 53), inhibited *C. neoformans* at the lowest concentration tested (MIC, 0.02 mg/ml). *M. capensis* (no. 53) also had very high activities against both of the Gram-positive bacteria (MIC's, 0.04 mg/ml). The extracts of *M. mellifera* and *M. capensis* had interesting activities (MIC's ≤ 0.16 mg/ml) against

various other pathogens and *M. mellifera* was one of the few species which yielded interesting activities against all six organisms (section 5.3.1.1).

Extracts of *Terminalia phanerophlebia,* sampled from three trees in different botanical gardens, had very high inhibitory effects at the lowest concentration of 0.02 mg/ml against at least one of the following organisms: *E. faecalis*, *P. aeruginosa* and *C. albicans.* Another *Terminalia* species (*T. sambesiaca*) also had very high activities against *C. albicans* with an MIC of 0.02 mg/ml. The inhibitory effects of the extracts of *T. phanerophlebia* and *T. sambesiaca* varied against the other pathogens. Extracts of *T. phanerophlebia* (no. 191) were exceptionally promising and inhibited all six pathogens at MIC's of 0.16 mg/ml and below.

**Table 5.2:** Acetone tree leaf extracts with very high activities (MIC's ≤ 0.04 mg/ml) against at least one of the six pathogens (MIC's ≤ 0.02 mg/ml in bold and underlined; MIC's ≤ 0.04 mg/ml in bold; No. = accession number in Phytomedicine Tree Database; SD = standard deviation; na = not analysed; *Ef = E. faecalis; Sa = S. aureus; Ec = E. coli; Pa = P. aeruginosa; Ca = C. albicans; Cn = C. neoformans*; [1] = non-indigenous species).

| Tree species | No. | MIC (mg/ml) ± SD | | | | | |
|---|---|---|---|---|---|---|---|
| | | Gram-positive bacteria | | Gram-negative bacteria | | Fungi | |
| | | *Ef* | *Sa* | *Ec* | *Pa* | *Ca* | *Cn* |
| *Azanza garckeana* | 726 | 1.25 ± 0.00 | 1.25 ± 0.00 | 0.08 ± 0.00 | **0.02 ± 0.00** | 0.08 ± 0.00 | 0.63 ± 0.00 |
| *Bolusanthus speciosus* | 580 | 0.63 ± 0.00 | 1.25 ± 0.00 | **0.02 ± 0.00** | 0.63 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 |
| *Bowkeria citrina* | 647 | 0.16 ± 0.00 | 0.04 ± 0.00 | 0.16 ± 0.00 | 0.16 ± 0.00 | **0.02 ± 0.00** | 0.08 ± 0.00 |
| *Brabejum stellatifolium* | 262 | 0.16 ± 0.00 | **0.02 ± 0.00** | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 2.50 ± 0.00 |
| *Calpurnia aurea* | 202 | **0.02 ± 0.00** | 0.16 ± 0.00 | 0.08 ± 0.00 | 0.13 ± 0.05 | 0.63 ± 0.00 | 0.13 ± 0.05 |
| *Capparis tomentosa* | 301 | 0.20 ± 0.09 | **0.02 ± 0.00** | 0.31 ± 0.00 | 0.63 ± 0.00 | 1.25 ± 0.00 | 0.79 ± 0.36 |
| *Cassine peragua* | 314 | 0.04 ± 0.00 | 0.16 ± 0.00 | 0.63 ± 0.00 | **0.02 ± 0.00** | 2.50 ± 0.00 | 0.10 ± 0.05 |
| *Diospyros natalensis* | 308 | 0.16 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | **0.02 ± 0.00** | 0.05 ± 0.02 | 0.31 ± 0.00 |
| *Elaeodendron croceum* | 11 | **0.02 ± 0.00** | **0.02 ± 0.00** | 0.04 ± 0.00 | 0.16 ± 0.00 | 0.31 ± 0.00 | 0.16 ± 0.00 |
| *Erythrophleum lasianthum* | 212 | **0.02 ± 0.00** | 0.31 ± 0.00 | 0.79 ± 0.36 | 0.79 ± 0.36 | 0.25 ± 0.09 | 0.63 ± 0.00 |
| *Grevillea robusta* | 488 | 0.04 ± 0.00 | **0.02 ± 0.00** | 0.63 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 |
| *Gymnosporia buxifolia* | 564 | 0.39 ± 0.18 | 0.08 ± 0.00 | 0.31 ± 0.00 | **0.02 ± 0.00** | na | na |
| *Haplocoelum foliolosum* | 303 | **0.02 ± 0.00** | **0.02 ± 0.00** | 0.31 ± 0.00 | 0.08 ± 0.00 | 0.31 ± 0.00 | 0.39 ± 0.18 |
| *Harpephyllum caffrum* | 324 | 0.08 ± 0.00 | 0.08 ± 0.00 | 0.16 ± 0.00 | 0.31 ± 0.00 | 0.04 ± 0.00 | **0.02 ± 0.00** |

| Tree species | No. | MIC (mg/ml) ± SD | | | | | |
|---|---|---|---|---|---|---|---|
| | | Gram-positive bacteria | | Gram-negative bacteria | | Fungi | |
| | | *Ef* | *Sa* | *Ec* | *Pa* | *Ca* | *Cn* |
| *Heteromorpha arborescens* | 491 | 0.63 ± 0.00 | 0.63 ± 0.00 | 0.02 ± 0.00 | 1.25 ± 0.00 | na | na |
| *Leucosidea sericea* | 288 | 0.02 ± 0.00 | 0.02 ± 0.00 | 0.50 ± 0.54 | 0.39 ± 0.18 | 2.50 ± 0.00 | 2.50 ± 0.00 |
| *L. sericea* | 609 | 0.02 ± 0.00 | 0.02 ± 0.00 | 0.16 ± 0.00 | 0.05 ± 0.02 | 0.63 ± 0.00 | 0.04 ± 0.00 |
| *Loxostylis alata* | 180 | 0.02 ± 0.00 | 0.06 ± 0.03 | 0.06 ± 0.03 | 0.03 ± 0.01 | 0.16 ± 0.00 | 0.04 ± 0.00 |
| *L. alata* | 614 | 0.08 ± 0.00 | 0.02 ± 0.00 | 0.03 ± 0.01 | 0.06 ± 0.03 | 0.31 ± 0.00 | 0.08 ± 0.00 |
| *Macaranga capensis* | 53 | 0.03 ± 0.01 | 0.03 ± 0.01 | 0.08 ± 0.00 | 0.31 ± 0.00 | 0.08 ± 0.00 | 0.02 ± 0.00 |
| *M. mellifera* | 54 | 0.13 ± 0.05 | 0.16 ± 0.00 | 0.08 ± 0.00 | 0.10 ± 0.05 | 0.05 ± 0.02 | 0.02 ± 0.00 |
| *Maclura africana* | 302 | 0.04 ± 0.00 | 0.02 ± 0.00 | 0.31 ± 0.00 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.16 ± 0.00 |
| *Maesa lanceolata* | 615 | 0.04 ± 0.00 | 0.02 ± 0.00 | 0.02 ± 0.00 | 0.02 ± 0.00 | 0.63 ± 0.00 | 0.16 ± 0.00 |
| *Millettia stuhlmannii* | 57 | 0.16 ± 0.00 | 0.25 ± 0.09 | 0.16 ± 0.00 | 0.02 ± 0.00 | 0.13 ± 0.05 | 0.31 ± 0.00 |
| *Morus mesozygia* | 58 | 0.08 ± 0.00 | 0.16 ± 0.00 | 0.08 ± 0.00 | 0.31 ± 0.00 | 0.20 ± 0.09 | 0.03 ± 0.01 |
| *Mundulea sericea* | 566 | 0.31 ± 0.00 | 0.02 ± 0.00 | 0.20 ± 0.09 | 0.02 ± 0.00 | na | na |
| *Schinziophyton rautanenii* | 455 | 2.50 ± 0.00 | 0.31 ± 0.00 | 2.50 ± 0.00 | 2.50 ± 0.00 | 0.04 ± 0.00 | 0.02 ± 0.00 |
| *Terminalia phanerophlebia* | 191 | 0.02 ± 0.00 | 0.08 ± 0.00 | 0.06 ± 0.03 | 0.11 ± 0.06 | 0.04 ± 0.00 | 0.10 ± 0.05 |
| *T. phanerophlebia* | 84 | 0.08 ± 0.00 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.50 ± 0.18 | 0.02 ± 0.00 | 1.00 ± 0.35 |
| *T. phanerophlebia* | 631 | 1.25 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.02 ± 0.00 | 0.63 ± 0.00 | 0.16 ± 0.00 |
| *T. sambesiaca* | 85 | 0.13 ± 0.05 | 0.50 ± 0.18 | 1.25 ± 0.00 | 0.63 ± 0.00 | 0.02 ± 0.00 | 1.25 ± 0.00 |
| *Trichilia emetica* | 87 | 0.16 ± 0.00 | 0.08 ± 0.00 | 0.50 ± 0.54 | 0.39 ± 0.18 | 0.02 ± 0.00 | 1.00 ± 0.35 |
| *Umtiza listeriana* | 311 | 0.20 ± 0.09 | 0.10 ± 0.05 | 0.16 ± 0.00 | 0.02 ± 0.00 | 0.16 ± 0.00 | 0.63 ± 0.00 |
| *Vitellariopsis dispar* | 226 | 0.08 ± 0.00 | 0.02 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 | 1.25 ± 0.00 | 0.63 ± 0.00 |
| [1]*Khaya anthotheca* | 215 | 0.02 ± 0.00 | 1.25 ± 0.00 | 1.25 ± 0.00 | 1.25 ± 0.00 | 0.79 ± 0.36 | 0.51 ± 0.18 |
| [1]*Bucida buceras* | 200 | 0.02 ± 0.00 | 0.10 ± 0.05 | 0.39 ± 0.18 | 0.20 ± 0.09 | 0.06 ± 0.03 | 0.13 ± 0.05 |

#### 5.3.2.3 Extracts of tree species with selective activities

##### 5.3.2.3.1 Selective activities against specific pathogens

The pathogens had different sensitivities against the range of extracts, for example *E. coli* was inhibited by 59 extracts compared to 124 extracts for *C. neoformans* (MIC's ≤ 0.16 mg/ml). Extracts that were selectively active against a single pathogen may be more selective in their activity by attacking a more restricted metabolic pathway possibly leading to a lower toxicity to the host cells. Our first tier selections were based on tree species of which extracts inhibited one of the pathogens at MIC's of 0.16 mg/ml and lower, while generating MIC's higher than 0.16 mg/ml against the other five pathogens.

Selective antimicrobial activities against bacteria

A large number of tree species against each of the pathogens were identified by this criterion: 31 extracts against *E. faecalis,* 20 extracts against *S. aureus*, 27 extracts against *E. coli* and 21 extracts against *P. aeruginosa*. Due to the large size, the tables are presented in Appendix C, Tables C.1 to C.4. The tree species with the most notable selectivity were *Strychnos madagascariensis* (no. 518) and *Lydenburgia cassinoides* (no. 104) against *E. faecalis* (Appendix C, Table C.1) as well as *Chionanthus foveolatus* (no. 315) and *Lippia javanica* (no. 435) against *P. aeruginosa* (Appendix C, Table C.4). Extracts selected on this basis may have selective activities and the identified tree species would be obvious choices for further detailed studies against the corresponding pathogens.

Selectivity antimicrobial activities against fungi

At least 37 extracts had interesting activities of 0.16 mg/ml and lower against *C. albicans* but with no interesting activities against the other pathogens (Appendix C, Table C.5). The most notable extracts with selective activities against *C. albicans* were that of *Vitellariopsis marginata* (no. 260)*, Sclerocarya birrea* (no. 130) and *Hymenodictyon parvifolium* (no. 494), all of which had very low activities (MIC's ≥ 1.25 mg/ml) against the other pathogens tested. Fifty three extracts yielded interesting MIC's of 0.16 mg/ml and lower against *C. neoformans* only (Appendix C, Table C.6). Among these, extracts of *Zanthoxylum capense* (no. 96) had the most notable selective activity against *C. neoformans* and only inhibited the other pathogens at MIC's of 2.50 mg/ml.

#### 5.3.2.3.2 Selective antimicrobial activities against specific pathogens classes

The results against the different pathogens were grouped together in classes: (1) Gram-positive bacteria, (2) Gram-negative bacteria, (3) fungi and (4) all four bacteria. Tree species were selected based on MIC's of 0.16 mg/ml and lower against either one of these classes while generating MIC's higher than 0.16 mg/ml against the rest of the pathogen classes. The extracts that met either of these criteria are listed respectively in Tables 5.3 to 5.6. Similar to the extracts identified in the previous selections, these extracts could be of medicinal importance because of their higher antimicrobial activity against one of the bacterial (either Gram-positive or Gram-negative) or fungal classes while having a lesser effect against the other test groups.

Table 5.3 shows the most effective tree extracts (MIC's ≤ 0.08 mg/ml) against both *E. faecalis* and *S. aureus* (Gram-positive bacteria) were *Leucosidea sericea* (no. 288), *Phoenix reclinata* (no. 292), *Vitellariopsis dispar* (no. 226) and the non-indigenous tree species *Grevillea robusta* (no. 488). Even though all the extracts listed in Table 5.3 inhibited Gram-positive bacteria at concentrations of 0.16 mg/ml and lower while inhibiting Gram-negative bacteria at concentrations higher than 0.16 mg/ml, the differences in activities were not profound. The MIC's of these extracts varied between 0.20 and 0.79 mg/ml against the Gram-negative bacteria with the exception of *Maurocenia frangula* (MIC of 1.25 mg/ml against *E. coli*). However, the extracts listed in Table 5.3, yielded noticeable lower activities against the fungal organisms and several of these extracts only inhibited both fungal strains at concentrations of 1.25 mg/ml and higher, e.g. *Allophylus chaunostachys* (no. 3), *A. rubifolius* (no. 2), *Gardenia volkensii* (no. 242), *Leucosidea sericea* (no. 288), *Loxostylis alata* (no. 498) and *Phoenix reclinata* (no. 292).

**Table 5.3:** Acetone tree leaf extracts with MIC's ≤ 0.16 mg/ml against both Gram-positive bacteria (*E. faecalis* and *S. aureus*) (MIC's ≤ 0.16 mg/ml in bold; MIC's > 0.31 mg/ml underlined; No. = accession number in Phytomedicine Tree Database; SD = standard deviation; na = not analysed; *Ef = E. faecalis; Sa = S. aureus; Ec = E. coli; Pa = P. aeruginosa; Ca = C. albicans; Cn = C. neoformans;* [1] = non-indigenous species).

| Tree species | No. | MIC (mg/ml) ± SD | | | | | |
|---|---|---|---|---|---|---|---|
| | | Gram-positive bacteria | | Gram-negative bacteria | | Fungi | |
| | | *Ef* | *Sa* | *Ec* | *Pa* | *Ca* | *Cn* |
| *Allophylus chaunostachys* | 3 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.20 ± 0.09 | 0.31 ± 0.00 | 2.50 ± 0.00 | 2.50 ± 0.00 |
| *A.rubifolius* | 2 | 0.16 ± 0.00 | 0.08 ± 0.00 | 0.31 ± 0.00 | 0.39 ± 0.18 | 2.50 ± 0.00 | 1.25 ± 0.00 |
| *Brabejum stellatifolium* | 262 | 0.16 ± 0.00 | 0.02 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 2.50 ± 0.00 |
| *Encephalartos natalensis* | 349 | 0.04 ± 0.00 | 0.16 ± 0.00 | 0.79 ± 0.36 | 0.79 ± 0.36 | 1.00 ± 0.35 | 0.31 ± 0.00 |
| *Gardenia volkensii* | 242 | 0.06 ± 0.02 | 0.16 ± 0.00 | 0.25 ± 0.09 | 0.50 ± 0.18 | 2.50 ± 0.00 | 1.98 ± 0.72 |
| *[1]Grevillea robusta* | 488 | 0.04 ± 0.00 | 0.02 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 |
| *Greyia sutherlandii* | 175 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.31 ± 0.00 | 0.50 ± 0.18 | 0.25 ± 0.09 | 0.31 ± 0.00 |
| *Leucosidea sericea* | 288 | 0.02 ± 0.00 | 0.02 ± 0.00 | 0.50 ± 0.54 | 0.39 ± 0.18 | 2.50 ± 0.00 | 2.50 ± 0.00 |
| *Loxostylis alata* | 498 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 | 1.25 ± 0.00 | 2.50 ± 0.00 |
| *Maurocenia frangula* | 500 | 0.16 ± 0.00 | 0.08 ± 0.00 | 1.25 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 | 1.25 ± 0.00 |
| *Maytenus peduncularis* | 305 | 0.05 ± 0.02 | 0.16 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 | 1.25 ± 0.00 | 0.79 ± 0.36 |
| *Phoenix reclinata* | 292 | 0.08 ± 0.00 | 0.08 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 2.50 ± 0.00 | 1.25 ± 0.00 |
| *Searsia pentheri* | 70 | 0.08 ± 0.00 | 0.16 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 | 2.50 ± 0.00 | 0.79 ± 0.36 |
| *Strychnos mitis* | 73 | 0.16 ± 0.00 | 0.08 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 2.50 ± 0.00 | 1.00 ± 0.35 |
| *Vitellariopsis dispar* | 226 | 0.08 ± 0.00 | 0.02 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 | 1.25 ± 0.00 | 0.63 ± 0.00 |

The results of the promising tree species extracts against Gram-negative bacteria are shown in Table 5.4. Similarly, the differences in antimicrobial activities were mostly small, especially between the Gram-negative and Gram-positive bacteria. Only one of the extracts (*Mackaya bella,* no. 616) had a notably lower activity (MIC ≥ 1.25 mg/ml) against the Gram-positive bacteria while the extracts of *Pseudosalacia streyi* (no. 507) and *Trichocladus ellipticus* (no. 521) had notable lower activities (MIC's between 1.25 and 2.50 mg/ml) against both fungal strains.

**Table 5.4**: Acetone tree leaf extracts with MIC's ≤ 0.16 mg/ml against both Gram-negative bacteria (*E. coli* and *P. aeruginosa*) (MIC's ≤ 0.16 mg/ml in bold; MIC's > 0.31 mg/ml underlined; No. = accession number in Phytomedicine Tree Database; SD = standard deviation; na = not analysed; *Ef = E. faecalis; Sa = S. aureus; Ec = E. coli; Pa =- P. aeruginosa; Ca = C. albicans; Cn = C. neoformans*).

| Tree species | No. | MIC (mg/ml) ± SD | | | | | |
|---|---|---|---|---|---|---|---|
| | | Gram-negative bacteria | | Gram-positive bacteria | | Fungi | |
| | | *Ec* | *Pa* | *Ef* | *Sa* | *Ca* | *Cn* |
| *Adansonia grandidieri* | 135 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 |
| *A. perrieri* | 136 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 |
| *Albizia tanganyicensis* | 554 | 0.16 ± 0.00 | 0.16 ± 0.00 | 1.25 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 |
| *Bauhinia bowkeri* | 142 | 0.10 ± 0.05 | 0.16 ± 0.00 | 0.20 ± 0.09 | 0.31 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 |
| *Brachylaena rotundata* | 555 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 | 1.25 ± 0.00 | 0.31 ± 0.00 |
| *Drypetes natalensis* | 663 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 |
| *Elaeodendron transvaalense* | 586 | 0.16 ± 0.00 | 0.16 ± 0.00 | 1.25 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 |
| *Grewia occidentalis* | 322 | 0.16 ± 0.00 | 0.08 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 2.50 ± 0.00 | 0.39 ± 0.18 |
| *Lagynias lasiantha* | 497 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 |
| *Mackaya bella* | 616 | 0.16 ± 0.00 | 0.16 ± 0.00 | 1.25 ± 0.00 | 1.25 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 |
| *Podocarpus henkelii* | 621 | 0.16 ± 0.00 | 0.08 ± 0.00 | 2.50 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 |
| *Pseudosalacia streyi* | 507 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.31 ± 0.00 | 2.50 ± 0.00 | 2.50 ± 0.00 | 2.50 ± 0.00 |
| *Pterocelastrus echinatus* | 509 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.31 ± 0.00 | 1.25 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 |
| *Strychnos pungens* | 571 | 0.16 ± 0.00 | 0.08 ± 0.00 | 0.39 ± 0.18 | 0.63 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 |
| *Tabernaemontana elegans* | 466 | 0.08 ± 0.00 | 0.13 ± 0.05 | 1.25 ± 0.00 | 0.63 ± 0.00 | 1.25 ± 0.00 | 0.63 ± 0.00 |
| *Trichocladus ellipticus* | 521 | 0.08 ± 0.00 | 0.16 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 2.50 ± 0.00 | 1.25 ± 0.00 |

The most effective antibacterial extracts (MIC ≤ 0.16 mg/ml) with no observed antifungal activity are listed in Table 5.5. Although *Euclea crispa* (no. 562) yielded MIC's of 0.16 mg/ml and lower against all the bacteria, it cannot be ruled out that it may also be effective against the fungi because the sampling material was in short supply and was not analysed against the fungal organisms. The most prominent selective activities against the bacteria were displayed by *Allophylus decipiens* (no. 312), *Ficus sur* (no. 44), *Gymnosporia buxifolia* (no. 323) and *Loxostylis alata* (no. 538).

**Table 5.5:** Acetone tree extracts with MIC's ≤ 0.16 mg/ml against all four bacterial organisms (*E. faecalis*, *S. aureus*, *E. coli* and *P. aeruginosa*) (MIC's ≤ 0.16 mg/ml in bold; MIC's > 0.31 mg/ml underlined; No. = accession number in Phytomedicine Tree Database; SD = standard deviation; na = not analysed; *Ef = E. faecalis; Sa = S. aureus; Ec = E. coli; Pa = P. aeruginosa; Ca = C. albicans; Cn = C. neoformans;* [1] = non-indigenous species).

| Tree species | No. | MIC (mg/ml) ± SD | | | | | |
|---|---|---|---|---|---|---|---|
| | | Gram-positive bacteria | | Gram-negative bacteria | | Fungi | |
| | | *Ef* | *Sa* | *Ec* | *Pa* | *Ca* | *Cn* |
| *Allophylus decipiens* | 312 | 0.16 ± 0.00 | 0.03 ± 0.01 | 0.13 ± 0.05 | 0.04 ± 0.00 | 2.50 ± 0.00 | 1.25 ± 0.00 |
| *Antidesma venosum* | 138 | 0.13 ± 0.05 | 0.16 ± 0.00 | 0.10 ± 0.05 | 0.16 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 |
| *Combretum caffrum* | 14 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.31 ± 0.00 | 0.20 ± 0.09 |
| *Dracaena mannii* | 29 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 |
| *Euclea crispa* | 562 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.08 ± 0.00 | na | na |
| *Ficus sur* | 44 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.16 ± 0.00 | 2.50 ± 0.00 | 2.50 ± 0.00 |
| *Gymnosporia buxifolia* | 323 | 0.04 ± 0.00 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.63 ± 0.00 | 2.50 ± 0.00 |
| *Loxostylis alata* | 538 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.10 ± 0.05 | 0.08 ± 0.00 | 2.50 ± 0.00 | 2.50 ± 0.00 |
| *Mimusops obovata* | 244 | 0.04 ± 0.00 | 0.08 ± 0.00 | 0.08 ± 0.00 | 0.04 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 |
| *Olea europaea* | 306 | 0.06 ± 0.02 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.05 ± 0.02 | 0.63 ± 0.00 | 0.50 ± 0.18 |
| *Syzygium legatii* | 80 | 0.16 ± 0.00 | 0.08 ± 0.00 | 0.10 ± 0.05 | 0.13 ± 0.05 | 0.20 ± 0.09 | 0.20 ± 0.09 |
| [1]*Baphiopsis parviflora* | 140 | 0.10 ± 0.05 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.13 ± 0.05 | 0.25 ± 0.09 | 0.63 ± 0.00 |

Extracts of the species listed in Table 5.6, had promising antifungal activities (MIC's ≤ 0.16 mg/ml) while exhibiting limited antibacterial activity. Among these extracts, *Brabejum stellatifolium* (no. 582), *Markhamia obtusifolia* (no. 55), *Newtonia hildebrandtii* (no. 307), *Polygala myrtifolia* (no. 293), *Psychotria capensis* (no. 706*), Schinziophyton rautanenii* (no. 455), *Schotia brachypetala* (no. 456), *Strelitzia nicolai* (no. 463), *Turraea floribunda (*no. 386) and the non-indigenous species *Quisqualis indica* (no. 741) had the highest antifungal activity compared to the other plant extracts with MIC's of 0.08 mg/ml and lower against both *C. albicans* and *C. neoformans,* while only inhibiting the other organisms at higher concentrations.

**Table 5.6:** Acetone tree leaf extracts with MIC's ≤ 0.16 mg/ml against both fungi (*C. albicans* and *C. neoformans*) (MIC's ≤ 0.16 mg/ml in bold; MIC's > 0.31 mg/ml underlined; No. = accession number in Phytomedicine Tree Database; SD = standard deviation; na =- not analysed; *Ef = E. faecalis; Sa = S. aureus; Ec = E. coli; Pa = P. aeruginosa; Ca = C. albicans; Cn = C. neoformans;* [1] = non-indigenous species).

| Tree species | No. | MIC (mg/ml) ± SD | | | | | |
|---|---|---|---|---|---|---|---|
| | | Fungi | | Gram-positive bacteria | | Gram-negative bacteria | |
| | | *Ca* | *Cn* | *Ef* | *Sa* | *Ec* | *Pa* |
| *Acridocarpus natalitius* | 390 | 0.16 ± 0.00 | 0.16 ± 0.00 | 1.25 ± 0.00 | 1.25 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 |
| *Alchornea hirtella* | 391 | 0.08 ± 0.00 | 0.16 ± 0.00 | 2.50 ± 0.00 | 0.31 ± 0.00 | 1.25 ± 0.00 | 1.25 ± 0.00 |
| *Azanza garckeana* | 364 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.63 ± 0.00 | 1.25 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 |
| *Brabejum stellatifolium* | 582 | 0.08 ± 0.00 | 0.08 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 |
| *B. stellatifolium* | 648 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.31 ± 0.00 | 2.50 ± 0.00 | 1.25 ± 0.00 | 0.63 ± 0.00 |
| *Brachylaena neriifolia* | 649 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.31 ± 0.00 | 1.25 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 |
| *Buddleja auriculata* | 650 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.63 ± 0.00 | 2.50 ± 0.00 | 0.63 ± 0.00 | 1.25 ± 0.00 |
| *Coptosperma rhodesiacum* | 304 | 0.10 ± 0.05 | 0.16 ± 0.00 | 1.25 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 |
| *Deinbollia oblongifolia* | 410 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.63 ± 0.00 | 2.50 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 |
| *Dombeya burgessiae* | 661 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 |
| *Ensete ventricosum* | 351 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.20 ± 0.09 | 0.31 ± 0.00 |
| *Hibiscus tiliaceus* | 426 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.31 ± 0.00 | 1.25 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 |
| *Ilex mitis* | 674 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.31 ± 0.00 | 1.25 ± 0.00 | 1.25 ± 0.00 | 0.63 ± 0.00 |

| Tree species | No. | MIC (mg/ml) ± SD | | | | | |
|---|---|---|---|---|---|---|---|
| | | Fungi | | Gram-positive bacteria | | Gram-negative bacteria | |
| | | *Ca* | *Cn* | *Ef* | *Sa* | *Ec* | *Pa* |
| *Inhambanella henriquesii* | 428 | 0.16 ± 0.00 | 0.08 ± 0.00 | 0.63 ± 0.00 | 1.98 ± 0.72 | 0.31 ± 0.00 | 0.31 ± 0.00 |
| *Jubaeopsis caffra* | 430 | 0.16 ± 0.00 | 0.08 ± 0.00 | 2.50 ± 0.00 | 2.50 ± 0.00 | 2.50 ± 0.00 | 0.31 ± 0.00 |
| *Maerua rosmarinoides* | 374 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 |
| *Markhamia obtusifolia* | 55 | 0.08 ± 0.00 | 0.05 ± 0.02 | 0.49 ± 0.54 | 1.25 ± 0.00 | 0.63 ± 0.00 | 2.50 ± 0.00 |
| *Newtonia hildebrandtii* | 307 | 0.08 ± 0.00 | 0.08 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 |
| *Oxyanthus pyriformis* | 252 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.63 ± 0.00 | 0.50 ± 0.18 | 0.39 ± 0.18 | 0.63 ± 0.00 |
| *Polygala myrtifolia* | 293 | 0.08 ± 0.00 | 0.08 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 | 1.25 ± 0.00 |
| *Pouzolzia mixta Solms* | 251 | 0.16 ± 0.00 | 0.13 ± 0.05 | 0.79 ± 0.36 | 1.57 ± 0.72 | 0.79 ± 0.36 | 0.39 ± 0.18 |
| *Pseudobersama mossambicensis* | 451 | 0.16 ± 0.00 | 0.08 ± 0.00 | 1.25 ± 0.00 | 2.50 ± 0.00 | 2.50 ± 0.00 | 1.25 ± 0.00 |
| *Pseudoscolopia polyantha* | 704 | 0.04 ± 0.00 | 0.16 ± 0.00 | 0.31 ± 0.00 | 1.25 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 |
| *Psychotria capensis* | 706 | 0.08 ± 0.00 | 0.04 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 |
| *Ptaeroxylon obliquum* | 186 | 0.06 ± 0.03 | 0.13 ± 0.05 | 0.31 ± 0.00 | 0.63 ± 0.00 | 0.11 ± 0.06 | 0.44 ± 0.23 |
| *Rhoicissus digitata* | 454 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.31 ± 0.00 | 1.25 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 |
| *Rothmannia capensis* | 708 | 0.16 ± 0.00 | 0.08 ± 0.00 | 0.63 ± 0.00 | 1.25 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 |
| *Schinziophyton rautanenii* | 455 | 0.04 ± 0.00 | 0.02 ± 0.00 | 2.50 ± 0.00 | 0.31 ± 0.00 | 2.50 ± 0.00 | 2.50 ± 0.00 |
| *Schotia brachypetala* | 456 | 0.06 ± 0.03 | 0.04 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 |
| *Strelitzia alba* | 460 | 0.16 ± 0.00 | 0.08 ± 0.00 | 1.25 ± 0.00 | 2.50 ± 0.00 | 1.25 ± 0.00 | 0.63 ± 0.00 |
| *S. nicolai* | 463 | 0.08 ± 0.00 | 0.08 ± 0.00 | 2.50 ± 0.00 | 1.25 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 |
| *Syncolostemon transvaalensis* | 285 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.50 ± 0.54 | 0.39 ± 0.27 | 0.31 ± 0.00 | 0.63 ± 0.00 |
| *Trilepisium madagascariense* | 551 | 0.16 ± 0.00 | 0.08 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 |
| *Turraea floribunda* | 386 | 0.08 ± 0.00 | 0.08 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 |
| *Uvaria lucida* | 388 | 0.16 ± 0.00 | 0.16 ± 0.00 | 2.50 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 | 1.25 ± 0.00 |
| *Vepris lanceolata* | 633 | 0.16 ± 0.00 | 0.08 ± 0.00 | 0.31 ± 0.00 | 2.50 ± 0.00 | 2.50 ± 0.00 | 0.31 ± 0.00 |
| *Xeroderris stuhlmannii* | 471 | 0.16 ± 0.00 | 0.16 ± 0.00 | 1.25 ± 0.00 | 2.50 ± 0.00 | 1.25 ± 0.00 | 1.25 ± 0.00 |
| [1]*Quisqualis indica* | 741 | 0.08 ± 0.00 | 0.08 ± 0.00 | 0.31 ± 0.00 | 1.25 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 |

### 5.3.3 Tree genera containing several promising species

Some genera contained a number of species that had interesting activities (MIC's ≤ 0.16 mg/ml) promising antimicrobial activities. Due to the large volume, only a few genera against each of the organisms are shown in Figures 5.5 to 5.10 and the most noteworthy genera are further discussed.

Extracts of several *Searsia* species yielded promising activities. Overall, the species yielded more promising activities against the Gram-positive organisms (Figures 5.5 and 5.6). As shown in Figures 5.5, 5.6 and 5.8, several of the seven species of the genus *Searsia* yielded MIC's of 0.16 mg/ml and lower against *E. faecalis* (4 species), *S. aureus* (5 species) and *P. aeruginosa* (2 species). Although not shown here, extracts of three *Searsia* species also had interesting activities against *E. coli* and an extract of one species had interesting activities against *C. neoformans*.

A number of the *Combretum* species yielded promising activities, especially against *S. aureus, E. coli* and *P. aeruginosa* (4 species each) (Figures 5.6, 5.7 and 5.8). The most noteworthy activities were from extracts of *C. caffrum* and *C. mkuzense*.

The genus *Leucadendron* contained 11 species of which numerous extracts had MIC's of 0.16 mg/ml and lower (Figures 5.5, 5.8, 5.9 and 5.10), especially against *C. albicans* (4 species) and *C. neoformans* (8 species).

Extracts of a number of species that belonged to the genus *Allophylus* had promising activities against the bacteria, especially against the Gram-positive bacteria (*E. faecalis* and *S. aureus*), as shown in Figures 5.5 and 5.6. *A. chaunostachys* and *A. rubifolius* had interesting activities of 0.16 mg/ml and lower against both Gram-positive bacteria. Extracts of *A. decipiens* had very high activities against *S. aureus* (MIC, 0.03 mg/ml) as shown in Figure 5.6 and *P. aeruginosa* (MIC, 0.04 mg/ml), not shown here. It also had interesting activities (MIC's ≤ 0.16 mg/ml) against the other two bacteria *E. faecalis* and *E. coli*.

As shown in Figure 5.10, extracts of all five *Strelitzia* species were very effective against *C. neoformans* while three extracts inhibited *C. albicans* at MIC's of 0.16 mg/ml and lower (Figure 5.9). These extracts were less effective against the bacteria, especially the Gram-positive bacteria (not shown).

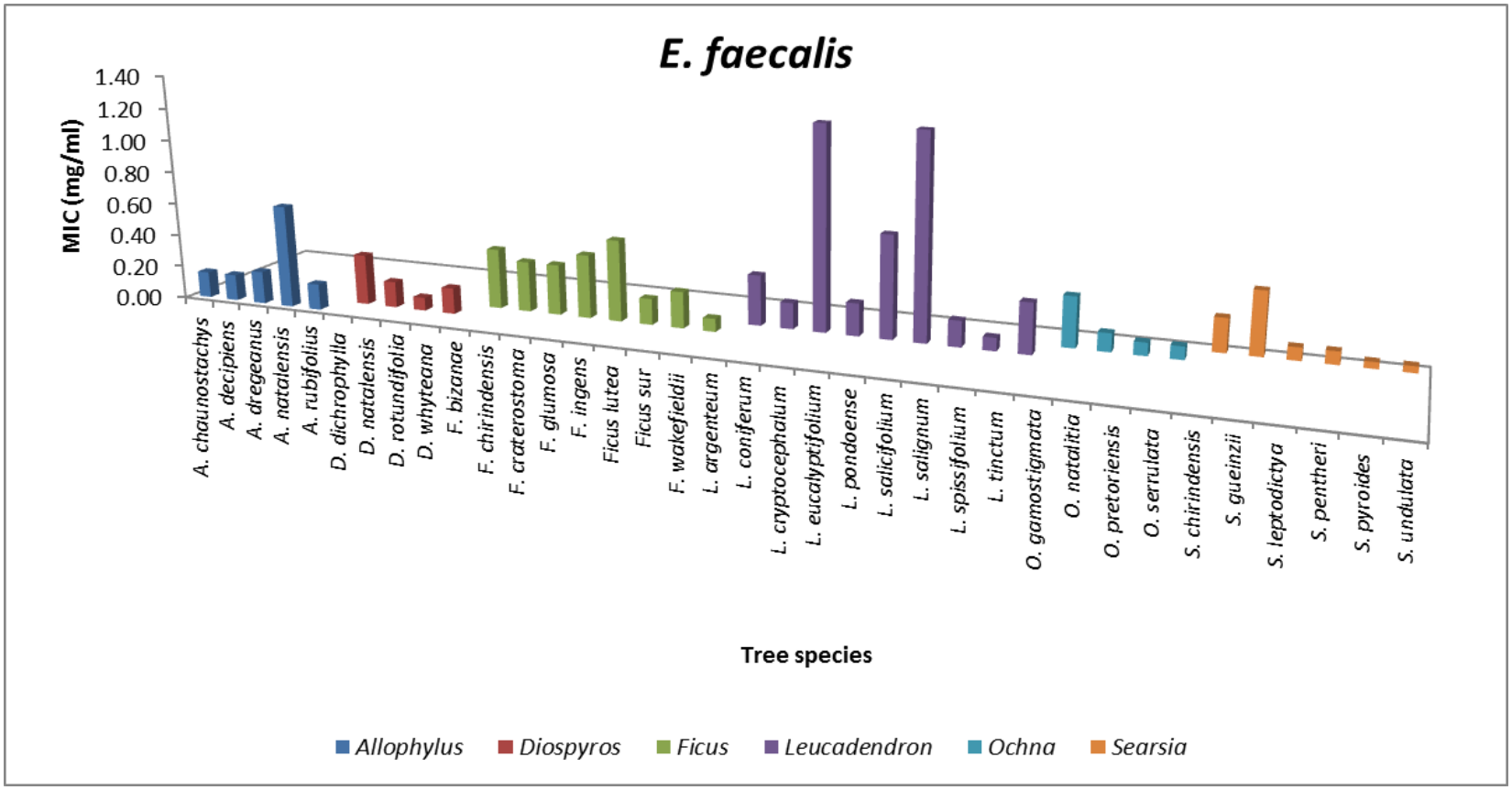


**Fig. 5.5:** Tree genera with several promising species against *E. faecalis*.

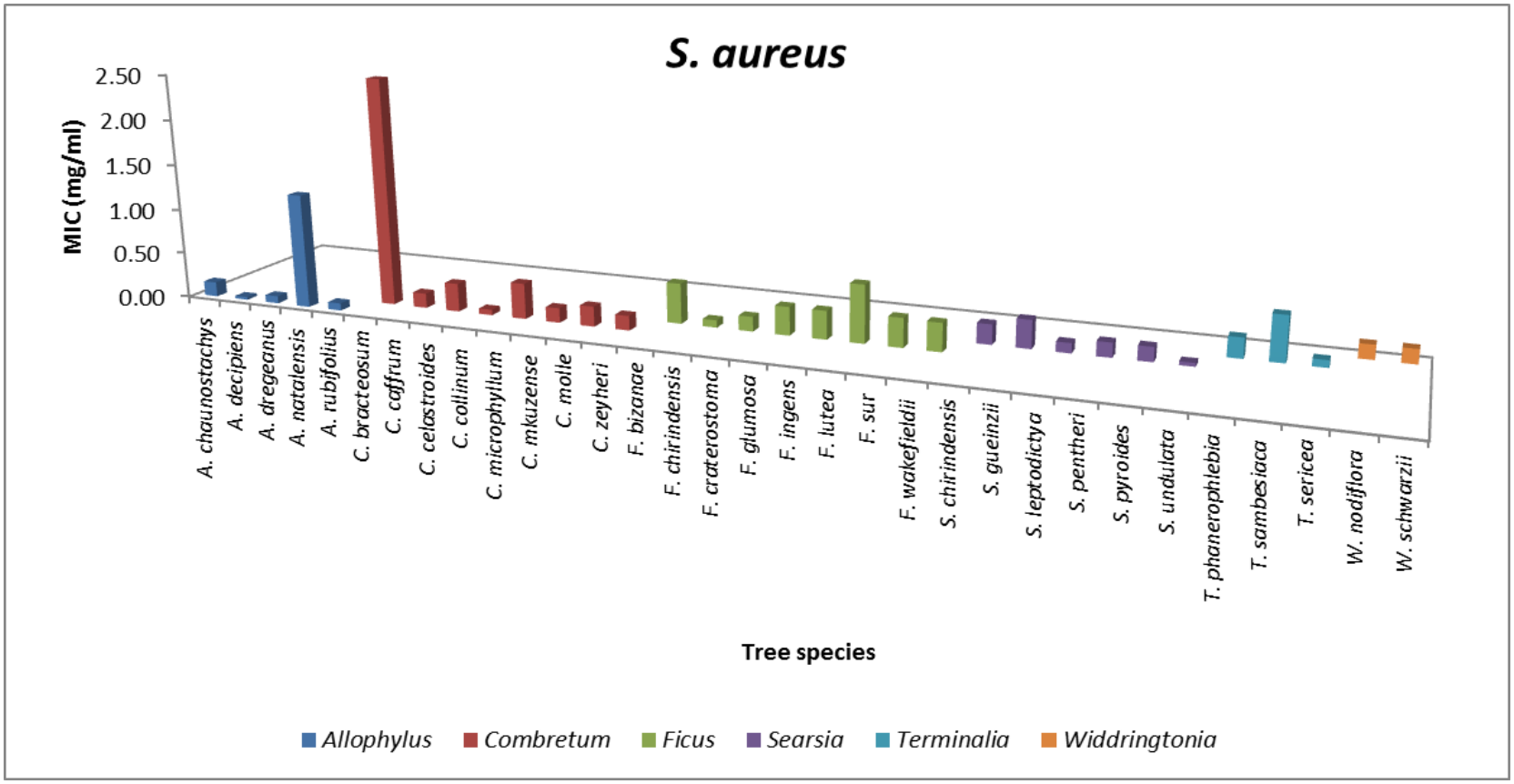


**Fig. 5.6:** Tree genera with several promising species against *S. aureus*.

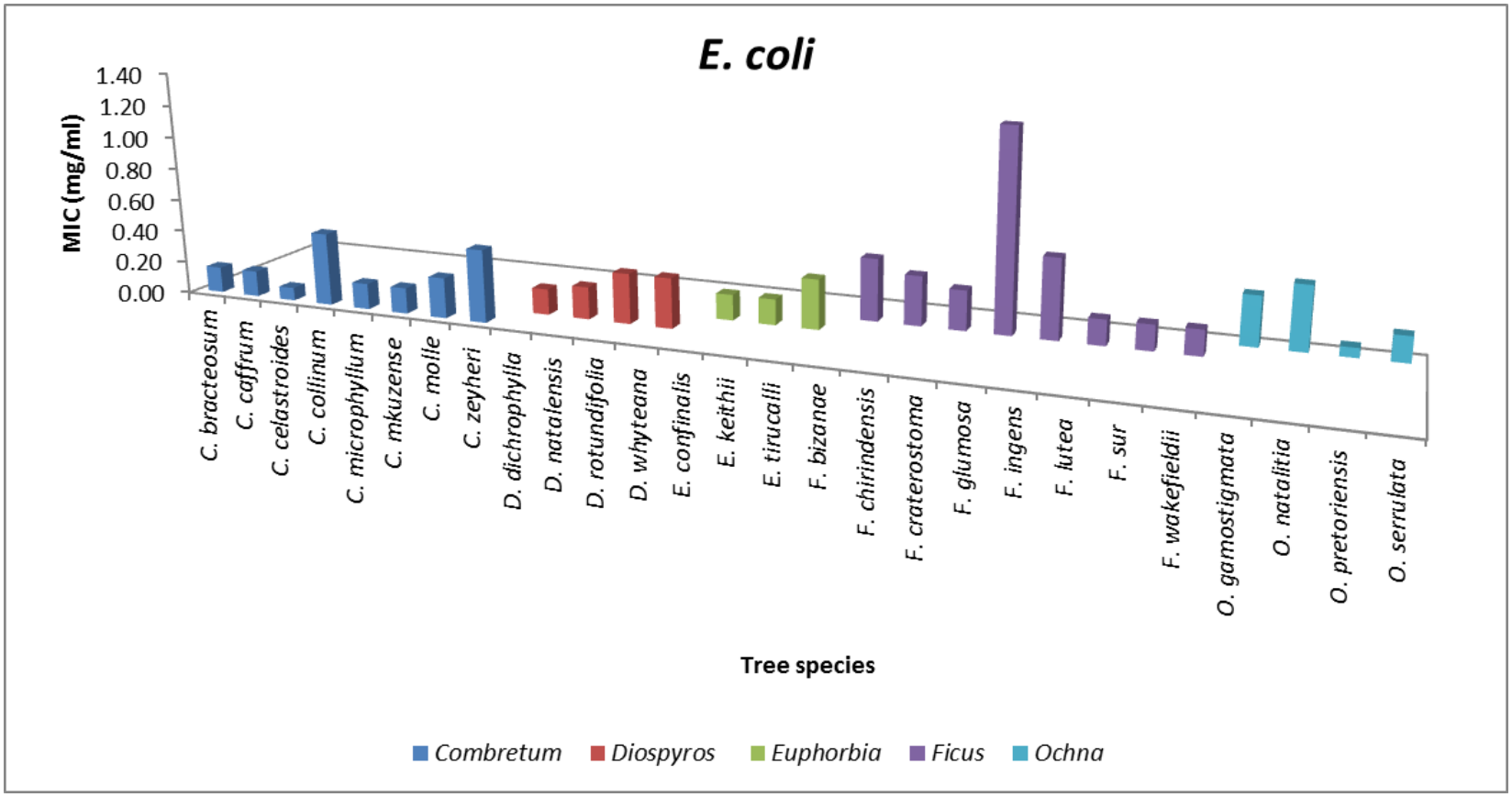


**Fig. 5.7:** Tree genera with several promising species against *E. coli*.

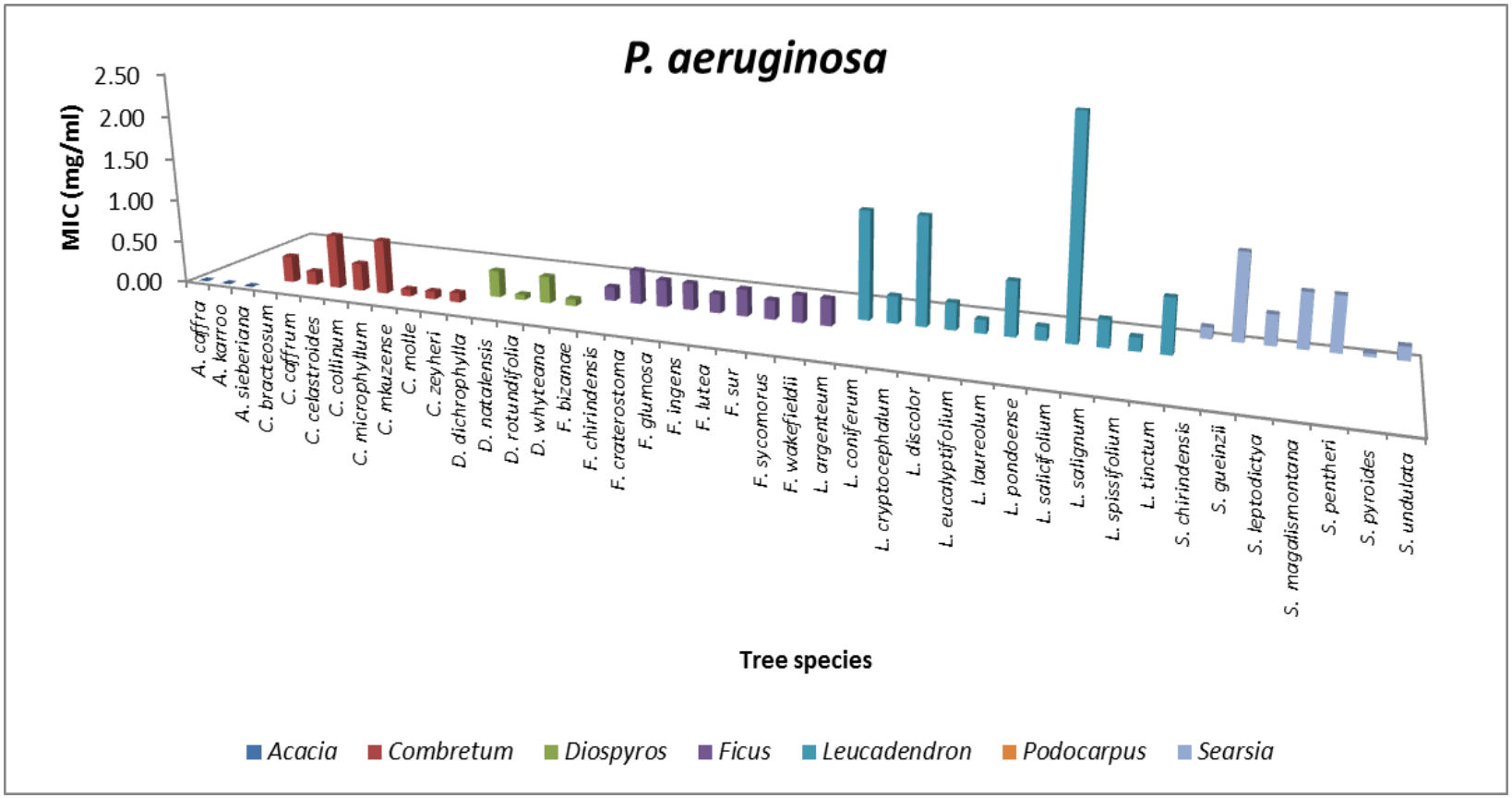


**Fig. 5.8:** Tree genera with several promising species against *P. aeruginosa*.

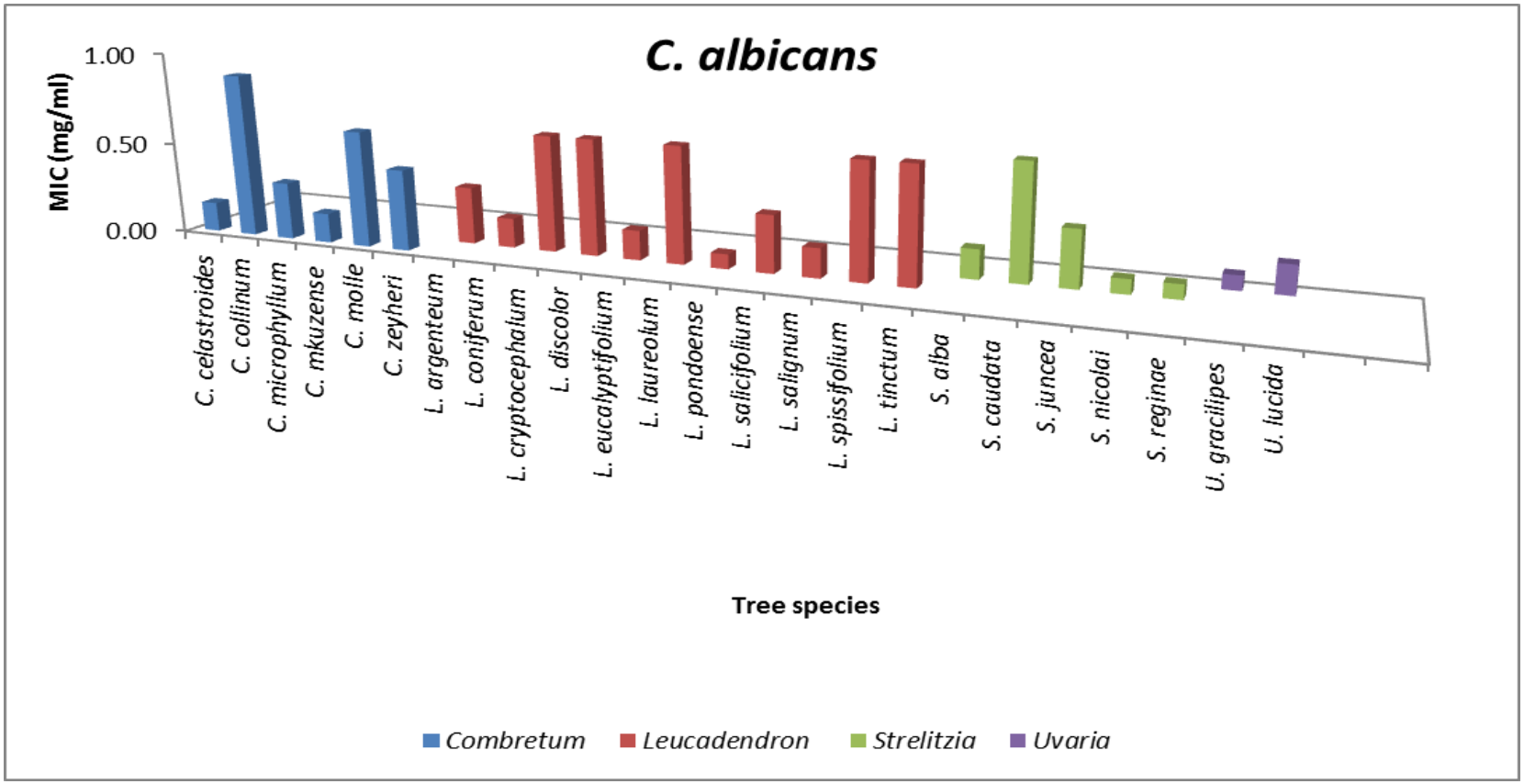


**Fig. 5.9:** Tree genera with several promising species against *C. albicans*.

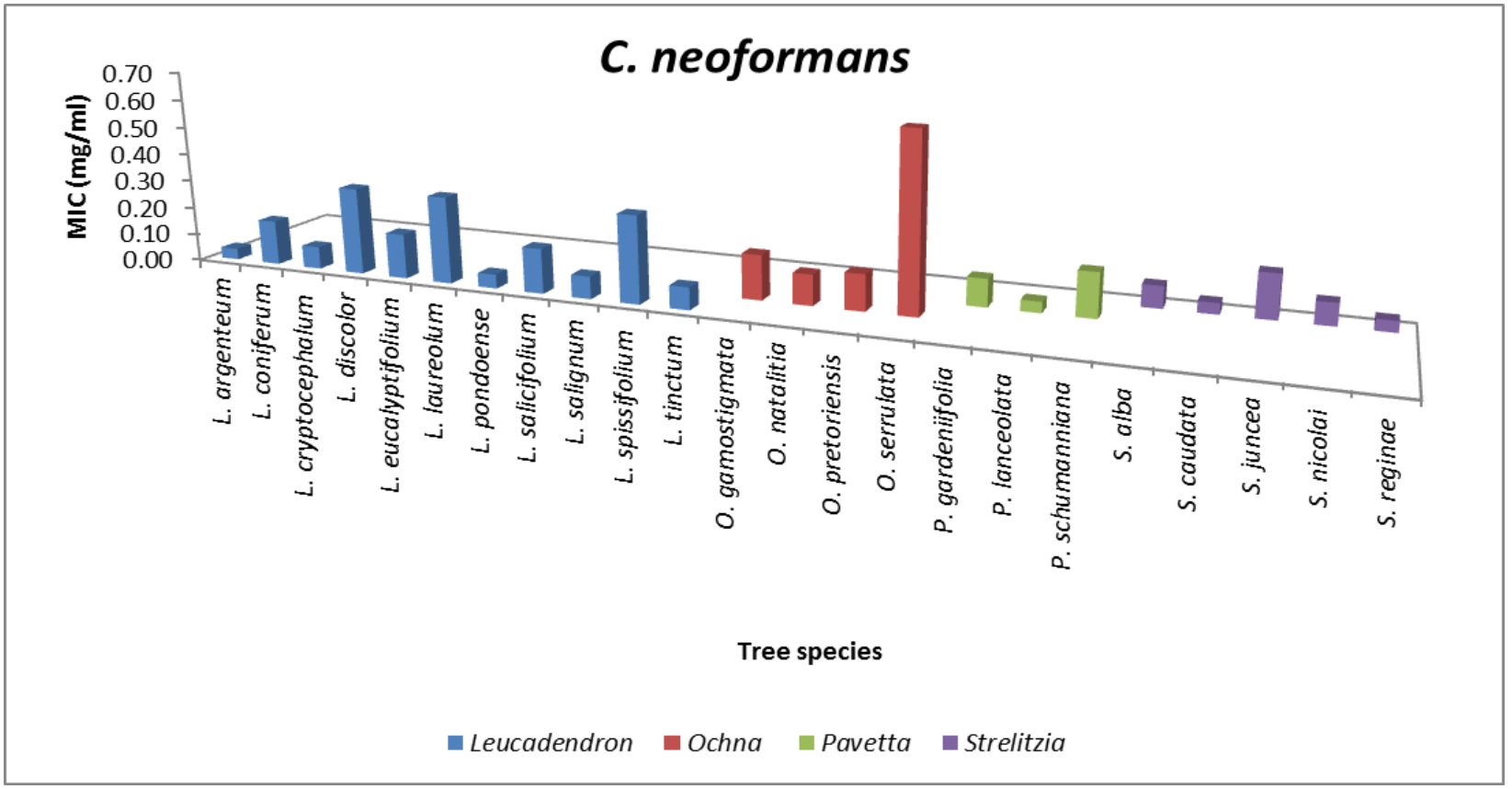


**Fig. 5.10:** Tree genera with several promising species against *C. neoformans*.

### 5.3.4 Parallels with previous antimicrobial studies and ethnomedicine

A large number of promising species were identified and although it was not in the scope of this project to compare the activities with previous findings and ethnomedicinal uses, a few of the promising species are highlighted hereafter to illustrate the potential of the project.

Some of our findings agreed with previously published results while other species have not yet been identified as promising before. In the present study, *Leucosidea sericea* was identified as a promising species using several criteria. In a previous study by Aremu *et al*. (2010), leaf extracts of *L. sericea* had a broad spectrum antibacterial activity, which was especially effective against *S. aureus* and *Bacillus subtilis*. They ascribed the higher activities of the leaf extracts possibly due to a higher level of phenolic compounds. Similar promising results by using the disc-diffusion method established that extracts of *L. sericea* was active against *S. aureus*, *Bacillus subtilis* and *C. albicans* (Bosman *et al*., 2004). These promising antimicrobial activities of *L. sericea* support the ethnomedicinal use of *L. sericea* extracts, among other the treatment of Ophthalmia, (Coates Palgrave, 2005). The published data on the antifungal activities of *L. sericea* is less promising compared to the antibacterial activities. Low activities were reported from petroleum ether, dichloromethane and ethanol extracts of leaf samples against *C. albicans* (Aremu *et al*., 2010) while moderate activities of 0.31 and 0.16 mg/ml against *C. albicans* and *C. neoformans* were reported for acetone leaf extracts (Adamu *et al*., 2012).

The promising results of extracts of several *Terminalia* species were substantiated by earlier reports. Extracts of leaves, roots and twigs of *T. phanerophlebia* also had good antibacterial activities against a range of organisms (Shai *et al*., 2008; Madikizela *et al*., 2013). Similarly, it was reported that extracts of *T. sambesiaca* was effective against a wide range of organisms (Fyhrquist *et al*., 2002, 2004). In the present study, other species of *Terminalia* also yielded promising activities for example extracts of non-indigenous *T. alata* yielded MIC's of 0.06 mg/ml and lower against *S. aureus, E. faecalis* and *C. neoformans*. More studies reported promising antibacterial and antifungal activities of several other southern African *Terminalia* species and various compounds have been isolated (Baba-Moussa, 1999; Eloff, 1999; Masoko *et al*., 2005; Masoko and Eloff, 2005; Samie and Mashau, 2013).

Traditionally, species of *Terminalia* are widely used throughout Africa as medicine (Fyhrquist *et al*., 2002, 2004) and the promising antimicrobial activities of *Terminalia* species validate their use in the treatment of numerous diseases in traditional medicine. Approximately twelve *Terminalia* species are found in southern Africa, of which the majority occur outside the borders of South Africa. Although only three indigenous species were analysed here, the numerous publications of research studies world-wide confirmed the potential use of the different species within this genus which warrant further studies, especially on the lesser known species.

No reports on the antimicrobial activities of *Macaranga mellifera* and *M. capensis* in the literature were found. However, other species of the genus *Macaranga* were found to have antibacterial and antifungal activities (Salah *et al*., 2003; Lim and Lim, 2009; Verma *et al*., 2013). *M. mellifera* and *M. capensis* are the only two southern African tree species found in the genus *Macaranga* and it should be worthwhile to investigate both species in more detail. The bark of *M. capensis* is used medicinally (Van Wyk and Van Wyk, 1997) and the roots are used for male impotence in south-central Zimbabwe and north-western Ethiopia (Muthuswamy and Mequente, 2009; Maroyi, 2013). The roots are also used to treat cancer in north-western Ethiopia (Muthuswamy and Mequente, 2009).

Although various species of *Allophylus* is used in traditional medicine in southern Africa, including *Allophylus decipiens* (Arnold *et al*., 2002), nothing has been reported on the antimicrobial activity of *A. decipiens*. However, other species such as *A. serratus* were found to have good antimicrobial activities against *Bacillus subtilis* using the agar diffusion method (Chavan and Gaikwad, 2013).

Many more promising species were identified but it was not in the scope of this project to investigate each of them in detail. However, the few examples above illustrate the potential of the project.

## 5.4 Conclusions

Between 704 and 717 extracts of 537 tree species that were tested had a wide range of activities against the pathogens. On average, 26% of the extracts inhibited the pathogens at MIC's of 0.16 mg/ml which highlighted the potential of the tree species of southern Africa as sources of antimicrobial activity. However, the large number of plant species recording interesting MIC's of 0.16 mg/ml may indicate that the concentration is not low enough to discriminate between promising species. The evidence from our wide-screening confirmed the emerging convention that an MIC of 0.1 mg/ml or lower would be a reasonable and practical standard to determine significant antimicrobial activity in screening procedures. The results also indicated that the benchmark could be modified for each of the pathogens because of the different sensitivities towards the extracts. In this case, the MIC benchmark could be lowered to 0.06 mg/ml for extracts tested against *C. neoformans* and increased to 0.01 mg/ml for extracts tested against *E. coli*. More stringent criteria could help to avoid the selection of false positives.

The study identified and short-listed several tree species and genera with promising activities that could be used in future in-depth studies or screening of related taxa. Among the extracts yielding MIC's of 0.16 mg/ml and lower, some inhibited the growth of several test pathogens. This may be indicative of a broad spectrum of antimicrobials. However, these extracts could

also be toxic to mammals. Some of the tree species were more effective against one of the pathogens or against a specific class of pathogens (bacteria or fungi or Gram-positive bacteria or Gram-negative bacteria). These species may have potential for the discovery of selective antimicrobial compounds.

The promising antimicrobial properties displayed by several plant extracts in this study provided a useful platform for future screening programmes. A number of the tree species that had high antimicrobial activities in this study have previously been shown to possess *in vitro* activity. Some of the most active extracts belonged to tree species which have been insufficiently explored to date and which may have been possibly identified for the first time; warranting further investigations as potential antimicrobial sources. For example, at this stage it is unclear whether the antimicrobial activities of the promising tree extracts result from the action of one individual compound or a number of compounds in combination. Furthermore, future studies should confirm if the activity is indeed antimicrobial activity or related to general toxicity.

Studies of the most active tree extracts are already underway in the Phytomedicine programme of the University of Pretoria, and would in some cases lead to patents. There is scope for more studies to confirm and extend the present results. These studies should include specific cytotoxic studies, phytochemical and pharmacological studies to determine the types of compounds responsible for the antimicrobial activities, mechanisms of action and eventually (but not necessarily) the isolation of bioactive compounds.

The next investigation that will be described in Chapter 6 was to identify the most susceptible pathogen and to determine if the antimicrobial activities against the respective pathogens are correlated.

# CHAPTER 6

# The sensitivity and correlation of six selected pathogens in relation to the antimicrobial activities of extracts of several hundred southern African tree species

## 6.1 Introduction

This chapter is linked to the previous Chapter 5 in which we screened several hundred southern African plant species against six medicinally important bacterial and fungal pathogens to determine significant antimicrobial activities based on the MIC values of the extracts and to identify the most promising tree extracts.

In this Chapter, our first aim was to determine which pathogen was the most sensitive to the inhibitory effect of tree extracts. Several authors state that bacteria are more sensitive to plant extracts than fungi (Oskay and Sari, 2007; Vaghasiya and Chanda, 2007; Zhang *et al*., 2013). Furthermore, several reports indicated that Gram-positive bacteria are generally more sensitive to plant extracts compared to Gram-negative bacteria (Recio *et al*., 1989; Vlietinck *et al*., 1995; Lall and Meyer, 2000; Samie *et al*., 2005; Sofidiya *et al*., 2012).

The high resistance that is often shown by Gram-negative bacteria could be related to the properties of the cell wall which makes it impermeable to lipophilic solutes and thus acts as a barrier against many substances including antibiotics (Nikaido, 1976). Most antibacterial compounds are non-polar and it is therefore more challenging to develop new drugs against Gram-negative bacteria (Eloff and McGaw, in press). Of the two fungi, *Candida albicans* was reported to be more resistant to plant extracts than *Cryptococcus neoformans* (Buwa and Van Staden, 2006; Samie *et al*. 2010; Rath and Ray, 2012).

The second aim of this chapter was to determine whether the antimicrobial activities of the tree extracts against different pathogens are correlated. If a strong correlation were to be determined between the antimicrobial activities of tree extracts against two pathogens, there would be a higher probability that extracts yielding promising activities against one pathogen may also have good activities against the correlated pathogen. Information about correlated activities could facilitate the selection of potential plant species for future testing against other pathogens based on results with the pathogens we used here.

## 6.2 Materials and methods

The study area, material and taxonomical arrangements of the trees of southern Africa were described in Chapter 2. The collection of tree samples and the description of the tree species represented in the Phytomedicine Tree Database were discussed in Chapter 3 and the preparation of general methods and data processing were discussed in Chapter 4.

## 6.3 Results and discussion

In Chapter 6.3.1, the antimicrobial activities of the extracts against the pathogens were compared to determine which pathogens were the most sensitive and this was followed by an investigation to determine if the antimicrobial activities of different pathogens and pathogen classes are correlated (6.3.2).

### 6.3.1 Sensitivity of the six pathogens

Two different approaches were followed to compare the pathogens. First we calculated for each pathogen the overall mean MIC from the spectrum of inhibition by the extracts (from low to high MIC values) against that pathogen (6.3.1.1). Mean MIC's were then compared between the pathogens.

The second approach was counting the number of extracts inhibiting the pathogens at different concentrations in the MIC spectrum (6.3.1.2) which enabled us to compare the pathogens based only on extracts with interesting activities (MIC ≤ 0.16 mg/ml). The total number of extracts that were applied per individual pathogen was used to calculate the proportion that inhibited each of the pathogens at MIC's of 0.16 mg/ml

#### 6.3.1.1 Sensitivity of the pathogens based on mean MIC values

In this approach, the sensitivity of the pathogen was compared based on the overall mean MIC values of all the extracts. The MIC values were compared statistically using the Friedman analysis of variance. The differences between the mean MIC's of the various organisms were small, mostly not significant, ranging from 0.36 mg/ml to 0.54 mg/ml (Table 6.1).

Of the six pathogens, *E. faecalis*, a Gram-positive bacterium, was the most susceptible pathogen, followed by *C. neoformans* one of the fungal pathogens. The analysis established that *E. faecalis* was significantly more sensitive compared to *S. aureus* and *C. albicans*. The Gram-positive bacterium, *S. aureus*, was the least susceptible pathogen and was significantly less sensitive to the extracts compared to all five the other pathogens. These results were unexpected and contrary to many reports asserting that Gram-positive bacteria are more sensitive compared to Gram-negative bacteria. However, there were some studies which found

that Gram-negative bacteria were more susceptible to the plant extracts tested compared to Gram-positive bacteria (Umeh *et al.,* 2005; Akter *et al*., 2010; Shai *et al*., 2013). This is supported by observations made by Obeidat *et al*. (2012), that most of their 11 plant extracts had promising activities against Gram-negative bacteria while having limited effects against Gram-positive bacteria.

**Table 6.1:** The mean minimum inhibitory concentration (MIC, mg/ml) ± standard deviation (SD) of all the extracts against each of the six pathogens (Mean MIC values followed by the same letter do not differ significantly at the 5% level).

| Pathogen | Number of extracts | MIC value (mg/ml) (± SD) |
|---|---|---|
| *E. faecalis* | 715 | 0.36 ± 0.77 [a] |
| *C. neoformans* | 717 | 0.38 ± 1.01[ab] |
| *P. aeruginosa* | 707 | 0.39 ± 0.75[ab] |
| *E. coli* | 714 | 0.40 ± 0.74[ab] |
| *C. albicans* | 708 | 0.42 ± 1.01 [b] |
| *S. aureus* | 717 | 0.54 ± 1.07 [c] |
| P - value | | < 0.0001 |

### 6.3.1.2 Sensitivity of the pathogens at different MIC cut-off points

In the second approach we counted the number of extracts (expressed as a percentage of the total number of extracts) that inhibited the individual pathogens at different concentrations in the MIC spectrum (Table 6.2). As explained before, MIC's of 0.16 mg/ml and lower were considered as interesting activities in this study and therefore we compared the sensitivity of the individual pathogens at this concentration and lower. At an MIC cut-off point of 0.16 mg/ml, the largest percentage of extracts was active against *C. neoformans, C. albicans* and *E. faecalis* (33%, 28% and 27% respectively).

**Table 6.2:** The number of extracts (percentage in brackets) inhibiting each of the pathogens at different concentrations in the MIC spectrum.

| Pathogen | MIC (mg/ml) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | < 0.02 | **≤ 0.04** | **≤ 0.08** | **≤ 0.16** | **≤ 0.31** | **≤ 0.63** | **≤ 1.25** | **≤ 2.50** |
| *S. aureus* | 12 (1.67%) | 23 (3.21%) | 57 (7.95%) | 136 (18.97%) | 275 (38%) | 457 (64%) | 586 (82%) | 717 |
| *E. faecalis* | 10 (1.40%) | 35 (4.90%) | 67 (9.37%) | 194 (27.13%) | 403 (56%) | 567 (79%) | 665 (93%) | 715 |
| *E. coli* | 3 (0.42%) | 6 (0.84%) | 38 (5.32%) | 178 (24.93%) | 389 (54%) | 557 (78%) | 667 (93%) | 714 |
| *P. aeruginosa* | 9 (1.26%) | 19 (2.65%) | 61 (8.51%) | 174 (24.27%) | 384 (54%) | 570 (79%) | 658 (92%) | 717 |
| *C. albicans* | 4 (0.56%) | 16 (2.26%) | 70 (9.89%) | 195 (27.54%) | 375 (53%) | 508 (72%) | 599 (85%) | 708 |
| *C. neoformans* | 4 (0.57%) | 25 (3.54%) | 103 (14.57%) | 232 (32.81%) | 383 (54%) | 520 (74%) | 598 (85%) | 707 |

The percentage (%) extracts that inhibited each of the individual bacterial organisms and fungal organisms at MIC's of 0.16 mg/ml and lower is presented visually in Figures 6.1 and 6.2 respectively.

At MIC's of 0.16 mg/ml (interesting activity), *E. faecalis* was the most sensitive bacterial pathogen (Figure 6.1). Only 19% of all the extracts inhibited *S. aureus* at this concentration. At an MIC cut-off point of 0.08 mg/ml (high activity), *E. faecalis* remained the most sensitive bacterium with 67 extracts (9.37%) inhibiting the growth of the organism. At this concentration, 61 extracts (8.51%) inhibited the growth of *P. aeruginosa* and 57 extracts (7.95%) inhibited the growth of *S. aureus*. Only 38 (5.32%) extracts inhibited the growth of *E. coli* at an MIC of 0.08 mg/ml.

At MIC's of 0.04 mg/ml (very high activity), 35 extracts of all the extracts (4.90%) inhibited *E. faecalis*, 23 extracts (3.21%) inhibited *S. aureus*, 19 extracts (2.65%) inhibited the growth of *P. aeruginosa*, while six (0.84%) extracts inhibited the growth of *E. coli*. At an MIC of 0.02 mg/ml (very high activity), *S. aureus* and *E. faecalis* were the most sensitive bacteria, respectively with twelve (1.67%) and ten extracts (1.40%) that inhibited the growth of the bacteria (Figure 6.1). In comparison, nine extracts (1.26%) inhibited the growth of *P. aeruginosa* and three (0.42%) inhibited the growth of *E. coli*.

The general higher sensitivity of both the Gram-positive bacteria at concentrations of 0.08 mg/ml and lower is in agreement with other studies (Recio *et al*., 1989; Vlietinck *et al*., 1995; Lall and Meyer, 2000; Stefanović *et al*., 2012). However, unexpectedly at concentrations higher than 0.08 mg/ml, *S. aureus* was more resistant compared to all other test bacteria.

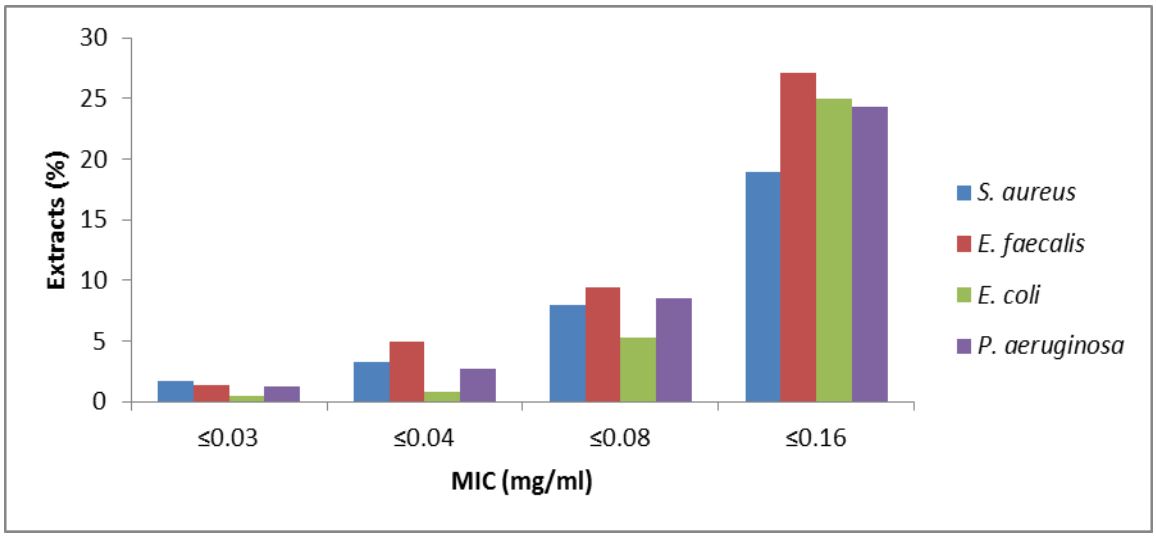


**Fig. 6.1:** Proportion of extracts (n = 714 - 717) that were active against bacterial pathogens at MIC's of 0.16 mg/ml and lower.

Among the fungi, *C. neoformans* was more sensitive compared to *C. albicans* at MIC's of 0.16 mg/ml and lower (Figure 6.2). The results correlated with a study by Samie *et al*. (2010) who found that more of the 76 extracts of 30 selected medicinal plants used in the Venda geographical region of South Africa were active against *C. neoformans* (43%) when compared to *C.albicans* (17%). Similarly, in a screening study using the agar diffusing method, *C. neoformans* was found to be more sensitive to several root extracts of *Terminalia, Pteleopsis* and *Combretum* species compared to *C. albicans* (Fyhrquist *et al*., 2004).

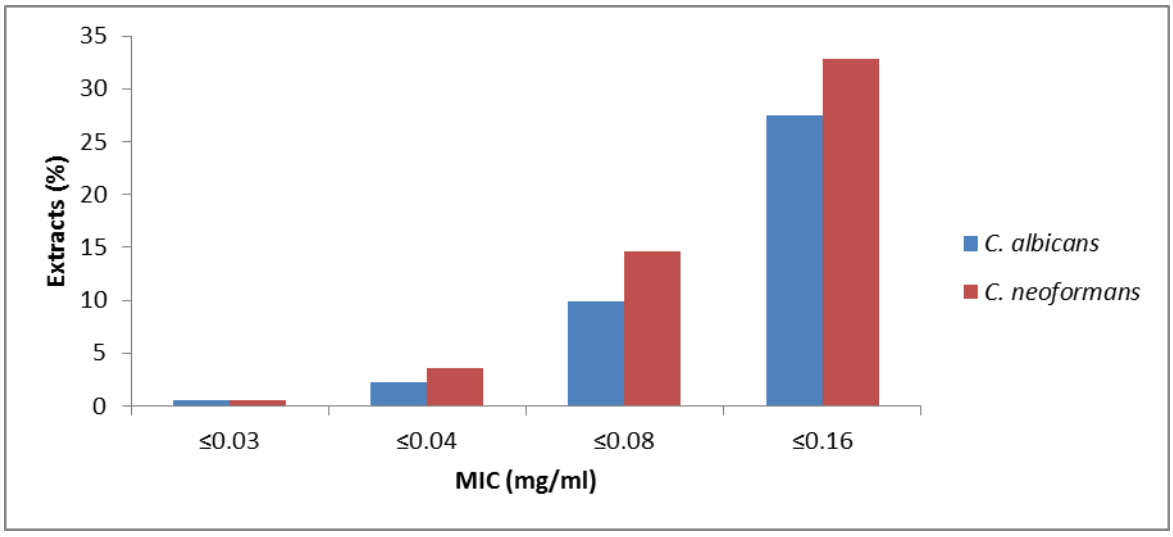


**Fig. 6.2:** Proportion of extracts (n = 707 - 708) that were active against fungal pathogens at MIC's of 0.16 mg/ml and lower.

### 6.3.2 Pathogen correlations

The next objective in this chapter was to compare the antimicrobial activities of 717 crude tree extracts to determine if a correlation existed between the pathogens (6.3.2.1) and between pathogen classes (6.3.2.2).

#### 6.3.2.1 Correlations between the reactions of pathogens to antimicrobials

To begin with, the antimicrobial activities of 717 crude tree extracts against the six pathogens were compared with one another by determining correlation coefficients (r) and coefficients of determination ($r^2$). The results are summarised in Table 6.3 indicating that all correlations were positive, ranging from moderate to weak. The three strongest and two weakest correlations are displayed in Figures 6.3 to 6.7 and will be discussed below.

The correlation between the two fungal pathogens was the strongest of all paired organisms. The correlation can be regarded as moderate (r = 0.49) indicating that only 24% of the variation in *C. albicans* could be explained by the variation in *C. neoformans* (Figure 6.3). As shown in Figures 6.4 and 6.5, moderate correlations were also found between the two corresponding Gram-positive bacteria (*S. aureus* and *E. faecalis*) and the two corresponding Gram-negative bacteria (*E. coli* and *P. aeruginosa*) with correlation coefficients of 0.42 and 0.45 respectively. The coefficient of determination indicated that 18% of the variance in *S. aureus* and *E. faecalis* is shared (Figure 6.4) while 19% of the variance of *E. coli* could be explained by the variation in *P. aeruginosa* (Figure 6.5). The correlation coefficients between the antimicrobial activities of combinations of organisms in different pathogen classes were lower, signifying weaker correlations. As shown in Figures 6.6 and 6.7, the weakest correlations were found between *C. albicans* when compared with *E. faecalis* (r = 0.03) and *S. aureus* (r = 0.02) respectively.

**Table 6.3:** Comparisons of the minimum inhibitory concentration (MIC) of 717 crude tree extracts to determine correlations between pathogens (*E. faecalis, S. aureus*, *E. coli, P. aeruginosa*, *C. albicans* and *C. neoformans*).

| Pathogens | Correlation coefficient (r) | Coefficient of determination ($r^2$) | Number of observations (n) |
|---|---|---|---|
| *C. neoformans - C. albicans* | 0.49 | 0.24 | 707 |
| *P. aeruginosa - E. coli* | 0.45 | 0.19 | 713 |
| *E. faecalis - S. aureus* | 0.42 | 0.18 | 714 |
| *E. coli - E. faecalis* | 0.38 | 0.14 | 714 |
| *E. coli - S. aureus* | 0.37 | 0.13 | 714 |
| *P. aeruginosa - E. faecalis* | 0.32 | 0.10 | 714 |
| *P. aeruginosa - S. aureus* | 0.27 | 0.07 | 716 |
| *C. neoformans - E. coli* | 0.19 | 0.04 | 701 |
| *C. albicans - E. coli* | 0.17 | 0.03 | 702 |
| *C. neoformans - P. aeruginosa* | 0.17 | 0.03 | 703 |
| *C. albicans - P. aeruginosa* | 0.13 | 0.02 | 706 |
| *C. neoformans - E. faecalis* | 0.12 | 0.01 | 701 |
| *C. neoformans - S. aureus* | 0.10 | 0.01 | 704 |
| *C. albicans - E. faecalis* | 0.03 | 0.00 | 702 |
| *C. albicans - S. aureus* | 0.02 | 0.00 | 705 |

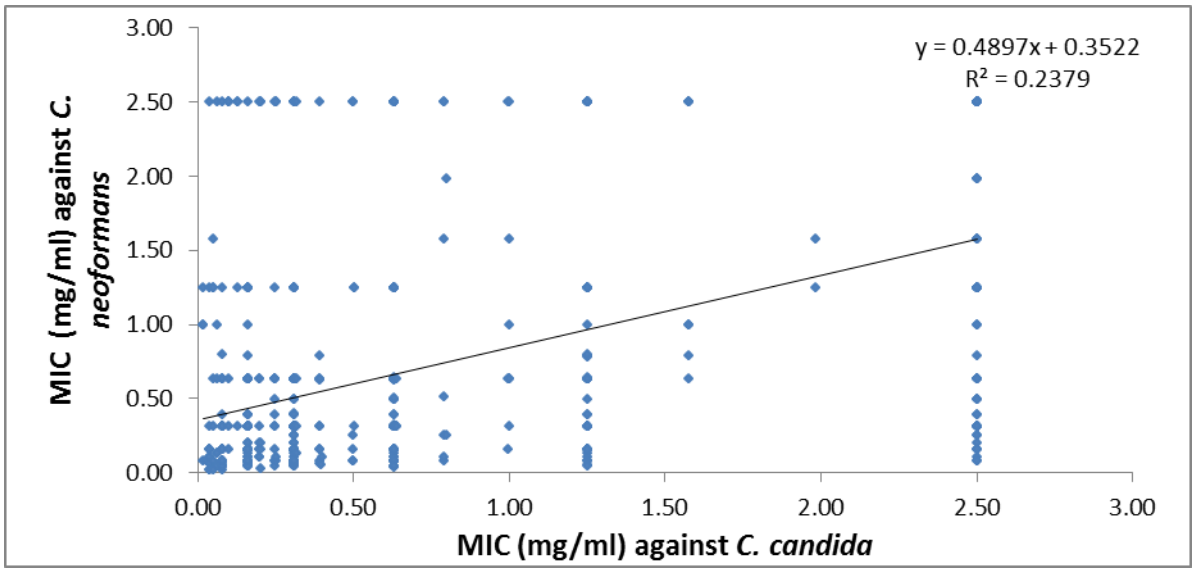


**Fig. 6.3:** Correlation of the minimum inhibitory concentrations (MIC in mg/ml) between *C. neoformans* and *C. albicans*.

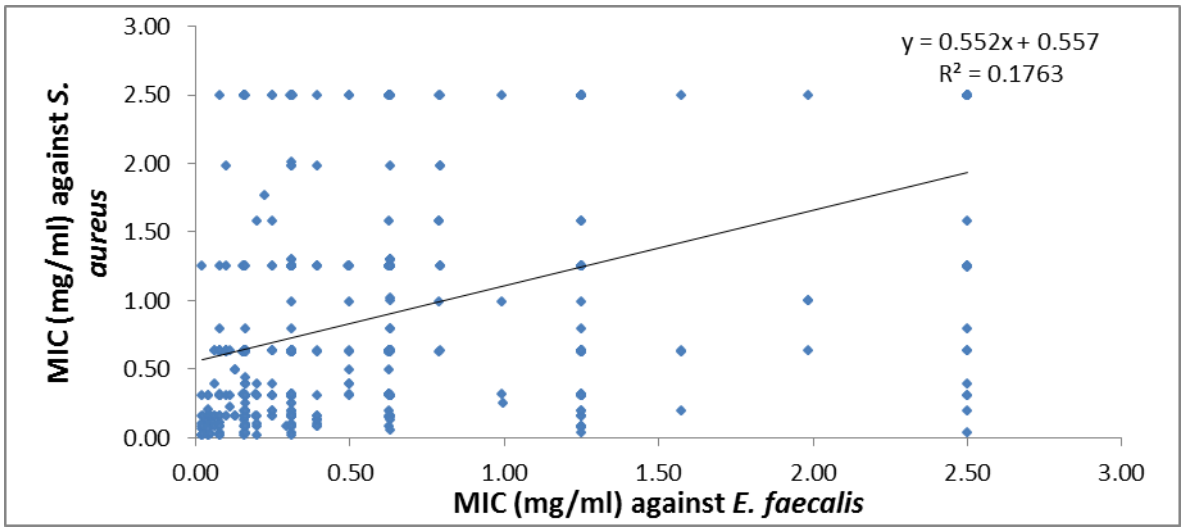


**Fig. 6.4:** Correlation of the minimum inhibitory concentrations (MIC in mg/ml) between *S. aureus* and *E. faecalis.*

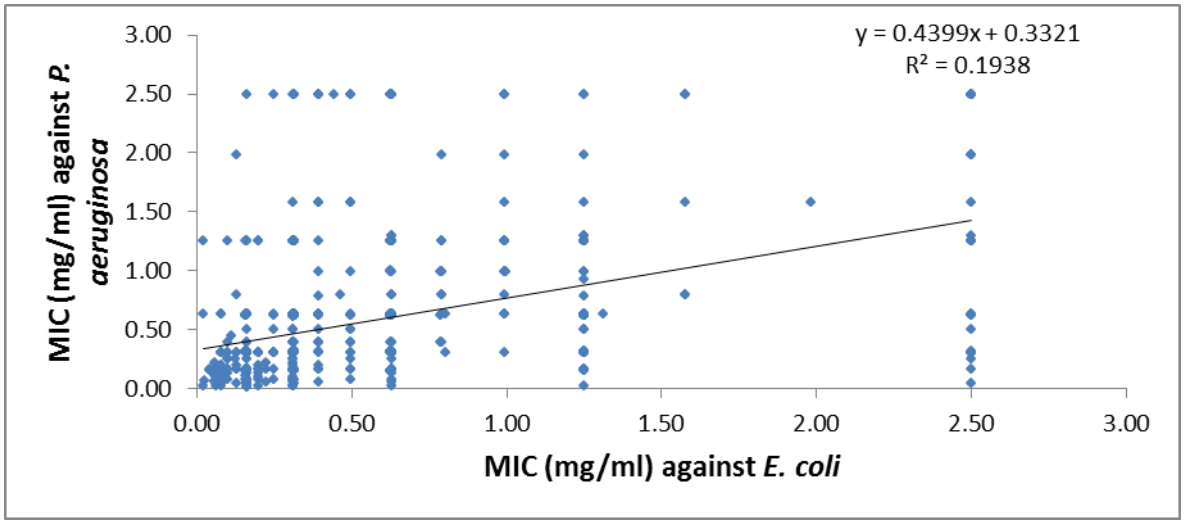


**Fig. 6.5:** The correlation of the minimum inhibitory concentrations (MIC in mg/ml) between *P. auruginosa* and *E.coli*.

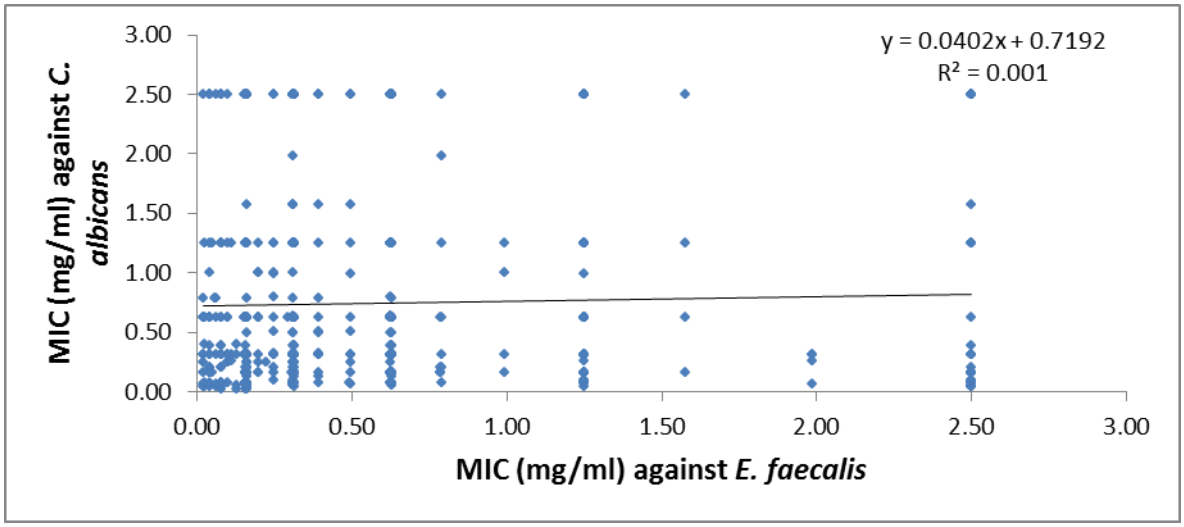


**Fig. 6.6:** The correlation of the minimum inhibitory concentrations (MIC in mg/ml) between *C. albicans* and *E. faecalis*.

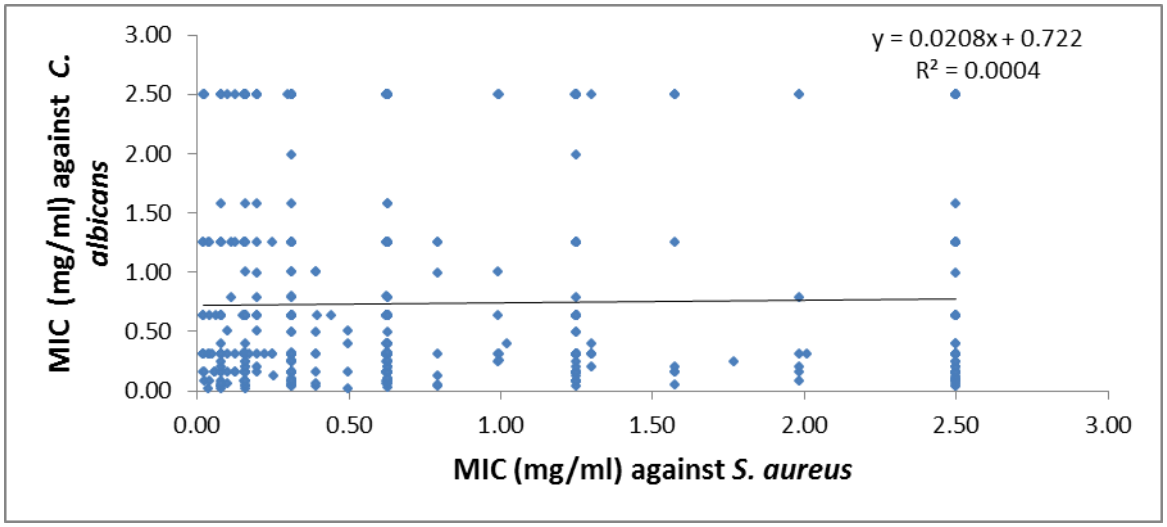


**Fig. 6.7:** Correlation of the minimum inhibitory concentrations (MIC in mg/ml) between *C. albicans* and *S. aureus*.

### 6.3.2.2 Correlations between the reactions of pathogen classes to antimicrobials

Secondly, we examined the correlation between the three classes of pathogens used (Gram-positive bacteria, Gram-negative bacteria and fungi) by determining correlation coefficients (r) and coefficients of determination ($r^2$) for each relationship as shown in Table 6.4. The correlations are visualised in Figures 6.8 to 6.10. Among these pathogen classes, the strongest correlation existed between the Gram-positive and Gram-negative bacterial classes while the other correlations were considerably weaker. Although it was the strongest correlation of the three, the correlation between bacterial classes was only moderate (r = 0.46).

The coefficient of determination indicated that only 21% of the activities against Gram-positive bacteria could be explained by the activities against Gram-negative bacteria. The correlation between the fungal class and the Gram-positive and Gram-negative bacterial classes respectively were 0.04 and 0.18. In these correlations, the activity of the extracts against the fungi was less than 3% in common with respectively Gram-positive and Gram-negative bacteria.

**Table 6.4:** Comparisons of the minimum inhibitory concentration (MIC) of between 700 and 713 crude tree extracts to determine correlations between Gram-positive bacteria, Gram-negative bacteria and fungi.

| Pathogen classes | Correlation coefficient (r) | Coefficient of determination ($r^2$) | Number of observations (n) |
|---|---|---|---|
| Gram-positive and Gram-negative bacteria | 0.46 | 0.21 | 713 |
| Gram-positive bacteria and fungi | 0.04 | 0.00 | 701 |
| Fungi and Gram-negative bacteria | 0.18 | 0.03 | 700 |

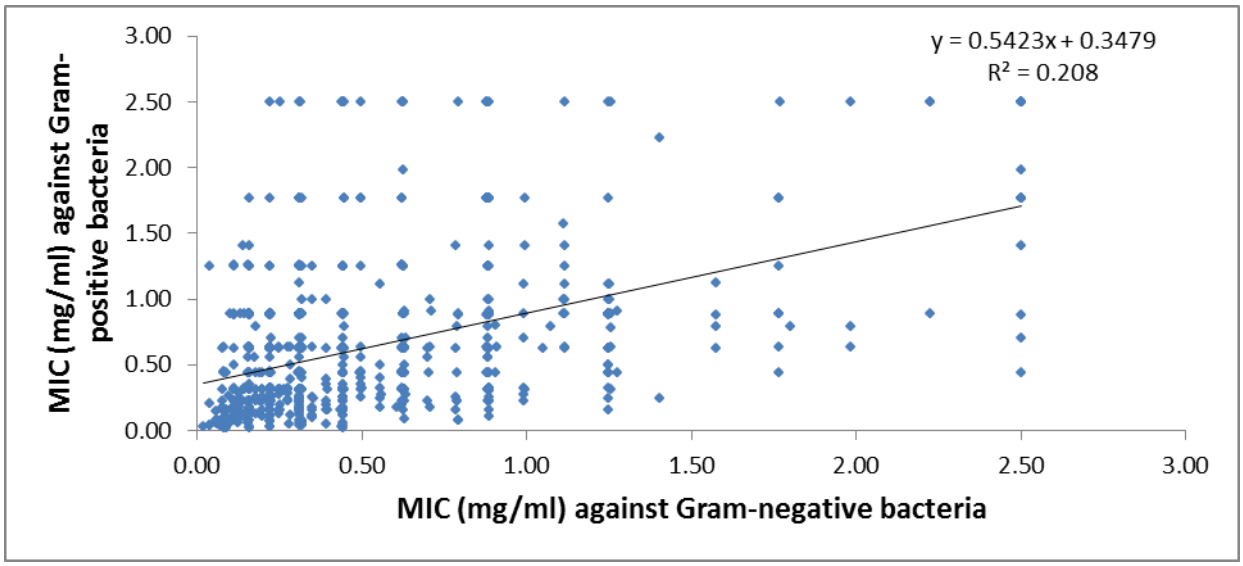


**Fig. 6.8:** The correlation of the minimum inhibitory concentrations (MIC in mg/ml) between Gram-positive and Gram-negative bacteria.

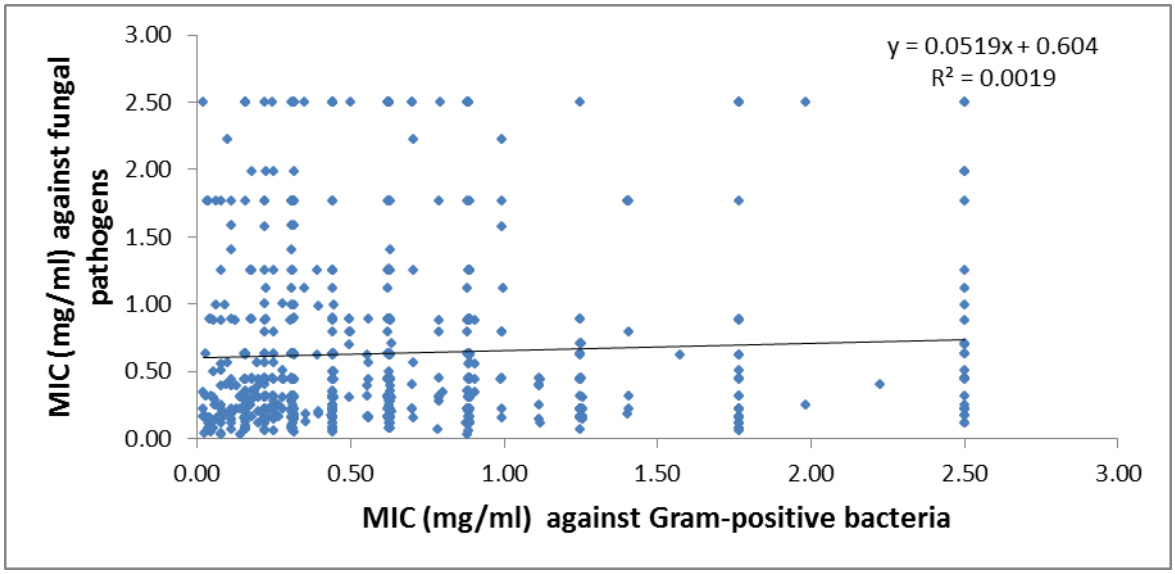


**Fig. 6.9:** Correlation of the minimum inhibitory concentrations (MIC in mg/ml) between Gram-positive bacterial and fungal pathogens.

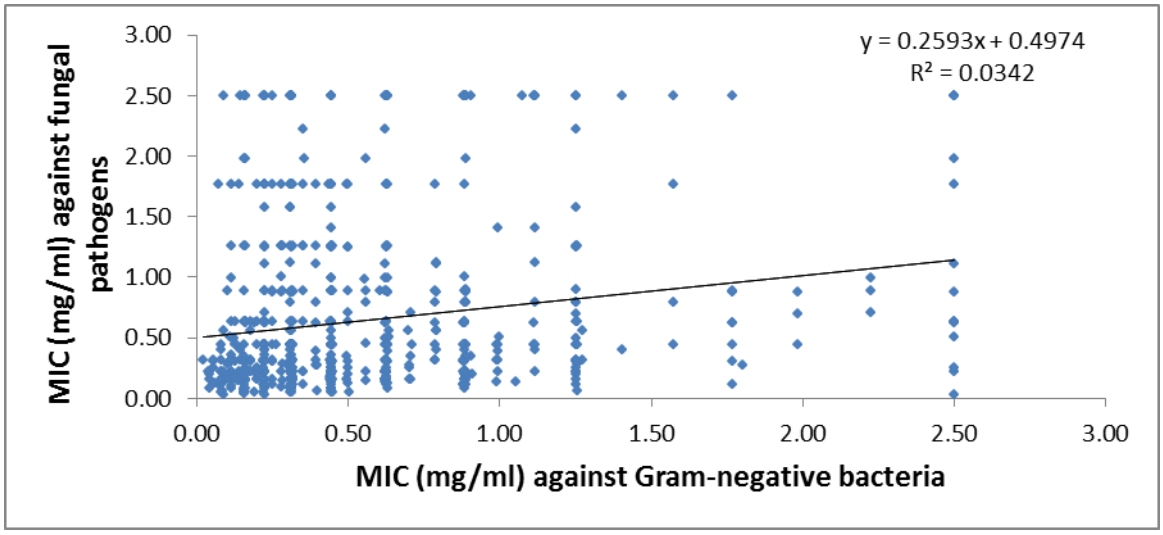


**Fig. 6.10:** Correlation of the minimum inhibitory concentrations (MIC in mg/ml) between Gram-negative bacterial and fungal pathogens.

## 6.4 Conclusions

We found small differences in the mean MIC values of all the extracts against each of the pathogens indicating small differences between the sensitivities of the pathogens. Among the bacteria, *E. faecalis* was the most sensitive bacterium while *C. neoformans* was the most sensitive fungal pathogen. The mean MIC value of the extracts against E*. faecalis* was significantly higher compared to the mean MIC values against both *S. aureus* and *C. albicans*.

Contrary to literature reports, *S. aureus,* a Gram-positive bacterium, was the least sensitive organism overall as recorded by the MIC values for all the extracts.

The susceptibility of the pathogens varied at the different MIC cut-off points. Among the bacteria, more extracts inhibited *E. faecalis* and *S. aureus* compared to *E. coli* and *P. aeruginosa* at an MIC's of 0.04 mg/ml and lower. At MIC's of 0.08 mg/ml (high activity), *E. faecalis* was the most sensitive and *E. coli* was the least sensitive bacterium (8.51% extracts inhibited *E. faecalis* compared to 5.32% for *E. coli*). However, at an MIC of 0.16 mg/ml, *S. aureus* was the most resistant bacterium. Among the fungi, *C. neoformans* was more sensitive compared to *C. albicans* at all the concentrations that were considered as interesting activity (MIC's ≤ 0.16 mg/ml). At higher concentrations the two fungi were equally sensitive to the extracts.

Reasons for the observations are not clear and further studies are needed to determine the mechanism of action to substantiate the basis of susceptibility to these plant extracts. Nevertheless, it was very promising that several extracts were active against Gram-negative *E. coli* and *P. aeruginosa.* These bacteria are usually resistant to many antibiotics and antimicrobial agents due to an outer membrane that provides an effective impermeable outer barrier (Nikaido, 1976). Equally important was that the results indicated that a large number of extracts were active against the fungi and therefore have the potential to treat fungal infections.

Generally, correlations between different pathogens based on the MIC values of the crude tree extracts, were all positive, varying from weak to moderate. The activities of the corresponding pathogens in each pathogen class were consistently better correlated than the activities of pathogens in different pathogen classes. Across pathogen classes, the relationship between the Gram-positive and Gram-negative bacteria was stronger compared to both their relationships with the fungal pathogens. However, the correlations were not strong enough to direct the selection of potential promising species for future studies.

The next step, as will be discussed in Chapter 7 and 8, was to determine whether some tree families and orders contain more promising species with high activity than others. Future collections could then strategically concentrate on close relatives of plants within these promising families and orders.

# CHAPTER 7

# Antimicrobial activities of southern African tree families

## 7.1 Introduction

Several studies showed that although certain plant families are preferred by traditional healers as medicinal plants, selection is not associated with family size and tree species are therefore traditionally selected on the basis of bioactivity (Moerman and Estabrook 2003; Douwes *et al*., 2008). Nonetheless, plant species in related taxa may have favourable traits or similar types of compounds (Heinrich *et al*., 2004). Therefore, evidence of chemotaxonomy relationships should enable predictions of certain characteristics of closely related taxa. This is based on the premises that: (1) secondary compounds are often specific to a given botanical family, genus or species and (2) there is a correlation between certain secondary compounds and antimicrobial activity.

In Chapter 6, we identified tree species with promising antimicrobial activities which belonged to several different families. The aim of this study was to investigate if some families generally contained tree species with higher antimicrobial activities compared to other families. If we were to find that certain families were more promising, then more species of that family than those analysed by this study may have similar activities when caused by the inheritance of chemical precursors from a common ancestor. Future collections may then concentrate on close relatives of plants in the promising families identified by this study. This approach may facilitate and optimise the selection of tree species for the discovery of new antimicrobial plant extracts by saving time and expenses.

To achieve the main objective, we compared the antimicrobial activities of the tree species at two taxonomic levels: suprageneric (family) and suprafamilial (order). In this chapter we compared the antimicrobial activities of families based on the average MIC of all the species that we have analysed within each family. This will be followed by an investigation of the antimicrobial activities at order level to be discussed in Chapter 8.

## 7.2 Materials and methods

### 7.2.1 General material and methods

A similar experimental design and dataset to the previous Chapters were used. The study area and taxonomical arrangements of the trees of southern Africa were described in Chapter 2. The collection of tree samples and the description of the tree species represented in the Phytomedicine Tree Database were discussed in Chapter 3 and the preparation of plant

extracts, microbial organisms, antimicrobial assays and data processing were discussed in Chapter 4.

### 7.2.2 Mean MIC values of the families

In this chapter the species of the database were grouped into their respective families and a mean MIC value was calculated for each of the 101 families against *S. aureus, E. faecalis, E. coli*, *P. aeruginosa, C. albicans* and *C. neoformans.* Furthermore, the results against the pathogens were grouped into three classes: Gram-positive bacteria (*S. aureus* and *E. faecalis*), Gram-negative bacteria (*E. coli* and *P. aeruginosa*) and fungi (*C. albicans* and *C. neoformans*), and a mean MIC value was calculated for each family against all three pathogen classes.

### 7.2.3 Family grouping

As discussed in Chapter 3, diversity of tree species is unevenly distributed between families resulting in substantial different family sizes. Furthermore, the range of family sizes is extremely skewed with smaller families outnumbering larger families. Such large differences in size complicate comparisons between the families. In addition, we could only sample a limited number of species per genus. We therefore resolved to lower the impact of the size differences between the families by clustering the families into groups according to size as shown in Table 7.1.

**Table 7.1:** Grouping of 101 plant families encompassing the tree species analysed for the Phytomedicine Tree Database (PMDB) into size classes.

| | Group 1 (one representative species) | Group 2 (small families) | Group 3 (medium families) | Group 4 (large families) |
|---|---|---|---|---|
| Number of tree species in family | 1 | 2 to 3 | 4 to 8 | ≥ 9 |
| Total number of families in group | 35 | 30 | 19 | 17 |

### 7.2.4 Statistical analysis

The data were partitioned by family size into their respective groups and each group was compared separately. The data was reported as mean ± standard deviation (SD) of the mean, except for families in Group 1 which reflected only the mean MIC of a single species.

The families in Group 1, 2 and 3 could not be compared statistically because those families did not contain sufficient species for a rational statistical analysis. Statistical tests normally require a larger sample size to be able to find significant relationships from the data.

For the comparisons of the families in Group 4, the mean MIC values calculated for each family against each of the three pathogen classes were log transformed after which an analysis of variance (ANOVA) was performed. In cases where statistical significances were established, the practical significance of differences was challenged, in accordance with the recommendations of Cohen (1988). If differences were found ($p < 0.05$), a *post hoc* test (least square means (LSM)) was implemented. Statistical computations were performed using Microsoft Excel, version 2010 and the statistical software program SAS (version 9.2).

## 7.3 Results and discussion

The results of the antibacterial activities of the families against the Gram-positive bacteria are presented in 7.3.1, against the Gram-negative bacteria in 7.3.2 and against the fungi in 7.3.3. A synthesis of the results against all three pathogen classes are presented in 7.3.4 and the antimicrobial activities of ethnomedicinal important families are discussed in 7.3.5. Finally, previous antimicrobial results from species in selected families are discussed in 7.3.6.

In each section, the results for the families were partitioned into their respective size groups. Comparisons between the small families were problematic because of the small sampling size. The majority of the 35 families in Group 1 contained a single genus and as a result only one representative species per family was collected. However, even the smallest families may contain a promising species. Moerman and Estabrook (2003) found that the largest proportion of medicinal species used in ethnomedicine were from the smaller families. For this reason, the antibacterial activities of members of these small families were documented as they could potentially contain tree species with promising activities that should be investigated further.

The families had different levels of efficacies against each of the pathogens. In the respective tables, mean MIC's against each of the pathogens as well as a mean MIC for each pathogen class (Gram-positive bacteria, Gram-negative bacteria and fungi) were listed. However, only the mean MIC against the pathogen class was used to compare the families in the discussion hereafter. High standard deviations indicated high diversities between the activities of the species analysed within each family and this will be investigated in Chapter 9.

### 7.3.1 Gram-positive antibacterial activities of the tree families

The families of Groups 1, 2 and 3 will be compared in section 7.3.1.1 and the families of Group 4 in section 7.3.1.2.

#### 7.3.1.1 Families in Groups 1, 2 and 3

The MIC values of the families in Group 1 against Gram-positive bacteria (*E. faecalis* and *S. aureus*) were listed in Table 7.2. Species representing the families Maesaceae (*Maesa lanceolata)* and Violaceae (*Rinorea angustifolia*) had the highest mean activities against the Gram-positive bacteria with mean MIC values of 0.03 and 0.08 mg/ml respectively. Representatives of the families Aphloiaceae (*Aphloia theiformis*) and Hernandiaceae (*Gyrocarpus americanus*) had a low mean activity against Gram-positive bacteria.

**Table 7.2:** Mean minimum inhibitory concentrations (MIC) of the families in Group 1 (one representative) against Gram-positive bacteria. The families were ranked from highest to lowest activity.

| Family | MIC (mg/ml) | | |
|---|---|---|---|
| | *E. faecalis* | *S. aureus* | Mean for Gram-positive bacteria |
| Maesaceae | 0.04 | 0.02 | 0.03 |
| Violaceae | 0.04 | 0.16 | 0.08 |
| Balanitaceae | 0.16 | 0.11 | 0.13 |
| Curtisiaceae | 0.13 | 0.16 | 0.14 |
| Dichapetalaceae | 0.16 | 0.16 | 0.16 |
| Rhizophoraceae | 0.07 | 0.42 | 0.17 |
| Crassulaceae | 0.16 | 0.31 | 0.22 |
| Heteropyxidaceae | 0.32 | 0.16 | 0.22 |
| Gentianaceae | 0.16 | 0.42 | 0.26 |
| Pittosporaceae | 0.16 | 0.44 | 0.26 |
| Cycadaceae | 0.31 | 0.26 | 0.28 |
| Musaceae | 0.31 | 0.31 | 0.31 |
| Bruniaceae | 0.22 | 0.44 | 0.31 |
| Oliniaceae | 0.16 | 0.84 | 0.37 |
| Iteaceae | 0.31 | 0.63 | 0.44 |
| Myricaceae | 0.63 | 0.31 | 0.44 |
| Lythraceae | 0.31 | 0.63 | 0.44 |
| Canellaceae | 0.45 | 0.54 | 0.49 |
| Dracaenaceae | 0.40 | 0.63 | 0.50 |
| Ptaeroxylaceae | 0.50 | 0.69 | 0.59 |
| Aquifoliaceae | 0.31 | 1.25 | 0.62 |

| Family | MIC (mg/ml) | | |
|---|---|---|---|
| | *E. faecalis* | *S. aureus* | Mean for Gram-positive bacteria |
| Cunoniaceae | 1.25 | 0.31 | 0.62 |
| Melastomataceae | 0.63 | 0.63 | 0.63 |
| Moringaceae | 0.83 | 0.63 | 0.72 |
| Asphodelaceae | 1.25 | 0.44 | 0.74 |
| Cecropiaceae | 0.63 | 1.25 | 0.89 |
| Portulacaceae | 0.93 | 1.48 | 1.17 |
| Chrysobalanaceae | 1.25 | 1.25 | 1.25 |
| Malpighiaceae | 1.25 | 1.25 | 1.25 |
| Cyathaceae | 1.25 | 2.50 | 1.77 |
| Helicteraceae | 2.50 | 1.25 | 1.77 |
| Pandanaceae | 1.25 | 2.50 | 1.77 |
| Salicaceae | 1.25 | 2.50 | 1.77 |
| Aphloiaceae | 2.50 | 2.50 | 2.50 |
| Hernandiaceae | 2.50 | 2.50 | 2.50 |

The range of mean MIC values of the families in Group 2 was smaller compared to the previous group (Table 7.3). The highest mean activities (lowest MIC's) in this group were present in extracts of the Rosaceae, Clusiaceae and Cupressaceae with mean MIC values of 0.14, 0.16 and 0.21 mg/ml respectively. Rosaceae is a large and important ethno-medicinal family. However, it is a poorly represented tree family in southern Africa with only three genera and seven tree species. Of all families in Group 2, Kiggelariaceae had the lowest mean antimicrobial activity.

Table 7.4 summarised the mean MIC values of the families in Group 3. Our aim of analysing at least one species per genera was met except for the families Ochnaceae and Scrophulariaceae of which samples of the genera *Brackenridgea* (Ochnaceae) and *Ixianthes* (Scrophulariaceae) were not collected. At a mean MIC of 0.20 mg/ml, it was the extracts of the family Ochnaceae that showed the highest mean antimicrobial activity when compared to the other families.

Other families in Group 3 which also had relatively high mean antibacterial activities were Rhamnaceae, Scrophulariaceae and Myrtaceae. The family with the lowest mean activity in Group 3 was Strelitziaceae which contains a single genus in the southern African region (*Strelitzia*).

Since the results of the families in Group 1 to 3 could not be substantiated by statistical analyses, differences among the calculated mean MIC's of the families may therefore be merely coincidental.

**Table 7.3:** Mean minimum inhibitory concentrations (MIC) ± standard deviations (SD) of the families in Group 2 against the Gram-positive bacteria. The families were ranked from highest to lowest activity (n = the number of tree species analysed in each family).

| Family | n (2-3) | MIC (mg/ml) (±SD) | | |
|---|---|---|---|---|
| | | *E. faecalis* | *S. aureus* | Mean for Gram-positive bacteria |
| Rosaceae | 2 | 0.09 ± 0.35 | 0.22 ± 2.48 | 0.14 ± 1.42 |
| Clusiaceae | 3 | 0.22 ± 0.42 | 0.12 ± 0.22 | 0.16 ± 0.33 |
| Cupressaceae | 3 | 0.35 ± 0.11 | 0.12 ± 0.06 | 0.21 ± 0.16 |
| Zamiaceae | 2 | 0.08 ± 0.68 | 0.63 ± 1.37 | 0.22 ± 1.29 |
| Myrsinaceae | 2 | 0.35 ± 0.43 | 0.20 ± 0.19 | 0.26 ± 0.33 |
| Greyiaceae | 2 | 0.32 ± 0.47 | 0.22 ± 0.15 | 0.27 ± 0.32 |
| Verbenaceae | 2 | 0.32 ± 0.47 | 0.22 ± 0.55 | 0.27 ± 0.52 |
| Buxaceae | 2 | 0.25 ± 0.11 | 0.30 ± 0.02 | 0.27 ± 0.08 |
| Strychnaceae | 3 | 0.25 ± 0.18 | 0.32 ± 0.50 | 0.28 ± 0.34 |
| Boraginaceae | 2 | 0.13 ± 0.14 | 0.62 ± 0.94 | 0.29 ± 0.64 |
| Picrodendraceae | 2 | 0.31 ± 0.01 | 0.31 ± 0.00 | 0.31 ± 0.01 |
| Polygalaceae | 2 | 0.26 ± 0.09 | 0.37 ± 0.40 | 0.31 ± 0.27 |
| Pentapetaceae | 2 | 0.26 ± 0.09 | 0.44 ± 0.31 | 0.34 ± 0.24 |
| Burseraceae | 2 | 0.10 ± 0.05 | 1.25 ±.0.00 | 0.35 ± 0.82 |
| Hamamelidaceae | 2 | 0.44 ± 0.32 | 0.31± 0.00 | 0.37 ± 0.20 |
| Lecythidaceae | 2 | 0.23 ± 0.06 | 0.70 ± 0.16 | 0.40 ± 0.35 |
| Putranjivaceae | 3 | 0.40 ± 0.16 | 0.43 ± 1.70 | 0.42 ± 0.98 |
| Erythroxylaceae | 2 | 0.28 ± 0.05 | 0.61 ± 1.56 | 0.42 ± 0.89 |
| Vitaceae | 3 | 0.23 ± 0.16 | 0.99 ± 0.53 | 0.47 ± 0.58 |
| Bombacaceae | 3 | 0.37 ± 0.17 | 0.62 ± 1.63 | 0.48 ± 0.95 |
| Urticaceae | 3 | 0.38 ± 0.45 | 0.86 ± 1.05 | 0.57 ± 0.79 |
| Acanthaceae | 3 | 0.53 ± 0.69 | 0.63 ± 0.13 | 0.58 ± 0.45 |
| Olacaceae | 3 | 0.39 ± 0.78 | 0.99 ± 1.58 | 0.62 ± 1.23 |
| Lauraceae | 3 | 0.50 ± 1.68 | 0.79 ± 1.56 | 0.62 ± 1.63 |
| Celtidaceae | 3 | 0.44 ± 0.75 | 1.01 ± 0.49 | 0.67 ± 0.72 |
| Ericaceae | 3 | 0.31 ± 0.33 | 1.57 ± 0.98 | 0.70 ± 0.97 |
| Apiaceae | 2 | 0.44 ± 0.32 | 1.25 ± 0.00 | 0.74 ± 0.62 |
| Melianthaceae | 3 | 1.03 ± 0.74 | 0.59 ± 0.70 | 0.78 ± 0.63 |
| Kirkiaceae | 2 | 0.57 ± 0.45 | 1.26 ± 1.32 | 0.85 ± 1.02 |
| Kiggelariaceae | 3 | 0.87 ± 0.44 | 1.35 ± 1.35 | 1.09 ± 0.91 |

**Table 7.4:** Mean minimum inhibitory concentrations (MIC) ± standard deviations (SD) of the families in Group 3 against the Gram-positive bacteria. The families were ranked from highest to lowest activity (n = the number of tree species analysed in each family).

| Family | n (4-8) | MIC (mg/ml) (±SD) | | |
|---|---|---|---|---|
| | | *E. faecalis* | *S. aureus* | Mean for Gram-positive bacteria |
| Ochnaceae | 4 | 0.12 ± 0.14 | 0.31 ± 0.39 | 0.20 ± 0.25 |
| Rhamnaceae | 6 | 0.12 ± 0.15 | 0.47 ± 1.32 | 0.24 ± 0.75 |
| Scrophulariaceae | 5 | 0.17 ± 0.30 | 0.36 ± 0.67 | 0.25 ± 0.49 |
| Myrtaceae | 6 | 0.23 ± 0.28 | 0.42 ± 0.64 | 0.31 ± 0.44 |
| Podocarpaceae | 4 | 0.25 ± 0.40 | 0.48 ± 0.23 | 0.35 ± 0.36 |
| Thymelaeaceae | 4 | 0.31 ± 0.27 | 0.42 ± 0.50 | 0.36 ± 0.33 |
| Sparrmanniaceae | 6 | 0.26 ± 0.10 | 0.52 ± 1.06 | 0.37 ± 0.60 |
| Ebenaceae | 8 | 0.23 ± 0.29 | 0.59 ± 0.89 | 0.37 ± 0.54 |
| Capparaceae | 6 | 0.36 ± 0.83 | 0.53 ± 1.19 | 0.44 ± 1.00 |
| Icacinaceae | 4 | 0.47 ± 0.87 | 0.44 ± 0.60 | 0.46 ± 0.70 |
| Flacourtiaceae | 8 | 0.36 ± 0.94 | 0.61 ± 0.98 | 0.47 ± 0.95 |
| Araliaceae | 6 | 0.53 ± 0.85 | 0.49 ± 1.18 | 0.51 ± 0.86 |
| Oleaceae | 6 | 0.45 ± 0.57 | 0.61 ± 0.91 | 0.53 ± 0.68 |
| Sterculiaceae | 4 | 0.22 ± 0.18 | 1.25 ± 1.32 | 0.55 ± 0.80 |
| Malvaceae | 7 | 0.44 ± 0.53 | 0.77 ± 0.70 | 0.58 ± 0.60 |
| Lamiaceae | 6 | 0.66 ± 0.91 | 0.54 ± 0.93 | 0.60 ± 0.85 |
| Arecaceae | 5 | 0.44 ± 1.26 | 0.92 ± 1.80 | 0.64 ± 1.46 |
| Buddlejaceae | 5 | 0.44 ± 0.22 | 1.25 ± 1.41 | 0.74 ± 0.94 |
| Strelitziaceae | 5 | 1.09 ± 1.13 | 2.18 ± 0.85 | 1.54 ± 1.03 |

### 7.3.1.2 Families in Group 4

The results of the mean MIC's of the families in Group 4 against Gram-positive bacteria are presented in Table 7.5. Although the range of antibacterial activities found in this group is much smaller compared to the previous groups, an ANOVA revealed significant differences between the mean MIC's of the families. The most effective families in this group against Gram-positive bacteria were Anacardiaceae and Moraceae. A least square *post-hoc* test (LSM) established that these two families had significantly higher activities ($p < 0.05$) compared to almost all the other families in Group 4, except Combretaceae and Celastraceae. Members of Combretaceae and Celastraceae had the third and fourth highest mean antibacterial activities in Group 4 which were significantly higher ($p < 0.05$) compared to the families Rubiaceae, Meliaceae, Proteaceae, Phyllanthaceae, Annonaceae, Bignoniaceae, Apocynaceae, Asteraceae and Rutaceae.

The least effective families against Gram-positive bacteria were Rutaceae, Asteraceae and Apocynaceae (Table 7.5). The antibacterial activities of these families were significantly lower compared to the families with the six highest mean activities, i.e. Anacardiaceae, Moraceae, Combretaceae, Celastraceae, Fabaceae and Euphorbiaceae. The relatively low activities displayed by members of Asteraceae were not expected as it is a family that is widely used in traditional medicine. It is a predominantly herbaceous family and consequently poorly represented in our study on trees. In addition, representatives of several genera within Asteraceae were not analysed either because the leaves of the specimen were too small to permit adequate sampling or representative trees were unavailable (Chapter 3.2). The low mean activities of Rutaceae and Apocynaceae were also surprising given that these families are also widely used in ethnomedicine (Moerman and Estabrook, 2003).

**Table 7.5:** Mean minimum inhibitory concentrations (MIC) ± standard deviation (SD) of the families in Group 4 against the Gram-positive bacteria. The families were ranked from highest to lowest activity (n = the number of tree species analysed in each family; mean MIC values followed by the same letter do not differ significantly at the 5% confidence level).

| Family | n (≥ 9) | MIC (mg/ml) (±SD) | | |
|---|---|---|---|---|
| | | *E. faecalis* | *S. aureus* | Mean for Gram-positive bacteria |
| Anacardiaceae | 17 | 0.16 ± 0.31 | 0.25 ± 0.70 | 0.20 ± 0.50 a |
| Moraceae | 12 | 0.22 ± 0.25 | 0.26 ± 0.79 | 0.24 ± 0.50 a |
| Combretaceae | 15 | 0.30 ± 0.95 | 0.25 ± 0.49 | 0.27 ± 0.62 abc |
| Celastraceae | 19 | 0.23 ± 0.41 | 0.37 ± 0.69 | 0.30 ± 0.47 abc |
| Fabaceae | 55 | 0.76 ± 0.41 | 0.87 ± 0.00 | 0.40 ± 0.83 bcd |
| Euphorbiaceae | 26 | 0.38 ± 0.84 | 0.51 ± 0.90 | 0.44 ± 0.72 cd |
| Sapindaceae | 19 | 0.40 ± 1.01 | 0.49 ± 1.21 | 0.45 ± 1.07 cde |
| Sapotaceae | 14 | 0.36 ± 0.62 | 0.63 ± 1.24 | 0.47 ± 0.95 cde |
| Rubiaceae | 41 | 0.38 ± 0.69 | 0.63 ± 1.04 | 0.49 ± 0.82 de |
| Meliaceae | 10 | 0.33 ± 0.93 | 0.79 ± 1.17 | 0.51 ± 1.04 de |
| Proteaceae | 28 | 0.38 ± 0.49 | 0.71 ± 1.05 | 0.52 ± 0.73 de |
| Phyllanthaceae | 13 | 0.52 ± 1.01 | 0.66 ± 1.17 | 0.58 ± 0.60 de |
| Annonaceae | 9 | 0.64 ± 1.40 | 0.63 ± 1.22 | 0.63 ± 1.26 de |
| Bignoniaceae | 9 | 0.53 ± 0.70 | 0.93 ± 1.10 | 0.70 ± 0.76 de |
| Apocynaceae | 19 | 0.54 ± 0.67 | 0.97 ± 1.05 | 0.73 ± 0.79 e |
| Asteraceae | 11 | 0.58 ± 0.42 | 1.11 ± 0.78 | 0.80 ± 0.60 e |
| Rutaceae | 11 | 0.79 ± 1.08 | 0.86 ± 1.00 | 0.83 ± 1.02 e |
| Degrees of freedom (DF) | | | | 16 |
| F value | | | | 2.86 |
| Pr > F | | | | 0.0002 |

### 7.3.2 Gram-negative antibacterial activities of the tree families

The families in Groups 1, 2 and 3 are compared in section 7.3.2.1 and the families in Group 4 in section 7.3.2.2.

#### 7.3.2.1 Families in Groups 1, 2 and 3

The number of species analysed in each of these families were too small for a statistical analysis. As shown in Table 7.6, the families in Group 1 had a wide range of mean MIC values against Gram-negative bacteria. The representative species analysed in Maesaceae and Curtisiaceae (respectively *Maesa lanceolata* and *Curtisia dentata*) had the highest Gram-negative antibacterial activities while the representative species of both Hernandiaceae and Salicaceae (respectively *Gyrocarpus americanus* and *Salix mucronata*) had the lowest activities in Group 1.

Group 2 consisted of families of which we analysed between two to three genera per family (Table 7.7). The families Hamamelidaceae, Strychnaceae and Clusiaceae had the highest activities while members of Ericaceae and Melianthaceae had very low activities with mean MIC's higher than 1.00 mg/ml against Gram-negative bacteria.

The families Buddlejaceae, Arecaceae and Lamiaceae had the lowest activities in Group 3 with MIC's of 0.71, 0.61 and of 0.59 mg/ml respectively.

**Table 7.6:** Mean minimum inhibitory concentrations (MIC) of the families in Group 1 (one representative) against Gram-negative bacteria. The families were ranked from highest to lowest activity.

| Family | Mean MIC (mg/ml) | | |
|---|---|---|---|
| | *P. aeruginosa* | *E. coli* | Mean for Gram-negative bacteria |
| Maesaceae | 0.02 | 0.02 | 0.02 |
| Curtisiaceae | 0.08 | 0.16 | 0.11 |
| Heteropyxidaceae | 0.22 | 0.11 | 0.16 |
| Oliniaceae | 0.08 | 0.31 | 0.16 |
| Crassulaceae | 0.16 | 0.16 | 0.16 |
| Balanitaceae | 0.31 | 0.11 | 0.18 |
| Ptaeroxylaceae | 0.27 | 0.15 | 0.20 |
| Pittosporaceae | 0.31 | 0.14 | 0.21 |
| Melastomataceae | 0.31 | 0.16 | 0.22 |
| Canellaceae | 0.16 | 0.31 | 0.22 |
| Portulacaceae | 0.20 | 0.31 | 0.25 |
| Dracaenaceae | 0.31 | 0.20 | 0.25 |

| Family | Mean MIC (mg/ml) | | |
|---|---|---|---|
| | *P. aeruginosa* | *E. coli* | Mean for Gram-negative bacteria |
| Cycadaceae | 0.31 | 0.21 | 0.26 |
| Musaceae | 0.31 | 0.21 | 0.26 |
| Cunoniaceae | 0.16 | 0.63 | 0.31 |
| Dichapetalaceae | 0.31 | 0.31 | 0.31 |
| Violaceae | 0.63 | 0.16 | 0.32 |
| Gentianaceae | 0.26 | 0.52 | 0.37 |
| Rhizophoraceae | 0.63 | 0.26 | 0.40 |
| Asphodelaceae | 0.63 | 0.31 | 0.44 |
| Myricaceae | 0.31 | 0.63 | 0.44 |
| Malpighiaceae | 0.63 | 0.31 | 0.44 |
| Lythraceae | 0.31 | 0.63 | 0.44 |
| Cecropiaceae | 1.25 | 0.16 | 0.45 |
| Helicteraceae | 1.25 | 0.16 | 0.45 |
| Pandanaceae | 0.16 | 1.25 | 0.45 |
| Bruniaceae | 0.63 | 0.88 | 0.74 |
| Aphloiaceae | 2.50 | 0.31 | 0.88 |
| Aquifoliaceae | 0.63 | 1.25 | 0.88 |
| Chrysobalanaceae | 0.63 | 1.25 | 0.88 |
| Cyatheaceae | 0.63 | 1.25 | 0.88 |
| Iteaceae | 0.63 | 1.25 | 0.88 |
| Moringaceae | 1.04 | 1.04 | 1.04 |
| Salicaceae | 1.25 | 2.50 | 1.77 |
| Hernandiaceae | 2.08 | 2.50 | 2.28 |

**Table 7.7:** Mean minimum inhibitory concentrations (MIC) ± standard deviation (SD) of the families in Group 2 against the Gram-negative bacteria. The families were ranked from highest to lowest activity (n = the number of tree species analysed in each family).

| Family | n (2-3) | Mean MIC (mg/ml) | | |
|---|---|---|---|---|
| | | *P. aeruginosa* | *E. coli* | Mean for Gram-negative bacteria |
| Hamamelidaceae | 2 | 0.16 ± 0.00 | 0.11 ± 0.08 | 0.13 ± 0.06 |
| Strychnaceae | 3 | 0.20 ± 0.21 | 0.29 ± 0.25 | 0.24 ± 0.23 |
| Clusiaceae | 3 | 0.42 ± 0.93 | 0.15 ± 0.16 | 0.25 ± 0.53 |
| Celtidaceae | 3 | 0.24 ± 0.39 | 0.37 ± 0.35 | 0.30 ± 0.37 |
| Polygalaceae | 2 | 0.20 ± 0.10 | 0.45 ± 0.31 | 0.30 ± 0.24 |
| Pentapetaceae | 2 | 0.37 ± 0.13 | 0.26 ± 0.09 | 0.31 ± 0.11 |
| Myrsinaceae | 2 | 0.39 ± 0.19 | 0.25 ± 0.11 | 0.31 ± 0.15 |

| Family | n (2-3) | Mean MIC (mg/ml) | | |
|---|---|---|---|---|
| | | *P. aeruginosa* | *E. coli* | Mean for Gram-negative bacteria |
| Verbenaceae | 2 | 0.27 ± 0.29 | 0.37 ± 0.13 | 0.31 ± 0.23 |
| Picrodendraceae | 2 | 0.31 ± 0.00 | 0.32 ± 0.47 | 0.31 ± 0.28 |
| Acanthaceae | 3 | 0.37 ± 0.50 | 0.27 ± 0.35 | 0.32 ± 0.43 |
| Bombacaceae | 3 | 0.38 ± 1.41 | 0.28 ± 0.51 | 0.32 ± 0.97 |
| Lecythidaceae | 2 | 0.46 ± 0.59 | 0.23 ± 0.27 | 0.32 ± 0.36 |
| Buxaceae | 2 | 0.33 ± 0.05 | 0.36 ± 0.42 | 0.35 ± 0.26 |
| Erythroxylaceae | 2 | 0.34 ± 0.30 | 0.36 ± 0.11 | 0.35 ± 0.22 |
| Olacaceae | 3 | 0.31 ± 0.00 | 0.40 ± 0.78 | 0.35 ± 0.44 |
| Vitaceae | 3 | 0.40 ± 0.41 | 0.36 ± 1.73 | 0.38 ± 1.06 |
| Burseraceae | 2 | 0.33 ± 0.53 | 0.44 ± 0.32 | 0.38 ± 0.34 |
| Apiaceae | 2 | 0.55 ± 0.14 | 0.27 ± 1.19 | 0.39 ± 0.81 |
| Kiggelariaceae | 3 | 0.44 ± 0.28 | 0.36 ± 0.55 | 0.40 ± 0.43 |
| Rosaceae | 2 | 0.39 ± 0.89 | 0.43 ± 0.34 | 0.41 ± 0.64 |
| Boraginaceae | 2 | 0.66 ± 0.06 | 0.29 ± 0.04 | 0.44 ± 0.26 |
| Putranjivaceae | 3 | 0.46 ± 0.74 | 0.44 ± 0.63 | 0.45 ± 0.68 |
| Greyiaceae | 2 | 0.57 ± 0.10 | 0.44 ± 0.32 | 0.50 ± 0.25 |
| Lauraceae | 3 | 0.99 ± 0.53 | 0.49 ± 0.72 | 0.70 ± 0.68 |
| Zamiaceae | 2 | 0.72 ± 0.15 | 0.72 ± 0.15 | 0.72 ± 0.21 |
| Cupressaceae | 3 | 0.67 ± 0.70 | 0.88 ± 0.44 | 0.77 ± 0.52 |
| Urticaceae | 3 | 0.69 ± 1.60 | 0.87 ± 0.44 | 0.77 ± 0.91 |
| Kirkiaceae | 2 | 1.36 ± 0.72 | 0.69 ± 0.87 | 0.97 ± 0.77 |
| Melianthaceae | 3 | 1.18 ± 0.17 | 1.25 ± 0.00. | 1.21 ± 0.11 |
| Ericaceae | 3 | 0.99 ± 0.53 | 1.57 ± 0.98 | 1.25 ± 0.66 |

**Table 7.8:** Mean minimum inhibitory concentrations (MIC) ± standard deviation (SD) of the families in Group 3 against the Gram-negative bacteria. The families were ranked from highest to lowest activity (n = the number of tree species analysed in each family).

| Family | n (4-8) | Mean MIC (mg/ml) | | |
|---|---|---|---|---|
| | | *P. aeruginosa* | *E. coli* | Mean for Gram-negative bacteria |
| Ebenaceae | 8 | 0.16 ± 0.27 | 0.25 ± 0.11 | 0.20 ± 0.20 |
| Ochnaceae | 4 | 0.32 ± 0.31 | 0.18 ± 0.22 | 0.24 ± 0.26 |
| Podocarpaceae | 4 | 0.26 ± 0.22 | 0.24 ± 0.14 | 0.25 ± 0.17 |
| Thymelaeaceae | 4 | 0.27 ± 0.30 | 0.33 ± 0.25 | 0.30 ± 0.22 |
| Sparrmanniaceae | 6 | 0.26 ± 1.11 | 0.42 ± 1.10 | 0.33 ± 1.11 |
| Araliaceae | 6 | 0.51 ± 0.82 | 0.28 ± 0.52 | 0.38 ± 0.55 |
| Scrophulariaceae | 5 | 0.43 ± 0.70 | 0.37 ± 0.39 | 0.40 ± 0.54 |
| Sterculiaceae | 4 | 0.39 ± 0.25 | 0.20 ± 0.12 | 0.41 ± 0.64 |

| Family | n (4-8) | Mean MIC (mg/ml) | | |
|---|---|---|---|---|
| | | *P. aeruginosa* | *E. coli* | Mean for Gram-negative bacteria |
| Malvaceae | 6 | 0.51 ± 0.45 | 0.34 ± 0.50 | 0.42 ± 0.48 |
| Myrtaceae | 6 | 0.45 ± 1.08 | 0.42 ± 1.11 | 0.43 ± 1.09 |
| Oleaceae | 6 | 0.48 ± 1.11 | 0.46 ± 0.31 | 0.47 ± 0.69 |
| Flacourtiaceae | 8 | 0.41 ± 0.75 | 0.52 ± 0.90 | 0.47 ± 0.82 |
| Strelitziaceae | 5 | 0.36 ± 0.30 | 0.62 ± 0.85 | 0.47 ± 0.59 |
| Icacinaceae | 4 | 0.57 ± 0.61 | 0.40 ± 1.39 | 0.48 ± 1.00 |
| Rhamnaceae | 6 | 0.47 ± 0.33 | 0.50 ± 0.96 | 0.48 ± 0.67 |
| Capparaceae | 6 | 0.59 ± 0.69 | 0.42 ± 0.47 | 0.49 ± 0.54 |
| Lamiaceae | 6 | 0.64 ± 0.55 | 0.54 ± 0.40 | 0.59 ± 0.42 |
| Arecaceae | 5 | 0.43 ± 0.34 | 0.87 ± 1.53 | 0.61 ± 0.86 |
| Buddlejaceae | 5 | 0.79 ± 0.93 | 0.64 ± 0.30 | 0.71 ± 0.63 |

### 7.3.2.2 Families in Group 4

The MIC values against Gram-negative bacteria of the families in Group 4 are shown in Table 7.9. The mean MIC values ranged from 0.26 to 0.69 mg/ml and although the differences were small, an ANOVA revealed significant differences ($p < 0.05$). A *post-hoc* least square means test (LSM) was applied to identify the families concerned.

Extracts from Combretaceae, Anacardiaceae, Moraceae and Fabaceae families had the highest activities. All these families had mean MIC's lower than 0.31 mg/ml against Gram-negative bacteria. Both Combretaceae and Anacardiaceae had significantly higher antibacterial activities compared to the families Euphorbiaceae, Rubiaceae, Sapotaceae, Annonaceae, Asteraceae, Apocynaceae, Meliaceae, Proteaceae and Rutaceae.

Both Moraceae and Fabaceae extracts had significantly higher activities than Asteraceae, Apocynaceae, Meliaceae, Proteaceae and Rutaceae families. The families Combretaceae, Anacardiaceae and Moraceae were also between the top families against Gram-positive bacteria while Combretaceae was also one of the more promising families against the fungi.

The antibacterial activity of the Rutaceae family members was significantly lower compared to several of the families with the highest activities (Table 7.9). Proteaceae, Meliaceae, Apocynaceae and Asteraceae members also had low activities. The activities of these families were significantly lower compared to the four families with the highest antimicrobial activities.

**Table 7.9:** Mean minimum inhibitory concentrations (MIC) ± standard deviation (SD) of the families in Group 4 against the Gram-negative bacteria. The families were ranked from highest to lowest activity (n = the number of tree species analysed in each family; mean MIC values followed by the same letter do not differ significantly at the 5% confidence level).

| Family | n (≥ 9) | Mean MIC (mg/ml) | | |
|---|---|---|---|---|
| | | *P. aeruginosa* | *E. coli* | Mean (Gram-negative bacteria) |
| Combretaceae | 15 | 0.25 ± 0.29 | 0.27 ± 0.36 | 0.26 ± 0.29 a |
| Anacardiaceae | 17 | 0.27 ± 0.70 | 0.26 ± 0.24 | 0.26 ± 0.45 a |
| Moraceae | 12 | 0.27 ± 0.11 | 0.28 ± 0.39 | 0.27 ± 0.24 ab |
| Fabaceae | 55 | 0.25 ± 0.44 | 0.33 ± 0.48 | 0.30 ± 0.52 ab |
| Phyllanthaceae | 13 | 0.27 ± 0.23 | 0.48 ± 0.85 | 0.36 ± 0.53 abc |
| Sapindaceae | 19 | 0.40 ± 0.74 | 0.39 ± 0.86 | 0.40 ± 0.78 abcd |
| Bignoniaceae | 9 | 0.56 ± 1.17 | 0.29 ± 0.30 | 0.40 ± 0.72 abcde |
| Celastraceae | 19 | 0.39 ± 0.96 | 0.44 ± 0.48 | 0.41 ± 0.68 abcde |
| Euphorbiaceae | 26 | 0.51 ± 0.75 | 0.35 ± 0.66 | 0.42 ± 0.67 bcde |
| Rubiaceae | 41 | 0.46 ± 0.64 | 0.41 ± 0.75 | 0.43 ± 0.66 cde |
| Sapotaceae | 14 | 0.44 ± 0.97 | 0.46 ± 0.84 | 0.45 ± 0.89 cde |
| Annonaceae | 9 | 0.58 ± 0.80 | 0.42 ± 0.43 | 0.49 ± 0.59 cde |
| Asteraceae | 11 | 0.44 ± 0.43 | 0.57 ± 0.82 | 0.50 ± 0.52 cde |
| Apocynaceae | 19 | 0.54 ± 0.99 | 0.51 ± 0.59 | 0.53 ± 0.75 cde |
| Meliaceae | 10 | 0.54 ± 0.48 | 0.54 ± 1.17 | 0.54 ± 0.81 cde |
| Proteaceae | 28 | 0.46 ± 0.78 | 0.78 ± 0.67 | 0.60 ± 0.70 de |
| Rutaceae | 11 | 0.80 ± 1.17 | 0.59 ± 1.11 | 0.69 ± 1.09 e |
| Degrees of freedom (DF) | | | | 16 |
| F value | | | | 2.86 |
| Pr > F | | | | 0.0002 |

### 7.3.3 Antifungal activities of the tree families

The families in Groups 1, 2 and 3 will be compared in section 7.3.3.1 and the families in Group 4 will be compared in section 7.3.3.2.

#### 7.3.3.1 Families in Groups 1, 2 and 3

Table 7.10 shows the mean MIC values against the fungal pathogens of the extracts of the representative species of Group 1. Members of Pandanaceae, Rhizophoraceae, Curtisiaceae and Crassulaceae had the highest mean antifungal activities while representatives of Melastomataceae, Iteaceae, Myricaceae, Asphodelaceae and Hernandiaceae had the lowest antifungal activities.

**Table 7.10:** Mean minimum inhibitory concentrations (MIC) of the families in Group 1 (one representative) against the fungal organisms. The families were ranked from highest to lowest activity.

| Family | Mean MIC (mg/ml) | | |
|---|---|---|---|
| | *C. albicans* | *C. neoformans* | Mean (Fungi) |
| Pandanaceae | 0.08 | 0.04 | 0.06 |
| Rhizophoraceae | 0.04 | 0.11 | 0.07 |
| Curtisiaceae | 0.06 | 0.14 | 0.09 |
| Crassulaceae | 0.26 | 0.08 | 0.14 |
| Aquifoliaceae | 0.16 | 0.16 | 0.16 |
| Dichapetalaceae | 0.16 | 0.16 | 0.16 |
| Helicteraceae | 0.16 | 0.16 | 0.16 |
| Malpighiaceae | 0.16 | 0.16 | 0.16 |
| Musaceae | 0.16 | 0.16 | 0.16 |
| Oliniaceae | 0.05 | 0.63 | 0.18 |
| Heteropyxidaceae | 0.40 | 0.10 | 0.20 |
| Canellaceae | 0.32 | 0.13 | 0.20 |
| Ptaeroxylaceae | 0.11 | 0.38 | 0.21 |
| Cyathaceae | 0.63 | 0.08 | 0.22 |
| Balanitaceae | 0.31 | 0.16 | 0.22 |
| Gentianaceae | 0.31 | 0.16 | 0.22 |
| Cunoniaceae | 1.25 | 0.04 | 0.22 |
| Bruniaceae | 0.45 | 0.16 | 0.27 |
| Cycadaceae | 0.31 | 0.31 | 0.31 |
| Cecropiaceae | 0.31 | 0.31 | 0.31 |
| Maesaceae | 0.63 | 0.16 | 0.32 |
| Moringaceae | 0.16 | 0.63 | 0.32 |
| Dracaenaceae | 0.32 | 0.39 | 0.35 |
| Portulacaceae | 0.47 | 0.31 | 0.38 |
| Chrysobalanaceae | 0.31 | 0.63 | 0.44 |
| Salicaceae | 0.63 | 0.31 | 0.44 |
| Aphloiaceae | 0.16 | 1.25 | 0.45 |
| Pittosporaceae | 0.63 | 0.37 | 0.48 |
| Lythraceae | 0.63 | 0.42 | 0.51 |
| Violaceae | 2.50 | 0.31 | 0.88 |
| Hernandiaceae | 0.42 | 2.50 | 1.02 |
| Asphodelaceae | 0.88 | 1.25 | 1.05 |
| Myricaceae | 1.25 | 1.25 | 1.25 |
| Iteaceae | 1.25 | 2.50 | 1.77 |
| Melastomataceae | 2.50 | 2.50 | 2.50 |

As shown in Table 7.11, among the 30 families in Group 2, Polygalaceae, Boraginaceae and Vitaceae had relatively high mean antifungal activities compared to the families Zamiaceae and Ericaceae which had the lowest antifungal activities.

Between four and eight representatives of the families in Group 3 were analysed and their mean MIC values are summarised in Table 7.12. The families Strelitziaceae, Malvaceae and Scrophulariaceae had very high antifungal activities. No sample of the genus *Ixianthes* (Scrophulariaceae) was collected and analysed and it may be rewarding to include samples of this genus in future research programs. Other families with relatively high activities were Capparaceae and Thymelaeaceae with mean MIC values of 0.21 mg/ml and lower. In contrast with the relatively high activities recorded against Gram-positive bacteria, members of both Rhamnaceae and Myrtaceae had low activities against the fungal organisms relative to the other families in Group 3.

**Table 7.11:** Mean minimum inhibitory concentrations (MIC) ± standard deviations (SD) of the families in Group 2 against the fungal organisms. The families were ranked from highest to lowest activity (n = the number of tree species analysed in each family).

| Family | n (2-3) | Mean MIC (mg/ml) | | |
|---|---|---|---|---|
| | | *C. albicans* | *C. neoformans* | Mean for fungi |
| Polygalaceae | 2 | 0.11 ± 0.08 | 0.22 ± 0.15 | 0.16 ± 0.14 |
| Boraginaceae | 2 | 0.28 ± 0.91 | 0.11 ± 0.08 | 0.18 ± 0.52 |
| Vitaceae | 3 | 0.19 ± 0.08 | 0.22 ± 0.38 | 0.20 ± 0.21 |
| Apiaceae | 2 | 0.18 ± 0.32 | 0.31 ± 0.00 | 0.24 ± 0.23 |
| Lecythidaceae | 2 | 0.29 ± 0.18 | 0.19 ± 0.08 | 0.24 ± 0.15 |
| Urticaceae | 3 | 0.24 ± 0.11 | 0.26 ± 0.21 | 0.25 ± 0.16 |
| Verbenaceae | 2 | 0.29 ± 0.04 | 0.22 ± 0.55 | 0.25 ± 0.33 |
| Pentapetaceae | 2 | 0.22 ± 0.15 | 0.32 ± 0.47 | 0.27 ± 0.33 |
| Kirkiaceae | 2 | 0.15 ± 0.21 | 0.53 ± 2.39 | 0.28 ± 1.24 |
| Kiggelariaceae | 3 | 0.26 ± 0.17 | 0.33 ± 0.24 | 0.29 ± 0.21 |
| Cupressaceae | 3 | 0.56 ± 0.84 | 0.16 ± 0.00 | 0.30 ± 0.51 |
| Greyiaceae | 2 | 0.21 ± 0.11 | 0.44 ± 0.32 | 0.30 ± 0.24 |
| Bombacaceae | 3 | 0.49 ± 0.72 | 0.25 ± 0.13 | 0.35 ± 0.40 |
| Celtidaceae | 3 | 0.24 ± 0.21 | 0.58 ± 0.31 | 0.37 ± 0.32 |
| Lauraceae | 3 | 0.40 ± 0.41 | 0.39 ± 0.25 | 0.40 ± 0.32 |
| Clusiaceae | 3 | 0.44 ± 1.08 | 0.40 ± 1.71 | 0.42 ± 1.40 |
| Putranjivaceae | 3 | 0.59 ± 0.48 | 0.29 ± 0.24 | 0.42 ± 0.33 |
| Hamamelidaceae | 2 | 0.63 ± 2.34 | 0.32 ± 1.17 | 0.45 ± 1.77 |
| Erythroxylaceae | 2 | 0.31± 0.20 | 0.75 ± 2.28 | 0.48 ± 1.31 |
| Myrsinaceae | 2 | 0.79 ± 2.25 | 0.32 ± 0.02 | 0.50 ± 1.25 |

| Family | n (2-3) | Mean MIC (mg/ml) | | |
|---|---|---|---|---|
| | | *C. albicans* | *C. neoformans* | Mean for fungi |
| Burseraceae | 2 | 0.69 ± 0.87 | 0.39 ± 0.39 | 0.52 ± 0.67 |
| Acanthaceae | 3 | 1.11 ± 1.47 | 0.31 ± 1.73 | 0.59 ± 1.70 |
| Rosaceae | 2 | 0.41 ± 1.12 | 0.89 ± 2.18 | 0.60 ± 1.27 |
| Picrodendraceae | 2 | 1.06 ± 1.14 | 0.44 ± 0.31 | 0.68 ± 0.85 |
| Olacaceae | 3 | 0.63 ± 1.82 | 0.79 ± 1.56 | 0.71 ± 1.69 |
| Melianthaceae | 3 | 0.79 ± 0.83 | 0.66 ± 0.44 | 0.72 ± 0.59 |
| Strychnaceae | 3 | 0.88 ± 1.55 | 0.59 ± 0.52 | 0.72 ± 1.06 |
| Buxaceae | 2 | 0.87 ± 1.72 | 0.66 ± 1.41 | 0.76 ± 1.57 |
| Ericaceae | 3 | 0.99 ± 1.58 | 0.99 ± 1.57 | 0.99 ± 1.58 |
| Zamiaceae | 2 | 1.62 ± 0.44 | 0.63 ± 0.69 | 1.01 ± 1.40 |

**Table 7.12:** Mean minimum inhibitory concentrations (MIC) ± standard deviations (SD) of the families in Group 3 against the fungal organisms. The families were ranked from highest to lowest activity (n = the number of tree species analysed in each family).

| Family | n (4-8) | Mean MIC (mg/ml) | | |
|---|---|---|---|---|
| | | *C. albicans* | *C. neoformans* | Mean for fungi |
| Strelitziaceae | 5 | 0.18 ± 0.30 | 0.07 ± 0.07 | 0.11 ± 0.18 |
| Malvaceae | 7 | 0.20 ± 0.12 | 0.16 ± 0.18 | 0.18 ± 0.13 |
| Scrophulariaceae | 5 | 0.14 ± 0.31 | 0.24 ± 0.37 | 0.18 ± 0.33 |
| Capparaceae | 6 | 0.24 ± 0.15 | 0.19 ± 0.40 | 0.21 ± 0.28 |
| Thymelaeaceae | 4 | 0.21 ± 0.47 | 0.22 ± 0.36 | 0.21 ± 0.41 |
| Sterculiaceae | 4 | 0.40 ± 0.41 | 0.18 ± 0.40 | 0.25 ± 0.34 |
| Podocarpaceae | 4 | 0.25 ± 0.45 | 0.33 ± 0.26 | 0.29 ± 0.35 |
| Ochnaceae | 4 | 0.44 ± 0.60 | 0.20 ± 0.31 | 0.30 ± 0.39 |
| Ebenaceae | 8 | 0.34 ± 1.04 | 0.34 ± 0.96 | 0.34 ± 0.99 |
| Flacourtiaceae | 8 | 0.38 ± 0.97 | 0.34 ± 0.94 | 0.36 ± 0.77 |
| Araliaceae | 6 | 0.51 ± 1.30 | 0.39 ± 1.25 | 0.39 ± 1.14 |
| Lamiaceae | 6 | 0.47 ± 1.16 | 0.39 ± 0.58 | 0.43 ± 0.87 |
| Icacinaceae | 4 | 0.67 ± 0.49 | 0.28 ± 0.34 | 0.43 ± 0.42 |
| Arecaceae | 5 | 0.36 ± 1.21 | 0.63 ± 1.74 | 0.47 ± 1.24 |
| Oleaceae | 6 | 0.51 ± 0.85 | 0.45 ± 0.52 | 0.48 ± 0.66 |
| Sparrmanniaceae | 6 | 0.55 ± 1.50 | 0.53 ± 1.52 | 0.54 ± 1.37 |
| Buddlejaceae | 5 | 0.69 ± 1.28 | 0.66 ± 1.24 | 0.68 ± 1.25 |
| Myrtaceae | 6 | 1.17 ± 1.58 | 0.53 ± 1.58 | 0.79 ± 1.57 |
| Rhamnaceae | 6 | 1.17 ± 1.58 | 0.68 ± 1.52 | 0.89 ± 1.55 |

### 7.3.3.2 Families in Group 4

The results of the mean MIC's of the families in Group 4 against the fungal organisms are presented in Table 7.13. Similar to the results against the Gram-positive and Gram-negative bacteria, the range of average activities found in this group was relatively small. An ANOVA revealed significant differences ($p < 0.05$) between the mean MIC's of some of the families.

**Table 7.13:** Mean minimum inhibitory concentrations (MIC) ± standard deviations (SD) of the families in Group 4 against the fungal organisms. The families were ranked from highest to lowest activity (n = the number of tree species analysed in each family; mean MIC values followed by the same letter do not differ significantly at the 5% confidence level).

| Family | n (≥ 9) | Mean MIC (mg/ml) | | |
|---|---|---|---|---|
| | | *C. albicans* | *C. neoformans* | Mean (Fungi) |
| Meliaceae | 10 | 0.29 ± 0.54 | 0.19 ± 0.37 | 0.23 ± 0.37 a |
| Combretaceae | 15 | 0.24 ± 0.41 | 0.36 ± 0.55 | 0.29 ± 0.42 ab |
| Euphorbiaceae | 26 | 0.34 ± 1.09 | 0.31 ± 1.01 | 0.32 ± 0.99 abc |
| Proteaceae | 28 | 0.38 ± 0.44 | 0.29 ± 0.75 | 0.33 ± 0.54 abc |
| Fabaceae | 55 | 0.36 ± 0.63 | 0.34 ± 0.92 | 0.35 ± 0.80 abcd |
| Annonaceae | 9 | 0.36 ± 0.99 | 0.35 ± 1.09 | 0.36 ± 0.92 abcde |
| Rubiaceae | 41 | 0.41 ± 0.93 | 0.32 ± 0.96 | 0.36 ± 0.88 abcde |
| Rutaceae | 11 | 0.31 ± 0.84 | 0.50 ± 0.50 | 0.39 ± 0.54 abcde |
| Bignoniaceae | 9 | 0.35 ± 0.56 | 0.52 ± 1.47 | 0.42 ± 0.99 abcde |
| Phyllanthaceae | 13 | 0.32 ± 0.85 | 0.59 ± 0.58 | 0.44 ± 0.61 bcde |
| Asteraceae | 11 | 0.48 ± 0.64 | 0.50 ± 0.94 | 0.49 ± 0.69 bcde |
| Moraceae | 12 | 0.68 ± 1.00 | 0.41 ± 0.70 | 0.53 ± 0.83 cde |
| Anacardiaceae | 17 | 0.60 ± 1.11 | 0.48 ± 1.15 | 0.54 ± 1.01 cde |
| Sapindaceae | 19 | 0.54 ± 1.36 | 0.58 ± 1.18 | 0.56 ± 1.22 de |
| Apocynaceae | 19 | 0.58 ± 1.17 | 0.55 ± 0.92 | 0.56 ± 0.97 de |
| Sapotaceae | 14 | 0.67 ± 1.08 | 0.48 ± 0.52 | 0.57 ± 0.74 e |
| Celastraceae | 19 | 0.66 ± 0.71 | 0.53 ± 0.71 | 0.59 ± 0.59 e |
| Degrees of freedom (DF) | | | | 16 |
| F value | | | | 1.55 |
| Pr > F | | | | 0.0801 |

In this group the family Meliaceae had the highest activity with a mean MIC of 0.23 mg/ml and a *post-hoc* least square mean test (LSM) established that it was significantly higher ($p < 0.05$) compared to the eight families with the lowest activities. Other families with high antifungal activities were Combretaceae, Euphorbiaceae and Proteaceae, all of which differed significantly from several of the families with the lowest MIC values (Table 7.13). The lowest mean antifungal activities were yielded by members of the families Celastraceae and Sapotaceae.

These activities were significantly lower ($p < 0.05$) compared to a number of the families with the highest activities as indicated in Table 7.13.

### 7.3.4 Synthesis of results

In this section, we integrated the results and compared the antimicrobial activities of families against all three pathogen classes to identify patterns of activities to support future selections of plants for further in-depth studies. The comparison was primarily based on the families in Groups 3 and 4. To support the discussion, the mean antimicrobial activities of the families in Groups 3 and 4 were ranked from high to low activity against each of the pathogen classes as shown in Table 7.14 and Table 7.15 respectively.

**Table 7.14:** Comparison of the ranking of the activities (high activity = low MIC and low rank) against each of the different pathogen classes of the families in Group 3 (families with the five highest rankings are highlighted in bold; families with the five lowest rankings are underlined; n = the number of tree species analysed in each family; observations with equal MIC values were ranked with similar numbers but separated by 'a', 'b', etc.).

| Family | n (4-8) | Rank | | |
|---|---|---|---|---|
| | | Gram-positive bacteria | Gram-negative bacteria | Fungi |
| Araliaceae | 6 | 12 | 6 | 11a |
| Arecaceae | 5 | 17 | 18 | 14 |
| Buddlejaceae | 5 | 18 | 19 | 17 |
| Capparaceae | 6 | 9 | 16 | 4a |
| Ebenaceae | 8 | 7b | 1 | 9 |
| Flacourtiaceae | 8 | 11 | 11b | 10 |
| Icacinaceae | 4 | 10 | 14a | 12a |
| Lamiaceae | 6 | 16 | 17 | 12b |
| Malvaceae | 7 | 15 | 9 | 2 |
| Myrtaceae | 6 | 4 | 10 | 18 |
| Ochnaceae | 4 | 1 | 2 | 8 |
| Oleaceae | 6 | 13 | 11a | 15 |
| Podocarpaceae | 4 | 5 | 3 | 7 |
| Rhamnaceae | 6 | 2 | 14b | 19 |
| Scrophulariaceae | 5 | 3 | 7 | 3 |
| Sparrmanniaceae | 6 | 7a | 5 | 16 |
| Sterculiaceae | 4 | 14 | 8 | 6 |
| Strelitziaceae | 5 | 19 | 11c | 1 |
| Thymelaeaceae | 4 | 6 | 4 | 4b |

**Table 7.15:** Comparison of the ranking of the activities (high activity = low MIC and low rank) against the different pathogen classes of the families in Group 4 (families with five highest rankings are highlighted in bold; families with the five lowest rankings are underlined; n = the number of tree species analysed in each family; observations with equal MIC values were ranked with similar numbers but separated by 'a', 'b', etc.).

| Family | n (≥ 9) | Rank | | |
|---|---|---|---|---|
| | | Gram-positive bacteria | Gram-negative bacteria | Fungi |
| Anacardiaceae | 17 | 1 | 1b | 13 |
| Annonaceae | 9 | 13 | 12 | 6a |
| Apocynaceae | 19 | 15 | 14 | 14b |
| Asteraceae | 11 | 16 | 13 | 11 |
| Bignoniaceae | 9 | 14 | 6b | 9 |
| Celastraceae | 19 | 4 | 8 | 17 |
| Combretaceae | 15 | 3 | 1a | 2 |
| Euphorbiaceae | 26 | 6 | 9 | 3 |
| Fabaceae | 55 | 5 | 4 | 5 |
| Meliaceae | 10 | 10 | 15 | 1 |
| Moraceae | 12 | 2 | 3 | 12 |
| Phyllanthaceae | 13 | 12 | 5 | 10 |
| Proteaceae | 28 | 11 | 16 | 4 |
| Rubiaceae | 41 | 9 | 10 | 6b |
| Rutaceae | 11 | 17 | 17 | 8 |
| Sapindaceae | 19 | 7 | 6a | 14a |
| Sapotaceae | 14 | 8 | 11 | 16 |

In some instances, families had high mean antimicrobial activities against all three pathogen classes. For example, the family Thymelaeaceae (Group 3) had relatively high mean activities against all three pathogen classes (Table 7.14). Among the larger families (Table 7.15), both Combretaceae and Fabaceae had relatively high mean antimicrobial activities against all three classes of pathogens.

A number of families had stronger antibacterial activities compared to antifungal activities. The most notable families were the Anacardiaceae and Moraceae (Table 7.15) which were both ranked higher against Gram-positive and Gram-negative bacteria compared to the fungi. The family Ochnaceae (Table 7.14) also had very strong antibacterial activities and were ranked first and second against Gram-positive and Gram-negative bacteria respectively. On the other hand the families Malvaceae, Strelitziaceae (Table 7.14), Proteaceae and Meliaceae (Table 7.15) had noticeable stronger antifungal activities compared to antibacterial activities.

The results also indicated that the activities of some families were ranked high against a single pathogen class only. This could indicate very useful selective activity. Members of the family Rhamnaceae (Table 7.14) had very good activity against Gram-positive bacteria while lower activities against the other two pathogen classes. Similarly, the family Celastraceae (Table 7.15) had stronger Gram-positive antibacterial activities and members of Phyllanthaceae (Table 7.15) had selective activity against the Gram-negative bacteria.

Some families had low activities against several pathogen classes. For example, Buddlejaceae (Table 7.14) and Apocynaceae (Table 7.14) were ranked low against all the pathogen classes. The families Lamiaceae (Table 7.14) and Asteraceae (Table 7.15) had relatively low activities against all the pathogen classes, most notably against the bacteria. The low activity of Asteraceae is interesting as the family is widely used in traditional medicine, possibly not for microbial infections. The family Rutaceae (Table 7.15) were less promising against the bacterial classes than to the fungi.

### 7.3.5 Antimicrobial activities of ethnomedicinal important families

Several of the species in the families with promising antimicrobial activities are widely used for medicinal purposes throughout southern Africa and world-wide. For example, Anacardiaceae, a family with particularly promising antibacterial activities in this study is one of the largest tree families in our region and 88% of the tree species that we have analysed were listed as ethnomedicinal plants in southern Africa (Arnold *et al*., 2002). However, the publication did not specify the disease that is treated by the specific plant and only recorded general medicinal usage. Similarly, a large proportion (77%) of the tree species that we have analysed of Combretaceae, a well-represented tree family in southern Africa, is listed as ethnomedicinal plants (Arnold *et al*., 2002).

The family Rutaceae had relatively low antimicrobial activities, especially against the Gram-positive and Gram-negative bacteria. This was unexpected, because many of the members of the family Rutaceae are used as medicinal plants (Moerman and Estabrook, 2003). Southern African tree species in the family Rutaceae such as *Toddalia asiatica* (Orwa *et al*., 2007; Arnold *et al*., 2002), *Zanthoxylum* capense (Watt and Breyer-Brandwijk, 1962; Hutchings *et al*., 1996) and *Clausena anisata* (Hutchings *et al*., 1996) are used as medicinal plants throughout Africa. Rutaceae is a family rich in alkaloids and was one of the families in the NAPRALERT database with the largest percentage of active antifungal species (Sortino *et al.,* 2012).

Of particular interest is Asteraceae which had relatively low activities compared to the other families in Group 4, especially against the Gram-positive and Gram-negative bacteria. Asteraceae is a family rich in secondary metabolites, especially sesquiterpene lactones (Goren

et al., 1996; Scott *et al*., 2004). Even though Asteraceae is a well-known medicinal plant family, it is the shrubs and herbs that are commonly used medicinally. Only 50% of the representative tree species of Asteraceae that we have analysed were listed as medicinal plants in Arnold *et al*. (2002). A similar low percentage (less than 50%) of the remainder of the tree species in Asteraceae that were not analysed in the current study was used as medicinal plants.

The relatively low mean antimicrobial activities of Apocynaceae and Lamiaceae against all the pathogen groups were also surprising given that these families are used word-wide in ethnomedicine (Moerman and Estabrook, 2003). Approximately 76% of Apocynaceae tree species analysed in this study is used as medicinal plants in southern Africa (Arnold *et al*., 2002).The family Lamiaceae is a large and diverse family that contains mainly herbs and shrubs. Only two of the six tree species analysed in Lamiaceae were listed as ethnomedicinal plants in southern Africa (Arnold *et al*., 2002).

Other well-known medicinal families such as Euphorbiaceae and Rubiaceae were ranked within the top ten families against each of the pathogen classes, but their mean antimicrobial activities did not differ significantly from the families with the most or least promising activities. Both Euphorbiaceae and Rubiaceae are relatively large tree families in southern Africa. In this study, a wide range of antimicrobial activities were found within both families as will be discussed in Chapter 9.

Araliaceae is considered by Heinrich *et al*. (2004) as a very important medicinal family. In southern Africa, several species is used in traditional medicine (Watt and Breyer-Brandwijk, 1962). In this study the family had mean activities ranging from 0.38 mg/ml (Gram-negative bacteria) to 0.51 mg/ml (Gram-positive bacteria). However, the different species in the family inhibited the organisms at a wide range of MIC's. Five of the nine species had interesting MIC's of 0.16 mg/ml against at least one of the six pathogens. *Cussonia transvaalensis* and *C. zuluensis* was the most effective species in the family Araliaceae with MIC's of 0.16 mg/ml and lower against respectively four and five of the pathogens.

Other medicinally important families like Asphodelaceae and Rosaceae (Moerman and Estabrook, 2003; Heinrich *et al*., 2004) contained relatively few tree genera and therefore only a small number of species were analysed within these families. The extracts of the representative of Asphodelaceae (*Aloe plicatilis*) had uninteresting antimicrobial activities ranging from 0.31 mg/ml against *E. coli* to 2.50 mg/ml against both *C. albicans* and *C. neoformans*. The mean MIC's of species analysed within Rosaceae in this study varied quite substantially, ranging from 0.14 mg/ml against Gram-positive bacteria to 0.60 mg/ml against the fungi.

There is no clear basis to explain why some of the families had higher mean activities. Plants contain numerous different secondary metabolites with antimicrobial activities (Cos *et al*., 2006) and a series of compounds is usually responsible for the chemical defences of plants (Verpoorte, 1998). Plants with the greatest chemical diversity may have a higher probability of producing active compounds (Jones and Firn, 1991). Mixtures of certain compounds may have a synergistic effect (Van Wyk and Wink, 2004) and/or plants may contain substances which improve the activity of the antimicrobials (Kalemba and Kunicka, 2003) and/or in other cases stimulate the immune system (Gilbert and Alves, 2003).

Several antimicrobial compounds have been isolated from all the families, irrespective of their level of activity recorded in the current study. For example, several types of secondary metabolites have been described in Fabaceae, a well-known medicinal plant family (Wink and Mohamed, 2003). Among these, all classes of terpenes, which are powerful defence compounds against microbes and herbivores, are common in Fabaceae (Wink and Mohamed, 2003). Other families are known to be rich in alkaloids such as Rubiaceae, Fabaceae, Boraginaceae, Apocynaceae, Asteraceae, Rutaceae, Papaveraceae, Amaryllidaceae, Berberidaceae, Ranunculaceae and Solanaceae (Van Wyk and Wink, 2004). Both Fabaceae and Anacardiaceae contain a large percentage of flavonoids (Douwes *et al*., 2008) which is one of the largest anti-staphylococcal (and broadly antibcterial) classes of metabolites (Gibbons, 2004).

### 7.3.6 Previous antimicrobial results of species in selected families

Numerous studies have investigated the antimicrobial activities of plant extracts and focused mainly on plants used in traditional medicine. In those studies, compounds with antimicrobial activities have been isolated both from families that we have identified with the highest antimicrobial activities and from families we have identified with the least promising antimicrobial activities. For example, compounds with antifungal activities have been isolated from Meliaceae, Combretaceae, Euphorbiaceae (families with very high antifungal activities) and from Celastraceae and Rhamnaceae (families with the least promising antifungal activities) (Abad *et al*., 2007).

Among the families in Group 3, Ochnaceae had very strong mean antibacterial activities. In a follow-up study by the Phytomedicine group, Makhafola and Eloff (2012), isolated several antibacterial compounds from species of one of the genera of Ochnaceae (*Ochna*), which confirmed the potential value of members of Ochnaceae as antibacterial agents. The other tree genus contained in Ochnaceae (not analysed) consists of a single species, *Brackenridgea zanguebarica*, which is used extensively in traditional African medicine, especially under the

Venda people in South Africa (Netshiungani and Van Wyk, 1980). *B. zanguebarica* has a restricted distribution in South Africa although it is more widespread in the rest of southern Africa. In a previous study, Möller *et al*. (2006) established that *B. zanguebarica* had a strong antibacterial activity against Gram-positive bacteria strains and thereby confirmed its use in traditional medicine.

The family Podocarpaceae (Group 3) had relatively strong antimicrobial activities, especially against Gram-negative bacteria. The promising results are in agreement with a study that recorded MIC values of less than 1.0 mg/ml for several southern African *Podocarpus* species against a number of bacterial organisms. In the same study, some *Podocarpus* species also had strong antifungal activities with MIC's as low as 0.03 mg/ml (Abdillahi *et al.,* 2008). In another study, Bagla (2012) found compounds in *P. henkellii* extracts that were active against several bacteria.

In this study, the family Ebenaceae (Group 3) had very strong Gram-negative antibacterial activities. Representatives of both tree genera in Ebenaceae (*Diospyros* and *Euclea*) were analysed. Previous studies on *Euclea* species reported antimicrobial results mainly for *E. natalensis,* one of the southern African tree species, which was not collected for this study. Lall and Meyer (2000) found that both water and acetone extracts of the root of *E. natalensis* inhibited the growth of mainly Gram-positive bacteria at concentrations from 0.1 mg/ml. Compounds with antimicrobial activities were isolated from the root bark of *E. natalensis* in phytochemical studies. One of the isolates inhibited Gram-positive bacteria and a drug-sensitive strain of *Mycobacterium tuberculosis* at concentrations of 0.1 mg/ml (Weigenand *et al*., 2004). No studies reported promising antimicrobial activities of southern African tree species of the genus *Diospyros*. However, other species of the genus were found to have promising antibacterial and antifungal activities. *Diospyros* species with promising antimicrobial activities were *D. bipindensis* against two streptococcus bacteria (Cesari, 2013), *D. bateri* and *D. monbuttensis* against a wide range of Gram-positive and Gram-negative bacteria (Odelola and Okorosobo, 1988) and *D.anisandra* against several microbial organisms including a *Mycobacterium* tuberculosis resistant strain (Borges-Argáez, 2007).

Among the larger families (Group 4), Combretaceae had relatively high mean antimicrobial activities against all the pathogen classes. This study therefore confirms that the Combretaceae is a very promising antimicrobial tree family. Several previous studies showed that southern African species of Combretaceae, especially of the genera *Combretum* and *Terminalia* had promising antibacterial and antifungal activities (Eloff, 1999; Baba-Moussa *et al*., 1999; McGaw, *et al*., 2001; Fyhrquist *et al*., 2004; Masoko *et al*., 2005). In studies performed in the

Phytomedicine Programme, several antimicrobial components were isolated from species of *Combretum*, the largest genus in Combretaceae (Martini and Eloff, 1998; Eloff *et al*., 2008).

## 7.4 Conclusions

A wide range of mean activities were recorded for all the tree families against the Gram-positive bacteria, Gram-negative bacteria and fungi. Comparisons of mean antibacterial activities of the families were problematic mainly because of size differences. Even though we sampled in accordance to our target, a large proportion of families contained only three or fewer genera and therefore only a small number of representatives were analysed for those families. Since statistical tests normally require a larger sample size, the results could not be substantiated statistically. Differences among the calculated mean MIC's of the families may therefore be based on inherent natural variation. Recommendations based on these results have to be treated with caution and more species will have to be screened to validate these provisional findings.

In evaluating the groups of larger families (Group 4) sufficient data was available to allow statistical analysis. The results provided an indication of a tree family's general effectiveness and enabled us to compile a list of promising families against respectively Gram-positive bacteria, Gram-negative bacteria and fungi. The potential of several members of these promising families were confirmed by other studies reported in the literature. The promising families may have more species with high antimicrobial activities based on the assumption that plants in related taxa often have similar compounds from common descent and therefore similar activities. Similarly, families with low antimicrobial activities may have fewer species with high activities and should receive less attention in future screening programmes.

The study also identified families with promising activities against more than one class of pathogen. This may indicate that members of these families have a broad spectrum of antimicrobial activity and may provide good leads. In other cases it may be more valuable that extracts of some families had much higher activities against a specific group of micro-organism while they were less promising against the other organisms. Future screening programmes directed at plants with either antibacterial or antifungal activities should consider screening the members of these highly selective families.

Several families that are widely used in ethnomedicine had promising activities in this study. On the other hand, several well-known important medicinal families had uninteresting activities. The low antibacterial activities of tree species in Asteraceae and Lamiaceae for example contradict several reports citing both as important medicinal families (Moerman and Estabrook, 2003;

Abad *et al*., 2007; Sortino *et al.,* 2012). This may be because the important medicinal families may have been used for other indications than microbial infections.

The species of promising families represent priority areas for further antimicrobial research in southern Africa. However, large standard deviations were recorded when we calculated the mean MIC for a family which indicated that there were large differences in the antimicrobial activities between species within a family. This variation will be investigated in Chapter 9. In the next chapter, similar analyses were performed at a higher taxonomic level (order level) to compare the antimicrobial activities of plant orders against the different pathogens.

# CHAPTER 8

# Antimicrobial activities of southern African tree orders

## 8.1 Introduction

The background to this chapter is the same as for Chapter 7 in which we focused on antimicrobial activities of tree families. In this chapter we analysed and compared the mean antibacterial activities of southern African trees at the order level.

Since orders are more inclusive compared to families, the mean MIC's calculated for each of orders should therefore be less affected by outliers. In addition, since the boundaries of orders are more accepted a comparison at this level may reduce variation caused by changes in classification systems or differences between classification systems.

## 8.2 Materials and methods

### 8.2.1 General material and methods

A similar experimental design and dataset to the previous Chapters were used. The study area and taxonomical arrangements of the trees of southern Africa were described in Chapter 2 and the collection of tree samples and the description of the tree species collected for the Phytomedicine Tree Database were discussed in Chapter 3. The preparation of plant extracts, microbial organisms, antimicrobial assays as well as general processing of the data were discussed in Chapter 4.

### 8.2.2 Mean MIC values of the orders

In this chapter we grouped the species into their respective orders and a mean MIC value was calculated for each of the 38 orders against *S. aureus, E. faecalis, E. coli*, *P. aeruginosa, C. albicans* and *C. neoformans.* Thereafter, a mean MIC value was calculated for extracts of the species examined in each order against all three pathogen classes: Gram-positive bacteria (*S. aureus* and *E. faecalis*), Gram-negative bacteria (*E. coli* and *P. aeruginosa*) and fungi (*C. albicans* and *C. neoformans*). The largest families (those with the most representatives analysed) in an order contributed the most to the mean value.

### 8.2.3 Order grouping

As discussed in Chapter 3, the sizes of orders varied substantially with many more small than large orders. These large differences complicate comparisons between the orders.

Consequently we decided to lower the impact of the size differences between orders by clustering them into three groups according to size (Table 8.1).

**Table 8.1:** Grouping of 38 plant orders encompassing the tree species analysed for the Phytomedicine Tree Database (PMDB) into size classes.

| | Group 1 (one representative species) | Group 2 (small orders) | Group 3 (large orders) |
|---|---|---|---|
| Number of tree species in order | 1 | 2 to 8 | ≥ 9 |
| Total number of orders in group | 11 | 13 | 14 |

### 8.2.4 Statistical analysis

The data were partitioned into their respective groups and compared separately. The data was reported as mean ± standard deviation (SD) of the mean, except for orders in Group 1 which reflected only the mean MIC of a single species.

The small orders (Groups 1 and 2) did not contain enough species to enable statistical analyses. For the comparisons between the larger orders (Group 3), the mean MIC values calculated for each order against the three pathogen classes were log transformed after which an ANOVA and a *post-hoc* test (LSM) were performed. Statistical computations were performed using Microsoft Excel, version 2010 and the statistical software program SAS (version 9.2). Statistical significance was defined as $p < 0.05$. In cases where statistical significances were established, the practical significance of differences was challenged, in accordance with the recommendations of Cohen (1988).

## 8.3 Results and discussion

Although orders are in general more inclusive than families, several of the orders contained only one or two families which in turn contained only a few genera or in some cases a single genus. For this reason, several of the orders in the database were represented by only a small number of species.

The results for the orders against the Gram-positive bacteria are presented in 8.3.1, against the Gram-negative bacteria in 8.3.2 and against the fungi in 8.3.3. A synthesis of the results against all three pathogen classes is given in 8.3.4.

In each section, the results for the orders were partitioned by size into their respective groups. The orders had different levels of efficacies against each of the pathogens. In the respective tables, mean MIC's against each of the pathogens as well as a mean MIC for each pathogen

class were listed. However, for the discussion the mean MIC against the pathogen class was used to compare the orders.

### 8.3.1 Gram-positive antibacterial activities of the orders

The representatives analysed in the orders placed in Group 1 (one representative species) yielded a wide range of antibacterial activities against the Gram-positive bacteria (Table 8.2). *Balanites maughamii* of the order Zygophyllales had the highest antibacterial activity compared to *Aphloia theiformis* of the order Crossomotales which had the lowest antibacterial activity.

**Table 8.2:** Mean minimum inhibitory concentrations (MIC) of the orders in Group 1 (one representative) against the Gram-positive bacteria, arranged from highest to lowest activity.

| Order | Mean MIC (mg/ml) | | |
|---|---|---|---|
| | *E. faecalis* | *S. aureus* | Mean (Gram-positive bacteria) |
| Zygophyllales | 0.16 | 0.11 | 0.13 |
| Cornales | 0.13 | 0.16 | 0.14 |
| Bruniales | 0.22 | 0.44 | 0.31 |
| Fagales | 0.63 | 0.31 | 0.44 |
| Canellales | 0.45 | 0.54 | 0.49 |
| Aquifoliales | 0.31 | 1.25 | 0.62 |
| Oxalidales | 1.25 | 0.31 | 0.62 |
| Caryophyllales | 0.93 | 1.48 | 1.17 |
| Cyatheales | 1.25 | 2.50 | 1.77 |
| Pandanales | 1.25 | 2.50 | 1.77 |
| Crossomotales | 2.50 | 2.50 | 2.50 |

Group 2 (small orders) contained 13 orders of which we analysed between two and eight representatives each. The MIC values of orders in Group 2 against the Gram-positive bacteria are shown in Table 8.3 and ranged from 0.21 to 1.18 mg/ml (Table 8.3). The orders Coniferales (Cupressaceae), Cycadales (Cycadaceae and Zamiaceae), Buxales (Buxaceae) and Saxifragales (Crassulaceae, Hamamelidaceae and Iteaceae) had the highest mean activities with mean MIC values of 0.34 mg/ml and lower. The orders with relatively low activities were Zingiberales (Musaceae and Strelitziaceae) and Laurales (Hernandiaceae and Lauraceae).

**Table 8.3:** Mean minimum inhibitory concentrations (MIC) ± standard deviation (SD) of the orders in Group 2 against the Gram-positive bacteria. The orders were arranged from highest to lowest activity (n = the number of tree species analysed in each order).

| Order | n (2 to 8) | MIC (mg/ml) (± SD) | | |
|---|---|---|---|---|
| | | *E. faecalis* | *S. aureus* | Mean (Gram-positive bacteria) |
| Coniferales | 3 | 0.34 ± 0.11 | 0.12 ± 0.06 | 0.21 ± 0.16 |
| Cycadales | 3 | 0.12 ± 1.04 | 0.47 ± 1.47 | 0.24 ± 0.92 |
| Buxales | 2 | 0.25 ± 0.11 | 0.30 ± 0.02 | 0.27 ± 0.08 |
| Saxifragales | 3 | 0.31 ± 0.33 | 0.31 ± 0.00 | 0.34 ± 0.20 |
| Pinales | 4 | 0.25 ± 0.40 | 0.48 ± 0.23 | 0.35 ± 0.36 |
| Brassicales | 7 | 0.41 ± 0.79 | 0.54 ± 1.08 | 0.47 ± 0.92 |
| Geraniales | 3 | 0.41 ± 0.41 | 0.40 ± 0.78 | 0.51 ± 0.63 |
| Vitales | 3 | 0.23 ± 0.16 | 0.99 ± 0.53 | 0.47 ± 0.58 |
| Asparagales | 2 | 0.71 ± 0.85 | 0.53 ± 0.19 | 0.61 ± 0.48 |
| Santalales | 4 | 0.37 ± 0.64 | 1.05 ± 1.30 | 0.62 ± 1.04 |
| Arecales | 5 | 0.44 ± 1.26 | 0.92 ± 1.80 | 0.64 ± 1.46 |
| Laurales | 4 | 0.74 ± 1.86 | 1.05 ± 1.69 | 0.88 ± 1.79 |
| Zingiberales | 6 | 0.88 ± 1.14 | 1.57 ± 1.54 | 1.18 ± 1.25 |

Nine and more representatives per order were analysed in the orders clustered in Group 3 (large orders). Several orders in this group such as Asterales, Celastrales, Magnoliales and Proteales contained only one family although each of these families contained several southern African tree species.

The differences between the mean MIC values of the 14 large orders in Group 3 ranged between 0.30 and 0.80 mg/ml (Table 8.4). An ANOVA revealed significant differences ($p < 0.05$) between the mean MIC of the orders in this group. Similar to the families, the high standard deviations indicated high diversities in activities of the species analysed in each order which will be further analysed in Chapter 9.

The orders with the highest Gram-positive antibacterial activities were Celastrales, Rosales and Myrtales, each yielding a mean MIC of 0.30 mg/ml. A *post-hoc* least square mean test (LSM) established that these three orders had significantly higher mean activities compared to the five orders with the lowest mean activities (Table 8.4). Furthermore, the extracts of species in the Fabales, Ericales and Sapindales had significantly higher activities compared to Asterales, the order with the lowest mean activity.

The order Celastrales comprises of two tree families of which only representatives of one (Celastraceae) is found in the southern African region. Therefore the activity of the order

reflects that of a single family although several representatives within the family were analysed. The family Celastraceae is one of the ten largest families in the region (Van Wyk *et al*., 1997) and contains several tree genera of which 19 tree species were analysed in this study. It may be interesting to evaluate the antimicrobial activities of representatives of the two tree species of the other family, Lepidobotryaceae, which naturally occurs in Central and East Africa as well as in South America.

The promising order Rosales comprises of six families in the southern African region (Van Wyk *et al.,* 2011) and representatives of all six were analysed. Against the Gram-positive bacteria, the core tree families in this order (Moraceae, Rhamnaceae and Rosaceae) had the highest activities compared to the other families with mean MIC's lower than 0.31 mg/ml. Considering that fewer than three species per family were analysed within the other families (Cecropiaceae, Celtidaceae and Urticaceae), it should be worthwhile to investigate more species of the genera in these families against the Gram-positive bacteria. It is interesting that all three core families (Moraceae, Rhamnaceae and Rosaceae) in the order Rosales yielded very low activities against the fungi (section 8.3.3).

The order Myrtales contained seven tree families and representatives of six were analysed. The Rhynchocalycaceae, a family that contained a single tree genus, was not collected. All the other families in this order had mean MIC values equal or lower than 0.63 mg/ml. These families were mostly small, consisting of a single genus, except for Combretaceae and Myrtaceae. The families in the order Myrtales with the highest activities against the Gram-positive bacteria were Heteropyxidaceae, Combretaceae and Myrtaceae.

The order Asterales, encompassing the family Asteraceae, had significantly lower activities ($p < 0.05$) in comparison with the seven orders with the highest activities in Group 3 (Table 8.4). Other orders of Group 3 with relatively low mean activities were Magnoliales, Gentianales, Lamiales and Proteales. The activities of these orders were significantly lower ($p < 0.05$) compared to Celastrales, Rosales and Myrtales.

**Table 8.4:** Mean minimum inhibitory concentrations (MIC) ± standard deviation (SD) of the orders in Group 3 against the Gram-positive bacteria. The orders are arranged from highest to lowest activity (n = the number of tree species analysed in each order; mean MIC values followed by the same superscript letter do not differ significantly at the 5% confidence level).

| Order | n (≥ 9) | MIC (mg/ml) (± SD) | | |
|---|---|---|---|---|
| | | *E. faecalis* | *S. aureus* | Mean (Gram-positive bacteria) |
| Celastrales | 19 | 0.23 ± 0.41 | 0.37 ± 0.69 | 0.30 ± 0.47 [a] |
| Rosales | 28 | 0.21 ± 0.35 | 0.41 ± 1.04 | 0.30 ± 0.66 [a] |
| Myrtales | 25 | 0.28 ± 0.74 | 0.31 ± 0.52 | 0.30 ± 0.53 [a] |
| Fabales | 57 | 0.38 ± 0.79 | 0.41 ± 0.92 | 0.40 ± 0.81 [ab] |
| Ericales | 30 | 0.28 ± 0.47 | 0.56 ± 1.10 | 0.40 ± 0.72 [ab] |
| Sapindales | 64 | 0.34 ± 0.88 | 0.52 ± 1.04 | 0.42 ± 0.92 [ab] |
| Malpighiales | 71 | 0.38 ± 0.84 | 0.53 ± 1.03 | 0.45 ± 0.86 [ab] |
| Malvales | 26 | 0.34 ± 0.57 | 0.65 ± 0.93 | 0.47 ± 0.67 [abc] |
| Apiales | 9 | 0.44 ± 0.71 | 0.59 ± 1.04 | 0.51 ± 0.75 [abc] |
| Proteales | 28 | 0.38 ± 0.49 | 0.71 ± 1.05 | 0.52 ± 0.73 [bc] |
| Lamiales | 35 | 0.44 ± 0.62 | 0.63 ± 1.00 | 0.53 ± 0.72 [bc] |
| Gentianales | 64 | 0.41 ± 0.67 | 0.69 ± 1.03 | 0.53 ± 0.80 [bc] |
| Magnoliales | 9 | 0.64 ± 1.40 | 0.63 ± 1.22 | 0.63 ± 1.26 [bc] |
| Asterales | 11 | 0.58 ± 0.42 | 1.11 ± 0.78 | 0.80 ± 0.60 [c] |
| Degrees of freedom (DF) | | | | 13 |
| F value | | | | 2.14 |
| Pr > F | | | | 0.0114 |

### 8.3.2 Gram-negative antibacterial activities of the orders

The mean MIC values of the orders in Groups 1 to 3 against Gram-negative bacteria are shown in Tables 8.5 to 8.7. Among the orders in Group 1 (Table 8.5), the representative species of respectively Cornales and Zygophyllales had the highest activities. Both these orders consisted of a single tree family, respectively Curtisiaceae and Balanitaceae. Members of the orders Canellales and Caryophyllales also yielded relatively high activities while, members of the orders Cyatheales, Crossomotales and Aquifoliales had the lowest activities.

Saxifragales and Pinales had the highest mean activities in comparison with the other orders in Group 2 (Table 8.6). The MIC's of the orders ranged from 0.22 to 0.94 mg/ml. The order Saxifragales contained two families, i.e. Crassulaceae and Hamamelidaceae while representatives of a single family, Podocarpaceae, were analysed in the order Pinales. The orders Laurales (Hernandiaceae and Lauraceae) and Geraniales (Greyiaceae and Melianthaceae) yielded the lowest mean activities compared to the other orders in Group 2.

**Table 8.5:** Mean minimum inhibitory concentrations (MIC) of orders in Group 1 (one representative) against the Gram-negative bacteria, arranged from highest to lowest activity.

| Order | Mean MIC (mg/ml) | | |
|---|---|---|---|
| | *P. aeruginosa* | *E. coli* | Mean (Gram-negative bacteria) |
| Cornales | 0.08 | 0.16 | 0.11 |
| Zygophyllales | 0.31 | 0.11 | 0.18 |
| Canellales | 0.16 | 0.31 | 0.22 |
| Caryophyllales | 0.20 | 0.31 | 0.25 |
| Oxalidales | 0.16 | 0.63 | 0.31 |
| Fagales | 0.31 | 0.63 | 0.44 |
| Pandanales | 0.16 | 1.25 | 0.45 |
| Bruniales | 0.63 | 0.88 | 0.74 |
| Aquifoliales | 0.63 | 1.25 | 0.88 |
| Crossomotales | 2.50 | 0.31 | 0.88 |
| Cyatheales | 0.63 | 1.25 | 0.88 |

**Table 8.6:** Mean minimum inhibitory concentrations (MIC) ± standard deviation (SD) of the orders in Group 2 against the Gram-negative bacteria. The orders were arranged from highest to lowest activity (n = the number of species analysed in each order).

| Order | n (2 to 8) | Mean MIC (mg/ml) | | |
|---|---|---|---|---|
| | | *P. aeruginosa* | *E. coli* | Mean (Gram-negative bacteria) |
| Saxifragales | 4 | 0.16 ± 0.00 | 0.12 ± 0.07 | 0.22 ± 0.50 |
| Pinales | 4 | 0.26 ± 0.22 | 0.24 ± 0.14 | 0.25 ± 0.17 |
| Asparagales | 2 | 0.44 ± 0.31 | 0.25 ± 0.11 | 0.33 ± 0.25 |
| Buxales | 2 | 0.33 ± 0.05 | 0.36 ± 0.42 | 0.35 ± 0.26 |
| Vitales | 3 | 0.40 ± 0.41 | 0.36 ± 1.73 | 0.38 ± 1.06 |
| Santalales | 4 | 0.37 ± 0.21 | 0.52 ± 0.66 | 0.40 ± 0.41 |
| Zingiberales | 6 | 0.35 ± 0.27 | 0.52 ± 0.80 | 0.43 ± 0.54 |
| Cycadales | 3 | 0.54 ± 0.42 | 0.48 ± 0.59 | 0.51 ± 0.43 |
| Brassicales | 7 | 0.64 ± 0.67 | 0.47 ± 0.53 | 0.55 ± 0.56 |
| Arecales | 5 | 0.43 ± 0.34 | 0.87 ± 1.53 | 0.61 ± 0.86 |
| Coniferales | 3 | 0.67 ± 0.70 | 0.88 ± 0.44 | 0.77 ± 0.52 |
| Geraniales | 3 | 0.74 ± 0.54 | 0.62 ± 0.66 | 0.85 ± 0.59 |
| Laurales | 4 | 1.20 ± 0.84 | 0.74 ± 1.39 | 0.94 ± 1.17 |

Group 3 included the orders with the largest number of representatives. The differences between the mean MIC of these orders against Gram-negative bacteria were relatively small, ranging from 0.29 to 0.60 mg/ml (Table 8.7). The orders which had the highest mean activities

in Group 3 were Myrtales and Fabales of which the mean MIC values were 0.29 and 0.30 mg/ml respectively. A *post-hoc* least square mean test (LSM) revealed that the activities of only a few orders differed significantly ($p < 0.05$). For example, the activities of the orders Myrtales and Fabales were significantly higher compared to the activities of Sapindales, Gentianales, Lamiales, Magnoliales, Asterales and Proteales. The order Myrtales contained families such as Combretaceae, Heteropyxidaceae, Lythraceae, Melastomataceae, Myrtaceae and Oliniaceae and the order Fabales enclosed families Fabaceae and Polygalaceae. Even though Malvales and Ericales yielded the third and fourth highest mean activities in Group 3, the activities were only significantly higher ($p < 0.05$) compared to the order Proteales.

The orders Proteales and Asterales yielded the lowest mean activities against Gram-negative bacteria compared to the other orders in Group 3. Although several species were analysed within each of these orders, they are contained within a single family: Proteaceae (Proteales) and Asteraceae (Asterales).

**Table 8.7:** Mean minimum inhibitory concentrations (MIC) ± standard deviation (SD) of the orders in Group 3 against the Gram-negative bacteria. The orders are arranged from highest to lowest activity (n = the number of tree species analysed in each order; mean MIC values followed by the same superscript letter do not differ significantly at the 5% confidence level).

| Order | n (≥ 9) | Mean MIC (mg/ml) | | |
|---|---|---|---|---|
| | | *P. aeruginosa* | *E. coli* | Mean (Gram-negative bacteria) |
| Myrtales | 25 | 0.28 ± 0.56 | 0.30 ± 0.59 | 0.29 ± 0.56 $^{a}$ |
| Fabales | 57 | 0.26 ± 0.55 | 0.34 ± 0.60 | 0.30 ± 0.51 $^{ab}$ |
| Malvales | 26 | 0.38 ± 0.73 | 0.31 ± 0.59 | 0.34 ± 0.64 $^{abc}$ |
| Ericales | 30 | 0.33 ± 0.77 | 0.36 ± 0.79 | 0.34 ± 0.75 $^{abc}$ |
| Apiales | 9 | 0.49 ± 0.66 | 0.26 ± 0.59 | 0.36 ± 0.53 $^{abcd}$ |
| Rosales | 28 | 0.37 ± 0.58 | 0.39 ± 0.58 | 0.38 ± 0.51 $^{abcd}$ |
| Malpighiales | 71 | 0.43 ± 0.66 | 0.37 ± 0.71 | 0.40 ± 0.62 $^{acd}$ |
| Celastrales | 19 | 0.39 ± 0.96 | 0.44 ± 0.48 | 0.41 ± 0.68 $^{abcd}$ |
| Sapindales | 64 | 0.45 ± 0.82 | 0.41 ± 0.84 | 0.43 ± 0.78 $^{cd}$ |
| Gentianales | 64 | 0.46 ± 0.75 | 0.43 ± 0.68 | 0.45 ± 0.67 $^{cd}$ |
| Lamiales | 35 | 0.52 ± 0.88 | 0.40 ± 0.36 | 0.46 ± 0.58 $^{cd}$ |
| Magnoliales | 9 | 0.58 ± 0.80 | 0.42 ± 0.43 | 0.49 ± 0.59 $^{cd}$ |
| Asterales | 11 | 0.44 ± 0.43 | 0.57 ± 0.82 | 0.50 ± 0.52 $^{cd}$ |
| Proteales | 28 | 0.46 ± 0.78 | 0.78 ± 0.67 | 0.60 ± 0.70 $^{d}$ |
| Degrees of freedom (DF) | | | | 13 |
| F value | | | | 2.27 |
| PR > F | | | | 0.0065 |

### 8.3.3 Antifungal activities of the orders

Group 1 consisted of 11 orders of which we analysed the MIC value of only one representative per order. As shown in Table 8.8, the representatives of these orders yielded a wide range of mean MIC values against the fungal organisms (MIC's ranging from 0.06 to 1.25 mg/ml). Representative species of the orders Pandanales (*Pandanus livingstonianus*) and Cornales (*Curtisia dentata*) yielded the highest activities compared to the very low activity (1.25 mg/ml) yielded by the representative species of Fagales (*Morella serrata*).

In Group 2, thirteen small orders with between two and eight representatives each were analysed (Table 8.9). The mean MIC values of these orders against the fungal organisms ranged from 0.12 to 0.76 mg/ml. The orders Zingiberales, Vitales and Brassicales had the highest antifungal activities while the orders Asparagales, Cycadales and Buxales had the lowest mean activities compared to the other orders in the group.

**Table 8.8:** Mean minimum inhibitory concentrations (MIC) of the orders in Group 1 (one representative) against the fungal organisms.

| Order | Mean MIC (mg/ml) | | |
|---|---|---|---|
| | *C. albicans* | *C. neoformans* | Mean (Fungi) |
| Pandanales | 0.08 | 0.04 | 0.06 |
| Cornales | 0.06 | 0.14 | 0.09 |
| Aquifoliales | 0.16 | 0.16 | 0.16 |
| Canellales | 0.32 | 0.13 | 0.20 |
| Cyatheales | 0.63 | 0.08 | 0.22 |
| Zygophyllales | 0.31 | 0.16 | 0.22 |
| Oxalidales | 1.25 | 0.04 | 0.22 |
| Bruniales | 0.45 | 0.16 | 0.27 |
| Caryophyllales | 0.47 | 0.31 | 0.38 |
| Crossomotales | 0.16 | 1.25 | 0.45 |
| Fagales | 1.25 | 1.25 | 1.25 |

**Table 8.9:** Mean minimum inhibitory concentrations (MIC) ± standard deviation (SD) of the orders in Group 2 against the fungal organisms. The orders were arranged from highest to lowest activity (n = the number of tree species analysed in each order).

| Order | n (2-8) | Mean MIC (mg/ml) | | |
|---|---|---|---|---|
| | | *C. albicans* | *C. neoformans* | Mean (Fungi) |
| Zingiberales | 6 | 0.18 ± 0.27 | 0.08 ± 0.08 | 0.12 ± 0.16 |
| Vitales | 3 | 0.19 ± 0.08 | 0.22 ± 0.38 | 0.20 ± 0.21 |
| Brassicales | 6 | 0.22 ± 0.15 | 0.23 ± 0.42 | 0.23 ± 0.28 |
| Pinales | 4 | 0.25 ± 0.45 | 0.33 ± 0.26 | 0.29 ± 0.35 |
| Coniferales | 3 | 0.56 ± 0.84 | 0.16 ± 0.00 | 0.30 ± 0.51 |
| Saxifragales | 3 | 0.47 ± 1.69 | 0.2 ± 0.85 | 0.31 ± 1.28 |
| Geraniales | 3 | 0.24 ± 0.11 | 0.5 ± 0.27 | 0.34 ± 0.23 |
| Arecales | 5 | 0.36 ± 1.21 | 0.63 ± 1.74 | 0.47 ± 1.24 |
| Laurales | 4 | 0.4 ± 0.34 | 0.63 ± 1.33 | 0.50 ± 0.77 |
| Santalales | 4 | 0.45 ± 1.53 | 0.63 ± 1.33 | 0.53 ± 1.43 |
| Asparagales | 2 | 0.53 ± 0.56 | 0.70 ± 0.86 | 0.61 ± 0.72 |
| Cycadales | 3 | 0.94 ± 1.04 | 0.5 ± 0.80 | 0.68 ± 1.16 |
| Buxales | 2 | 0.87 ± 1.72 | 0.66 ± 1.41 | 0.76 ± 1.57 |

Fourteen large orders were clustered in Group 3 and their mean MIC values are shown in Table 8.10. Among these orders, Malvales had the highest mean antifungal activity. The differences between the mean MIC values of the orders in Group 3 were relatively small. However, an ANOVA performed on the data set followed by a *post-hoc* least square mean test (LSM) established that the activity was significantly higher ($p < 0.05$) compared to those recorded for the orders Gentianales, Sapindales, Ericales, Asterales, Rosales and Celastrales. Malvales contained families such as Bombaceae, Helicteraceae, Malvaceae, Pentapetaceae, Sparrmanniaceae, Sterculiaceae and Thymelaeaceae.

Members of the order Proteales (Proteaceae) yielded the second highest activity, but it was significantly higher ($p < 0.05$) compared to only the order with the lowest activity (Celastrales). Although members of the order Proteales yielded promising activities against the fungi, they had very low activities against both Gram-positive and Gram-negative bacteria.

As shown in Table 8.10, the order Celastrales had the lowest mean activity against the fungi which differed significantly ($p < 0.05$) from Malvales, Proteales and Fabales. The order Celastrales enclosed a single tree family, Celastraceae. The order Rosales also had a relatively low mean activity but it was only significantly lower compared to Malvales. All three core families in Rosales (Moraceae, Rhamnaceae and Rosaceae) yielded very low activities against the fungi (Chapter 7.3.3). Different levels of activities were found against the Gram-positive

bacteria (Section 8.3.1) where both Celastrales and Rosales had relatively high mean activities compared to the other orders in Group 3.

**Table 8.10:** Mean minimum inhibitory concentrations (MIC) ± standard deviation (SD) of the orders in Group 3 against the fungal organisms. The orders were arranged from highest to lowest activity (n = the number of tree species analysed in each order; mean MIC values followed by the same superscript letter do not differ significantly at the 5% confidence level).

| Orders | n (≥ 9) | Mean MIC (mg/ml) | | |
|---|---|---|---|---|
| | | *C. albicans* | *C. neoformans* | Mean (Fungi) |
| Malvales | 26 | 0.31 ± 0.76 | 0.25 ± 0.74 | 0.28 ± 0.68 [a] |
| Proteales | 27 | 0.38 ± 0.44 | 0.29 ± 0.75 | 0.33 ± 0.54 [ab] |
| Fabales | 56 | 0.37 ± 0.82 | 0.34 ± 0.90 | 0.35 ± 0.79 [ab] |
| Apiales | 9 | 0.41 ± 1.06 | 0.37 ± 0.94 | 0.35 ± 0.92 [abc] |
| Magnoliales | 9 | 0.36 ± 0.99 | 0.35 ± 1.09 | 0.36 ± 0.92 [abc] |
| Malpighiales | 71 | 0.36 ± 0.91 | 0.36 ± 0.86 | 0.36 ± 0.79 [abc] |
| Myrtales | 25 | 0.38 ± 1.06 | 0.41 ± 0.98 | 0.40 ± 0.97 [abc] |
| Lamiales | 35 | 0.42 ± 0.91 | 0.41 ± 1.01 | 0.42 ± 0.90 [abc] |
| Gentianales | 64 | 0.47 ± 1.02 | 0.38 ± 0.93 | 0.42 ± 0.90 [bc] |
| Sapindales | 63 | 0.44 ± 1.04 | 0.44 ± 0.97 | 0.44 ± 0.91 [bc] |
| Ericales | 30 | 0.55 ± 1.11 | 0.42 ± 0.77 | 0.48 ± 0.89 [bc] |
| Asterales | 11 | 0.48 ± 0.64 | 0.50 ± 0.94 | 0.49 ± 0.69 [bc] |
| Rosales | 28 | 0.57 ± 1.07 | 0.46 ± 0.94 | 0.51 ± 0.94 [bc] |
| Celastrales | 19 | 0.66 ± 0.71 | 0.53 ± 0.71 | 0.59 ± 0.59 [c] |
| Degrees of freedom (DF) | | | | 13 |
| F value | | | | 1.29 |
| Pr > F | | | | 0.2149 |

### 8.3.4 Synthesis of results

In the previous sections, orders with high activities against each of the pathogen classes were identified. In this section, we integrated the results of the orders in Group 3 against all three pathogen classes to identify patterns of activities in support of future selection of plants for further in-depth studies.

The mean antimicrobial activities of the orders in Group 3 were ranked from high to low activity against each of the pathogen classes and compared in Table 8.11. Species of the order Fabales had relatively high activities against all pathogen classes. Fabales enclosed the families Fabaceae and Polygalaceae (Table 8.11). The family Fabaceae contains most of the diversity in this order in the southern African region and is a family widely used in traditional medicine (Wink and Mohamed, 2003). The family Polygalaceae made up a much smaller

proportion of the diversity of the family. Both families yielded mean MIC's lower than 0.41 mg/ml against all three pathogen groups.

The antibacterial activities of the orders Ericales and Myrtales were ranked high against both Gram-positive and Gram-negative bacteria. Ericales contained families such as Ebenaceae, Ericaceae, Myrsinaceae and Sapotaceae. Well-known families analysed in Myrtales were Combretaceae, Heteropyxidaceae and Oliniaceae. On the other hand, orders such as Malvales and Apiales performed better against the Gram-negative bacteria and the fungi compared to the Gram-positive bacteria.

Some orders had low activities against more than one of the pathogen classes and members of these orders would be less promising candidates for further screening tests. For example, the order Asterales, which contained a single family (Asteraceae) were ranked relatively low against all pathogen classes while Gentianales and Lamiales had relatively low rankings against both Gram-positive and Gram-negative bacterial classes.

**Table 8.11:** Comparison of the ranking of activities (high activity = low MIC and low rank) against each of the pathogen classes of the orders in Group 3. Orders with the highest and lowest activities in each class were highlighted (n = the number of tree species analysed in each family; four highest rankings are highlighted in bold; four lowest rankings are underlined; observations with equal MIC values were ranked with similar numbers but separated by 'a', 'b', etc.).

| Order | n (≥ 9) | Rank of activity | | |
|---|---|---|---|---|
| | | Gram-positive bacteria | Gram-negative bacteria | Fungi |
| Apiales | 9 | 9 | 5 | 3b |
| Asterales | 11 | 14 | 13 | 12 |
| Celastrales | 19 | 1a | 8 | 14 |
| Ericalis | 30 | 4b | 3b | 11 |
| Fabales | 55 | 4a | 2 | 3a |
| Gentianales | 64 | 11b | 10a | 8b |
| Lamiales | 35 | 11a | 10b | 8a |
| Magnoliales | 9 | 13 | 12 | 5a |
| Malpighiales | 71 | 7 | 7 | 5b |
| Malvales | 26 | 8 | 3a | 1 |
| Myrtales | 25 | 1c | 1 | 7 |
| Proteales | 28 | 10 | 14 | 2 |
| Rosales | 28 | 1b | 6 | 13 |
| Sapindales | 64 | 6 | 9 | 10 |

A number of orders had relatively high activities against one of the pathogen classes while their activities were notably lower against the other two pathogen classes which could indicate a selective activity. For example the order Celastrales were ranked higher against Gram-positive bacteria compared to the other two pathogen classess while Magnoliales and Proteales had relatively higher antifungal activities.

## 8.5 Conclusions

A wide range of mean activities were recorded for each of the orders against the three pathogen classes. Orders are in general more inclusive and could therefore provide stronger evidence of patterns of activities. However, some of the orders enclosed a single tree family of which a few families enclosed a single genus.

Considering that only one to eight species per order in Group 1 and 2 were analysed, the data of these two groups provided limited information at order level. These comparisons reflected mean activities of a small number of tree species and the results could not be analysed statistically because of the small sampling number. Therefore, differences among the calculated mean MIC's of the orders in Group 1 and 2 could be merely coincidental.

The comparisons between the larger groups of orders (Group 3) summarised more data and were substantiated with statistical analyses. Among the orders in Group 3, we found that Celastrales, Rosales and Myrtales had significantly higher mean activities against Gram-positive bacteria while the order Asterales had the lowest mean activity and species within this order may therefore be less suitable candidates for future antimicrobial investigations. Against the Gram-negative bacteria, members of the orders Fabales, Malvales, Ericales and Myrtales in Group 3 were more promising while members of Proteales and Asterales were less promising comparatively. The orders in Group 3 with the strongest antifungal activities were Malvales and Proteales. The activities of these orders were significantly higher compared to the activities of a few other families in the group and may yield more promising candidates for further antifungal studies. On the other hand, species in the orders Rosales and Celastrales may be less likely to yield prospective antifungal candidates. Both orders had relatively low mean activities compared to the other orders in Group 3, some of which differed significantly.

As was the case in the family analysis, some of the orders yielded promising activities against more than one pathogen class which may indicate that members of these orders contain a broad spectrum of antimicrobials. Some orders yielded low mean activities against more than one class of pathogen or had high activities exclusively against a specific class of micro-organism. Future screening programmes, focused to find plants with either antibacterial or antifungal activities, should consider screening members of these families.

These results supported us to compile a list of promising orders as well as less effective orders against each of the pathogen classes. The species in the promising orders represent priority areas for further antimicrobial research in southern Africa. These orders could yield more species with interesting antimicrobial activities based on the assumption that plants in related taxa often have similar compounds and therefore similar activities. On the other hand, orders and families identified with low activities may yield fewer species with high activities. However, high standard deviations were recorded when we calculated the mean MIC of the orders (Chapter 8) and families (Chapter 7). This indicated that there are pronounced differences in activities between the species within a family and order which will be investigated in Chapter 9.

# CHAPTER 9

# Intra-taxa variation in the context of a wide screening approach

## 9.1 Introduction

Plant species in the same genus, family and order may have inherited properties for defence against other organisms from common ancestors (Wink, 2003; Heinrich *et al*., 2004). Taxonomic relationships may therefore enable predictions of antimicrobial activities of closely related taxa due to similarities in secondary metabolites which are often specific to a given botanical family, genus or species. In Chapters 7 and 8, we discerned promising from less promising families and orders based on antimicrobial activities of tree leaf extracts against Gram-positive bacteria, Gram-negative bacteria and fungi. The initial screening performed in this study may be followed by also investigating close relations of promising taxa to facilitate the discovery of new antimicrobial plant extracts.

However, high variability could limit conclusive findings mainly because species within promising genera, families or orders will exhibit variable levels of antimicrobial activity. It was therefore necessary to examine the extent of variation between related taxa included in this study. This was achieved by examining the range of antimicrobial activities within selected families (intra-family) and orders (intra-order) in the first part of this chapter.

In the second part of this chapter, the families that were identified in Chapter 7 as having the five highest mean activities were compared with the families with the five lowest antimicrobial activities in order to determine the predictive value of the taxonomic approach to wide screening to deliver leads. Similar comparisons were performed at order level.

## 9.2 Material and methods

### 9.2.1 General material and methods

A similar experimental design and dataset as for the previous Chapters were used. The study area and taxonomical arrangements of the trees of southern Africa that were sampled was discussed in Chapter 2. The collection of tree samples and the description of the tree species represented in the Phytomedicine Tree Database were discussed in Chapter 3. The preparation of plant extracts, microbial organisms used, antimicrobial assays as well as the processing of the data was discussed in Chapter 4.

### 9.2.2 Investigation of intra-family and intra-order variation

For this evaluation, we selected the five largest families from the Phytomedicine Tree Database (PMDB) to examine the intra-family variation in antimicrobial activities of the trees analysed in the current study. The families Fabaceae, Euphorbiaceae, Rubiaceae and Proteaceae were selected on the basis of highest species representation.

Five large orders containing four or more tree families were selected for the intra-order comparison of the mean antimicrobial activities of the families within orders. The orders selected were Rosales, Myrtales, Malpighiales, Malvales and Gentianales.

### 9.2.3 Comparison of the distribution of the MIC values of extracts of tree species within the most promising and least promising families and orders

We used a range of MIC categories and MIC cut-off points to compare the distribution of representative species of the families and orders. We firstly compared the antimicrobial activities of the representative species of the families with the five most promising activities in Group 4 ($n \geq 9$) with the antimicrobial activities of representative species of the families with the five least promising activities. The distribution of antimicrobial activities of representative species of the orders with the four most promising activities in Group 3 ($n \geq 9$) was then compared with the antimicrobial activities of the representative species in the orders with the four least promising activities. We selected fewer orders because the total number of orders in Group 3 was less compared to the total number of families in Group 4.

The ranges of the categories of MIC's were: < 0.03 mg/ml; 0.03 to 0.04 mg/ml; 0.05 to 0.08 mg/ml; 0.09 to 0.16 mg/ml; 0.17 to 0.31 mg/ml; 0.32 to 0.63 mg/ml; 0.64 to 1.25 mg/ml and 1.26 to 2.50 mg/ml. The range of MIC cut-off points were: ≤ 0.02 mg/ml; ≤ 0.04 mg/ml; ≤ 0.08 mg/ml; ≤ 0.16 mg/ml; ≤ 0.31 mg/ml; ≤ 0.63mg/ml; ≤ 1.25mg/ml and ≤ 2.50mg/ml. All values were expressed as a percentage. The results against each of the three pathogen classes were compared separately.

## 9.3 Results and discussion

The intra-family and intra-order variation in antimicrobial activities of the tree species will be discussed in section 9.3.1 and 9.3.2 respectively. The MIC distribution of the families with the five highest mean antimicrobial activities was compared with the five families with the lowest mean antimicrobial activities in section 9.3.3. Similarly, the MIC distribution of the orders was investigated in 9.3.4.

### 9.3.1 Intra-family variation in antimicrobial activities

The range of antimicrobial activities of closely related species within families varied considerably as demonstrated by the variation in the selected families (Figures 9.1 to 9.6). The selected families each had a large number of species and therefore the species names are not labeled in the figures but are represented by the same colour bar.

The first family that was selected (Fabaceae) is the largest tree family in southern Africa. Fifty-five Fabaceae tree species, belonging to forty-six genera were analysed. Approximately 22 genera were not represented in the study, largely because their distribution ranges are outside the South African borders. Figure 9.1 shows that a wide range of activities were recorded within the family. The MIC's ranged from 0.06 to 2.50 mg/ml (Gram-positive bacteria), 0.06 to 1.25 mg/ml (Gram-negative bacteria) and from 0.05 to 2.50 mg/ml (fungi). The number of species with interesting activities (MIC's ≤ 0.16 mg/ml) ranged from 19% (fungi), 22% (Gram-positive bacteria) to 29% (Gram-negative bacteria) and the number of species with very low activities (MIC's ≥ 1.25 mg/ml) ranged from 5% (Gram-negative bacteria), 15% (Gram-positive bacteria) to 16% (Gram-positive bacteria).

Fabaceae has three distinct subfamilies, though which are treated by some classification systems as separate families. Representative species of all three subfamilies were analysed and the MIC's of the species within each subfamily are shown in Figures 9.2(a) to (c). No noteworthy differences were found between the mean MIC's of the subfamilies. However, the range of MIC's against each of the pathogen groups were slightly smaller in Mimosoideae compared to the other two subfamilies. The large variation of activities within Fabaceae may have resulted from the early evolution of genes that control secondary metabolites being switched on or off according to ecological needs (Wink and Mohamed, 2003).

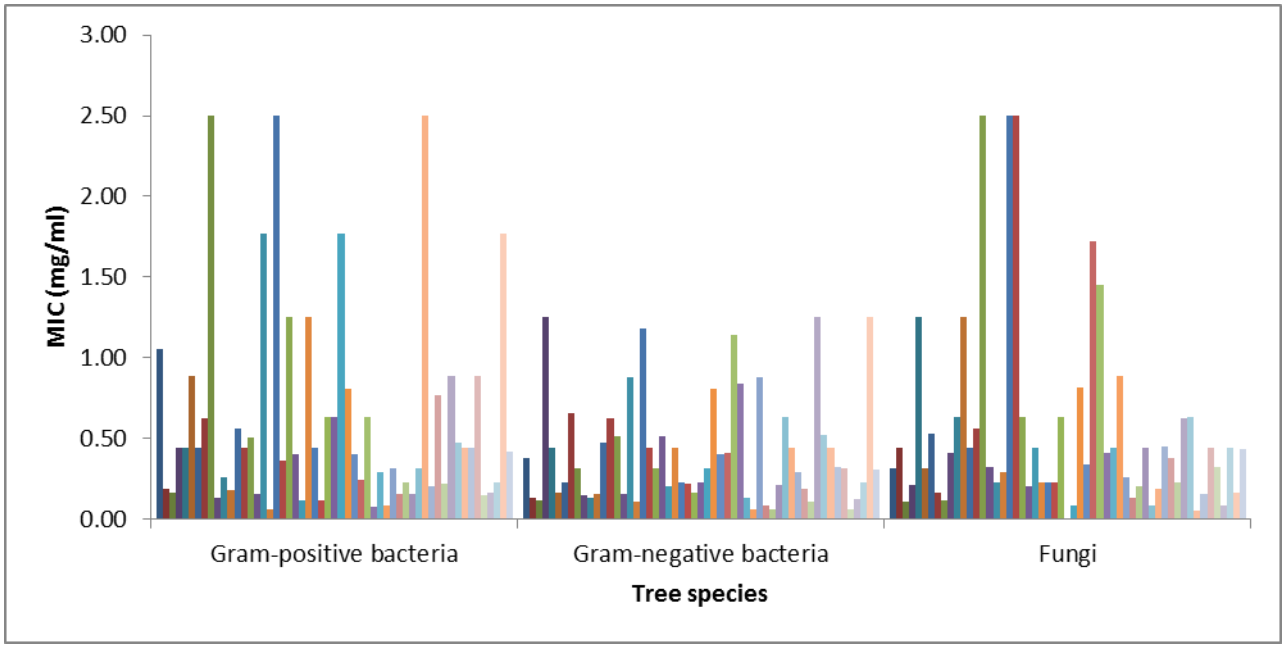


**Fig. 9.1:** The mean MIC values (mg/ml) of the tree species within Fabaceae against the Gram-positive bacteria, Gram-negative bacteria and the fungal organisms (the same colour bar represents the same species).

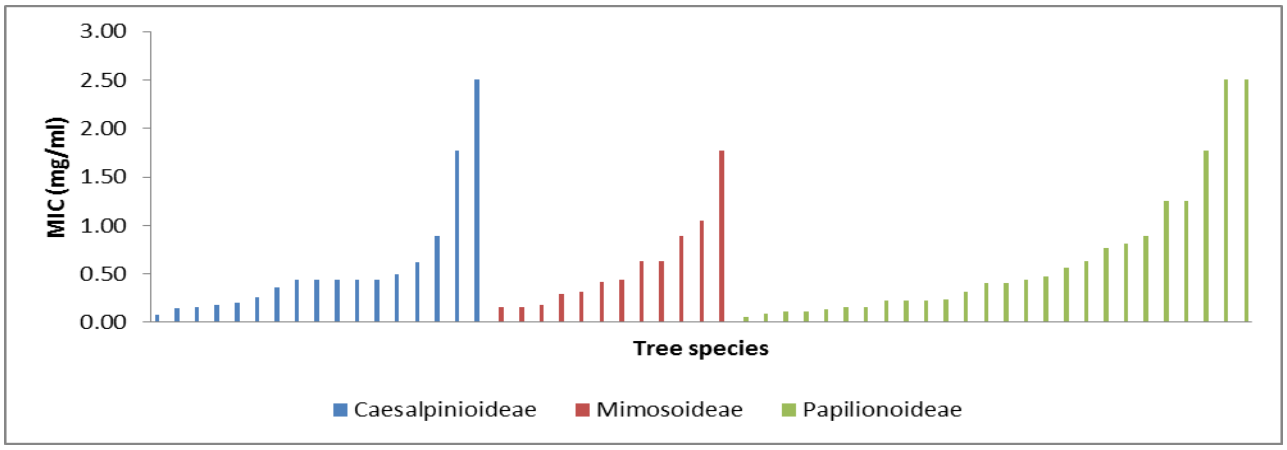


**Fig. 9.2 (a):** The mean MIC values (mg/ml) of the tree species within the Fabaceae subfamilies (Caesalpinioideae, Mimosoideae and Papilionoideae) against the Gram-positive bacteria.

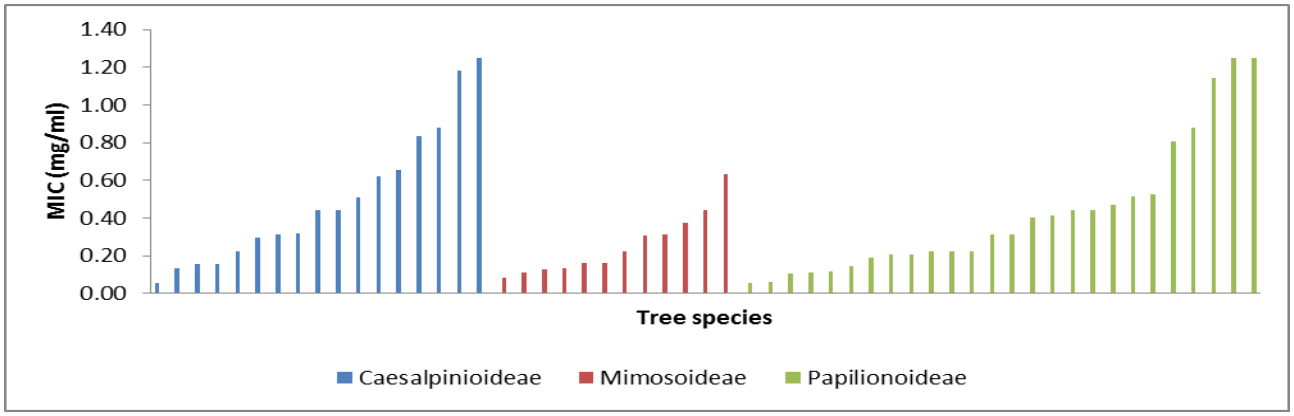


**Fig. 9.2(b):** The mean MIC values (mg/ml) of the tree species within the Fabaceae subfamilies (Caesalpinioideae, Mimosoideae and Papilionoideae) against the Gram-negative bacteria.

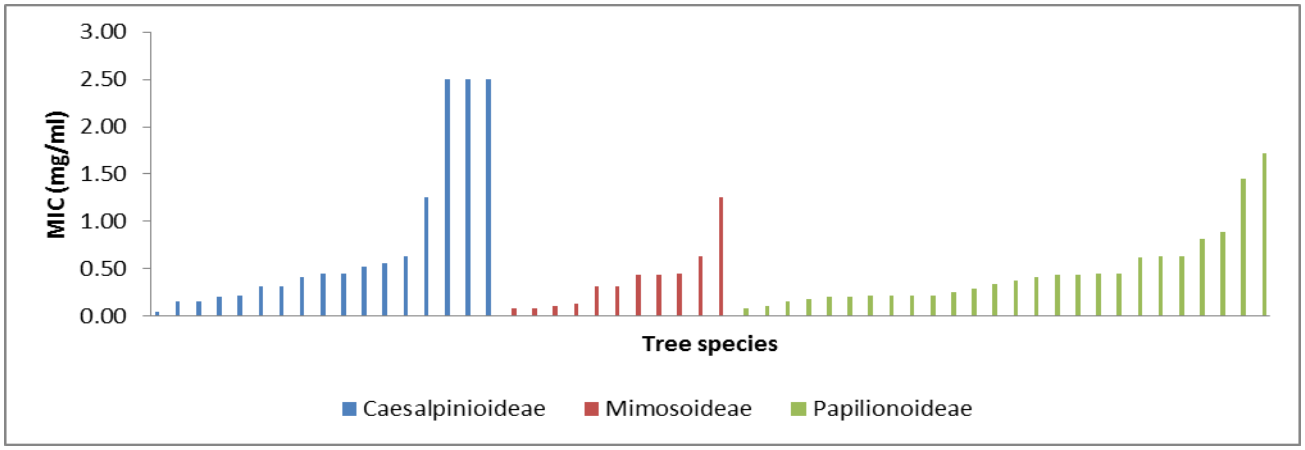


**Fig. 9.2(c):** The mean MIC values (mg/ml) of the tree species within the Fabaceae subfamilies (Caesalpinioideae, Mimosoideae and Papilionoideae) against the fungal organisms.

Rubiaceae, the second largest woody family in southern Africa, is a rich source of alkaloids and is widely used as medicine (Van Wyk and Van Wyk, 1997). The family contains approximately 51 tree genera of which a large number of representative species occurs naturally outside South Africa. Consequently, at least 26 of these genera were not collected and analysed. We have analysed 41 Rubiaceae species belonging to 25 genera (Figure 9.3). The MIC's of the species within Rubiaceae varied from 0.05 to 2.50 mg/ml (Gram-positive bacteria), from 0.11 to 2.50 mg/ml (Gram-negative bacteria) and from 0.06 to 2.50 mg/ml (fungi). Among the species within Rubiaceae, a few had interesting activities (≤ 0.16 mg/ml) as well as very low activities (≥ 1.25 mg.ml) against each of the pathogen groups.

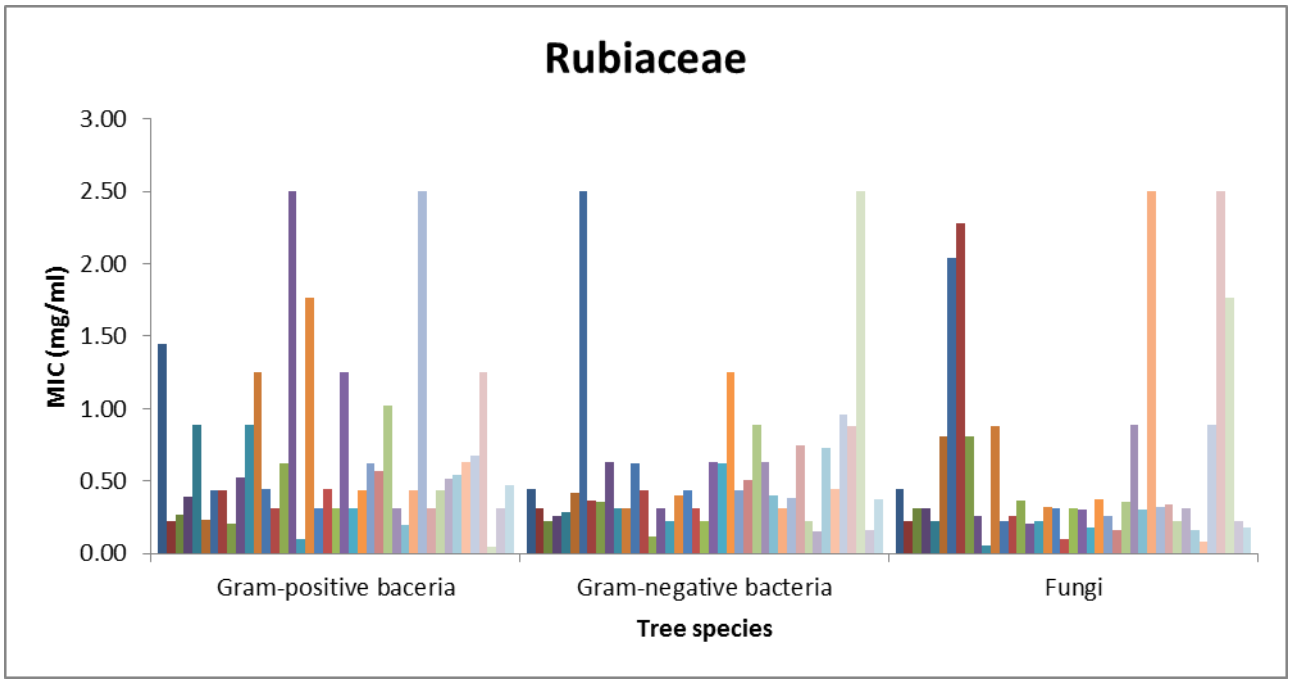


**Fig. 9.3:** The mean MIC values (mg/ml) of the tree species within Rubiaceae against the Gram-positive bacteria, Gram-negative bacteria and the fungal organisms (the same colour bar represents the same species).

Euphorbiaceae is the third largest tree family in southern Africa and contain 26 tree genera. Many of the tree species have medicinal uses and/or are poisonous (Van Wyk and Van Wyk, 1997). We analysed approximately 27 tree species belonging to 22 genera. The species had a wide range of antibacterial activities (Figure 9.4). The MIC's of the Euphorbiaceae species ranged from 0.03 to 1.77 mg/ml against the Gram-positive bacteria, from 0.09 to 2.50 mg/ml against the Gram-negative bacteria and from 0.03 to 2.50 mg/ml against the fungal organisms. It is known that a large number of secondary metabolites are found within Euphorbiaceae (Mwine and Van Damme, 2011) which possibly explains the wide range in antibacterial activities.

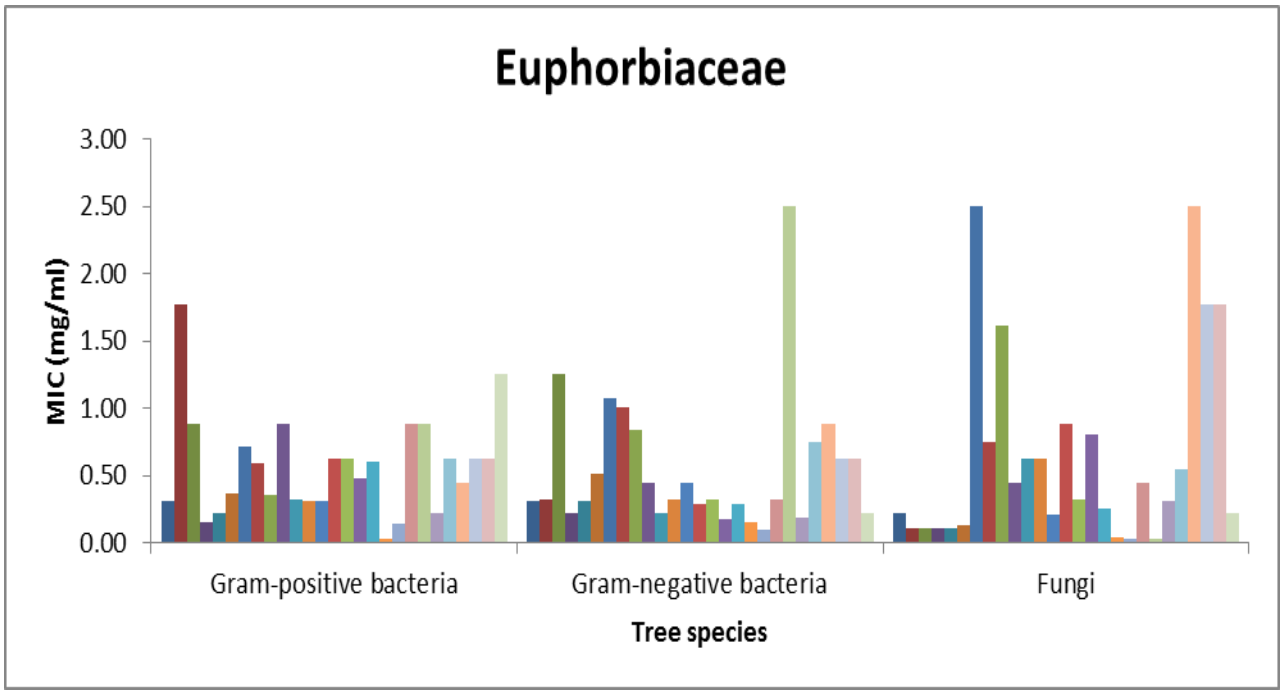


**Fig. 9.4:** The mean MIC values (mg/ml) of the tree species within Euphorbiaceae against the Gram-positive bacteria, Gram-negative bacteria and the fungal organisms (the same colour bar represents the same species).

Proteaceae is a southern hemisphere woody plant family that is best presented in the southern parts of South Africa, more specifically in the fynbos vegetation type in the Cape floristic region (Van Wyk and Van Wyk, 1997; Van Wyk *et al*., 2011). In the family Proteaceae, the genera *Brabejum, Faurea, Leucadendron, Leucospermum, Mimetes, Paranomus, Protea* contain southern African tree species of which *Leucadendron* (18 species) and *Protea* (35 species) are the two largest genera. We have analysed approximately 28 Proteaceae species of which 11 were *Leucadendron* species and 7 were *Protea* species. Members of Proteaceae had good antifungal activities but were less promising against the bacteria (Chapter 7). Notwithstanding, the differences in the grade of activities between the pathogen groups, the Proteaceae species had a large range of MIC's against all three pathogen groups (Figure 9.5). The MIC's varied from 0.03 to 1.77 mg/ml (Gram-positive bacteria), from 0.22 to 2.50 mg/ml (Gram-negative bacteria) and from 0.07 to 1.77 mg/ml (fungi). At least five species had interesting antifungal activities (MIC ≤ 0.16 mg/ml) compared to two species with interesting activities against the Gram-positive bacteria. Not one of the species had interesting antimicrobial activities of 0.16 mg/ml or lower against the Gram-negative bacteria. Up to five species had very low antimicrobial activities (MIC ≥ 1.25 mg/ml) against all the pathogen classes.

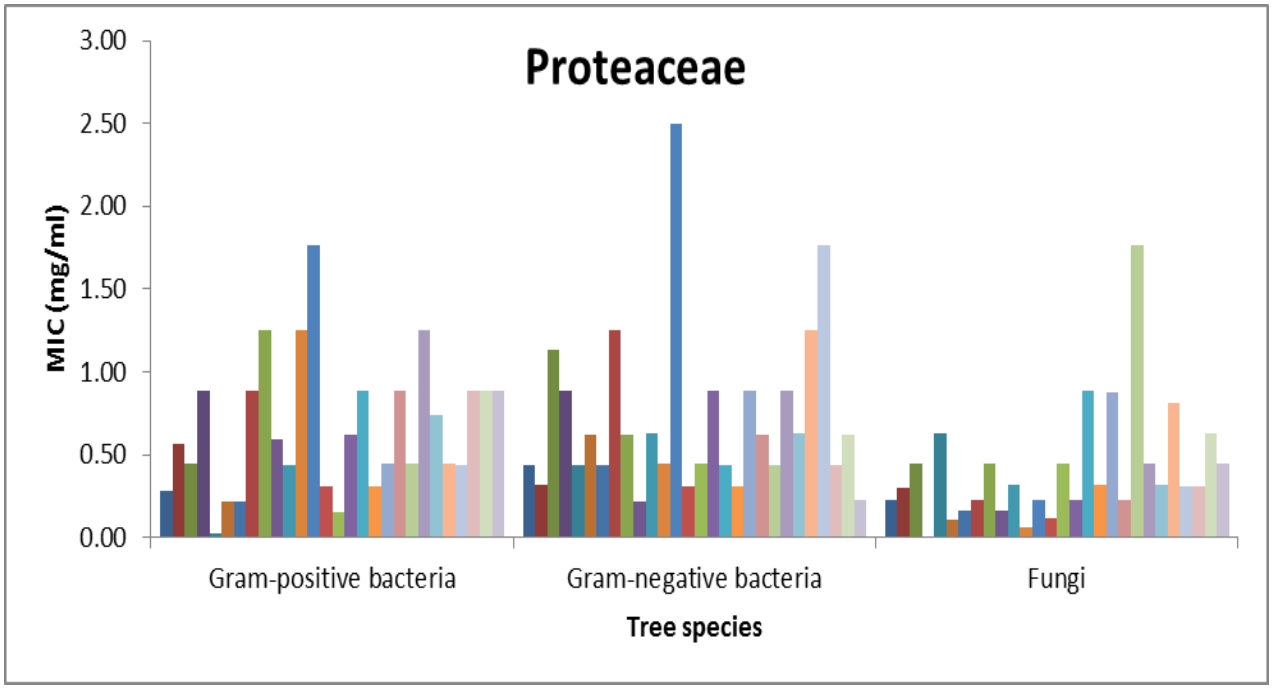


**Fig. 9.5:** The mean MIC values (mg/ml) of the tree species within Proteaceae against the Gram-positive bacteria, Gram-negative bacteria and the fungal organisms (the same colour bar represents the same species).

The family Celastraceae encloses 19 tree genera of which we have analysed 19 species belonging to 13 genera. Well-known genera of the family include *Cassine, Catha, Elaeodendron, Gymnosporia, Maytenus and Putterlickia*. The MIC's of the species of Celastraceae ranged from 0.08 to 0.74 mg/ml against the Gram-positive bacteria (Figure 9.6). The species also had a wide range of activities against the Gram-negative bacteria with MIC's ranging from 0.11 to 1.77 mg/ml and against the fungi the MIC's ranged from 0.16 to 1.25 mg/ml. The family had a relatively high mean activity against Gram-positive bacteria but was less promising against the other two pathogens. Extracts of species with interesting activities (MIC ≤ 0.16 mg/ml) were found against all three pathogen classes. However, as expected, more species had interesting activities (MIC ≤ 0.16 mg/ml) against the Gram-positive bacteria (5 species) and very few species had interesting activities against the Gram-negative bacteria (2 species) and fungi (1 species).

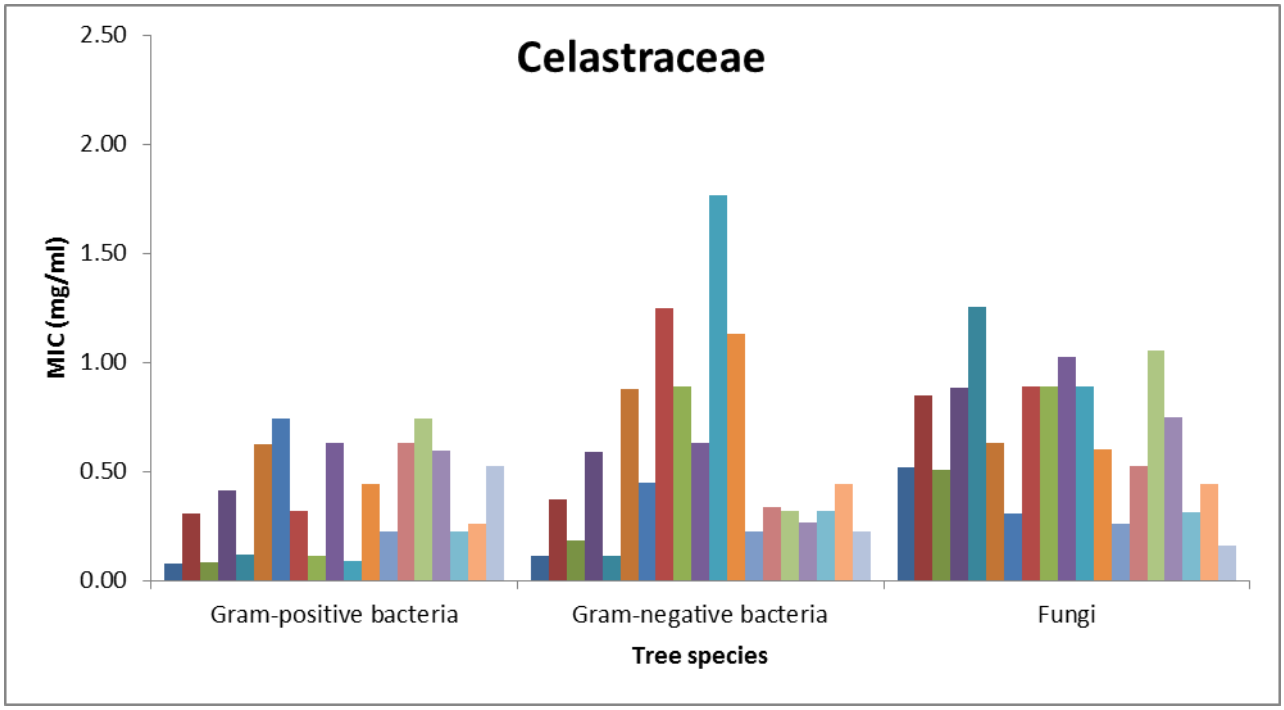


**Fig. 9.6:** The mean MIC values (mg/ml) of the tree species within Proteaceae against the Gram-positive bacteria, Gram-negative bacteria and the fungal organisms (the same colour bar represents the same species).

### 9.3.2 Intra-order variation in antimicrobial activities

Similar to the variation of the antimicrobial activities of species within families, the range of antimicrobial activities of families in the same order also varied considerably. The orders Rosales, Myrtales, Malpighiales, Malvales and Gentianales were selected to compare the mean antimicrobial activities of the families within an order.

As shown in Figure 9.7, the mean MIC's of the Rosales families varied from 0.14 mg/ml to 0.89 mg/ml (Gram-positive bacteria), from 0.27 to 0.77 mg/ml (Gram-negative bacteria) and from 0.25 mg/ml to 0.89 mg/ml (fungi). The order Rosales is composed of six southern African tree families of which Moraceae, Rhamnaceae and Rosaceae were the core families in the southern African region.

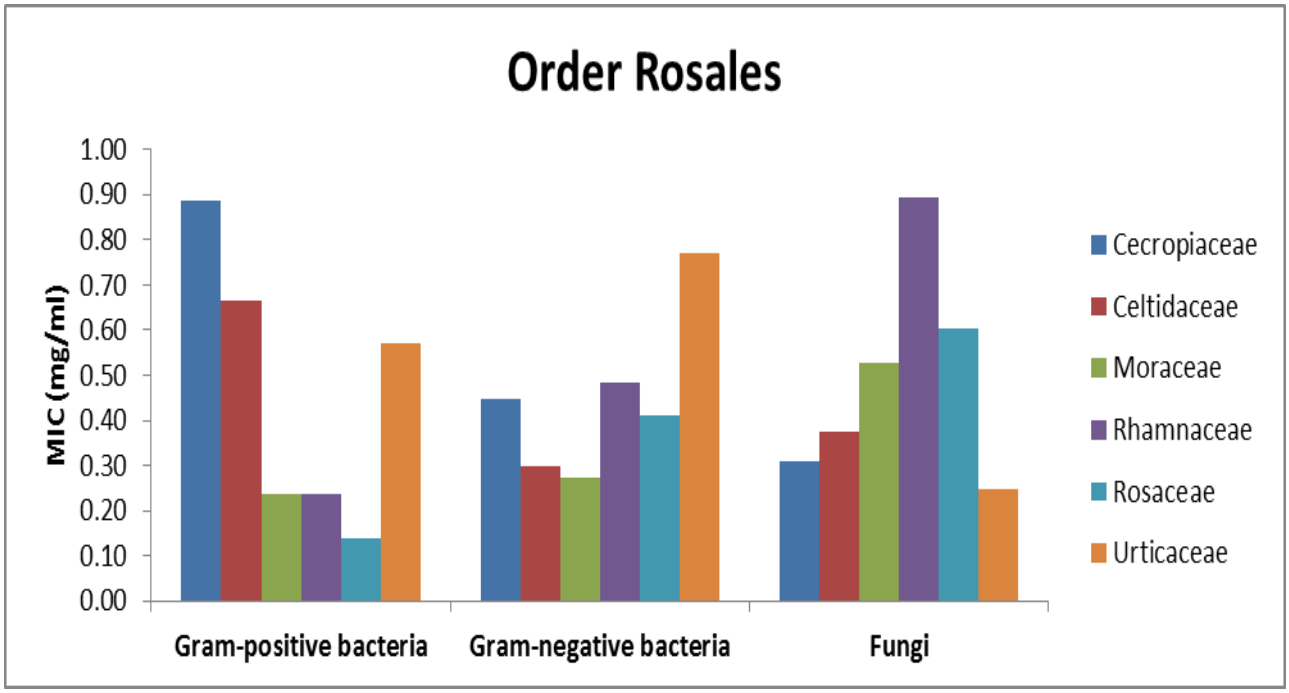


**Fig. 9.7:** The mean MIC values (mg/ml) of the families of the order Rosales against the Gram-negative bacteria, Gram-negative bacteria and the fungal organisms.

In the order Myrtales, the families Combretaceae and Myrtaceae contains most of the species diversity of the order in the southern African region. Other families, for example Heteropyxidaceae, Lythraceae, Melastomataceae, Oliniaceae, Rhynchocalycaceae and Sonneratiaceae, have a comparatively smaller proportion of the species diversity of the order. The mean MIC's of the families within Myrtales, excluding Rhynchocalycaceae and Sonneratiaceae, of which no samples were collected, are shown in Figure 9.8. Their mean MIC's ranged from 0.22 mg/ml to 0.63 mg/ml (Gram-positive bacteria), from 0.16 mg/ml to 0.44 mg/ml (Gram-negative bacteria) and from 0.18 mg/ml to 2.50 mg/ml (fungi). Myrtales had the highest mean activity in comparison with all other orders against Gram-positive bacteria and Gram-negative bacteria, but was less active against the fungi.

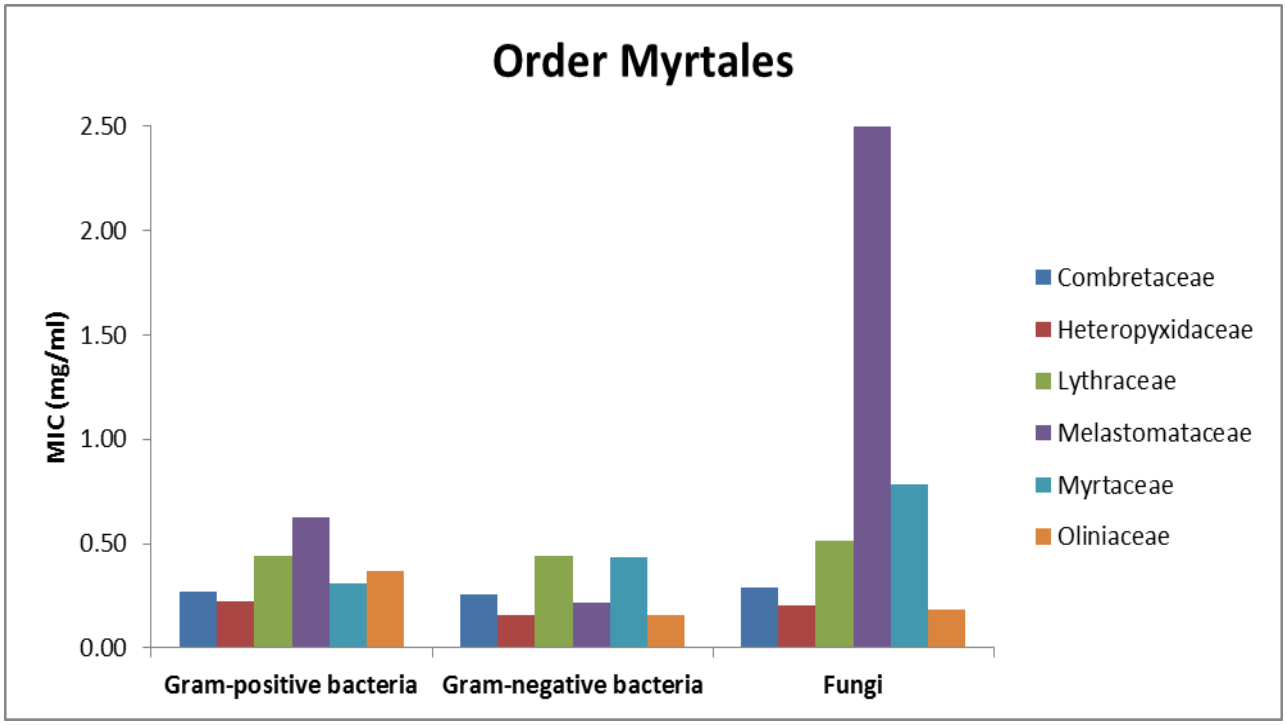


**Fig. 9.8:** The mean MIC values (mg/ml) of the families of the order Myrtales against the Gram-negative bacteria, Gram-negative bacteria and the fungal organisms.

Relatively large differences were found between the activities of the families of Malpighiales (Figure 9.9). The order Malpighiales is one of the largest orders and very diverse enclosing 19 southern African tree families. Members of 15 of those families were analysed. Some of the well-known families included Euphorbiaceae, Flacourtiaceae, Kiggelariaceae, Ochnaceae and Phyllanthaceae. The mean MIC's of the families within the order ranged from 0.08 to 1.77 mg/ml (Gram-positive bacteria) and from 0.24 to 1.77 mg/ml (Gram-negative bacteria). The variation was smaller against the fungi, with mean MIC's ranging from 0.07 to 0.88 mg/ml.

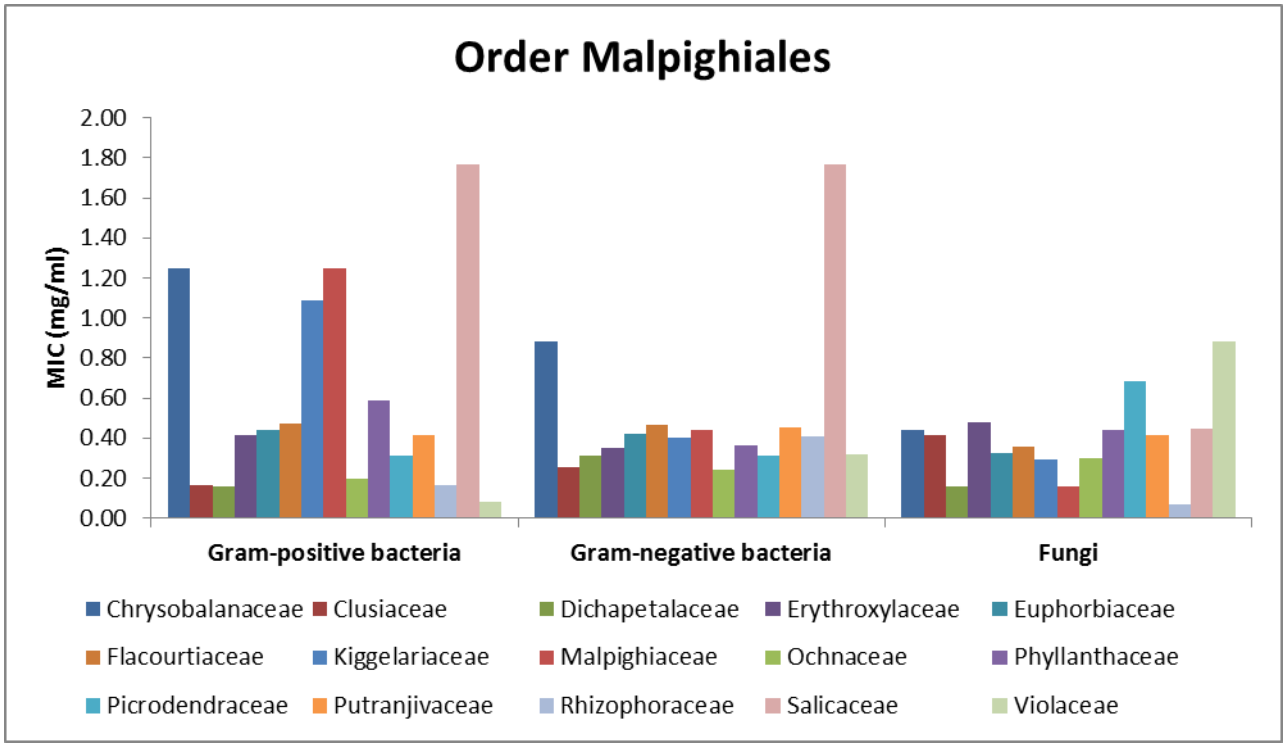


**Fig. 9.9:** The mean MIC values (mg/ml) of the families of the order Malpighiales against the Gram-negative bacteria, Gram-negative bacteria and the fungal organisms.

The boundaries of the families of the order Malvales, the fourth order chosen for the comparative analyses, are problematic and contain many taxonomically difficult groups. Several classification systems and in particular the most recent APG III expanded the Malvaceae family to include the families Bombacaceae, Sterculiaceae and Tiliaceae. However, in this study we retained Bombacaceae and Sterculiaceae as separate families and grouped some members of the order, formerly classified under Tiliaceae, into the family Sparrmanniaceae (Van Wyk *et al.,* 2011). In addition, Helicteraceae and Pentapetaceae are each treated as a family and are not grouped within Sterculiaceae. The mean MIC values of the nine tree families in the order Malvales are shown in Figure 9.10. All the families had MIC's ranging between 0.16 mg/ml and 0.58 mg/ml, with the exception of the family Helicteraceae against the Gram-positive bacteria. In the order analysis (Chapter 8), Malvales had high mean activities against both the fungi and Gram-negative bacteria but was less promising against the Gram-positive bacteria.

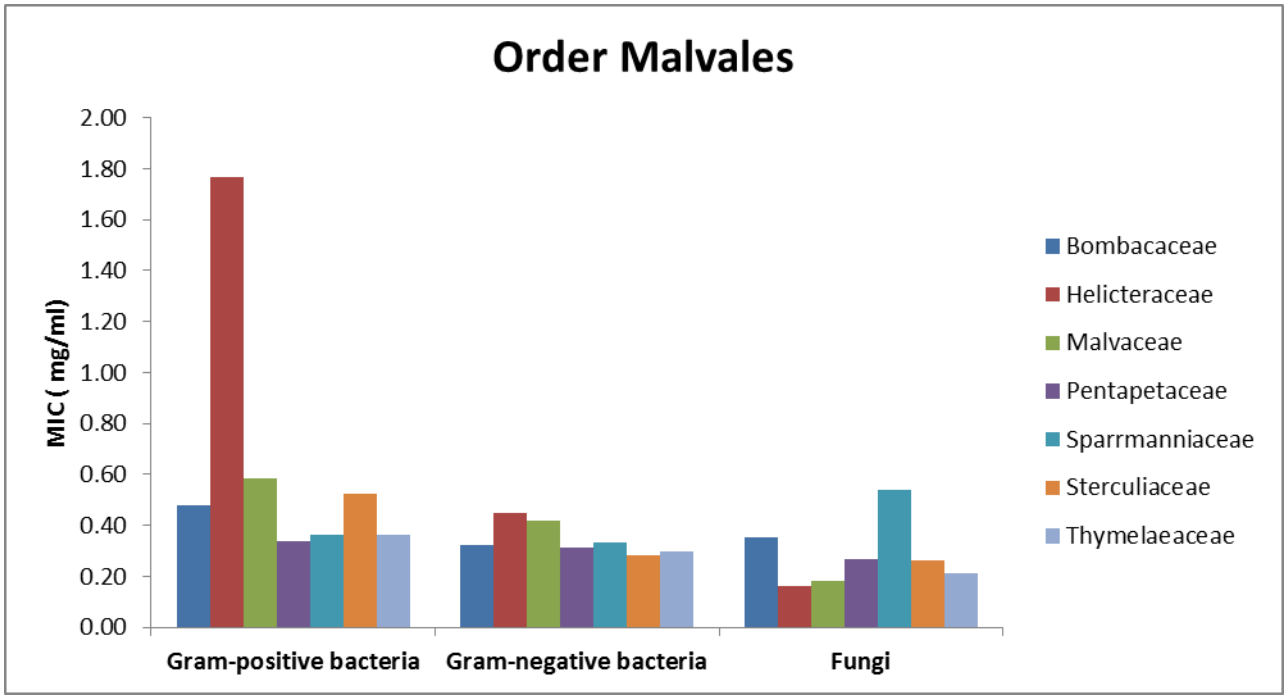


**Fig. 9.10:** The mean MIC values (mg/ml) of the families of the order Malvales against the Gram-negative bacteria, Gram-negative bacteria and the fungal organisms.

The families within the order Gentianales displayed a diverse range of MIC's (Figure 9.11). In the current study we recognised five families: Apocynaceae, Asclepiadaceae (not represented), Gentianaceae, Rubiaceae and Strychnaceae, of which Rubiaceae is the second largest tree family in southern Africa. The family Strychnaceae was accepted as a separate family in this study although it is placed under Loganiaceae by the APG III system. In the order analysis (Chapter 8), Gentianales had relatively low mean MIC's (high activities) against all three pathogen groups. The MIC's of the families within the order varied from 0.26 to 0.73 mg/ml (Gram-positive bacteria), from 0.24 to 0.53 mg/ml (Gram-negative bacteria) and from 0.22 to 0.72 mg/ml (fungi). Alkaloids are found in several of the families contained in Gentianales and a large number of its species are poisonous and some are used medicinally (Van Wyk and Van Wyk, 1997).

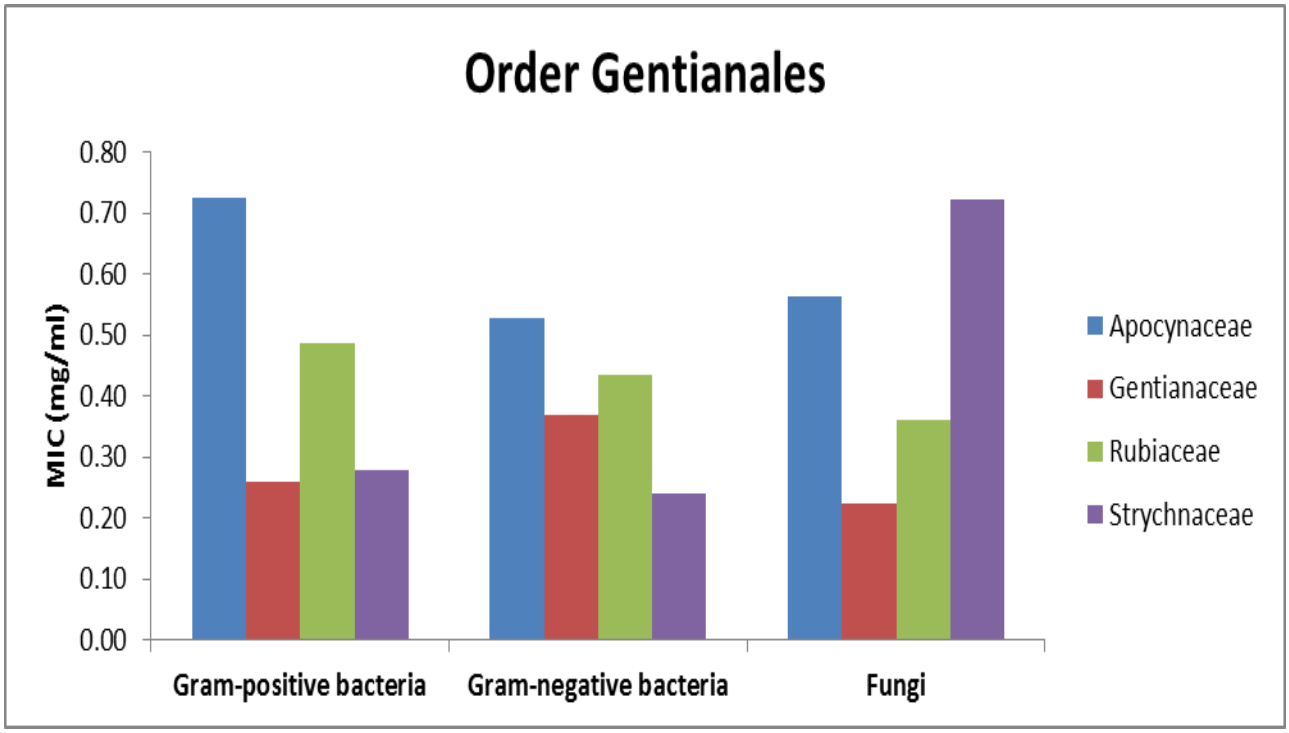


**Fig. 9.11:** The mean MIC values (mg/ml) of the families of the order Gentianales against the Gram-negative bacteria, Gram-negative bacteria and the fungal organisms.

### 9.3.3 Comparison of the antimicrobial activities of tree species within families with the highest and lowest mean antimicrobial activities

The results against each of the different pathogen classes were compared independently.

#### 9.3.3.1 Gram-positive bacteria

The antimicrobial activities (MIC in mg/ml) against Gram-positive bacteria of the families in Group 4 with the five highest activities are matched with the families with the five lowest activities in Figures 12 (a) and (b). The five most promising large families, based on their low mean MIC's against Gram-positive bacteria, were Anacardiaceae, Moraceae, Combretaceae, Celastraceae and Fabaceae and the five families with the lowest activities against Gram-positive bacteria were Rutaceae, Asteraceae, Apocynaceae, Bignoniaceae and Annonaceae. Among the most promising families, almost 32% of the species inhibited Gram-positive bacteria at concentrations of 0.16 mg/ml and lower, which was considered interesting activities. To the contrary, among the five least promising families, only 4% of the species had interesting activities of 0.16 mg/ml and lower. None of the species in the families with the five lowest activities against the Gram-positive bacteria had high activities (MIC's ≤ 0.08 mg/ml) compared to 11% of species in the families with the five highest mean activities. In the five most promising families, only 13% of the species had very low activities with MIC's of 1.25 mg/ml and higher

while at least 62% of the species in the least promising families had very low activities (MIC's ≥ 1.25 mg/ml).

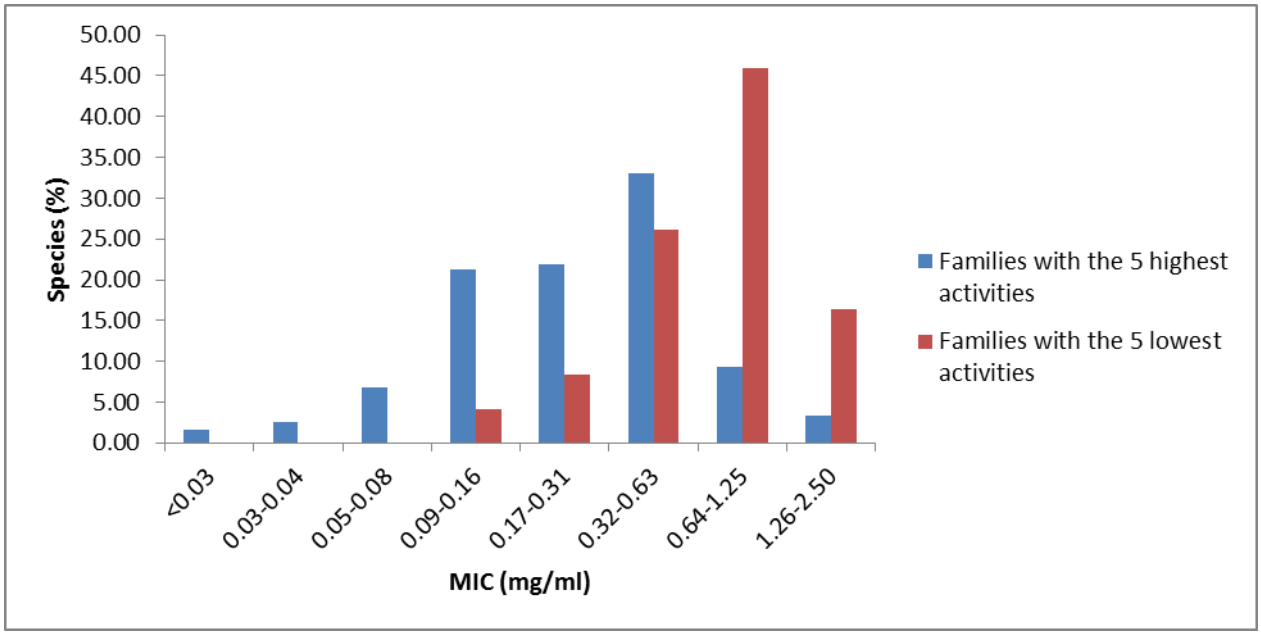


**Fig. 9.12(a):** The percentage of tree species inhibiting Gram-positive bacteria across a range of MIC categories for comparison between families with the five highest and five lowest mean activities.

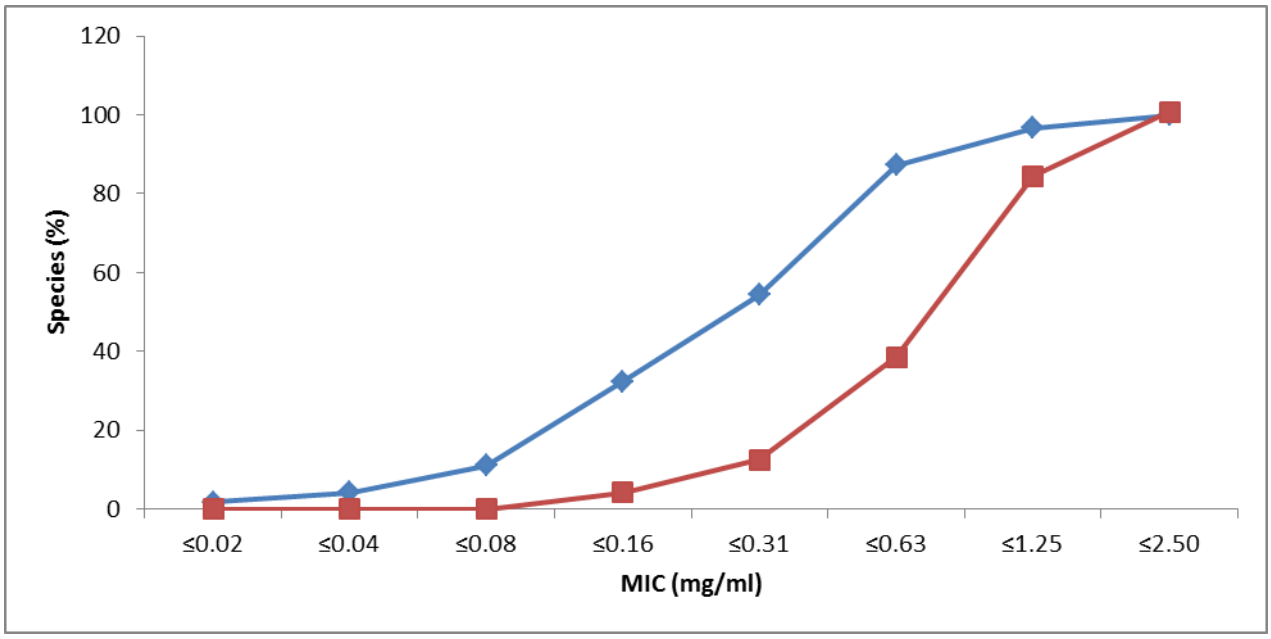


**Fig. 9.12(b):** The percentage of tree species inhibiting Gram-positive bacteria at MIC cut-off values for comparison between families with the five highest (top) and five lowest (bottom) mean activities.

### 9.3.3.2 Gram-negative bacteria

The antimicrobial activities (MIC in mg/ml) of the families in Group 4 with the five highest activities were compared to the five families with the lowest activities against Gram-negative bacteria in Figures 9.13(a) and (b). The five most promising families were Combretaceae, Anacardiaceae, Moraceae, Fabaceae and Sparrmanniaceae while the five families with the lowest activities were Asteraceae, Apocynaceae, Meliaceae, Proteaceae and Rutaceae. Amongst the families with the highest mean activities, 24% had interesting activities based on the inhibition of Gram-negative bacteria at MIC's of 0.16 mg/ml and lower. Among these promising species, 4% had high activities of 0.08 mg/ml. Conversely, among the least promising families, far fewer species (6%) had interesting activities with MIC's of 0.16 mg/ml and lower. Only a small percentage (10%) of the species in these promising families had very low activities (MIC's ≥ 1.25 mg/ml) compared to 34% in the least promising families.

The results disclosed that species within the most and least promising families, had activities over a large range of MIC's. However, the majority of species within the most promising families had overall higher activities. None of the species in the least promising families had high activities (MIC's ≤ 0.08 mg/ml).

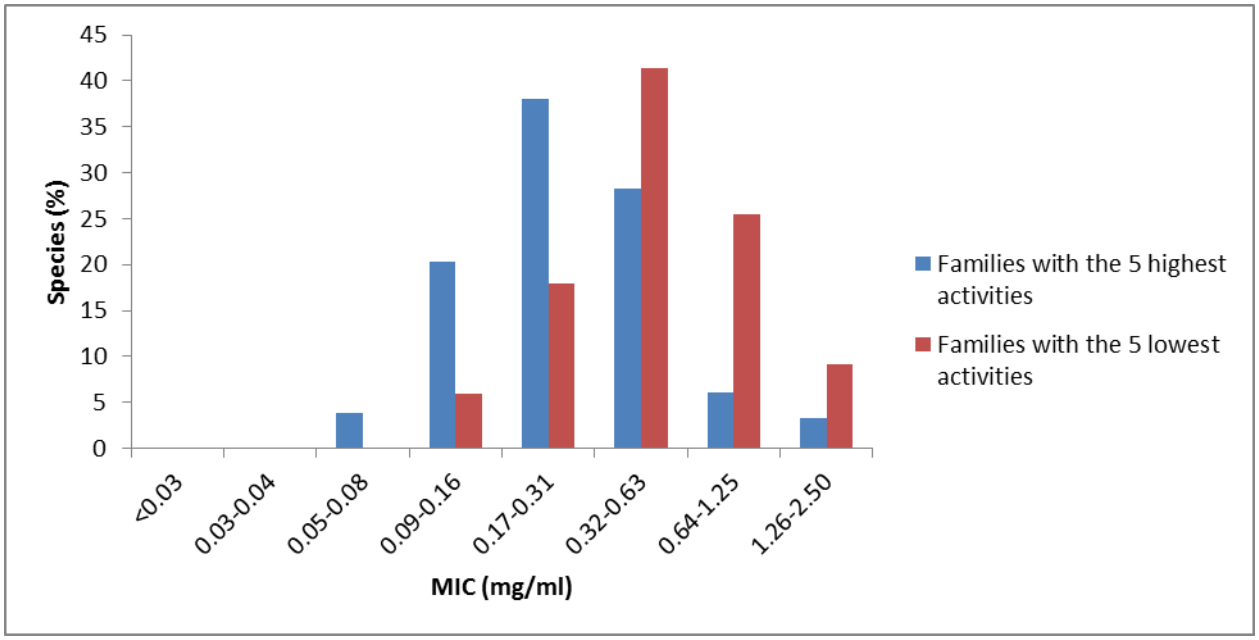


**Fig. 9.13(a):** The percentage of tree species inhibiting Gram-negative bacteria across a range of MIC categories for comparison between families with the five highest and five lowest mean activities.

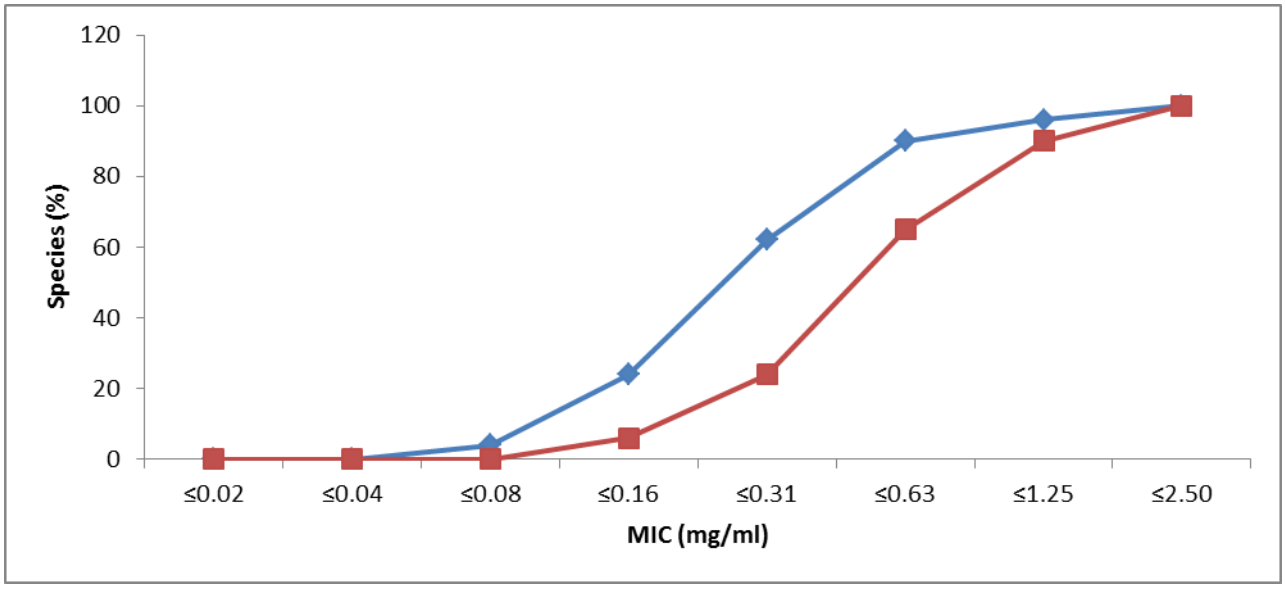


**Fig. 9.13(b):** The percentage of tree species inhibiting Gram-negative bacteria at MIC cut-off values for comparison between families with the five highest (top) and five lowest (bottom) mean activities.

### 9.3.3.3 Fungal organisms

In Figures 9.14 (a) and (b), we compared the antimicrobial activities of the families in Group 4 with the five highest (Malvaceae, Meliaceae, Combretaceae, Euphorbiaceae and Proteaceae) and five lowest (Sapindaceae, Apocynaceae, Sapotaceae, Celastraceae and Rhamnaceae) mean activities against the fungi. Among the families with the highest activities, 30% of the species had activities considered as interesting against the fungi (MIC's ≤ 0.16 mg/ml). Among the families with the least promising activities, less species (8%) inhibited the fungi at MIC's of 0.16 mg/ml (interesting activities). Only 16% of the species in the promising families had very low activities (MIC's ≥ 1.25 mg/ml) compared to 45% of the species in the least promising families.

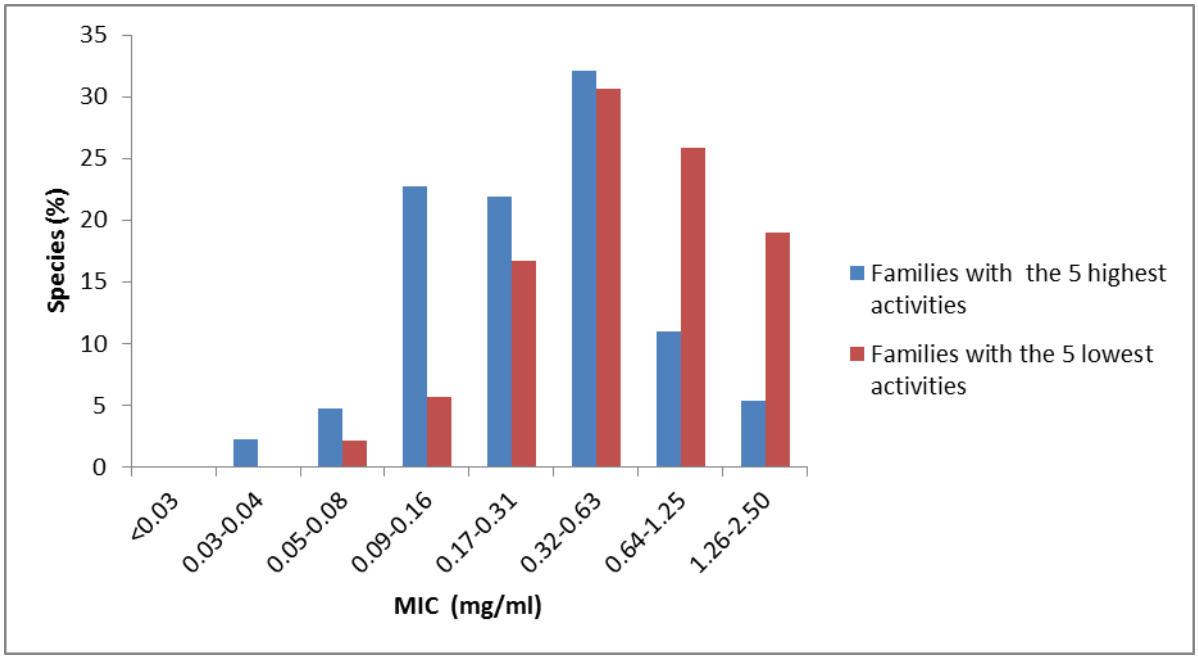


**Fig. 9.14(a):** The percentage of tree species inhibiting fungi across a range of MIC categories for comparison between families with the five highest and five lowest mean activities.

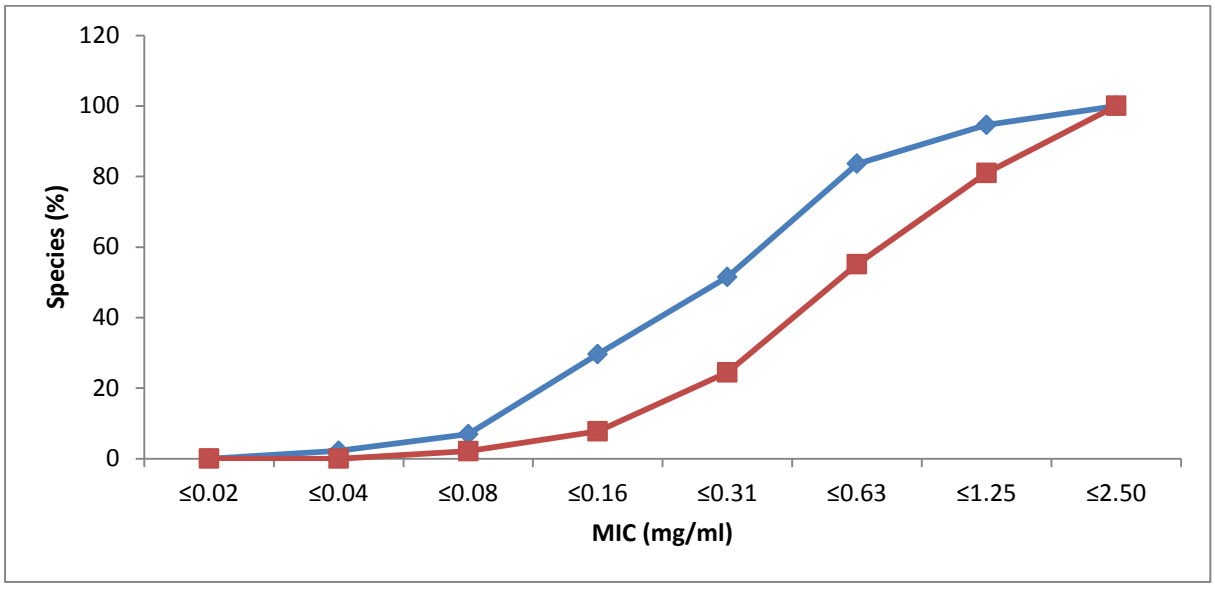


**Fig. 9.14(b):** The percentage of tree species inhibiting fungi at MIC cut-off values for comparison between families with the five highest (top) and five lowest (bottom) mean activities.

### 9.3.4 Comparison of the antimicrobial activities of tree species within orders with the highest and lowest mean antibacterial activities

The results of the orders against each of the different pathogen classes were compared independently.

#### 9.3.4.1 Gram-positive bacteria

The antimicrobial activities (MIC in mg/ml) against Gram-positive bacteria of the orders in Group 3 with the four highest activities are compared with the orders with the four lowest mean antimicrobial activities in Figures 9.15 (a) and (b). The four most promising large orders were Celastrales, Rosales, Myrtales and Fabales and the four orders with the lowest mean activities were Asterales, Magnoliales, Gentianales and Lamiales. Similar trends in variation were noticed among the orders compared to that of the families.

Among the most promising orders, 24% of the species had interesting activities with mean MIC's of 0.16 mg/ml and lower compared to 7% of the species in the orders with the lowest mean activities. None of the orders with the least promising activities had species with very active MIC's (MIC's ≤ 0.04). To the contrary, among the most promising orders, fewer species had very low activities (MIC's ≥ 1.25 mg/ml) compared to the least promising orders.

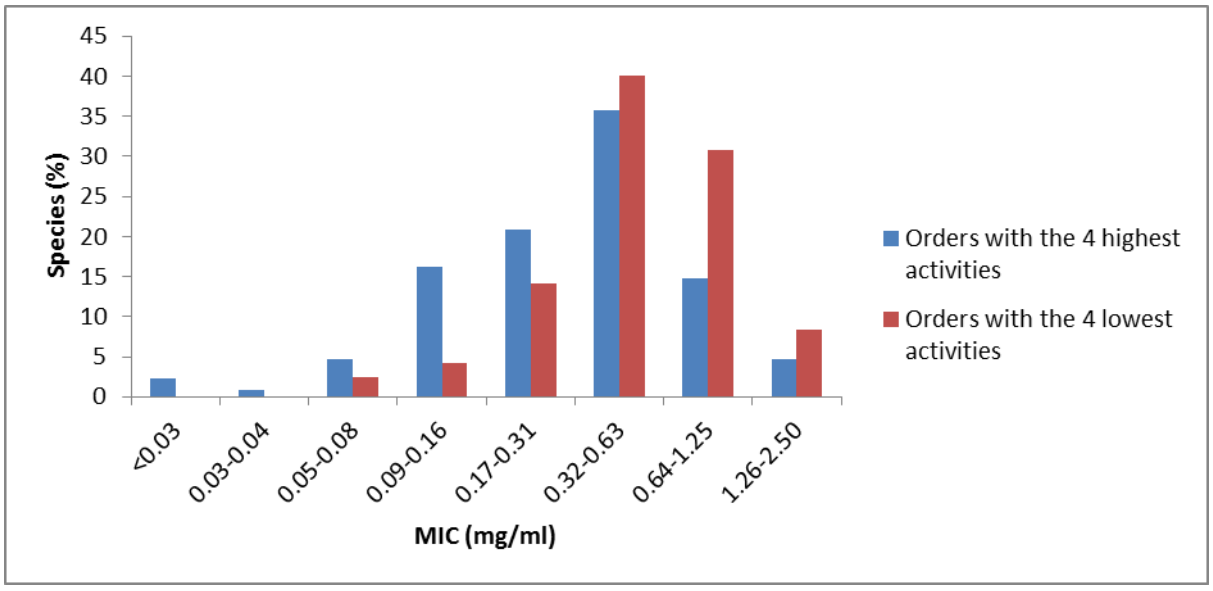


**Fig. 9.15(a):** The percentage of tree species inhibiting Gram-positive bacteria across a range of MIC categories for comparison between orders with the four highest and four lowest mean activities.

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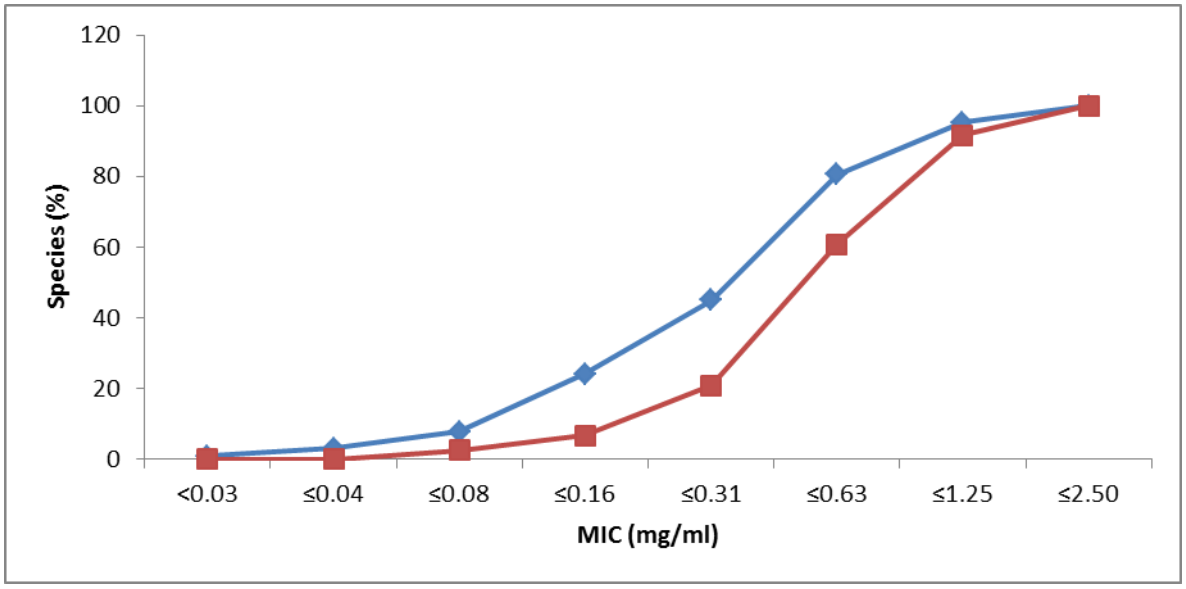


**Fig. 9.15(b):** The percentage of tree species inhibiting Gram-positive bacteria at MIC cut-off values for comparison between families with the four highest (top) and four lowest (bottom) mean activities.

### 9.3.4.2 Gram-negative bacteria

In Figures 9.16(a) and (b), the antibacterial activities of the species in the four most promising orders (Myrtales, Fabales, Malvales and Ericales) were compared with the antibacterial activities of the species in the four orders with the lowest mean activities against Gram-negative bacteria (Proteales, Asterales, Magnoliales and Lamiales). Amongst the orders with the highest mean activities against Gram-negative bacteria, 24% of the species had interesting activities (MIC's ≤ 0.16 mg/ml) and 17% of the species had very low activities (MIC's ≥ 1.25 mg/ml). In comparison, among the orders with the lowest mean activities, 5% of the species had MIC's of 0.16 mg/ml and lower (interesting activities) and 27% of the species had MIC of 1.25 and higher (very low activities). None of the species in the orders with the least promising activities had high activities (MIC's ≤ 0.08 mg/ml).

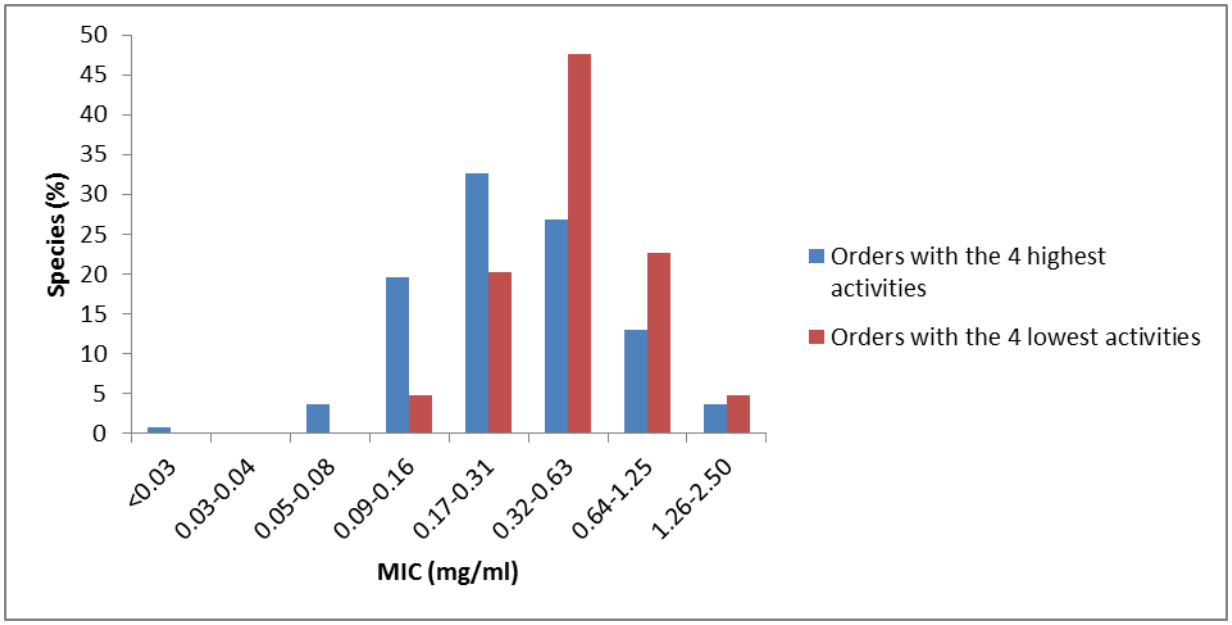


**Fig. 9.16(a):** The percentage of tree species inhibiting Gram-negative bacteria across a range of MIC categories for comparison between orders with the four highest and four lowest mean activities.

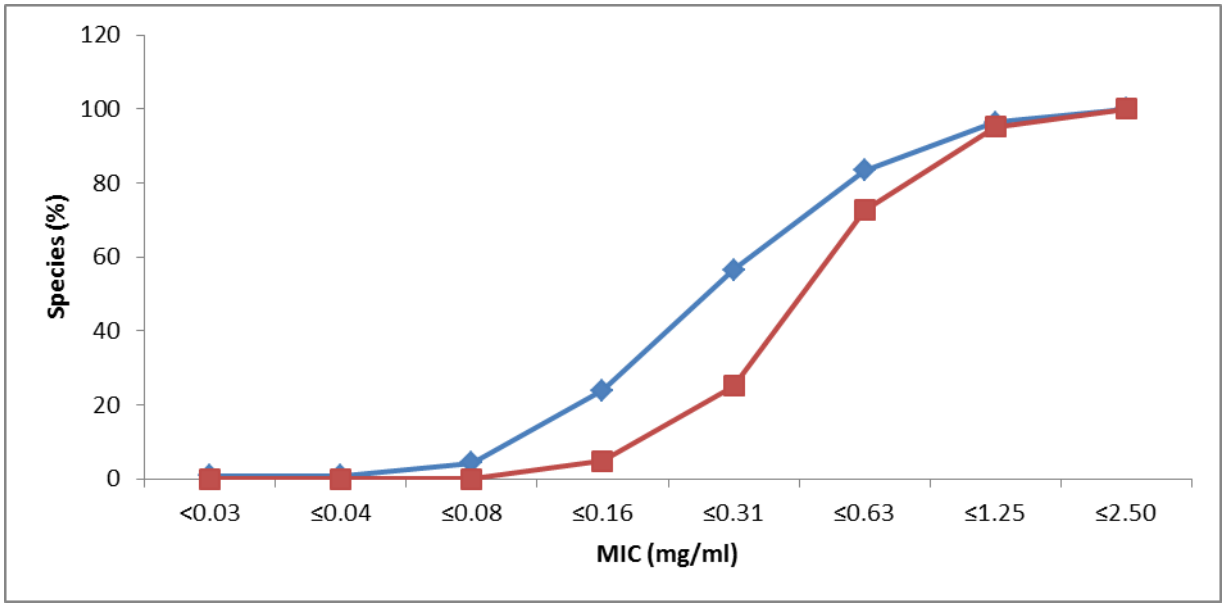


**Fig. 9.16(b):** The percentage of tree species inhibiting Gram-negative bacteria at MIC cut-off values for comparison between families with the four highest (top) and four lowest (bottom) mean activities.

### 9.3.4.3 Fungal organisms

The antifungal activities of the orders in Group 3 with the four highest (Malvales, Proteales, Fabales and Apiales) and four lowest (Celastrales, Rosales, Asterales and Ericales) activities were compared in Figures 9.17(a) and (b). Among the orders with the highest activities, 23% of the species had interesting activities with MIC's of 0.16 mg/ml and lower. Of these, 6% of the species had high activities (MIC's ≤ 0.08 mg/ml) against the fungi. In contrast, the orders with the least promising activities had 11% species with interesting activities (MIC's ≤ 0.16 mg/ml) of which 2% had high activities (MIC's ≤ 0.08 mg/ml). In the case of the orders with promising activities, only 16% of the species had very low activities (MIC's ≥ 1.25 mg/ml) compared to 39% of the species in the least promising orders.

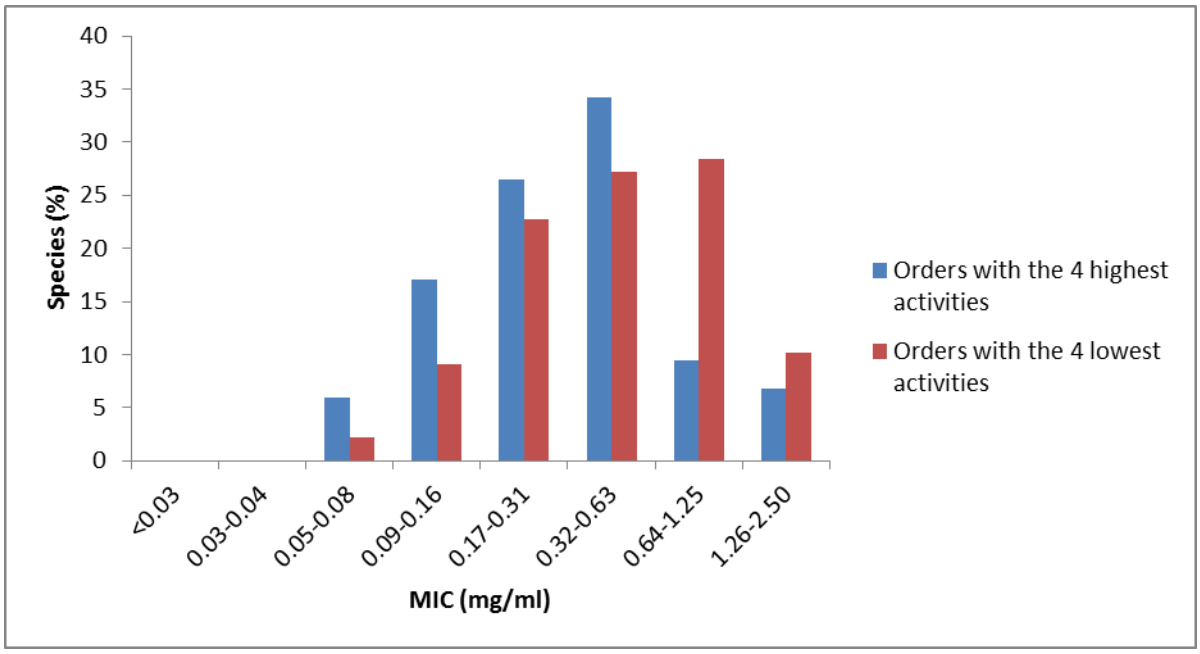


**Fig. 9.17(a):** The percentage of tree species inhibiting fungi across a range of MIC categories for comparison between orders with the four highest and four lowest mean activities.

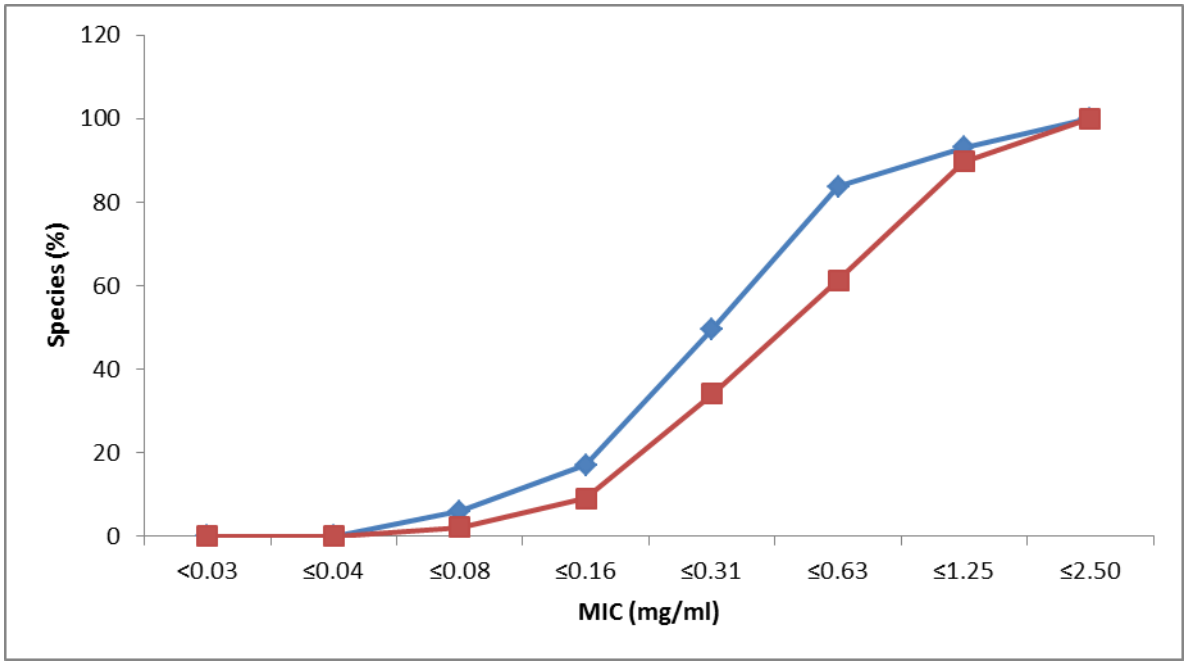


**Fig. 9.17(b):** The percentage of tree species inhibiting fungi at MIC cut-off values for comparison between families with the four highest (top) and four lowest (bottom) mean activities.

## 9.4 Conclusions

The study established that there is substantial variation in antimicrobial activities of closely related species within families and between closely related families within orders. It was also established that large variations occurred between the antimicrobial activities of closely related families within orders. It appears that there is a normal distribution of activity within families/orders (Figures 9.12(a), 9.13(a), 9.14(a), 9.15(a), 9.16(a) and 9.17(a)), but that the means of families/orders are close and that there is substantial intersection across all families and all orders.

These large variations in antimicrobial activities were not restricted to comparisons at family and order levels. We also found large differences between species within the same genus. To illustrate, seven *Protea* species inhibited the six pathogens at MIC's ranging from 0.16 mg/ml to 2.50 mg/ml while eleven *Leucadendron* species had MIC's ranging between 0.04 and 2.50 mg/ml. A previous study also found a large variation in the antibacterial activity and chemistry of antibacterial compounds among the different genera and species in the Combretaceae (Eloff, 1999).

These differences may be ascribed to inconsistent presence of antimicrobial secondary metabolites produced by the species of the same genus. A thorough study of the genera may

indicate differences in activity between subgeneric designations. This situation occurred within the subgeneric classification of generic species (Eloff *et al*., 2008).

The variation within the families and orders may be an indication of the large diversity of secondary metabolites in plants, even in closely related species. In a family such as Fabaceae, several types of secondary metabolites including alkaloids, flavonoids, isoflavonoids, coumarins, non-protein amino acids, amines, phenylpropanoids, anthraquinones, di-sesqui-and triterpenes, cyanogenic glycosides, protease inhibitors and lectins have been described (Wink and Mohammed, 2003). The astounding diversity of secondary metabolites in plants may be an indication that secondary metabolites may have originated early in the evolution of plants (Theis and Lerdau, 2003). These compounds may change between different development stages and environmental conditions such as pathogen pressure, daily and seasonal changes and soil structure (Benli *et al.,* 2007). The bioactivity of plants may also be influenced by genotype (Verpoorte, 1998).

Among the five most promising families and the families with the five least promising activities, a number of species had interesting activities (≤ 0.16 mg/ml) while others had very low mean activities (≥ 1.25 mg/ml). The results indicated that potential valuable species (≤ 0.16 mg/ml) were found in both groups, although fewer were found in the less promising families. Similar results were found when we compared the antimicrobial activities of the orders. Consequently, if future screening research projects evaluate only the most promising families, these families will most likely include a number of less promising species. On the other hand, the least promising families also contained species with relatively high activities (≤ 0.16 mg/ml), albeit a lower percentage. Therefore, prospective species from less promising families may be overlooked should future research projects only focus on the most promising families.

The high variability confounds the identification of superior plant orders or families against a given pathogen class because the taxa contain species with varying degrees of antimicrobial activity. Taking the high intra-taxa variation into consideration, it may be difficult to predict with reasonable confidence the likelihood that species will exhibit promising activities on the basis of their relatedness. Nevertheless some families and orders did show a statistically significant ($P < 0.05$) higher or lower activity.

# CHAPTER 10

# General conclusions

## 10.1 Introduction

The main aim of this study was to facilitate the discovery of plant extracts with high activities that may yield products that can be used to combat microbial infections in animals and humans. To contribute towards this aim a number of objectives were formulated. Results obtained with these objectives are presented under different headings below.

## 10.2 Screen several hundred tree species leaf extracts for antimicrobial activity against six important pathogens

We have screened approximately 717 extracts from tree leaves for their ability to inhibit selected pathogens. The panel of test organisms included two Gram-positive bacteria, two Gram-negative bacteria and two fungi. Our wide-screening has led to the identification of several tree species with interesting to very high antimicrobial activities. Several of these promising species have already been subjected to further studies in the Phytomedicine Programme of the University of Pretoria and have led to highly cited papers and to patents. These follow-up studies corroborate the value of our wide-screening approach and the accuracy of the techniques applied.

## 10.3 Determine a standard to establish at what concentration an extract may be considered to have significant antimicrobial activity based on the antimicrobial activities of a large number of tree leaf extracts

Several authors have designated plant extracts to be active at such high concentrations that the results are meaningless in pharmacological terms (Ríos and Recio, 2005; Cos *et al*., 2006). Many authors have used a thumb suck to propose an MIC of 0.1 mg/ml as a reasonable measure of antimicrobial activity (Eloff, 2004; Ríos and Recio, 2005; Cos *et al*., 2006; Gertsch, 2009). Therefore, the objective was to determine a standard MIC based on more than 4 000 assays against six different microbes. We found that on average 13% of all extracts were active against the six pathogens at an MIC of 0.1 mg/ml. The concentrations at which 10 and 5% of extracts were showing activity against all six pathogens was 0.084 and 0.056 mg/ml respectively. At an MIC of 0.1 mg/ml and lower one out of eight plants would be considered active. A value of 0.08 mg/ml would identify about one out of 10 plants examined and may be a better guideline.

Individual pathogens also had different sensitivities towards plant extracts and adjusted MIC's could be considered for individual pathogens. We therefore recommend that the benchmark for each pathogen should be adjusted according to its general sensitivity.

### 10.4 Identify tree species and genera with high antibacterial and/or antifungal activity against six important pathogens

The objective was to identify tree species with promising antimicrobial activities. Future studies on these species and examining different populations may lead to the development of effective extracts to protect humans or animals against infections. Related species within the same genus may also be investigated since it is well known that related plant species contain similar chemical compounds and therefore may have comparable biological activities. Selection of species for analyses which is guided by knowledge of promising closely related species should rapidly expand the pool of promising tree species.

An extensive range of trees belonging to a wide variety of plant families had interesting antimicrobial activities. We considered extracts with an MIC of 0.16 mg/ml and lower as interesting activity, an MIC of 0.08 mg/ml and lower as high activity and an MIC of 0.04 mg/ml and lower as very high antimicrobial activity. Promising species in this study were short-listed based on a number of criteria. Some of the tree genera contained a substantial number of promising species against each of the pathogens. The study found that extracts of *Acacia sieberiana, Bowkeria citrina, Curtisia dentata, Dodonaea viscosa, Hypericum roeperianum, Macaranga mellifera, Smodingium argutum, Terminalia phanerophlebia* and *Loxostylis alata* had interesting activities (≤ 0.16 mg/ml) against all six pathogens. Several of these extracts had very high activities with MIC's of 0.04 mg/ml and lower.

Extracts of some of the tree species with interesting activities inhibited the growth of several test pathogens which may be indicative of broad spectrum of antimicrobials. Some of the other tree species were more effective against certain pathogens or pathogen classes (bacteria or fungi or Gram-positive bacteria or Gram-negative bacteria). Those species may have potential for the discovery of selective antimicrobial compounds.

The results showed that besides those species of which their traditional use have been published already, there are also several other tree species offering interesting antimicrobial activity. The additional tree species that we have identified could be important sources of antimicrobial extracts, underlining the potential of southern African tree extracts as sources of antimicrobials to be used in the treatment of bacterial and fungal infections. The study provided a good platform for future research projects. Future studies should include cytotoxicity and

animal toxicity studies of promising extracts at an early stage to determine the safety and to establish if antimicrobial activity is not related to the presence of a general metabolic toxin.

### 10.5 Evaluate the overall susceptibility of the six different pathogens to acetone leaf extracts of several hundred southern African tree species

This study set out to evaluate the susceptibility of the six pathogens used in the study to the inhibitory effect of the tree leaf extracts. There is an urgent need to find new drugs, especially against fungal pathogens and Gram-negative bacteria, mainly due to their increase in resistance to antibiotics. Firstly, the mean MIC values (whole spectrum of inhibition from low to high MIC values) of all the extracts against each of the pathogens were compared. Small and mostly insignificant differences were found. *E. faecalis* was the most sensitive bacterium while *C. neoformans* was the most sensitive fungal pathogen. Only the mean MIC value of the extracts against E*. faecalis* was statistically significant higher compared to the mean MIC value against both *S. aureus* and *C. albicans*. Of all the pathogens, *S. aureus* was the least sensitive. This is in contradiction with most literature reports in which Gram-positive bacteria such as *S. aureus* are found to be more sensitive.

We compared the susceptibility of the different organisms to the extracts at different MIC cut-off points. At an MIC of 0.16 mg/ml, *C. neoformans, C. albicans* and *E. faecalis* were sensitive to the largest number of extracts. At MICs between 0.02 and 0.04 mg/ml, more extracts inhibited *E. faecalis, C. neoformans* and *S. aureus* compared to *P. aeruginosa, C. albicans* and *E. coli*.

### 10.6 Determine if there are correlations between the activities of tree leaf extracts against different pathogens

It would be very useful if, based on the activity of an extract against one pathogen, one could predict the activity against other pathogens. We therefore determined the correlation between the activities of extracts against different pathogens based on the MIC values of 717 crude tree extracts against the six pathogens. The best correlation was between the two fungi, *C. albicans* and *C. neoformans* (r = 0.49), followed by the correlation between the two Gram negative bacteria, *E. coli* and *P. aeruginosa* (r = 0.45). The third best correlation was between the two Gram positive bacteria *E. faecalis* and *S. aureus* (r = 0.42).

## 10.7 Identify tree families with best likelihoods of delivering extracts with high antimicrobial activity against Gram-positive bacteria, Gram-negative bacteria and fungi

We examined whether antimicrobial activity is associated with plant taxonomy. The antimicrobial activities of the tree species were compared firstly at suprageneric (family) level. If good correlations in antimicrobial activity are found between related taxa, it could provide a lead in selecting and screening related tree species from promising families for continuing studies. This is based on the assumption that closely related species contain similar active biochemical substances and therefore similar activities.

For this investigation, the species were grouped into their respective families. A large proportion of families consisted of only three or fewer genera and since these statistical analyses required a larger sample size, only the activities of larger families ($n \geq 9$) were analysed statistically.

Among the larger families, the differences between the mean MIC values of the families seemed generally small, but statistical analyses demonstrated that certain families have significant higher activities compared to some other families. The larger families yielding most active extracts against Gram-positive bacteria were Anacardiaceae, Moraceae, Combretaceae and Celastraceae. Tree leaf extracts from Combretaceae, Anacardiaceae, Moraceae and Fabaceae families had the highest activities against Gram-negative bacteria. The families Meliaceae, Combretaceae, Euphorbiaceae and Proteaceae extracts had the highest antifungal activities. Although the current study is based on a small sample of species, the results suggest that these families may provide good leads in selecting tree species. Further screening of related taxa in these families may yield promising results.

We also found that extracts from some families had relatively high activities against more than one class of pathogen. In particular, the family Combretaceae had high antimicrobial activities against all pathogen classes and the family Anacardiaceae had stronger antibacterial activities (high activities against both Gram-positive and Gram-negative bacteria). This may be an indication that members of these families have a broad spectrum of antimicrobial activity and may provide good leads. However, the wide level of activity may have been caused by a general metabolic toxin that could be harmful to the host cells as well.

It may be more valuable to identify families with more selective activity against a specific pathogen class such as Celastraceae (Gram-positive bacteria), Phyllanthaceae (Gram-negative bacteria), Proteaceae (fungi) and Meliaceae (fungi). Extracts of the species in these families may be more selective in their activity by attacking a more restricted metabolic pathway possibly leading to a lower toxicity to the host cells. Future screening programmes directed at

plants with either antibacterial or antifungal activities should consider screening members of these families.

The results also indicated that extracts from some families were significantly less active than others. The least effective families against Gram-positive bacteria were Rutaceae, Asteraceae and Apocynaceae. The family Rutaceae also had significantly lower activities against Gram-negative bacteria and the families Celastraceae and Sapotaceae had the lowest mean antifungal activities. These families may have fewer species with high activities, and based on the assumption that plants in related taxa often have similar activities, these should not be prioritised in future screening programmes.

### 10.8 Identify tree orders with best likelihoods of delivering extracts with high antimicrobial activity against Gram-positive bacteria, Gram-negative bacteria and fungi

The second taxonomical comparison was based on the activities (mean MIC's) of southern African tree species at the suprafamilial (order) level. Since the boundaries of orders are generally more widely accepted, a comparison at this level may reduce variation caused by modifications in classification systems. Our premise was that because orders are more inclusive compared to families, the mean MIC's of orders should therefore be less affected by outliers.

The analysis of the data enhanced our understanding of the antimicrobial activities of tree orders. The study found that some of the differences in antimicrobial activity among the larger orders ($n \geq 9$) were significant ($p < 0.05$). The orders with the highest Gram-positive antibacterial activities were Celastrales, Rosales, Myrtales and Fabales while the orders with the highest Gram-negative antibacterial activities were Myrtales and Fabales. The orders with the highest antifungal activities were Malvales and Proteales. An order such as Fabales had relatively high activities against all pathogen classes while the order Myrtales had high activities against both Gram-positive and Gram-negative bacteria.

The results also indicated that some of the orders such as Asterales had lower activities against Gram-positive and Gram-negative bacteria while the order Celastrales had a low antifungal activity. Even though members of the order Proteales had high activities against the fungi, they were less promising against the bacteria, especially against the Gram-negative bacteria.

## 10.9 Analyse and interpret intra-taxa variation in the context of a wide screening approach

The study investigated the intra-taxa variation in antimicrobial activity of the tree families and orders analysed in the study. We examined the range of antimicrobial activities within selected families (intra-family) and orders (intra-order) and found large variations between related species within a family and order. It may therefore be difficult to predict with reasonable confidence the likelihood that species within a family or even a genus will exhibit similar activities due to the high intra-taxa variation.

Our results also show that in families with the least promising activities, some tree species still had interesting activities (MIC's ≤ 0.16 mg/ml). However, the less promising families had fewer species with interesting activities than the promising families and *vice versa*. Similar results were found when we compared the antimicrobial activities of the orders. Consequently, if future screening projects evaluate only the most promising families and orders, these families and orders will include a number of less promising species also. On the other hand, extracts of species within the least promising families and orders also contained some species with interesting activities (MIC's ≤ 0.16 mg/ml). The findings imply that prospective species in less promising families may be overlooked should only samples of promising families be considered for future research projects. The high intra-taxa variability influences the identification of superior plant orders or families against a given pathogen class because the taxa may contain species with varying degrees of antimicrobial activity. Nevertheless, some families do have a statistically significant ($p < 0.05$) overall higher or lower mean activity than others.

## 10.10 Interpretation and implications for future research

This study is at present by far the largest to document antimicrobial activities of southern African tree species. The study was a wide-screening research project and therefore limited by a number of factors. It was not possible to sample all the tree species in southern Africa and therefore we sampled species over a wide range of genera, families and orders (i.e. at least one species per genera). However, due to the extent of species found within southern Africa, the number of species analysed per family and order is still relatively small and would therefore be only partially representative of the taxa. Selections may have been too small in some cases to generalise from this study. Our findings need to be interpreted in this light. Despite its exploratory nature, this study offers new and extensive insights about the antimicrobial effectiveness of families and orders of the tree species of southern Africa, a vast and largely understudied field of research.

It may be useful to compare activities of extracts of trees used traditionally to combat infections with our results to compare species selected on a random basis with trees selected on an ethnic use basis. Unfortunately, especially older literature is not necessarily trustworthy because agar diffusion assays were mainly used and quantitative data was frequently not provided. In some cases MIC values as high as 7 mg/ml were considered as active.

The wide-screening established that at an MIC of 0.1 mg/ml, growth of about one in eight plants was inhibited. An even more discerning standard would be an MIC of 0.08 mg/ml at which level one in ten species was inhibited. Because different pathogens have different susceptibilities, 0.1 mg/ml, as suggested by many authors, would be a reasonable and practical standard to determine significant antimicrobial activity in screening procedures. These important results should serve as a basis for a classification system for future antimicrobial activities.

It is interesting that there was a reasonable correlation between activities of the two Gram-negative bacteria, the two Gram-positive bacteria and the two fungi. It may be very interesting to determine if the correlation also holds for other groups of fungi.

Selection of species for future work will depend on the purpose of the intended study and literature searches could assist the selection of species. Other criteria such as the availability of plants, traditional use, presence of antimicrobial tannins in extracts and quantities extracted could also assist the selection of species for further studies. There is scope for more studies to confirm and extend the present results and should include specific cytotoxic studies so that activities related to a general toxicity could be excluded. Further phytochemical and pharmacological studies are required to determine the types of compounds responsible for the antimicrobial activities, mechanisms of action and eventually (but not necessarily) the isolation of bioactive compounds.

This research enhanced our understanding of the efficacy of families and orders and demonstrated that certain taxa do have significant higher or lower activities compared to other taxa. The results suggest that future research should therefore concentrate on the investigation of more tree species in these promising families and orders. This could maximise the number of leads that are found in the screens in a shorter time than random collections. Some of the families with promising activities have indeed been identified before, in particular the family Combretaceae, but the low activities of members of the family Asteraceae were unexpected.

The high intra-taxa variability in antimicrobial activities complicates the identification of superior plant orders or families against a given pathogen class because the taxa may contain species with diverse levels of antimicrobial activity. Nonetheless, by generating and organising relevant information about antimicrobial activities of tree leaf extracts, the results of our study have made

a contribution in our aim to facilitate the discovery of plant extracts with high activities that may yield products that can be used to combat microbial infections in animals and humans.

# Chapter 11

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# APPENDIX A

**Table A.1:** The families, genera and orders of the trees of southern Africa. Families are arranged alphabetically. Family classification of the angiosperm plants followed Van Wyk *et al.* (2011) (Bold = no species were collected in the entire tree genus/family/order; [1] = the genus/family/order is found outside the borders of South Africa; [2] = the family is grouped in a different family under APG III; [3] = non-indigenous genus).

| FAMILY | COMMENTS | ORDER | GENERA |
|---|---|---|---|
| Acanthaceae Juss. | | Lamiales Bromhead | ***Anisotes, Brillantaisia,*** *Duvernoia,* ***Justicia,*** *Mackaya, Metarungia, Sclerochiton* |
| Anacardiaceae R.Br. | | Sapindales Juss. ex Bercht. & J.Presl | *Harpephyllum, Heeria, Lannea,* ***Laurophyllus,*** *Loxostylis, Ozoroa, Protorhus, Searsia, Sclerocarya, Smodingium* |
| Annonaceae Juss. | | Magnoliales Juss. ex Bercht. & J.Presl | *Annona, Artabotrys,* ***Cleistochlamys,*** *Friesodielsia, Hexalobus, Monanthotaxis, Monodora,* ***Sphaerocoryne,*** *Uvaria, Xylopia* |
| Aphloiaceae Takht. | *Aphloia* sp. was originally classified under Flacourtiaceae, order Malpighiales. Now classified under its own family, Aphloiaceae, a family with one species.<br>APG III - under order Crossosomatales, Eurosids II, Eudicots. In APG II, the family was unplaced in rosids clade | Crossosomatales Takht. ex Reveal | *Aphloia* |
| Apiaceae Lindl. | | Apiales Nakai | *Heteromorpha,* ***Polemannia, Polemanniopsis,*** *Steganotaenia* |
| Apocynaceae Juss. | | Gentianales Juss. ex Bercht. & J.Presl | *Acokanthera,* ***Adenium, Anchylobotrys, Baissea, Callichilia,*** *Carissa, Diplorhynchus, Furtumia, Gonioma, Holarrhena,* ***Landolphia,*** *Mascarenhasia,* ***Mondia,*** *Oncinotus, Pachypodium, Pleiocarpa,* ***Pleioceras,*** *Rauvolfia, Strophantus, Tabernaemontana, Voacanga, Wrightia, [3]Pachypodium* |
| Aquifoliaceae Bercht. & J.Presl | | Aquifoliales Senft | *Ilex* |
| Araliaceae Juss. | | Apiales Nakai | *Cussonia, Polyscias, Schefflera,* ***Seemannaralia*** |
| Arecaceae Bercht. & J.Presl | | Arecales Bromhead | ***Borassus,*** *Hyphaene, Jubaeopsis, Phoenix, Raphia* |
| [1]Asclepiadaceae R.Br. | Alternatively under Apocynaceae (in broad sense) | Gentianales Juss. ex Bercht. & J.Presl | [1]***Fockea*** |
| [2]Asphodelaceae Juss | Alternatively under Aloaceae<br>APG III – under Xanthorrhoeaceae Dumort | Asparagales Link | *Aloe* |
| Asteraceae Bercht. & J.Presl | | Asterales Link | ***Berkheya,*** *Brachylaena, Chrysanthemoides,* ***Didelta, Distephanus,*** *Euryops,* ***Lopholaena,*** *Metalasia,* ***Microglossa,*** *Oldenburgia,* ***Osmitopsis, Othonna, Psiadia, Senecio,*** *Solanecio,Tarchonanthus,* ***Vernonia, Zoutpansbergia*** |
| [2]Avicenniaceae End.ex Schnizl | Formerly under Verbenaceae | Lamiales Bromhead | ***Avicennia*** |

| FAMILY | COMMENTS | ORDER | GENERA |
|---|---|---|---|
| | APG III – under Acanthaceae Juss. | | |
| Balanitaceae Endl | Alternatively under Zygophyllaceae R.Br.<br>APG III – under Zygophyllaceae R.Br. | Zygophyllales Link | *Balanites* |
| Bignoniaceae Juss. | | Lamiales Bromhead | *Catophractes*, *Dolichandrone*, *Fernandoa*, *Kigelia*, *Markhamia*, *Podranea*, *Rhigozum*, *Stereospermum*, *Tecomaria (=Tecoma)* |
| [2]Bombaceae Kunth | Alternatively under Malvaceae (in broad sense)<br>APG III – under Malvaceae Juss. | Malvales Juss. ex Bercht. & J.Presl | *Adansonia* |
| Boraginaceae Juss. | | Unplaced | *Cordia*, *Ehretia* |
| Brownlowiaceae | Formerly under Tiliaceae (in broad sense); alternatively under Malvaceae (in broad sense)<br>APG III – under Malvaceae Juss. | Malvales Juss. ex Bercht. & J.Presl | [1]*Carpodiptera* |
| Bruniaceae R.Br. ex DC. | | Bruniales Dumort. | *Berzelia*, *Raspalia* |
| [2]Buddlejaceae Wilhelm | Alternatively under Scrophulariaceae (in broad sense); formerly under Loganiaceae<br>APG III – under Scrophulariaceae Juss. | Lamiales Bromhead | *Buddleja*, *Gomphostigma*, *Nuxia* |
| Burseraceae Kunth | | Sapindales Juss. ex Bercht. & J.Presl | *Commiphora* |
| Buxaceae Dumort. | | Buxales Takht. ex Reveal | *Buxus* |
| Canellaceae Mart. | | Canellales Cronquist | Warburgia |
| Capparaceae Juss. | | Brassicales Bromhead | *Bachmannia*, *Boscia*, *Cadaba*, *Capparis*, *Cladostemon*, *Maerua*, *Thilachium* |
| [2]Cecropiaceae C.Berg | Formerly under Moraceae (in broad sense); alternatively under Urticaceae (in broad sense)<br>APG III – under Urticaceae Juss. | Rosales Bercht. & J.Presl | *Myrianthus* |
| Celastraceae R.Br. | | Celastrales Link | *Allocassine*, *Brexia*, *Cassine*, *Catha*, *Elaeodendron*, *Gloveria*, *Gymnosporia*, *Hippobromus*, *Lauridia*, *Lydenburgia*, *Maurocenia*, *Maytenus*, *Mystroxylon*, *Pleurostylia*, *Pseudosalacia*, *Pterocelastrus*, *Putterlickia*, *Robsonodendron*, *Salacia* |
| [2]Celtidaceae Engl. | Formerly under Ulmaceae<br>APG III – under Cannabaceae Martinov | Rosales Bercht. & J.Presl | *Celtis*, *Chaetacme*, *Trema* |
| [2]Chenopodiaceae Vent | APG III – under Amaranthaceae Juss. | Caryophyllales Juss. ex Bercht. & J.Presl | *Salsola* |
| Chrysobalanaceae R.Br. | | Malpighiales Juss. ex Bercht. & J.Presl | *Maranthes*, *Parinari* |
| [2]Clusiaceae Lindl. | Alternatively under Hypericaceae Juss.<br>APG III – under Hypericaceae Juss. | Malpighiales Juss. ex Bercht. & J.Presl | *Garcinia*, *Harungana*, *Hypericum*, *Psorospermum* |
| Combretaceae R.Br. | | Myrtales Juss. ex Bercht. & J.Presl | *Combretum*, *Pteleopsis*, *Quisqualis*, *Terminalia*, *Lumnitzera*, [3]*Quisqualis*, [3]*Bucida* |

| FAMILY | COMMENTS | ORDER | GENERA |
|---|---|---|---|
| Connaraceae R.Br. | | Oxalidales Bercht. & J.Presl | *Cnestis, Rourea* |
| [1]Cornaceae Takht. | | Cornales Link. | [1]*Afrocrania*, [1]*Alangium* |
| Crassulaceae J.St.-Hil. | | Saxifragales Bercht. & J.Presl | *Crassula, Tylecodon* |
| Cunoniaceae R.Br. | | Oxalidales Bercht. & J.Presl | *Cunonia, Platylophus* |
| Cupressaceae Bartlett | Gymnosperms; alternatively placed in order Pinales or Cupressales (Stevens, 2001 onwards; Christenhusz *et al.*, 2011) | Coniferales | *Widdringtonia, Juniperus* |
| Curtisiaceae Takht | Formerly under Cornaceae (in broad sense) | Cornales Link. | *Curtisia* |
| Cyatheaceae | Tree ferns | Cyatheales | *Cyathea* |
| Cycadaceae Persoon | Gymnosperms | Cycadales | *Cycas* |
| Dichapetalaceae Baill. | | Malpighiales Juss. ex Bercht. & J.Presl | *Tapura* |
| Dipterocarpaceae Blume | | Malvales Juss. ex Bercht. & J.Presl | *Monotes* |
| [2]Dracaenaceae R.A. Salisbury | Formerly under Agavaceae; sometimes under Convallariaceae<br>APG III – under Asparagaceae Juss. | Asparagales Link | *Dracaena* |
| Ebenaceae Gürke | | Ericales Bercht. & J.Presl | *Diospyros, Euclea* |
| Ericaceae Juss. | | Ericales Bercht. & J.Presl | *Erica, Vaccinium* |
| Erythroxylaceae Kunth | | Malpighiales Juss. ex Bercht. & J.Presl | *Erythroxylum, Nectaropetalum* |
| Euphorbiaceae Juss. | | Malpighiales Juss. ex Bercht. & J.Presl | *Acalypha, Alchornea, Argomuellera, Cavacoa, Croton, Erythrococca, Euphorbia, Excoecaria, Jatropha, Macaranga, Maprounea, Micrococca, Necepsia, Sapium, Schinziophyton,Sclerocroton, Shirakiopsis, Spirostachys, Suregada, Synadenium, Tannodia,* [3]*Jatropha* |
| Fabaceae Lindl. (in broad sense) | Van Wyk *et al*. (2011) place them in three families: Caesalpiniaceae, Fabaceae (in narrow sense) and Mimosaceae | Fabales Bromhead | Subclass Caesalpinioideae *Adenolobus, Afzelia, Baikiaea, Bauhinia, Brachystegia, Burkea, Caesalpinia, Cassia, Colophospermum, Dialum, Erythrophleum, Guibourtia, Haematoxylon, Hymenaea, Julbernardia, Parkinsonia, Peltophorum, Piliostigma, Pterolobium, Schotia, Senna, Umtiza, Tamarindus,* [3]*Tamarindus*<br>Subclass Mimosoideae *Acacia, Adenopodia, Albizia, Amblygonocarpus, Dichrostachys, Elephantorrhiza, Entada, Faidherbia, Newtonia, Xylia,* [3]*Leucaenia*<br>Subclass Papilionoideae: *Aeschynomene, Baphia,* [3]*Baphiopsis, Bolusanthus, Calpurnia, Cordyla, Craibia, Crotalaria, Cyclopia, Crotalaria, Dalbergia, Erythrina, Flemingia, Hypocalyptus, Indigofera, Millettia, Mundulea, Ormocarpum, Otholobium, Philenoptera, Podalyria, Psoralea, Pterocarpus, Rhynchosia, Sesbania, Sophora, Stirtonanthus, Swartzia, Tephrosia, Virgilia, Wiborgia, Xanthocercis, Xeroderris* |
| [2]Flacourtiaceae (in narrow sense) | Alternatively under Salicaceae (in broad sense)<br>APG III - under Salicaceae Mirb. | Malpighiales Juss. ex Bercht. & J.Presl | *Casearia, Dovyalis Flacourtia, Homalium, Oncoba, Pseudoscolopia, Scolopia, Trimeria* |

| FAMILY | COMMENTS | ORDER | GENERA |
|---|---|---|---|
| Gentianaceae Juss. | | Gentianales Juss. ex Bercht. & J.Presl | *Anthocleista* |
| [1]Gerrardinaceae Alford | Formerly under Flacourtiaceae (in narrow sense); alternatively under Salicaceae (in broad sense) | Huerteales Doweld | [1]*Gerrardina* |
| [2]Greyiaceae (Gürke) Hutch | Alternatively under Melianthaceae<br>APG III – under Melianthaceae Horan. | Geraniales Juss. ex Bercht. & J.Presl | *Greyia* |
| Hamamelidaceae R.Br. | | Saxifragales Bercht. & J.Presl | *Trichocladus* |
| [2]Helicteraceae J. Agardh | Formerly under Sterculiaceae (in broad sense); alternatively under Malvaceae (in broad sense)<br>APG III – under Malvaceae Juss. | Malvales Juss. ex Bercht. & J.Presl | [1]*Triplochiton* |
| Hernandiaceae Blume | | Laurales Juss. ex Bercht. & J.Presl | *Gyrocarpus* |
| [2]Heteropyxidaceae Engler & Gilg | Alternatively under Myrtaceae<br>APG III - under Myrtaceae | Myrtales Juss. ex Bercht. & J.Presl | *Heteropyxis* |
| Icacinaceae Miers | | Unplaced | *Apodytes*, *Cassinopsis* |
| Iteaceae J.Agardh | (Formerly under Escalloniaceae) | Saxifragales Bercht. & J.Presl | *Choristylis* |
| [2]Kiggelariaceae Link | Formerly under Flacourtiaceae; alternatively under Achariaceae (in broad sense)<br>APG III – under Achariaceae Harms | Malpighiales Juss. ex Bercht. & J.Presl | *Kiggelaria, Rawsonia, Xylotheca* |
| Kirkiaceae Takht. | | Sapindales Juss. ex Bercht. & J.Presl | *Kirkia* |
| Lamiaceae Martinov | | Lamiales Bromhead | *Achyrospermum*,*Clerodendrum*, *Hemizygia*, *Karomia*, *Plectranthus*, *Premna*, *Rotheca*, *Syncolostemon*, *Tetradenia*, *Tinnea*, *Vitex* |
| Lauraceae Juss. | | Laurales Juss. ex Bercht. & J.Presl | *Cryptocarya*, *Dahlgrenodendron*, *Ocotea* |
| Lecythidaceae A.Rich. | | Ericales Bercht. & J.Presl | *Barringtonia* |
| Linaceae DC. ex Perleb | | Malpighiales Juss. ex Bercht. & J.Presl | *Hugonia* |
| Lythraceae J.St.-Hil. | | Myrtales Juss. ex Bercht. & J.Presl | *Galpinia* |
| [2]Maesaceae Anderb.,B. Stähl & Källersjö | Alternatively under Primulaceae (in broad sense)<br>APG III – under Primulaceae Batsch ex Borkh. | Ericales Bercht. & J.Presl | *Maesa* |
| Malpighiaceae Juss. | | Malpighiales Juss. ex Bercht. & J.Presl | *Acridocarpus*, *Triaspis* |
| Malvaceae Juss. | | Malvales Juss. ex Bercht. & J.Presl | *Abutilon, Azanza, Hibiscus*, [3]*Pavonia*, *Thespesia* |
| Melastomataceae Juss. | | Myrtales Juss. ex Bercht. & J.Presl | *Dissotis, Memecylon, Warneckea* |

| | | | |
|---|---|---|---|
| Meliaceae Juss. | | Sapindales Juss. ex Bercht. & J.Presl | *Ekebergia, Entandrophragma, Khaya, Lovoa, Nymania, Pseudobersama, Trichilia, Turraea, Xylocarpus,* [3]*Khaya* |
| Melianthaceae Horan. | | Geraniales Juss. ex Bercht. & J.Presl | *Bersama* |
| Menispermaceae Juss. | | Ranunculales Juss. ex Bercht. & J.Presl | *Cocculus, Tiliacora, Tinospora* |
| [2]Mesembryanthemaceae Fenzl | APG III – under *Aizoaceae* Martinov | Caryophyllales Juss. ex Bercht. & J.Presl | *Stoeberia, Mestoklema* |
| Monimiaceae Juss. | | Laurales Juss. ex Bercht. & J.Presl | *Xymalos* |
| Montiniaceae Nakai | | Solanales Juss. ex Bercht. & J.Presl | *Montinia* |
| Moraceae Gaudich. | | Rosales Bercht. & J.Presl | *Ficus, Maclura, Morus, Trilepisium* |
| Moringaceae Martinov | | Brassicales Bromhead | *Moringa* |
| Musaceae Juss. | | Zingiberales Griseb. | *Ensete* |
| Myricaceae A.Rich. ex Kunth | | Fagales Engl. | *Morella* |
| [2]Myrsinaceae R.Br. | Alternatively under Primulaceae (in broad sense)<br>APG III – under Primulaceae Batsch ex Borkh. | Ericales Bercht. & J.Presl | *Embelia, Myrsine, Rapanea* |
| Myrtaceae Juss. | | Myrtales Juss. ex Bercht. & J.Presl | *Eugenia, Metrosideros, Syzygium* |
| Nyctaginaceae Juss. | | Caryophyllales Juss. ex Bercht. & J.Presl | *Phaeoptilum, Pisonia* |
| Ochnaceae DC. | | Malpighiales Juss. ex Bercht. & J.Presl | *Brackenridgea, Ochna* |
| Olacaceae R.Br. | | Santalales R.Br. ex Bercht. & J.Presl | *Olax, Strombosia, Ximenia* |
| Oleaceae Hoffmanns. & Link | | Lamiales Bromhead | *Chionanthus,* [3]*Jasminum, Olea Schrebera* |
| [2]Oliniaceae Arn.ex Sond. | APG III – under Penaeaceae Sweet ex Guill. | Myrtales Juss. ex Bercht. & J.Presl | *Olinia* |
| Opiliaceae Valeton | | Santalales R.Br. ex Bercht. & J.Presl | *Opilia* |
| Pandanaceae R.Br. | non-indigenous | Pandanales R.Br. ex Bercht. & J.Presl | *Pandanus* |
| Passifloraceae Juss. ex Roussel | | Malpighiales Juss. ex Bercht. & J.Presl | *Paropsia, Adenia* |
| Pedaliaceae R.Br. | | Lamiales Bromhead | *Sesamothamnus* |
| [2]Pentapetaceae Bercht & J. Presl. | Formerly under Sterculiaceae (in broad sense); alternatively under Malvaceae (in broad sense)<br>APG III - under Malvaceae Juss. | Malvales Juss. ex Bercht. & J.Presl | *Dombeya* |

| | | | |
|---|---|---|---|
| Phyllanthaceae Martinov | | Malpighiales Juss. ex Bercht. & J.Presl | *Andrachne, Antidesma Bridelia, Cleistanthus, Flueggea, Heywoodia, Hymenocardia, Lachnostylis, Margaritaria, Phyllanthus, Pseudolachnostylis, Uapaca* |
| Picrodendraceae Small | | Malpighiales Juss. ex Bercht. & J.Presl | *Androstachys, Hyaenanche* |
| Piperaceae C.A. Agardh | | Piperales Bercht. & J.Presl | *Piper* |
| Pittosporaceae R.Br. | | Apiales Nakai | *Pittosporum* |
| Poaceae Barnhart | | Poales Small (commelinids) | *Thamnocalamus, Oreobambos, Oxytenanthera* |
| Podocarpaceae Endl. | Gymnosperms: alternatively placed in order Araucariales (Christenhusz *et al.*, 2011) | Pinales | *Podocarpus* |
| Polygalaceae Hoffmanns. & Link | | Fabales Bromhead | *Carpolobia, Nylandtia, Polygala, Securidaca* |
| Portulacaceae Juss. | | Caryophyllales Juss. ex Bercht. & J.Presl | *Ceraria, Portulacaria* |
| Proteaceae Juss. | | Proteales Juss. ex Bercht. & J.Presl | *Brabejum, Faurea, Leucadendron, Leucospermum, Mimetes, Paranomus, Protea* |
| [2]Ptaeroxylaceae Sonder | Alternatively under Rutaceae<br>APG III - under Rutaceae Juss. | Sapindales Juss. ex Bercht. & J.Presl | *Ptaeroxylon* |
| Putranjivaceae Meisn. | | Malpighiales Juss. ex Bercht. & J.Presl | *Drypetes* |
| Rhamnaceae Juss. | | Rosales Bercht. & J.Presl | *Berchemia, Colubrina, Helinus, Noltea, Lasiodiscus, Phylica, Rhamnus, Scutia, Ziziphus* |
| Rhizophoraceae Pers. | | Malpighiales Juss. ex Bercht. & J.Presl | *Bruguiera, Cassipourea, Ceriops, Rhizophora* |
| [2]Rhynchocalycaceae L.A.S.Johnson | Formerly under Lythraceae<br>APG III - under Penaeaceae Sweet ex Guill. | Myrtales Juss. ex Bercht. & J.Presl | *Rhynchocalyx* |
| Rosaceae Juss. | | Rosales Bercht. & J.Presl | *Cliffortia, Leucosidea, Prunus* |
| Rubiaceae Juss. | | Gentianales Juss. ex Bercht. & J.Presl | *Afrocanthium, Aidia, Anthospermum, Alberta, Breonadia, Burchellia, Burttdavya, Canthium (=Plectroniella), Carphalea, Catunaregam, Cephalanthus, Chassalia, Coddia, Coffea, Coptosperma, Craterispermum, Cremaspora, Crossopteryx, Didymosalpinx, Feretia, Gardenia, Guettarda, Heinsenia, Hymenodictyon, Hyperacanthus, Ixora, Keetia, Kraussia, Lagynias, Lasianthus, Leptactina, Mitriostigma, Multidentia, Mussaenda, Oxyanthus, Pachystigma, Pauridiantha, Pavetta, Polysphaeria, Psychotria, Psydrax, Pyrostria, Rothmannia, Rytigynia, Rutidea, Tapiphyllum, Tarenna, Tricalysia (=Empogona), Sericanthe, Vangueria, Vangueriopsis* |
| Rutaceae Juss. | | Sapindales Juss. ex Bercht. & J.Presl | *Calodendrum, Citropsis, Clausenia, Coleonema, Empleurum, Fagaropsis, Oricia, Teclea, Toddalia, Toddaliopsis, Vepris, Zanthoxylum* |
| Salicaceae Mirb. | | Malpighiales Juss. ex Bercht. & J.Presl | *Salix* |
| Salvadoraceae Lindl. | | Brassicales Bromhead | *Azima, Salvadora* |
| *Santalaceae* R.Br. | | Santalales R.Br. ex Bercht. & J.Presl | *Osyris* |

| | | | |
|---|---|---|---|
| Sapindaceae Juss. | | Sapindales Juss. ex Bercht. & J.Presl | *Allophylus, Aporrhiza, Atalaya, Blighia, Deinbollia, Dodonaea, Erythrophysa, Filicium, Glenniea, Haplocoelum, Hippobromus, Lecaniodiscus, Lepisanthes, Macphersonia, Pancovia, Pappea, Smelophyllum, Stadmannia, Zanha* |
| Sapotaceae Juss. | | Ericales Bercht. & J.Presl | *Chrysophyllum, Englerophytum, Inhambanella, Manilkara, Mimusops, Pouteria, Sideroxylon Synsepalum, Vitellariopsis* |
| Scrophulariaceae Juss. | | Lamiales Bromhead | *Anastrabe, Antherothamnus, Bowkeria, Manuleopsis, Freylina, Halleria, Ixianthes* |
| Solanaceae Juss. | | Solanales Juss. ex Bercht. & J.Presl | *Solanum* |
| [1]Sonneratiaceae Engl. & Gilg | | Myrtales Juss. ex Bercht. & J.Presl | [1]*Sonneratia* |
| [2]Sparrmanniaceae J. Agardh | Formerly under Tiliaceae (in broad sense); alternatively under Malvaceae (in broad sense)<br>APG III - under Malvaceae Juss. | Malvales Juss. ex Bercht. & J.Presl | *Glyphaea, Grewia, Sparrmannia* |
| [2]Sterculiaceae Vent. | Alternatively under Malvaceae (in broad sense)<br>APG III under Malvaceae Juss. | Malvales Juss. ex Bercht. & J.Presl | *Cola, Heritiera, Sterculia* |
| Strelitziaceae Hutch. | | Zingiberales Griseb. | *Strelitzia* |
| [2]Strychnaceae Link. | Formerly under Loganiaceae (in broad sense)<br>APG III – under Loganiaceae R.Br. ex Mart. | Gentianales Juss. ex Bercht. & J.Presl | *Strychnos* |
| Tamaricaceae Link | | Caryophyllales Juss. ex Bercht. & J.Presl | *Tamarix* |
| Thymelaeaceae Juss. | | Malvales Juss. ex Bercht. & J.Presl | *Dais, Englerodaphne, Passerina, Peddiea, Synaptolepis* |
| [1]Turneraceae DC. | Previously in order Violaceae<br>APG III – under Violaceae | Malpighiales Juss. ex Bercht. & J.Presl | [1]*Turnera* |
| Urticaceae Juss. | | Rosales Bercht. & J.Presl | *Obetia,* [3]*Pouzolzia, Urera* |
| Verbenaceae J.St.-Hil. (in narrow sense) | | Lamiales Bromhead | *Lippia, Lantana* |
| Violaceae Batsch | | Malpighiales Juss. ex Bercht. & J.Presl | *Rinorea* |
| Vitaceae Juss. | | Vitales Juss. ex Bercht. & J.Presl | *Cissus, Cyphostemma, Rhoicissus* |
| [1]Welwitschiaceae | Gymnosperms | Welwitchiales | [1]*Welwitschia* |
| Zamiaceae Horaninow | Gymnosperms | Cycadales | *Encephalartos* |
| [1]Zygophyllaceae R.Br. | | Zygophyllales Link | [1]*Neoluederitzia* |

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# APPENDIX B

**Table B.1:** Acetone tree leaf extracts with very high activity (MIC ≤ 0.04 mg/ml) against at least one of the test organisms (MIC's ≤ 0.04 mg/ml in bold; No. = accession number in Phytomedicine Tree Database; SD = standard deviation; na = not analysed; *Ef = E. faecalis; Sa = S. aureus; Ec = E. coli; Pa = P. aeruginosa; Ca = C. albicans; Cn = C. neoformans*; [1] = non-indigenous species).

| Tree species | No. | Pathogen | | | | | |
|---|---|---|---|---|---|---|---|
| | | *Ef* | *Sa* | *Ec* | *Pa* | *Ca* | *Cn* |
| *Allophylus decipiens* (Sond.) Radlk. | 312 | 0.16 ± 0.00 | 0.03 ± 0.01 | 0.13 ± 0.05 | 0.04 ± 0.00 | 2.50 ± 0.00 | 1.25 ± 0.00 |
| *Azanza garckeana* (F.Hoffm.) Exell & Hillc. | 726 | 1.25 ± 0.00 | 1.25 ± 0.00 | 0.08 ± 0.00 | 0.02 ± 0.00 | 0.08 ± 0.00 | 0.63 ± 0.00 |
| *Bolusanthus speciosus* (Bolus) Harms | 580 | 0.63 ± 0.00 | 1.25 ± 0.00 | 0.02 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 |
| *Bowkeria citrina* Thode | 647 | 0.16 ± 0.00 | 0.04 ± 0.00 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.02 ± 0.00 | 0.08 ± 0.00 |
| *Brabejum stellatifolium* L. | 262 | 0.16 ± 0.00 | 0.02 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 2.50 ± 0.00 |
| *Breonadia salicina* (Vahl) Hepper & J.R.I.Wood | 199 | 0.16 ± 0.00 | 0.63 ± 0.00 | 0.13 ± 0.05 | 1.98 ± 0.72 | 0.03 ± 0.01 | 0.08 ± 0.00 |
| *Burchellia bubalina* (L.f.) Sims | 313 | 0.10 ± 0.05 | 0.63 ± 0.00 | 1.25 ± 0.00 | 0.03 ± 0.01 | 0.63 ± 0.00 | 0.50 ± 0.18 |
| *Calpurnia aurea* (Aiton) Benth. subsp. aurea | 202 | 0.02 ± 0.00 | 0.16 ± 0.00 | 0.08 ± 0.00 | 0.13 ± 0.05 | 0.63 ± 0.00 | 0.13 ± 0.05 |
| *Capparis tomentosa* Lam. | 528 | 0.39 ± 0.18 | 0.16 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.08 ± 0.00 | 0.04 ± 0.00 |
| *Capparis tomentosa* Lam. | 301 | 0.20 ± 0.09 | 0.02 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 | 1.25 ± 0.00 | 0.79 ± 0.36 |
| *Cassine peragua* L. | 314 | 0.04 ± 0.00 | 0.16 ± 0.00 | 0.63 ± 0.00 | 0.02 ± 0.00 | 2.50 ± 0.00 | 0.10 ± 0.05 |
| *Cassipourea gummiflua* Tul. | 203 | 0.06 ± 0.02 | 0.39 ± 0.18 | 0.25 ± 0.09 | 0.63 ± 0.00 | 0.04 ± 0.00 | 0.10 ± 0.05 |
| *Cladostemon kirkii* (Oliv.) Pax & Gilg | 205 | 0.06 ± 0.02 | 0.63 ± 0.00 | 0.16 ± 0.00 | 0.39 ± 0.18 | 0.31 ± 0.00 | 0.04 ± 0.00 |
| *Combretum collinum* Fresen. subsp. *suluense* (Engl. & Diels) Okafor | 406 | 2.50 ± 0.00 | 0.04 ± 0.00 | 0.16 ± 0.00 | 0.63 ± 0.00 | 1.25 ± 0.00 | 2.50 ± 0.00 |
| *Combretum zeyheri* Sond. | 558 | 1.25 ± 0.00 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.04 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 |
| *Crotalaria capensis* Jacq. | 318 | 0.31 ± 0.00 | 0.04 ± 0.00 | 0.13 ± 0.05 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 |
| *Cunonia capensis* L. | 656 | 1.25 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 | 0.16 ± 0.00 | 1.25 ± 0.00 | 0.04 ± 0.00 |
| *Curtisia dentata* (Burm.f.) C.A.Sm. | 26 | 0.03 ± 0.01 | 0.08 ± 0.00 | 0.08 ± 0.00 | 0.08 ± 0.00 | 0.04 ± 0.00 | 0.06 ± 0.02 |
| *Diospyros natalensis* (Harv.) Brenan subsp nummelaria | 308 | 0.16 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.02 ± 0.00 | 0.05 ± 0.02 | 0.31 ± 0.00 |
| Eckl. & Zeyh. *Cussonia paniculata* | 657 | 0.31 ± 0.00 | 0.16 ± 0.00 | 2.50 ± 0.00 | 0.04 ± 0.00 | 1.25 ± 0.00 | 2.50 ± 0.00 |
| *Elaeodendron croceum* (Thunb.) DC. | 11 | 0.02 ± 0.00 | 0.02 ± 0.00 | 0.04 ± 0.00 | 0.16 ± 0.00 | 0.31 ± 0.00 | 0.16 ± 0.00 |
| *Encephalartos natalensis* R.A.Dyer & I.Verd. | 349 | 0.04 ± 0.00 | 0.16 ± 0.00 | 0.79 ± 0.36 | 0.79 ± 0.36 | 1.00 ± 0.35 | 0.31 ± 0.00 |
| *Entada* sp | 413 | 1.25 ± 0.00 | 2.50 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.04 ± 0.00 | 0.16 ± 0.00 |
| *Erythrophleum lasianthum* Corbishley | 212 | 0.02 ± 0.00 | 0.31 ± 0.00 | 0.79 ± 0.36 | 0.79 ± 0.36 | 0.25 ± 0.09 | 0.63 ± 0.00 |
| *Freylinia visseri* Van Jaarsv. | 672 | 0.04 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.16 ± 0.00 |
| *Grevillea robusta* A.Cunn. / *Grevillea robusta* A.Cunn. Ex R.Br. | 488 | 0.04 ± 0.00 | 0.02 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 |
| *Gymnosporia buxifolia* (L.) Szyszyl. | 323 | 0.04 ± 0.00 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.63 ± 0.00 | 2.50 ± 0.00 |
| *Gymnosporia buxifolia* (L.) Szyszyl. | 564 | 0.39 ± 0.18 | 0.08 ± 0.00 | 0.31 ± 0.00 | 0.02 ± 0.00 | na | na |
| *Haplocoelum foliolosum* (Hiern) Bullock | 303 | 0.02 ± 0.00 | 0.02 ± 0.00 | 0.31 ± 0.00 | 0.08 ± 0.00 | 0.31 ± 0.00 | 0.39 ± 0.18 |
| *Harpephyllum caffrum* Bernh. ex Krauss | 324 | 0.08 ± 0.00 | 0.08 ± 0.00 | 0.16 ± 0.00 | 0.31 ± 0.00 | 0.04 ± 0.00 | 0.02 ± 0.00 |
| *Heteromorpha arborescens* (Spreng.) Cham. & Schltdl. | 491 | 0.63 ± 0.00 | 0.63 ± 0.00 | 0.02 ± 0.00 | 1.25 ± 0.00 | na | na |
| *Heteropyxis natalensis* Harv. | 354 | 0.16 ± 0.00 | 0.04 ± 0.00 | 0.04 ± 0.00 | 0.16 ± 0.00 | 0.31 ± 0.00 | 0.04 ± 0.00 |

| Tree species | No. | Pathogen | | | | | |
|---|---|---|---|---|---|---|---|
| | | *Ef* | *Sa* | *Ec* | *Pa* | *Ca* | *Cn* |
| *Hexalobus monopetalus* (A.Rich.) Engl. & Diels | 492 | 0.31 ± 0.00 | 0.63 ± 0.00 | 0.16 ± 0.00 | 0.31 ± 0.00 | 0.16 ± 0.00 | 0.04 ± 0.00 |
| *Hypericum roeperianum* G.W.Schimp. ex A.Rich. | 356 | 0.05 ± 0.02 | 0.03 ± 0.01 | 0.08 ± 0.00 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.08 ± 0.00 |
| *Keetia gueinzii* (Sond.) Bridson | 432 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.25 ± 0.09 | 0.04 ± 0.00 |
| *Kigelia africana* (Lam.) Benth. | 124 | 2.50 ± 0.00 | 0.79 ± 0.36 | 0.79 ± 0.36 | 1.25 ± 0.00 | 0.04 ± 0.00 | 2.50 ± 0.00 |
| *Leucadendron argenteum* (L.) R.Br. | 682 | 0.31 ± 0.00 | 0.16 ± 0.00 | 0.31 ± 0.00 | 1.25 ± 0.00 | 0.31 ± 0.00 | 0.04 ± 0.00 |
| *Leucosidea sericea* Eckl. & Zeyh. | 609 | 0.02 ± 0.00 | 0.02 ± 0.00 | 0.16 ± 0.00 | 0.05 ± 0.02 | 0.63 ± 0.00 | 0.04 ± 0.00 |
| *Leucosidea sericea* Eckl. & Zeyh. | 288 | 0.02 ± 0.00 | 0.02 ± 0.00 | 0.50 ± 0.54 | 0.39 ± 0.18 | 2.50 ± 0.00 | 2.50 ± 0.00 |
| *Loxostylis alata* A.Spreng. ex Rchb. | 180 | 0.02 ± 0.00 | 0.06 ± 0.03 | 0.06 ± 0.03 | 0.03 ± 0.01 | 0.16 ± 0.00 | 0.04 ± 0.00 |
| *Loxostylis alata* A.Spreng. ex Rchb. | 736 | 0.04 ± 0.00 | 0.08 ± 0.00 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.08 ± 0.00 | 0.08 ± 0.00 |
| *Loxostylis alata* A.Spreng. ex Rchb. | 614 | 0.08 ± 0.00 | 0.02 ± 0.00 | 0.03 ± 0.01 | 0.06 ± 0.03 | 0.31 ± 0.00 | 0.08 ± 0.00 |
| *Macaranga capensis* (Baill.) Benth. ex Sim | 53 | 0.03 ± 0.01 | 0.03 ± 0.01 | 0.08 ± 0.00 | 0.31 ± 0.00 | 0.08 ± 0.00 | 0.02 ± 0.00 |
| *Macaranga mellifera* Prain. | 54 | 0.13 ± 0.05 | 0.16 ± 0.00 | 0.08 ± 0.00 | 0.10 ± 0.05 | 0.05 ± 0.02 | 0.02 ± 0.00 |
| *Maclura africana* (Bureau) Corner | 302 | 0.04 ± 0.00 | 0.02 ± 0.00 | 0.31 ± 0.00 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.16 ± 0.00 |
| Maerua sp. | 499 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 | 0.16 ± 0.00 | 0.04 ± 0.00 |
| *Maesa lanceolata* Forssk. | 615 | 0.04 ± 0.00 | 0.02 ± 0.00 | 0.02 ± 0.00 | 0.02 ± 0.00 | 0.63 ± 0.00 | 0.16 ± 0.00 |
| *Millettia stuhlmannii* Taub. | 57 | 0.16 ± 0.00 | 0.25 ± 0.09 | 0.16 ± 0.00 | 0.02 ± 0.00 | 0.13 ± 0.05 | 0.31 ± 0.00 |
| *Mimusops obovata* Nees ex Sond. | 244 | 0.04 ± 0.00 | 0.08 ± 0.00 | 0.08 ± 0.00 | 0.04 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 |
| *Monodora junodii* Engl. & Diels | 218 | 0.03 ± 0.01 | 0.08 ± 0.00 | 0.63 ± 0.00 | 0.16 ± 0.00 | 1.25 ± 0.00 | 0.63 ± 0.00 |
| *Morus mesozygia* Stapf ex A.Chev. | 58 | 0.08 ± 0.00 | 0.16 ± 0.00 | 0.08 ± 0.00 | 0.31 ± 0.00 | 0.20 ± 0.09 | 0.03 ± 0.01 |
| *Mundulea sericea* (Willd.) A.Chev. | 473 | 1.25 ± 0.00 | 0.04 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 |
| *Mundulea sericea* (Willd.) A.Chev. | 566 | 0.31 ± 0.00 | 0.02 ± 0.00 | 0.20 ± 0.09 | 0.02 ± 0.00 | na | na |
| *Mystroxylon aethiopicum* (Thunb.) Loes. | 10 | 0.04 ± 0.00 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.08 ± 0.00 | 0.20 ± 0.09 | 0.10 ± 0.05 |
| *Ozoroa paniculosa* (Sond.) R.& A.Fern. | 568 | 0.31 ± 0.00 | 0.63 ± 0.00 | 0.16 ± 0.00 | 0.04 ± 0.00 | na | na |
| *Pandanus livingstonianus* Rendle | 446 | 1.25 ± 0.00 | 2.50 ± 0.00 | 1.25 ± 0.00 | 0.16 ± 0.00 | 0.08 ± 0.00 | 0.04 ± 0.00 |
| *Pavetta lanceolata* Eckl. | 697 | 0.63 ± 0.00 | 0.31 ± 0.00 | 0.16 ± 0.00 | 1.25 ± 0.00 | 0.16 ± 0.00 | 0.04 ± 0.00 |
| *Peddiea africana* Harv. | 699 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.16 ± 0.00 | 0.08 ± 0.00 | 0.04 ± 0.00 |
| *Philenoptera nelsii* (Schinz) Schrire | 612 | 0.63 ± 0.00 | 0.63 ± 0.00 | 0.16 ± 0.00 | 0.04 ± 0.00 | 0.16 ± 0.00 | 0.08 ± 0.00 |
| *Pittosporum viridiflorum* Sims | 184 | 0.04 ± 0.00 | 0.31 ± 0.00 | 0.06 ± 0.03 | 0.22 ± 0.11 | 0.16 ± 0.00 | 0.20 ± 0.09 |
| *Podalyria calyptrata* (Retz.) Willd. | 759 | 0.08 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 | 0.04 ± 0.00 | 0.31 ± 0.00 | 0.16 ± 0.00 |
| *Podocarpus falcatus* (Thunb.) R.Br. ex Mirb. | 329 | 0.04 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.39 ± 0.18 | 0.04 ± 0.00 | 0.31 ± 0.00 |
| *Pseudoscolopia polyantha* Gilg | 704 | 0.31 ± 0.00 | 1.25 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.04 ± 0.00 | 0.16 ± 0.00 |
| *Psychotria capensis* (Eckl.) Vatke | 706 | 0.63 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.08 ± 0.00 | 0.04 ± 0.00 |
| *Rapanea melanophloeos* (L.) Mez | 330 | 0.08 ± 0.00 | 0.04 ± 0.00 | 0.16 ± 0.00 | 0.63 ± 0.00 | 0.08 ± 0.00 | 0.80 ± 0.35 |
| *Rhamnus prinoides* L'Hér. | 332 | 0.04 ± 0.00 | 0.03 ± 0.01 | 0.16 ± 0.00 | 0.31 ± 0.00 | 2.50 ± 0.00 | 1.25 ± 0.00 |
| *Rinorea angustifolia* (Thouars) Baill. | 221 | 0.04 ± 0.00 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.63 ± 0.00 | 2.50 ± 0.00 | 0.31 ± 0.00 |
| *Rothmannia manganjae* (Hiern) Keay | 223 | 0.03 ± 0.01 | 0.08 ± 0.00 | 0.31 ± 0.00 | 0.50 ± 0.18 | 0.63 ± 0.00 | 0.16 ± 0.00 |
| Schinziophyton rautanenii (Schinz) Radcl.-Sm. | 455 | 2.50 ± 0.00 | 0.31 ± 0.00 | 2.50 ± 0.00 | 2.50 ± 0.00 | 0.04 ± 0.00 | 0.02 ± 0.00 |
| *Schotia brachypetala* Sond. | 456 | 0.31 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 | 0.06 ± 0.03 | 0.04 ± 0.00 |
| *Searsia chirindensis* (Baker f.) Moffett | 333 | 0.03 ± 0.01 | 0.16 ± 0.00 | 0.08 ± 0.00 | 0.31 ± 0.00 | 0.40 ± 0.18 | 0.05 ± 0.02 |
| *Searsia leptodictya* (Diels) T.S.Yi, A.J.Mill. & J.Wen | 255 | 0.04 ± 0.00 | 0.08 ± 0.00 | 0.08 ± 0.00 | 0.20 ± 0.09 | 0.39 ± 0.18 | 0.63 ± 0.00 |
| *Searsia pyroides* (Burch.) Moffett | 570 | 0.04 ± 0.00 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.04 ± 0.00 | 0.31 ± 0.00 | 0.16 ± 0.00 |
| *Searsia undulata* (Jacq.) T.S.Yi, A.J.Mill. & J.Wen | 628 | 0.04 ± 0.00 | 0.04 ± 0.00 | 0.63 ± 0.00 | 0.16 ± 0.00 | 1.25 ± 0.00 | 2.50 ± 0.00 |

| Tree species | No. | Pathogen | | | | | |
|---|---|---|---|---|---|---|---|
| | | *Ef* | *Sa* | *Ec* | *Pa* | *Ca* | *Cn* |
| *Smodingium argutum* E.Mey. ex Sond. | 188 | 0.04 ± 0.00 | 0.06 ± 0.03 | 0.06 ± 0.03 | 0.06 ± 0.03 | 0.16 ± 0.00 | 0.10 ± 0.05 |
| *Sparrmannia africana* L.f. | 189 | 0.16 ± 0.00 | 0.31 ± 0.00 | 0.22 ± 0.11 | 0.16 ± 0.00 | 0.08 ± 0.00 | 0.04 ± 0.00 |
| *Strelitzia caudata* R.A.Dyer | 461 | 1.25 ± 0.00 | 2.50 ± 0.00 | 1.25 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 | 0.04 ± 0.00 |
| *Strelitzia reginae* Banks ex Aiton | 361 | 0.31 ± 0.00 | 2.50 ± 0.00 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.08 ± 0.00 | 0.04 ± 0.00 |
| *Terminalia phanerophlebia* Engl. & Diels | 191 | 0.02 ± 0.00 | 0.08 ± 0.00 | 0.06 ± 0.03 | 0.11 ± 0.06 | 0.04 ± 0.00 | 0.10 ± 0.05 |
| *Terminalia phanerophlebia* Engl. & Diels | 631 | 1.25 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.02 ± 0.00 | 0.63 ± 0.00 | 0.16 ± 0.00 |
| *Terminalia phanerophlebia* Engl. & Diels | 84 | 0.08 ± 0.00 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.50 ± 0.18 | 0.02 ± 0.00 | 1.00 ± 0.35 |
| *Terminalia sambesiaca* Engl. & Diels | 85 | 0.13 ± 0.05 | 0.50 ± 0.18 | 1.25 ± 0.00 | 0.63 ± 0.00 | 0.02 ± 0.00 | 1.25 ± 0.00 |
| *Trichilia emetica* Vahl | 87 | 0.16 ± 0.00 | 0.08 ± 0.00 | 0.50 ± 0.54 | 0.39 ± 0.18 | 0.02 ± 0.00 | 1.00 ± 0.35 |
| *Umtiza listeriana* Sim | 311 | 0.20 ± 0.09 | 0.10 ± 0.05 | 0.16 ± 0.00 | 0.02 ± 0.00 | 0.16 ± 0.00 | 0.63 ± 0.00 |
| *Vangueria infausta* Burch. | 632 | 0.16 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.08 ± 0.00 | 0.04 ± 0.00 |
| *Virgilia divaricata* Adamson | 192 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.22 ± 0.11 | 0.06 ± 0.03 | 0.04 ± 0.00 | 0.16 ± 0.00 |
| *Vitellariopsis dispar* (N.E.Br.) Aubrév. | 226 | 0.08 ± 0.00 | 0.02 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 | 1.25 ± 0.00 | 0.63 ± 0.00 |
| *Zanthoxylum leprieurii* Guill. & Perr. | 229 | 2.50 ± 0.00 | 2.50 ± 0.00 | 0.31 ± 0.00 | 1.25 ± 0.00 | 0.04 ± 0.00 | 1.25 ± 0.00 |
| *Ziziphus rivularis* Codd | 194 | 0.04 ± 0.00 | 0.20 ± 0.09 | 0.08 ± 0.00 | 0.20 ± 0.09 | 0.20 ± 0.09 | 0.16 ± 0.00 |
| [1]*Bucida buceras* L. | 200 | 0.02 ± 0.00 | 0.10 ± 0.05 | 0.39 ± 0.18 | 0.20 ± 0.09 | 0.06 ± 0.03 | 0.13 ± 0.05 |
| [1]*Khaya anthotheca* (Welw.) C.DC. | 215 | 0.02 ± 0.00 | 1.25 ± 0.00 | 1.25 ± 0.00 | 1.25 ± 0.00 | 0.79 ± 0.36 | 0.51 ± 0.18 |
| [1]*Terminalia alata* Herb.Madr. Ex Wall. (T. tomentosa) | 225 | 0.03 ± 0.01 | 0.06 ± 0.02 | 0.16 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 | 0.04 ± 0.00 |

# APPENDIX C

**Table C.1:** Acetone tree extracts with MIC's ≤ 0.16 mg/ml against *E. faecalis* and MIC's > 0.16 mg/ml against the rest of the pathogens (No. - accession number in Phytomedicine Tree Database (PMDB); *Ef = E. faecalis; Sa = S. aureus; Ec = E. coli; Pa = P. aeruginosa; Ca = C. albicans; Cn = C. neoformans*; [1] = non-indigenous species).

| Tree species | No. | Pathogen | | | | | |
|---|---|---|---|---|---|---|---|
| | | *Ef* | *Sa* | *Ec* | *Pa* | *Ca* | *Cn* |
| *Erythrophleum lasianthum* Corbishley | 212 | 0.02 ± 0.00 | 0.31 ± 0.00 | 0.79 ± 0.36 | 0.79 ± 0.36 | 0.25 ± 0.09 | 0.63 ± 0.00 |
| [1]*Khaya anthotheca* (Welw.) C.DC. | 215 | 0.02 ± 0.00 | 1.25 ± 0.00 | 1.25 ± 0.00 | 1.25 ± 0.00 | 0.79 ± 0.36 | 0.51 ± 0.18 |
| *Croton sylvaticus* Hochst. | 25 | 0.06 ± 0.02 | 0.63 ± 0.00 | 0.31 ± 0.00 | 1.25 ± 0.00 | 0.79 ± 0.36 | 0.25 ± 0.09 |
| *Ochna natalitia* (Meisn.) Walp. | 60 | 0.08 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 | 1.25 ± 0.00 | 0.39 ± 0.18 | 0.06 ± 0.02 |
| *Diospyros rotundifolia* Hiern | 109 | 0.08 ± 0.00 | 2.50 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 |
| *Eugenia woodii* Dummer | 114 | 0.08 ± 0.00 | 0.79 ± 0.36 | 0.63 ± 0.00 | 0.39 ± 0.18 | 1.25 ± 0.00 | 1.25 ± 0.00 |
| *Trimeria grandifolia* (Hochst.) Warb. | 336 | 0.10 ± 0.05 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.20 ± 0.09 |
| *Maytenus undata* (Thunb.) Blakelock | 56 | 0.16 ± 0.00 | 0.63 ± 0.00 | 0.39 ± 0.18 | 2.50 ± 0.00 | 0.31 ± 0.00 | 0.06 ± 0.02 |
| *Strychnos madagascariensis* Poir. | 72 | 0.16 ± 0.00 | 0.63 ± 0.00 | 1.25 ± 0.00 | 0.63 ± 0.00 | 2.50 ± 0.00 | 2.50 ± 0.00 |
| *Lydenburgia cassinoides* N.Robson | 104 | 0.16 ± 0.00 | 0.63 ± 0.00 | 1.25 ± 0.00 | 1.25 ± 0.00 | 1.25 ± 0.00 | 0.63 ± 0.00 |
| *Hyphaene coriacea* Gaertn. | 123 | 0.16 ± 0.00 | 2.50 ± 0.00 | 0.63 ± 0.00 | 0.79 ± 0.36 | 0.20 ± 0.09 | 2.50 ± 0.00 |
| *Apodytes dimidiata* E.Mey. ex Arn. subsp. dimidiata | 139 | 0.16 ± 0.00 | 0.31 ± 0.00 | 0.20 ± 0.09 | 0.20 ± 0.09 | 0.63 ± 0.00 | 0.63 ± 0.00 |
| *Celtis africana* Burm.f. | 167 | 0.16 ± 0.00 | 0.20 ± 0.09 | 0.39 ± 0.18 | 0.31 ± 0.00 | 0.63 ± 0.00 | 2.50 ± 0.00 |
| *Harpephyllum caffrum* Bernh. ex Krauss | 176 | 0.16 ± 0.00 | 0.20 ± 0.09 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 |
| *Manilkara discolor* (Sond.) J.H.Hemsl. | 243 | 0.16 ± 0.00 | 0.20 ± 0.09 | 0.46 ± 0.22 | 0.79 ± 0.36 | 1.25 ± 0.00 | 0.63 ± 0.00 |
| *Lachnostylis hirta* (L.f.) Müll.Arg. | 325 | 0.16 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 | 1.57 ± 0.72 | 0.63 ± 0.00 |
| *Scolopia zeyheri* (Nees) Harv. | 334 | 0.16 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 | 0.50 ± 0.18 |
| *Mundulea sericea* (Willd.) A.Chev. | 352 | 0.16 ± 0.00 | 2.50 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 |
| *Gymnosporia nemorosa* (Eckl. & Zeyh.) Szyszyl. | 371 | 0.16 ± 0.00 | 2.50 ± 0.00 | 0.31 ± 0.00 | 2.50 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 |
| *Mystroxylon aethiopicum* (Thunb.) Loes. | 375 | 0.16 ± 0.00 | 2.50 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 |
| *Putterlickia retrospinosa* A.E.van Wyk & Mostert | 381 | 0.16 ± 0.00 | 0.39 ± 0.19 | 0.63 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 |
| *Androstachys johnsonii* Prain | 476 | 0.16 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 | 2.50 ± 0.00 | 0.31 ± 0.00 |
| *Hyperacanthus* sp. | 535 | 0.16 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 2.50 ± 0.00 | 2.50 ± 0.00 |
| *Ximenia caffra* Sond. | 574 | 0.16 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 2.50 ± 0.00 | 2.50 ± 0.00 |
| *Cryptocarya woodii* Engl. | 655 | 0.16 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 | 1.25 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 |
| *Phylica buxifolia* L. | 701 | 0.16 ± 0.00 | 2.50 ± 0.00 | 1.25 ± 0.00 | 0.63 ± 0.00 | 1.25 ± 0.00 | 0.31 ± 0.00 |
| *Protea lacticolor* Salisb. | 703 | 0.16 ± 0.00 | 1.25 ± 0.00 | 1.25 ± 0.00 | 1.25 ± 0.00 | 1.25 ± 0.00 | 0.50 ± 0.18 |
| *Flueggea virosa* (Roxb. ex Willd.) Voigt | 711 | 0.16 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 |

| Tree species | No. | Pathogen | | | | | |
|---|---|---|---|---|---|---|---|
| | | *Ef* | *Sa* | *Ec* | *Pa* | *Ca* | *Cn* |
| *Encephalartos paucidentatus* Stapf & Burtt Davy | 729 | 0.16 ± 0.00 | 2.50 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 | 2.50 ± 0.00 | 1.25 ± 0.00 |
| *Synadenium cupulare* (Boiss.) L.C.Wheeler | 742 | 0.16 ± 0.00 | 2.50 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 | 1.25 ± 0.00 | 2.50 ± 0.00 |
| *Heeria argentea* (Thunb.) Meisn. | 751 | 0.16 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 |

**Table C.2:** Acetone tree extracts with MIC's ≤ 0.16 mg/ml against *S. aureus* and MIC's > 0.16 mg/ml against the rest of the pathogens (No. = accession number in Phytomedicine Tree Database (PMDB); *Ef = E. faecalis; Sa = S. aureus; Ec = E. coli; Pa = P. aeruginosa; Ca = C. albicans; Cn = C. neoformans*).

| Tree species | No. | Pathogen | | | | | |
|---|---|---|---|---|---|---|---|
| | | *Sa* | *Ef* | *Ec* | *Pa* | *Ca* | *Cn* |
| *Capparis tomentosa* Lam. | 301 | 0.02 ± 0.00 | 0.20 ± 0.09 | 0.31 ± 0.00 | 0.63 ± 0.00 | 1.25 ± 0.00 | 0.79 ± 0.36 |
| *Mundulea sericea* (Willd.) A.Chev. | 473 | 0.04 ± 0.00 | 1.25 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 |
| *Erythrina caffra* Thunb. | 280 | 0.05 ± 0.02 | 0.63 ± 0.00 | 0.79 ± 0.36 | 0.63 ± 0.00 | 0.31 ± 0.00 | 0.40 ± 0.18 |
| *Allophylus dregeanus* (Sond.) De Winter | 4 | 0.08 ± 0.00 | 0.20 ± 0.09 | 0.20 ± 0.09 | 0.31 ± 0.00 | 0.31 ± 0.00 | 2.50 ± 0.00 |
| *Ficus chirindensis* C.C.Berg | 38 | 0.08 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.39 ± 0.18 | 0.63 ± 0.00 | 0.63 ± 0.00 |
| *Faurea rochetiana* (A.Rich.) Chiov. ex Pic.Serm. | 282 | 0.08 ± 0.00 | 1.25 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 |
| *Indigofera frutescens* L.f. | 675 | 0.08 ± 0.00 | 1.25 ± 0.00 | 0.31 ± 0.00 | 2.50 ± 0.00 | 1.25 ± 0.00 | 0.63 ± 0.00 |
| *Drypetes mossambicensis* Hutch. | 30 | 0.10 ± 0.05 | 0.39 ± 0.18 | 0.50 ± 0.54 | 0.31 ± 0.00 | 0.50 ± 0.18 | 0.31 ± 0.00 |
| *Ficus glumosa* Delile | 40 | 0.13 ± 0.05 | 0.39 ± 0.18 | 1.25 ± 0.00 | 0.31 ± 0.00 | 1.25 ± 0.00 | 0.63 ± 0.00 |
| *Searsia gueinzii* (Sond.) F.A.Barkley | 68 | 0.13 ± 0.05 | 0.39 ± 0.18 | 0.63 ± 0.00 | 1.00 ± 1.08 | 2.50 ± 0.00 | 0.50 ± 0.18 |
| *Peltophorum africanum* Sond. | 291 | 0.16 ± 0.00 | 0.20 ± 0.09 | 0.50 ± 0.54 | 0.63 ± 0.00 | 0.63 ± 0.00 | 1.25 ± 0.00 |
| *Breonadia salicina* (Vahl) Hepper & J.R.I.Wood | 1 | 0.16 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 2.50 ± 0.00 | 1.25 ± 0.00 |
| *Ficus craterostoma* Warb. ex Mildbr. & Burret | 39 | 0.16 ± 0.00 | 0.31 ± 0.00 | 0.25 ± 0.09 | 0.31 ± 0.00 | 1.57 ± 0.72 | 1.00 ± 0.35 |
| *Buxus natalensis* (Oliv.) Hutch. | 718 | 0.16 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 |
| *Podocarpus latifolius* (Thunb.) R.Br. ex Mirb. | 250 | 0.16 ± 0.00 | 0.39 ± 0.18 | 0.39 ± 0.18 | 0.79 ± 0.36 | 0.31 ± 0.00 | 0.63 ± 0.00 |
| *Antidesma venosum* E.Mey. ex Tul. | 5 | 0.16 ± 0.00 | 0.63 ± 0.00 | 0.79 ± 0.36 | 0.39 ± 0.18 | 2.50 ± 0.00 | 1.57 ± 1.08 |
| *Brachystegia spiciformis* Benth. | 7 | 0.16 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 | 0.25 ± 0.09 | 2.50 ± 0.00 | 1.25 ± 0.00 |
| *Carissa bispinosa* (L.) Desf. ex Brenan | 300 | 0.16 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 | 1.25 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 |
| *Chionanthus peglerae* (C.H.Wright) Stearn | 237 | 0.16 ± 0.00 | 1.25 ± 0.00 | 0.31 ± 0.00 | 1.25 ± 0.00 | 0.63 ± 0.00 | 0.39 ± 0.18 |
| *Rauvolfia caffra* Sond. | 331 | 0.16 ± 0.00 | 1.25 ± 0.00 | 1.25 ± 0.00 | 0.31 ± 0.00 | 2.50 ± 0.00 | 0.39 ± 0.18 |

**Table C.3:** Acetone tree extracts with MIC's ≤ 0.16 mg/ml against *E.coli* and MIC's > 0.16 mg/ml against the rest of the pathogens (No. = accession number in Phytomedicine Tree Database (PMDB); *Ef = E. faecalis; Sa = S. aureus; Ec = E. coli; Pa = P. aeruginosa; Ca = C. albicans; Cn = C. neoformans*).

| Tree species | No. | Pathogen | | | | | |
|---|---|---|---|---|---|---|---|
| | | *Ec* | *Ef* | *Sa* | *Pa* | *Ca* | *Cn* |
| *Bolusanthus speciosus* (Bolus) Harms | 580 | 0.02 ± 0.00 | 0.63 ± 0.00 | 1.25 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 |
| *Heteromorpha arborescens* (Spreng.) Cham. & Schltdl. | 491 | 0.02 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 | 1.25 ± 0.00 | na | na |
| *Rhigozum obovatum* Burch. | 513 | 0.08 ± 0.00 | 0.31 ± 0.00 | 1.25 ± 0.00 | 0.63 ± 0.00 | 0.50 ± 0.18 | 2.50 ± 0.00 |
| *Dichrostachys cinerea* (L.) Wight & Arn. | 276 | 0.10 ± 0.05 | 0.63 ± 0.00 | 2.50 ± 0.00 | 0.25 ± 0.09 | 2.50 ± 0.00 | 2.50 ± 0.00 |
| *Garcinia livingstonei* T.Anderson | 118 | 0.10 ± 0.05 | 0.31 ± 0.00 | 0.20 ± 0.09 | 1.25 ± 0.00 | 1.57 ± 0.72 | 2.50 ± 0.00 |
| *Catunaregam spinosa* (Thunb.) Tirveng. subsp. spinosa | 365 | 0.16 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 |
| *Chrysophyllum viridifolium* J.M.Wood & Franks | 168 | 0.16 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.25 ± 0.09 | 1.25 ± 0.00 | 2.50 ± 0.00 |
| *Coleonema pulchellum* I.Williams | 170 | 0.16 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.39 ± 0.18 | 0.31 ± 0.00 | 0.31 ± 0.00 |
| *Combretum bracteosum* (Hochst.) Engl. & Diels | 480 | 0.16 ± 0.00 | 1.25 ± 0.00 | 2.50 ± 0.00 | 0.31 ± 0.00 | 2.50 ± 0.00 | 2.50 ± 0.00 |
| *Combretum microphyllum* Klotzsch | 407 | 0.16 ± 0.00 | 2.50 ± 0.00 | 0.39 ± 0.18 | 0.63 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 |
| *Cordia caffra* Sond. | 591 | 0.16 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 |
| *Diospyros dichrophylla* (Gand.) De Winter | 173 | 0.16 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 |
| *Dissotis princeps* (Kunth) Triana | 660 | 0.16 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 | 2.50 ± 0.00 | 2.50 ± 0.00 |
| *Englerophytum magalismontanum* (Sond.) T.D.Penn. | 532 | 0.16 ± 0.00 | 2.50 ± 0.00 | 2.50 ± 0.00 | 0.31 ± 0.00 | 1.25 ± 0.00 | 1.25 ± 0.00 |
| *Euphorbia keithii* R.A.Dyer | 417 | 0.16 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 |
| *Ficus sur* Forssk. | 487 | 0.16 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 |
| *Filicium decipiens* (Wight & Arn.) Thwaites of Filicium decipiens Thwaites | 420 | 0.16 ± 0.00 | 1.25 ± 0.00 | 1.25 ± 0.00 | 0.31 ± 0.00 | 2.50 ± 0.00 | 0.20 ± 0.09 |
| *Heritiera littoralis* Aiton | 490 | 0.16 ± 0.00 | 0.31 ± 0.00 | 1.25 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 |
| *Hyaenanche globosa* (Gaertn.) Lamb. & Vahl | 177 | 0.16 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 |
| *Markhamia zanzibarica* (Bojer ex DC.) K.Schum. | 641 | 0.16 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 | 2.50 ± 0.00 | 2.50 ± 0.00 |
| *Myrianthus holstii* Engl. | 441 | 0.16 ± 0.00 | 0.63 ± 0.00 | 1.25 ± 0.00 | 1.25 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 |
| *Podocarpus elongatus* (Aiton) L'Hér. ex Pers. | 620 | 0.16 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 |
| *Rhigozum zambesiacum* Baker | 514 | 0.16 ± 0.00 | 0.31 ± 0.00 | 2.50 ± 0.00 | 0.31 ± 0.00 | 1.25 ± 0.00 | 2.50 ± 0.00 |
| *Schefflera umbellifera* (Sond.) Baill. | 515 | 0.16 ± 0.00 | 0.63 ± 0.00 | 1.25 ± 0.00 | 0.31 ± 0.00 | 2.50 ± 0.00 | 0.63 ± 0.00 |
| *Syzygium cordatum* Hochst. ex C.Krauss subsp. cordatum | 519 | 0.16 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 | 2.50 ± 0.00 | 2.50 ± 0.00 |
| *Vangueria infausta* Burch. | 573 | 0.16 ± 0.00 | 0.31 ± 0.00 | 1.25 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 |
| *Ximenia americana* L. | 526 | 0.16 ± 0.00 | 0.31 ± 0.00 | 1.25 ± 0.00 | 0.31 ± 0.00 | 1.25 ± 0.00 | 0.63 ± 0.00 |

**Table C.4:** Acetone tree extracts with MIC's ≤ 0.16 mg/ml against *P. aeruginosa* and MIC's > 0.16 mg/ml against the rest of the pathogens (No. = accession number in Phytomedicine Tree Database (PMDB); *Ef = E. faecalis; Sa = S. aureus; Ec = E. coli; Pa = P. aeruginosa; Ca = C. albicans; Cn = C. neoformans*).

| Tree species | No | Pathogen | | | | | |
|---|---|---|---|---|---|---|---|
| | | *Pa* | *Ef* | *Sa* | *Ec* | *Ca* | *Cn* |
| *Englerophytum magalismontanum* (Sond.) T.D.Penn. | 561 | 0.06 ± 0.03 | 1.25 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 | na | na |
| *Searsia chirindensis* (Baker f.) Moffett | 627 | 0.06 ± 0.03 | 0.63 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 | 1.25 ± 0.00 |
| *Euclea crispa* (Thunb.) Gürke | 637 | 0.08 ± 0.00 | 0.31 ± 0.00 | 1.25 ± 0.00 | 0.31 ± 0.00 | 1.25 ± 0.00 | 0.63 ± 0.00 |
| *Flueggea virosa* (Roxb. ex Willd.) Voigt subsp. virosa | 241 | 0.08 ± 0.00 | 1.25 ± 0.00 | 1.57 ± 0.72 | 0.25 ± 0.09 | 1.25 ± 0.00 | 2.50 ± 0.00 |
| *Combretum molle* R.Br. ex G.Don | 19 | 0.10 ± 0.05 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.20 ± 0.09 | na | na |
| *Clausena anisata* (Willd.) Hook.f. ex Benth. | 317 | 0.13 ± 0.05 | 0.31 ± 0.00 | 0.79 ± 0.36 | 0.63 ± 0.00 | 1.25 ± 0.00 | 0.63 ± 0.00 |
| *Toddalia asiatica* (L.) Lam. | 467 | 0.13 ± 0.05 | 1.25 ± 0.00 | 0.63 ± 0.00 | 0.20 ± 0.09 | 0.63 ± 0.00 | 0.63 ± 0.00 |
| *Androstachys johnsonii* Prain | 393 | 0.16 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 | 1.25 ± 0.00 | 1.25 ± 0.00 |
| *Annona senegalensis* Pers. | 137 | 0.16 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.20 ± 0.09 | 0.63 ± 0.00 | 0.63 ± 0.00 |
| *Chionanthus foveolatus* (E.Mey.) Stearn | 315 | 0.16 ± 0.00 | 0.79 ± 0.36 | 0.63 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 | 2.50 ± 0.00 |
| *Dovyalis rhamnoides* (Burch. ex DC.) Burch. & Harv. | 28 | 0.16 ± 0.00 | 0.25 ± 0.09 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.80 ± 0.35 | 0.25 ± 0.09 |
| *Elephantorrhiza burkei* Benth. | 560 | 0.16 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 | na | na |
| *Ficus bizanae* Hutch. & Burtt Davy | 36 | 0.16 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 | 0.50 ± 0.54 | 0.31 ± 0.00 | 0.25 ± 0.09 |
| *Ficus ingens* (Miq.) Miq. | 41 | 0.16 ± 0.00 | 0.50 ± 0.54 | 0.31 ± 0.00 | 0.50 ± 0.54 | 1.57 ± 0.72 | 1.00 ± 0.35 |
| *Heywoodia lucens* Sim | 50 | 0.16 ± 0.00 | 1.25 ± 0.00 | 0.31 ± 0.00 | 1.25 ± 0.00 | 2.50 ± 0.00 | 0.63 ± 0.00 |
| *Lippia javanica* (Burm.f.) Spreng. | 611 | 0.16 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 | na | na |
| *Podocarpus latifolius* (Thunb.) R.Br. ex Mirb. | 506 | 0.16 ± 0.00 | 0.31 ± 0.00 | 1.25 ± 0.00 | 0.31 ± 0.00 | 1.25 ± 0.00 | 0.63 ± 0.00 |
| *Protea rubropilosa* Beard | 624 | 0.16 ± 0.00 | 0.63 ± 0.00 | 1.25 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 1.25 ± 0.00 |
| *Smelophyllum capense* (Sond.) Radlk. | 712 | 0.16 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 |
| *Synsepalum kassneri* (Engl.) T.D.Penn | 465 | 0.16 ± 0.00 | 0.31 ± 0.00 | 1.25 ± 0.00 | 0.31 ± 0.00 | 2.50 ± 0.00 | 1.25 ± 0.00 |
| *Vangueria infausta* Burch. subsp. infausta | 90 | 0.16 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 |

**Table C.5:** Acetone tree extracts with MIC's ≤ 0.16 mg/ml against *C. albicans* and MIC's > 0.16 mg/ml against the rest of the pathogens (No. = accession number in Phytomedicine Tree Database (PMDB); *Ef = E. faecalis; Sa = S. aureus; Ec = E. coli; Pa = P. aeruginosa; Ca = C. albicans; Cn = C. neoformans*).

| Tree species | No. | Pathogen | | | | | |
|---|---|---|---|---|---|---|---|
| | | *Ca* | *Ef* | *Sa* | *Ec* | *Pa* | *Cn* |
| *Kigelia africana* (Lam.) Benth. | 124 | 0.04 ± 0.00 | 2.50 ± 0.00 | 0.79 ± 0.36 | 0.79 ± 0.36 | 1.25 ± 0.00 | 2.50 ± 0.00 |
| *Zanthoxylum leprieurii* Guill. & Perr. | 229 | 0.04 ± 0.00 | 2.50 ± 0.00 | 2.50 ± 0.00 | 0.31 ± 0.00 | 1.25 ± 0.00 | 1.25 ± 0.00 |
| *Uapaca nitida* Müll.Arg | 88 | 0.05 ± 0.02 | 0.31 ± 0.00 | 0.31 ± 0.00 | 2.50 ± 0.00 | 0.63 ± 0.00 | 1.57 ± 1.08 |
| *Vepris reflexa* I.Verd. | 91 | 0.05 ± 0.02 | 0.63 ± 0.00 | 0.31 ± 0.00 | 0.50 ± 0.54 | 0.50 ± 0.18 | 1.25 ± 0.00 |
| *Vitellariopsis marginata* (N.E.Br.) Aubrév. | 92 | 0.05 ± 0.02 | 2.50 ± 0.00 | 1.57 ± 0.72 | 2.50 ± 0.00 | 2.50 ± 0.00 | 1.25 ± 0.00 |
| *Friesodielsia obovata* (Benth.) Verdc. | 117 | 0.06 ± 0.03 | 1.98 ± 0.72 | 2.50 ± 0.00 | 0.99 ± 0.36 | 1.98 ± 0.72 | 2.50 ± 0.00 |
| *Vangueria infausta* Burch. subsp. infausta | 89 | 0.06 ± 0.03 | 0.50 ± 0.54 | 0.39 ± 0.18 | 0.50 ± 0.54 | 0.99 ± 0.36 | 1.00 ± 0.35 |
| *Curtisia dentata* (Burm.f.) C.A.Sm. | 319 | 0.08 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 1.25 ± 0.00 | 0.31 ± 0.00 |
| *Euryops linearis* Harv. | 669 | 0.08 ± 0.00 | 0.63 ± 0.00 | 1.25 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 |
| *Kirkia acuminata* Oliv. | 125 | 0.08 ± 0.00 | 0.79 ± 0.36 | 1.98 ± 0.72 | 1.25 ± 0.00 | 0.99 ± 0.36 | 2.50 ± 0.00 |
| *Steganotaenia araliacea* Hochst. | 724 | 0.08 ± 0.00 | 0.31 ± 0.00 | 1.25 ± 0.00 | 1.25 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 |
| *Strombosia scheffleri* Engl. | 464 | 0.08 ± 0.00 | 1.25 ± 0.00 | 2.50 ± 0.00 | 1.25 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 |
| *Xylotheca kraussiana* Hochst. | 228 | 0.08 ± 0.00 | 2.50 ± 0.00 | 2.50 ± 0.00 | 0.39 ± 0.18 | 0.99 ± 0.36 | 0.39 ± 0.18 |
| *Sclerocarya birrea* (A.Rich.) Hochst. subsp. *caffra* (Sond.) Kokwaro | 130 | 0.10 ± 0.05 | 2.50 ± 0.00 | 2.50 ± 0.00 | 2.50 ± 0.00 | 2.50 ± 0.00 | 2.50 ± 0.00 |
| *Sideroxylon inerme* L. subsp. *inerme* | 131 | 0.10 ± 0.05 | 1.25 ± 0.00 | 2.50 ± 0.00 | 1.00 ± 1.08 | 0.99 ± 0.36 | 2.50 ± 0.00 |
| *Prunus africana* (Hook.f.) Kalkman | 128 | 0.13 ± 0.05 | 0.31 ± 0.00 | 2.50 ± 0.00 | 0.63 ± 0.00 | 0.99 ± 0.36 | 2.50 ± 0.00 |
| *Aphloia theiformis* (Vahl) Benn. | 231 | 0.16 ± 0.00 | 2.50 ± 0.00 | 2.50 ± 0.00 | 0.31 ± 0.00 | 2.50 ± 0.00 | 1.25 ± 0.00 |
| *Blighia unijugata* Baker | 234 | 0.16 ± 0.00 | 2.50 ± 0.00 | 2.50 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.39 ± 0.18 |
| *Clausena anisata* (Willd.) Hook.f. ex Benth. | 590 | 0.16 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 |
| *Cyphostemma juttae* (Dinter & Gilg) Desc. | 659 | 0.16 ± 0.00 | 0.31 ± 0.00 | 1.25 ± 0.00 | 2.50 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 |
| *Englerodaphne pilosa* Burtt Davy | 664 | 0.16 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 |
| *Erythrococca menyharthii* (Pax) Prain | 486 | 0.16 ± 0.00 | 0.31 ± 0.00 | 2.50 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 | 1.25 ± 0.00 |
| *Greyia flanaganii* Bolus | 673 | 0.16 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 |
| *Haplocoelum foliolosum* (Hiern) Bullock | 424 | 0.16 ± 0.00 | 0.63 ± 0.00 | 1.25 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 |
| *Heteromorpha arborescens* (Spreng.) Cham. & Schltdl. | 735 | 0.16 ± 0.00 | na | 1.25 ± 0.00 | na | 0.31 ± 0.00 | 0.31 ± 0.00 |
| *Hymenodictyon parvifolium Oliv. subsp. parvifolium* | 494 | 0.16 ± 0.00 | 1.25 ± 0.00 | 1.25 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 |
| *Hymenodictyon parvifolium* Oliv. subsp. parvifolium | 121 | 0.16 ± 0.00 | 2.50 ± 0.00 | 2.50 ± 0.00 | 2.50 ± 0.00 | 2.50 ± 0.00 | 2.50 ± 0.00 |
| *Jasminum multipartitum* Hochst. | 676 | 0.16 ± 0.00 | 0.31 ± 0.00 | 1.25 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 |

| Tree species | No. | Pathogen | | | | | |
|---|---|---|---|---|---|---|---|
| | | *Ca* | *Ef* | *Sa* | *Ec* | *Pa* | *Cn* |
| *Margaritaria discoidea* (Baill.) G.L. Webster | 438 | 0.16 ± 0.00 | 0.31 ± 0.00 | 2.50 ± 0.00 | 1.25 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 |
| *Metalasia muricata* (L.) D.Don | 686 | 0.16 ± 0.00 | 0.63 ± 0.00 | 2.50 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 |
| *Mimetes cucullatus* (L.) R.Br. | 689 | 0.16 ± 0.00 | 0.63 ± 0.00 | 1.25 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 |
| *Mimetes cucullatus* (L.) R.Br. | 757 | 0.16 ± 0.00 | 1.25 ± 0.00 | 0.63 ± 0.00 | 1.25 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 |
| *Phyllanthus engleri* Pax | 447 | 0.16 ± 0.00 | 2.50 ± 0.00 | 2.50 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 |
| *Pseudolachnostylis maprouneifolia* Pax | 452 | 0.16 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 | na | 0.63 ± 0.00 |
| *Strophanthus speciosus* (Ward & Harv.) Reber | 256 | 0.16 ± 0.00 | 2.50 ± 0.00 | 2.50 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 |
| *Vepris reflexa* I.Verd. | 259 | 0.16 ± 0.00 | 1.57 ± 1.08 | 0.63 ± 0.00 | 0.50 ± 0.54 | 2.50 ± 0.00 | 1.25 ± 0.00 |
| *Vitellariopsis marginata* (N.E.Br.) Aubrév. | 260 | 0.16 ± 0.00 | 0.99 ± 1.08 | 0.31 ± 0.00 | 0.12 ± 0.05 | 0.25 ± 0.09 | 0.63 ± 0.00 |

**Table C.6:** Acetone tree extracts with MIC's ≤ 0.16 mg/ml against *C. neoformans* and MIC's > 0.16 mg/ml against the rest of the pathogens (No. = accession number in Phytomedicine Tree Database (PMDB); *Ef = E. faecalis; Sa = S. aureus; Ec = E. coli; Pa = P. aeruginosa; Ca = C. albicans; Cn = C. neoformans*).

| Tree species | No. | *Pathogen* | | | | | |
|---|---|---|---|---|---|---|---|
| | | *Cn* | *Ef* | *Sa* | *Ec* | *Pa* | *Ca* |
| *Keetia gueinzii* (Sond.) Bridson | 432 | 0.04 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.25 ± 0.09 |
| *Strelitzia caudata* R.A.Dyer | 461 | 0.04 ± 0.00 | 1.25 ± 0.00 | 2.50 ± 0.00 | 1.25 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 |
| *Polyscias fulva* (Hiern) Harms | 450 | 0.06 ± 0.02 | 0.63 ± 0.00 | 2.50 ± 0.00 | 0.31 ± 0.00 | 1.25 ± 0.00 | 0.31 ± 0.00 |
| *Alsophila dregei* (Kunze) R.M.Tryon | 658 | 0.08 ± 0.00 | 1.25 ± 0.00 | 2.50 ± 0.00 | 1.25 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 |
| *Berzelia lanuginosa* (L.) Brongn. | 748 | 0.08 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 1.25 ± 0.00 | 0.63 ± 0.00 | 1.25 ± 0.00 |
| *Englerophytum magalismontanum* (Sond.) T.D.Penn. | 600 | 0.08 ± 0.00 | 0.50 ± 0.54 | 1.25 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 | 2.50 ± 0.00 |
| *Euclea undulata* Thunb. | 415 | 0.08 ± 0.00 | 0.63 ± 0.00 | 0.79 ± 0.36 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 |
| *Euphorbia tirucalli* L. | 341 | 0.08 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 | 0.50 ± 0.18 |
| *Euryops tysonii* E.Phillips | 670 | 0.08 ± 0.00 | 0.63 ± 0.00 | 1.25 ± 0.00 | 0.63 ± 0.00 | 1.25 ± 0.00 | 0.50 ± 0.18 |
| *Kirkia wilmsii* Engl. | 179 | 0.08 ± 0.00 | 0.22 ± 0.11 | 1.57 ± 0.88 | 0.44 ± 0.23 | 2.50 ± 0.00 | 0.25 ± 0.09 |
| *Leucadendron cryptocephalum* Guthrie | 677 | 0.08 ± 0.00 | 1.25 ± 0.00 | 0.63 ± 0.00 | 1.25 ± 0.00 | 1.25 ± 0.00 | 0.63 ± 0.00 |
| *Leucadendron tinctum* I.Williams | 681 | 0.08 ± 0.00 | 0.31 ± 0.00 | 1.25 ± 0.00 | 1.25 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 |
| *Lippia javanica* (Burm.f.) Spreng. | 435 | 0.08 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 | 1.25 ± 0.00 | 0.31 ± 0.00 |
| *Mackaya bella* Harv. | 181 | 0.08 ± 0.00 | 0.11 ± 0.06 | 0.22 ± 0.11 | 0.22 ± 0.11 | 0.22 ± 0.11 | 0.31 ± 0.00 |
| *Metrosideros angustifolia* (L.) Sm. | 688 | 0.08 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 |
| *Monanthotaxis caffra* (Sond.) Verdc. | 690 | 0.08 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 |
| *Oncoba spinosa* Forssk. | 740 | 0.08 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 |
| *Oxyanthus pyriformis* (Hochst.) Skeels | 445 | 0.08 ± 0.00 | 0.79 ± 0.36 | 1.25 ± 0.00 | 0.63 ± 0.00 | 0.79 ± 0.36 | 0.31 ± 0.00 |
| *Pavonia columella* Cav. | 698 | 0.08 ± 0.00 | 1.25 ± 0.00 | 1.25 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 |
| *Trema orientalis* (L.) Blume | 469 | 0.08 ± 0.00 | 0.31 ± 0.00 | 1.25 ± 0.00 | 1.25 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 |
| *Ziziphus mucronata* Willd. | 576 | 0.08 ± 0.00 | 0.31 ± 0.00 | 1.25 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 | 1.25 ± 0.00 |
| *Craibia zimmermannii* (Harms) Dunn | 209 | 0.13 ± 0.05 | 0.25 ± 0.09 | 0.63 ± 0.00 | 0.20 ± 0.09 | 1.25 ± 0.00 | 0.31 ± 0.00 |
| *Adansonia digitata* L. | 97 | 0.16 ± 0.00 | 0.50 ± 0.54 | 2.50 ± 0.00 | 0.79 ± 0.36 | 1.98 ± 0.72 | 1.25 ± 0.00 |
| *Afzelia quanzensis* Welw. | 195 | 0.16 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 | 1.25 ± 0.00 | 1.25 ± 0.00 | 0.25 ± 0.09 |
| *Allophylus natalensis* (Sond.) De Winter | 644 | 0.16 ± 0.00 | 0.63 ± 0.00 | 1.25 ± 0.00 | 0.63 ± 0.00 | 1.25 ± 0.00 | 0.31 ± 0.00 |
| *Brachystegia spiciformis* Benth. | 400 | 0.16 ± 0.00 | 1.25 ± 0.00 | 2.50 ± 0.00 | 2.50 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 |
| *Caesalpinia rostrata* N.E.Br. | 472 | 0.16 ± 0.00 | 2.50 ± 0.00 | 1.25 ± 0.00 | 2.50 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 |
| *Canthium armatum* (K.Schum.) Lantz | 377 | 0.16 ± 0.00 | 1.25 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 |
| *Cephalanthus natalensis* Oliv. | 651 | 0.16 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 | 0.39 ± 0.18 |
| *Chionanthus battiscombei* (Hutch.) Stearn | 653 | 0.16 ± 0.00 | 0.63 ± 0.00 | 1.25 ± 0.00 | 0.31 ± 0.00 | 1.25 ± 0.00 | 0.63 ± 0.00 |
| *Crotalaria monteiroi* Taub. ex Baker f. | 409 | 0.16 ± 0.00 | 1.25 ± 0.00 | 1.25 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 |
| *Drypetes gerrardii* Hutch. | 63 | 0.16 ± 0.00 | 0.50 ± 0.54 | 2.50 ± 0.00 | 0.99 ± 0.36 | 1.25 ± 0.00 | 0.99 ± 0.36 |
| *Entandrophragma caudatum* (Sprague) Sprague | 368 | 0.16 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 |

| Tree species | No. | *Pathogen* | | | | | |
|---|---|---|---|---|---|---|---|
| | | *Cn* | *Ef* | *Sa* | *Ec* | *Pa* | *Ca* |
| *Erythrophysa transvaalensis* I.Verd. | 667 | 0.16 ± 0.00 | 0.39 ± 0.18 | 1.25 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 |
| *Faurea macnaughtonii* E.Phillips | 671 | 0.16 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 |
| *Kirkia wilmsii* Engl. | 608 | 0.16 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 1.25 ± 0.00 | 0.31 ± 0.00 |
| *Leucadendron salicifolium* (Salisb.) I.Williams | 679 | 0.16 ± 0.00 | 1.25 ± 0.00 | 2.50 ± 0.00 | 2.50 ± 0.00 | 2.50 ± 0.00 | 0.31 ± 0.00 |
| *Lovoa swynnertonii* Baker f. | 436 | 0.16 ± 0.00 | 2.50 ± 0.00 | 2.50 ± 0.00 | 2.50 ± 0.00 | 0.63 ± 0.00 | 1.25 ± 0.00 |
| *Ochna gamostigmata* Du Toit | 692 | 0.16 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 |
| *Pavetta schumanniana* F.Hoffm. ex K.Schum. | 504 | 0.16 ± 0.00 | 0.31 ± 0.00 | 0.17 ± 0.10 | 0.31 ± 0.00 | 0.25 ± 0.09 | 0.31 ± 0.00 |
| *Podranea brycei* Sprague | 449 | 0.16 ± 0.00 | 1.25 ± 0.00 | 0.63 ± 0.00 | 0.39 ± 0.18 | 2.50 ± 0.00 | 0.63 ± 0.00 |
| *Protea caffra* Meisn. subsp. *caffra* | 569 | 0.16 ± 0.00 | 0.63 ± 0.00 | 2.50 ± 0.00 | 1.25 ± 0.00 | 0.63 ± 0.00 | 1.25 ± 0.00 |
| *Protea cynaroides* (L.) L. | 702 | 0.16 ± 0.00 | 0.31 ± 0.00 | 1.25 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 | 0.63 ± 0.00 |
| *Raphia australis* Oberm. & Strey | 453 | 0.16 ± 0.00 | 1.25 ± 0.00 | 0.63 ± 0.00 | 2.50 ± 0.00 | 0.50 ± 0.18 | 0.31 ± 0.00 |
| *Sideroxylon inerme* L. subsp. *inerme* | 360 | 0.16 ± 0.00 | 0.31 ± 0.00 | 1.25 ± 0.00 | 0.63 ± 0.00 | 0.31 ± 0.00 | 1.25 ± 0.00 |
| *Spirostachys africana* Sond. | 458 | 0.16 ± 0.00 | 1.25 ± 0.00 | 0.63 ± 0.00 | 1.25 ± 0.00 | 0.63 ± 0.00 | 1.25 ± 0.00 |
| *Strelitzia juncea* Link | 462 | 0.16 ± 0.00 | 1.25 ± 0.00 | 2.50 ± 0.00 | 1.25 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 |
| *Strophanthus* DC. sp | 190 | 0.16 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.22 ± 0.11 | 1.25 ± 0.00 |
| *Tecomaria capensis* (Thunb.) Lindl. | 362 | 0.16 ± 0.00 | 0.63 ± 0.00 | 2.50 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 | 0.31 ± 0.00 |
| *Trema orientalis* (L.) Blume | 257 | 0.16 ± 0.00 | 0.39 ± 0.18 | 2.50 ± 0.00 | 0.31 ± 0.00 | 0.39 ± 0.18 | 0.31 ± 0.00 |
| *Vitex harveyana* H.Pearson | 227 | 0.16 ± 0.00 | 1.98 ± 0.72 | 0.99 ± 0.36 | 0.99 ± 0.36 | 0.79 ± 0.36 | 0.31 ± 0.00 |
| *Zanthoxylum capense* (Thunb.) Harv | 96 | 0.16 ± 0.00 | 2.50 ± 0.00 | 2.50 ± 0.00 | 2.50 ± 0.00 | 2.50 ± 0.00 | 2.50 ± 0.00 |
| *Ziziphus mucronata* Willd. | 345 | 0.16 ± 0.00 | 0.25 ± 0.09 | 0.31 ± 0.00 | 0.50 ± 0.54 | 0.63 ± 0.00 | 0.31 ± 0.00 |