ETHNOPHARMACOLOGY AND PHYTOCHEMISTRY OF SOME SELECTED MEDICINAL PLANTS IN KWAZULU NATAL

BY

SIYABULELA S.B.N. NTULI, SNR

THESIS SUBMITTED IN FULFILMENT FOR THE REQUIREMENTS OF THE MASTER OF SCIENCE DEGREE IN THE FACULTY OF SCIENCE AND AGRICULTURE, SCHOOL OF BIOLOGICAL AND CONSERVATION SCIENCES, DISCIPLINE OF BOTANY IN THE UNIVERSITY OF KWAZULU NATAL, R.S.A.

2006

SUPERVISOR : PROF. G. NAIDOO

TABLE OF CONTENTS

PREFACE

This thesis contains experimental work that was carried out in the Disciplines of Botany and Chemistry, University of KwaZulu-Natal, Westville campus, under the supervision of Professors G. Naidoo and F.O. Shode

These studies represent original work by the author and have not been submitted in any form to another University. I acknowledge that I have consulted many publications in compiling this work and the references are all listed.

NTULI, SIYABULELA SBONISO BRIGHTSON NOEL

ABSTRACT

In this ethnopharmacological study to isolate, purify, identify and test crude and isolated compounds from organic and aqueous extracts from stem and leaves of *Protorhus longifolia* and *Sclerocarya birrea*, stem bark of *Hibiscus cannabinus* and *Heteropyxis natalensis*, leaves of *Acokanthera venenata*, *Carissa marcrocarpa* and *Syzygium cordatum*, seeds of *Chiononthus foveolatus* and calyces of *Hibiscus sabdariffa* were tested against seven pathogenic microorganisms which included six bacterial species [*Klebsiella pneumoniae* (ATCC 12265), *Bacillus cereus* (ATCC 11778), *Salmonella typhimurium* (ATCC 13311), *Pseudomonas aeruginosa*(ATCC 27853), *Escherichia coli* (ATCC 35219), *Staphylococcus aureus* (ATCC 29213)) and fungal yeast *Candida albicans* (ATCC 10231)]. Both organic and aqueous extracts from these medicinal plants exhibited antimicrobial properties against one or more microorganisms.

The extracts of stem bark and leaves were tested for antimicrobial properties. Crude extracts that showed the highest activity were analysed through chromatographic and spectroscopic techniques to isolate, purify and characterise their active components. The highly active antimicrobial extracts were further tested for antimicrobial activity. Chromatographic (TLC and CC) spectroscopic (IR, ^{13}C, $^{1}HNMR$) analyses of *Syzygium cordatum* leaf extract in ethylacetate revealed the presence of C-30 triterpenes, ursolic (3β-hydroxyurs-12-en-28-oic acid) (UA) and oleanolic (3-hydroxylean-12-en-28-oic acid) (OA) acids; a mixture of methyl maslinate (2α, 3β-dihydroxyolean-12-en-28-oic acid methyl ester) (MM) and methyl corosolate (MC).

Analyses of *Protorhus longifolia* leaf extract in hexane and ethylacetate revealed the presence of the alkaloid lupeol (lup-20(29)-en-3β-ol), lupenone [lup - 20 (29) - en - 3 - one or lup - 20 (30) - en - 3 – one], lupinine (octahydro-2H-quinolizine-1-methanol), lupulon (3,5-dihydroxy-2,6,6-tris(3-methyl-2-butenyl)-4-(3-methyl-1-oxobutyl)-2,4-yclohexadien-1-one) or (3,5-dihdroxy-4-isovaleryl-2,6,6-tris(3-methyl-2-butenyl)-2,4-cyclohexadien-1-one) and luteolin [(2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4H-1-benzopyran-4-one), 3',4',5,7-tetrahydroxyflavone or 5,7,3',4'-tetrahydroxyflavone] and other compounds to be characterised in future studies.

Sclerocarya birrea bark extract in methanol was found to contain mixtures of compounds that could not be separated due to solvent complications. *Heteropyxis natalensis* stem bark in ethylacetate gave betunilic acid (3β-hydroxy-20(29)-lupaene-28-oic acid) as a major compound.

ACKNOWLEDGEMENTS

Firstly I would like to thank Prof. G. Naidoo (my supervisor) for his constant assistance and encouragement throughout the project.

I'd also like to thank Prof. F.O. Shode (my co-supervisor) for his assistance in this project and more especially the chemistry side.

I'd like to express my gratitude to the following:

The NRF for its funding via grant-holder bursary from Prof. Naidoo,

The staff from the department of Botany and Microbiology in the Westville campus, UKZN

The staff from the Biotechnology department of Durban Institute of Technology in the Sultan campus,

The staff from the Virology department in the Inkosi Albert Luthuli hospital,

Dillip from the Durban campus of UKZN Chemistry department who helped with the NMR and MS spectra.

I'd also like to thank all my friends and family for standing by me and believing in me.

I'd also like to thank Dr David Katerere (of MRC, Cape Town) for his constructive criticism in the writing of this thesis

Lastly I'd like to express my sincere gratitude to my late mother, for everything she could do in her power to make me what I am today, may her soul rest in peace.

This thesis is dedicated to my late mother Ntombikayise Goodness Ntuli.

LIST OF ABBREVIATIONS

AA	Arjunolic acid
AV	*Acokanthera venenata*
BA	Betulinic acid
BC	*Bacillus cereus*
CA	*Candida albicans*
$CHCL_3$	Chloroform
CM	*Carissa macrocarpa*
^{13}CNMR	Carbon Nuclear Magnetic Resonance
COSY	Correlated Spectroscopy
DCM	Dichloromethane
DEPT	Destortionless Enhancement by Polarization Transfer
DMSO	Dimethylsulfoxide
EA	Ellagic acid
EC	*Escherichia coli*
EtOAc	Ethylacetate
GA	Gallic acid
HC	*Hibiscus cannabinus*
HEX	Hexane
HEX:EA	Heaxane:Ethylacetate
HN	*Hibiscus natalensis*
^{1}H-^{1}HNMR	Proton Nuclear Magnetic Resonance
HS	*Hibiscus sabdariffa*
IR	Infra Red
KP	*Klebsiella pneumoniae*
MC	Methyl corosolate
MeOH	Methanol
MM	Methyl maslinate
OA	Oleanolic acid
PA	*Pseudomonas aeruginosa*
PL	*Protorhus longifolia*
SA	*Staphylococcus aureus*
SB	*Sclerocarya birrea*
SC	*Syzygium cordatum*

ST	*Salmonella typhimurium*
β-ST	β-sitosterol
UA	Ursolic acid

CHAPTER 1

1. INTRODUCTION

1.1 Use of medicinal plants

Medicinal plants are an important aspect of the daily lives of many people and an important part of the South African cultural heritage. South Africa is rich in cultural diversity that reflects in both formal and informal systems of medicine presently practiced throughout the country. The informal oral-traditional medical systems of the Khoi-San, the Nguni and the Sotho-speaking people have not yet been systematized, and have been passed on by word of mouth from generation to generation (van Wyk *et al.*, 1997). Herbal medicines are traded all over southern Africa and some have also found market places abroad. However, scientific investigation and information on their therapeutic potential are limited. With the possibility of traditional practitioners being integrated into the state health delivery system, there is an urgent need to systematically and empirically evaluate plants used in herbal medicine. Such research could also lead to new drug discovery or advance the use of indigenous herbal medicines for orthodox treatment (Lin *et al.*, 1999).

Medicinal plants have become the focus of intense study recently in terms of conservation and to whether their traditional uses are supported by actual pharmacological effects or merely based on folklore (Cunningham, 1988; Locher *et al.*, 1995; Jager *et al.*, 1996; Williams, 1996). Plants are a primary source of all medicines and they still continue to provide new remedies. Natural products and their derivatives represent more than 50% of all chemical drugs while higher plants contribute up to 25% of the total (Kinghorn and Balandrin, 1993). Well-known examples of plant-derived medicines include the following: quinine, morphine, codeine, aspirin, atropine, reserpine and cocaine. Recently, important anti-cancer drugs such as taxol and vincristine have been developed. In South Africa and elsewhere, there is a growing interest in natural and herbal medicines as a source of new commercial products (Robbers *et al.*, 1996; van Wyk *et al.*, 1997; Kaufman *et al.*, 1998). This part of natural health science dealing with the use of traditional medicinal plants is known as pharmacognosy, which has developed rapidly due to

improvements in technology of isolation processes which include the development of chromatographic techniques such as column, paper, thin layer, gas-liquid, high performance and droplet counter current. These methods have allowed the rapid isolation of substances previously difficult to obtain by classical procedures (Kaufman *et al.*, 1998). The development of new spectroscopic techniques opened up whole new avenues in the field of herbal medicine. Simultaneous advancement in other fields such as chemistry, biochemistry, biosynthesis and pharmacology have also developed pharmacognosy (Puniani, 2003). Advancement of synthetic organic chemistry has led to the synthesis of most active constituents from medicinal plants. Over the past decade, research in ethnobotany has intensified worldwide, thus the World Health Organization in 2001 recognized the contribution of herbal medicine to poor communities (Good, 1987). Recently, ethnobotanical research has shifted from studying medicinal plants to the screening and documentation of their chemical constituents. This screening has concentrated largely on the flora of the developing world, but with some emphasis on plants of the tropics. This search for drugs, however, concentrated largely on lower plants, fungi and bacteria. Less research was focused on higher plants although identified plant compounds such as sanguinarine, emetine and berberine still find specialized uses (Mitscher *et al.*, 1987).

The birth of the pharmaceutical industry in the late fifteenth century, and its growth especially since the mid twentieth century, have widened the search for natural products possessing antibacterial, antiviral, cytotoxic, fungicidal, insecticidal or pesticidal activity, leading to the development of natural product chemistry (Koskinen, 1995; Lesney, 2000). The isolation of pure components from complex extracts was quite difficult to achieve since chromatographic methods were still in their early stages of development. Elucidation of chemical structures were difficult due to the absence of sophisticated techniques such as nuclear magnetic resonance (NMR), mass spectrometry (MS) and single crystal x-ray diffraction (XRD), with structure determination purely based on chemical degradation studies (Simonsen & Ross, 1957; Carlisle & Ladd, 1966). Such studies required substantial amounts of material, very careful analysis and interpretation of results. Structural determination of natural products required many years of dedicated work (Koskinen, 1995). Today, such structure determinations are easily achieved.

This field is now much more interdisciplinary than before, and with the introduction of pharmacogenomics, the potential of natural products continues to grow (Henry, 2000). Many phytochemical studies make use of accumulated ethnobotanical knowledge of traditional or empirical local practitioners. Ten to fifteen percent of approximately 250 000 species (and the number keeps growing) of higher plants have been systematically investigated for the presence of bioactive phytochemicals (Cragg & Newmann, 1997) and the world of plants is still virtually an untapped reservoir of novel bioactive agents. It has been estimated that at least 119 drugs derived from 90 different plant species are currently used in one or more countries, with 77% of these being derived from plants used in herbal medicine (Cragg & Newmann, 1997). Plants provide a cheaper alternative treatment for native people who cannot afford, or who have no access to commercially available medicines.

1.2 Aims and objectives of this study

In this study, plant species widely used medicinally for different ailments were selected on the basis of their usage in traditional medicine (Watt & Breyer-Brandwijk, 1962; Gelfand *et al.*, 1985; Pujol, 1990; Mander *et al.*, 1995; Shode *et al.*, 2001). This study investigates nine medicinal plants, three of which (given under the section of the review of the plants species used) have been studied previously by the author at honours level and the other six (with their extracts) were donated by the chemistry department to the author for antimicrobial, chromatographic and spectroscopic analyses. All nine species were, *Acokanthera venenata* (G. Don.) *Carissa macrocarpa* (Eckl.) A. DC., both from the family Apocynaceae; *Chionanthus foveolatus* (E. Mey.), from Oleaceae; *Hibiscus cannabinus* (L.); *Heteropyxis natalensis* (Harvey) and *Hibiscus sabdariffa* (L.), from Malvaceae; *Protorhus longifolia* (Bernh.) Engl. and *Sclerocarya birrea* (Hochst.), both from Anacardiaceae and *Syzygium cordatum* (Hochst.) from Myrtaceae. The main aims and objectives of this study were to:

- test for antimicrobial activity of crude extracts of the leaves and bark of *P. longifolia*, *S. birrea*, leaves of *S. cordatum*, crude methanolic leaf extract and ethylacetate leaf extractive of *A. venenata*, crude methanolic leaf extract and ethylacetate extractive of *C. macrocarpa*, crude methanolic bark extract of

H. canabinus, ethylacetate bark extractive of *H. natalensis* and crude methanolic calyx extract of *H. sabdariffa* and methanolic seed extractive of *C. foveolatus*.

- isolate, purify and identify the major compounds from the leaves and stem bark of *S. birrea* and *P. longifolia* and from the leaves of *S. cordatum*;
- test the isolated compounds and mixtures for possible antimicrobial activities.

CHAPTER 2

2. LITERATURE RIVIEW

2.1 Plants and plant parts used in herbal medicine

The active compounds in leaves, bark and roots are often quite different, one part may be very toxic and another quite harmless. The entire plant is therefore rarely used in herbal medicine. Usually specific plant organs are used for specific illnesses. The harvest of roots may be very destructive because the whole plant is often removed. Generally a specific part of the plant is used and examples include the secondary tubers of devil's claw, *Harpagophytum procumbens* and of *Xysmalobium undulatum.* Bulbs are also important for medicinal use e.g. those of *Scilla maritime, S. natalensis* and *Urginea sanguinea.* Some bulbs are not swollen and may be mistaken for rhizomes (underground stems), such as wild garlic, *Tulbaghia violacea.* Rhizomes utilised in herbal medicine include *Agapanthus spp.*, *Typha* capensis, *Sansevieria hyacinthoides* and *Alepidea amatymbica.* Tubers used medicinally include *Dioscorea dregeana* and *Stangeria aeriopus.* Examples of African herbal medicine derived from bark include *Warburgia salutaris* and *Ocotea bullata.* Often the young vigorously growing tips of stems and leaves are preferred over the thicker basal parts. Thick stems or wood are only rarely used, e.g. *Ptaeroxylon obliquum.* Flowers may be included or may sometimes be considered an essential part of the medication, as in traditional honey-bush tea, *Cyclopia spp.*

Fruits and seeds are rarely used for medicinal purposes. Examples include the small dry fruits of fennel, *Foeniculum vulgare*, the fleshy fruits of *Gethyllis spp.*, and the true seeds or nuts of the castor oil plant, *Ricinus communis* and purging nut, *Jatropha curcas* (Hutchings *et al.*, 1994; Hutchings, 1996; Hutchings *et al.*, 1996; van Wyk *et al.*, 1997;). Gums exudes from damaged stems as a defense mechanism to stop wood-boring insects and to seal off wounds so that wood-rotting fungi and bacteria are excluded. An example is Cape gum from *Acacia karroo*, used in the pharmaceutical industry for insecticidal and antimicrobial purposes. The yellow exudate flowing from a cut leaf of bitter aloe, *Aloe ferox*, is dried to a resinous medicinal product used in the treatment of skin ailments. Syrups or strong sugar solutions are used in cough remedies or to mask the unpleasant taste of medicines, e.g. the syrup produced by

boiling the nectar of the sugar-brush, *Protea repens* (Robbers & Speedie 1996; van Wyk *et al.*, 1997).

2.2 Plant species used in the study

There were three species selected for the study (*Protorhus longifolia*, *Sclerocarya birrea* and *Syzygium cordatum*), the other six from chemistry department (whose extracts were already prepared) were *Acokanthera venenata*, *Carissa macrocarpa*, *Chionanthus foveolatus*, *Hibiscus cannabinus*, *Hibiscus natalensis* and *Hibiscus sabdariffa*.

1. *Protorhus longifolia*:

Figure 1: *Protorhus longifolia* tree growing in the University of KwaZulu Natal, Westville campus

Distribution:

This plant is widely distributed in South Africa, occurring from the north in Limpopo and Gauteng, the west in Mpumalanga and south in KwaZulu-Natal and coastal to Swaziland and Mozambique (van Wyk, 1972).

Ethnobotany:

This species is used traditionally for a variety of ailments. Stem bark, root bark and roots are used by herbalists as medicine to strengthen the heart (Gerstner, 1939). The pulverized bark is injected to cure hemiphlegic paralysis believed to be caused by witchcraft (Gerstner, 1941).

Bark decoctions are taken in 200ml doses as emetics to relieve heartburn and bleeding from the stomach (Pujol, 1990). The bark yields 10.2-18% tanning material and 7% of tannin (Watt & Breyer-Brandwijk, 1962). The gum, which exudes from the cut bark of the stem, was used by the Zulus to fix assegai blades in the handle. Gum and latex from the stem are also used as a depilatory by smearing the fingers with either the gum or the latex and grasping the hair firmly and pulling it out by the roots (Watt & Breyer-Brandwijk, 1962).

2. *Sclerocarya birrea* (Marula):

Figure 2: *Sclerocarya birrea* tree growing in the University of KwaZulu Natal, Westville campus

Distribution:

This species has a wide distribution from Ethiopia and Sudan in the North to the lowlands of KwaZulu-Natal in the south, and from the southern parts of Namibia and Botswana in the west, to Gauteng and Mozambique in the east. In Gauteng it is found in the central regions from Magaliesberg mountains to the Limpopo, and throughout

the Lowveld. *S. birrea* is found in every ecological niche in the area, is seldom dominant but one of the most important of the woody components. The greatest concentration of this species occur on the basalt plains where it is closely associated with *Acacia nigrescens* and usually is the subdominant species. According to van Wyk *et al.*, 1997, the species is widely distributed in the African continent but in southern Africa it is only the subspecies *caffra* that is found.

Description:

It is moderately large (15 to 18m in height), deciduous (leaves turn yellow-green to pale yellow before being shed), single-stemmed tree with a rather dense, wide-spreading, round crown. The normally straight, round stem generally subdivides about three or four metres above ground level, and often grows as much as 60cm in diameter (van Wyk, 1974; van Wyk *et al.*, 1997).

Leaves:

The leaf aqueous extracts show low toxicity and are thought to have a direct action on the uptake of glucose, in particular by the muscular tissues. However, no hyperglycaemic effects were observed from patients to whom hot water leaf extracts were administered (Ampofo, 1977). Leaf decoctions are taken and also inhaled for malaria in Madagascar

Bark:

In north eastern Gauteng a brandy tincture of the stem bark is taken in small doses as a prophylactic against malaria and the powdered bark taken in teaspoonful doses. Extracts of the bark have however, given negative results in antimalarial experiments (Spencer *et al.*, 1947). Infusions of the bark are used as a remedy for dysentery, diarrhoea and, when mixed with brandy, for malaria. Sometimes decoctions of the bark or roots are taken orally as enemas (Watt & Breyer-Brandwijk, 1962; Hutchings, 1989; Pujol, 1990) and leaf infusions or decoctions are used for diabetes (Iwu, 1993). Zulus use a decoction of the bark, externally, as a prophylactic against gangrenous rectitis (Bryant, 1909). The Venda people use the bark for fevers, stomach ailments, headaches, ulcers, toothache, backache and infertility (Mabogo, 1990). Bark from *S. birrea* found in Madagascar contains 3,5% tannin (Heim de Balsac, 1928) and from trees in southern Africa, collected during October before the appearance of the leaves, contains 20.5% of tannin and a trace of alkaloids (Brandwijk, 1928). About 10% tannin has been found from this bark of *S. birrea*.

The bark is stringent and may prove of value in diarrhoeas but is extremely unlikely to produce benefit in malaria. The bark also contains procyanidins (Galvez *et al.*, 1993). These procyanidins inhibit peristaltic reflexes in guinea pig colon and show anti-diarrhoeal activity on isolated guinea pig ileum and against various induced diarrhoeas in mice (Galvez *et al.*, 1991, 1993). Extracts from dried stem bark have shown antibacterial activity against *Cornebacterium diphtheriae*, and various other organisms including *Neisseria*, *Streptococcus*, *Streptobacillus* and *Salmonella* species but were inactive against *Staphylococcus aureus* (Hussein and Deeni, 1991). Controversially, Ntuli (2000) showed crude extracts from dried stem bark of *Sclerocarya birrea* were highly active against *Staphylococcus aureus* and was found to be the case in this current study. The whole plant is reported to contain gallotanins, flavonoids and cathechins (Watt & Breyer-Brandwijk, 1962; Iwu, 1993), 10 to 20% tannin, traces of alkaloids and is used as a coagulant as is reported effective for diarrhoea treatment (van Wyk, 1974; van Wyk *et al.*, 1997).

Fruit:

The fruit is aromatic and edible. It is freely eaten by human beings and is much sought after by baboons, monkeys and elephants. The outer skin of the fruit has a rather pungent apple-like odour and the flavour of the skin has been described as resembling that of the litchi, the apple, the guava and the pineapple. The pulp contains citric and malic acids and a sugar (Marloth, 1913). Amongst the Africans and some Europeans, fruits from this plant are used to produce an alcoholic drink, known among the Shangaans as *ukanya* and this beer may be very intoxicating but is highly anti-scorbic. In East Africa a very potent spirit is brewed from the fruit. In Mozambique, the fruit is used universally for the making of a national fermented beverage. The fermented fruit juice, as prepared by the Pedi (Quin, 1954), contains 1,5% citric acid. The fruits also make a delicious pale-yellow to red jelly. The fruit is rich in ascorbic acid and the juice extracts yield 33 sesquiterpene hydrocarbons (Pretorius *et al.*, 1985). This fruit juice contains four times the amount of vitamin C than that of orange juice. The Zulus use the decoction of the fruit or raw fruits for the destruction of ticks and as an insecticide (Bryant, 1909). The Thongas celebrate the feast of the first fruits by pouring the fresh juice of the fruit on the tomb of deceased chiefs in the sacred wood. Branches of the tree are also used in funeral rites of the Thongas.

The dice of the Shangaan diviners include a *Sclerocarya* nut which represents the vegetable kingdom or 'Medicine' (Ferreira, personal communication to John Mitchel Watt). Also bark decoctions are taken for dysentery and diarrhoea in some parts of southern Africa (Watt & Breyer-Brandwijk, 1962). Bark has also been used as a prophylactic and cure for malaria and for proctitis.

Roots:

Among the African population in dry areas, chewing the roots of the marula tree provides a source of water. Ground roots are used for many purposes in Zimbabwe, including menorrhagia, schistosomiasis, sore eyes, heart pain and to arouse or prevent possession from spirits (Gelfand *et al.*, 1985). In East Africa, roots are an ingredient in an alcoholic medicine taken to treat an internal ailment known as *kati* while bark is used for toothache, constipation and stomach disorders (Kokwaro, 1976). In Senegal, leaves and root bark of *S. birrea* are used together with leaves and root bark of *Securidaca longependunculata* Fresen., and sometimes with other plants, for snake bite and other venoms (Kerharo & Adam, 1974; Rasoanaivo *et al.*, 1992).

Diseases:

A decoction of the bark of *S. birrea* is taken in 250ml doses by patients suffering from dysebteris and diarrhoea (Watt & Breyer-Brandwijk, 1962). This decoction has also been used by Europeans prophylactically and curatively, in treating malaria. It is thought that, for this purpose, the bark is most active if gathered just before the leaf appears on the tree. Medicinally, bark decoctions are administered as enemas for malaria and diarrhoea, and are also taken as teas twice a day to strengthen the heart, or as blood-cleansing emetics before marriage (Gerstner, 1938; Pujol, 1990). Newly-born baby girls and their mothers are traditionally washed in water heated on a fire made from the twigs so that the baby may be endued with fertility, softness, tenderness and early maturity (Palmer and Pitman, 1972b). Traditional healers wash in bark decoctions before treating patients with gangrenous rectitis and also administer the decoction to the patient (Bryant, 1966).

Seeds:

The seeds are eaten raw, or are cooked and eaten with maize porridge. Seeds are also frequently sold by local street vendours to tourists. Kernels yield about 50% of non-drying oil and contain as much as 28% protein and some iodine (Watt & Breyer-Brandwijk, 1962). The oil-rich seeds contain 64% oleic acid, myristic, stearic and

amino acids with a predominance of glutamic and arginine (Busson, 1965).

3. *Syzygium cordatum*:

Figure 3: *Syzygium cordatum* tree growing in the Univeristy of KwaZulu Natal, Westville campus

Distribution:

This hydrophilic species is cosmopolitan, occurring along the coast of South Africa from eastern Cape to KwaZulu-Natal and Natal bushveld regions to Swaziland as well as the central and northern Gauteng (van Wyk, 1972). It is common on the sandy lowlands along the Mozambique coast and also occurs in Botswana, Zimbabwe, Zambia and Angola. According to van Wyk (1997) this species is a rather small to moderately large tree. Can grow up to 15m in height with stems up to 50cm in diameter, evergreen with a dense, spreading, round crown. Numerous lateral twigs are produced and they are initially green but later become pale brown to grey brown (van Wyk, 1972).

Bark:

Central Africans use an extract of bark and leaves as a purgative and also for the treatment of diarrhoea and general stomach indisposition. In South Africa the bark is

used to cure tuberculosis and also used as an emetic (Cunningham, 1988). Bark and leaves from *Syzygium* species have previously shown antibacterial activity against the Herpes simplex virus in concentrations as small as 10μg ml^{-1} (Takechi and Tanaka, 1981). A single dose of an aqueous extract of the seeds of *Syzygium* species can reduce blood sugar levels by between 15-25% in four to five hours (Sepaha and Bhose, 1956; Jain and Sharma, 1967). The exact pharmacological action of *Syzygium cordatum* is not known, but the presence of phenolic compounds may be the source of antidiarrhoeal activity exhibited by this species (Bruneton, 1995).

Chemistry:

Triterpenoids from leaves and bark of *Syzygium* species show a wide range of antimicrobial activities (Bruneton, 1995). Clove oil, from crude extracts from another species, *S. aromaticum* is used for toothache and mouth infections. The active compound is eugenol, a widely used dental analgesic (Merck index, 1989) that appears to be absent from *S. cordatum*. Sixteen constituents from *Syzygium* species have been identified using different solvents as extractants, these include friedelin, epi-friedelinol, β-sitosterol, arjunolic, gallic and ellagic acids (Merck index, 1989). Hot mineral-acid extraction of fresh and dried bark is reported to extract delphinidin derived from leucodelphinidin and other non-essential compounds and oils (Merck, 1989).

4. *Acokanthera venenata*:

Figure 4: *Acokanthera venenata* tree growing in the field

Distribution, description and traditional uses:

This shrub and other species of the genus are found in South Africa and Ethiopia. *Acokanthera venenata* is used traditionally as a source of arrow poison by Bushmen (Watt & Breyer-Brandwijk, 1962). This plant is known to contain cardiac glycosides toxic enough to cause death (www.wikipedia.com)

5. *Carissa macrocarpa*:

Figure 5: *Carissa macrocarpa* growing in the University of KwaZulu-Natal, Westville campus

Distribution and description:

C. macrocarpa, affectionately known as the Natal plum is found naturally growing in KwaZulu-Natal. It is a dense, closely branched spiny evergreen shrub growing up to 6m. It produces white star-like flowers with five thich waxy petals, with a sweet fragrance. The fruits are edible red berries which taste like cranberries (Watt & Breyer-Brandwijk, 1962).

Traditional uses and chemistry:

All parts of this plant are poisonous and have not previously shown any antibacterial properties. Fruits contain vitamin C, calcium, magnesium and phosphorus. The leaves and fruits have both given positive tests for tannins, sterol, organic acids and phenols (Pooley, 1993).

6. *Chionanthus foveolatus*:

Figure 6: *Chionanthus foveolatus* tree growing in the field

Distribution and description:

This is a small to medium-sized evergreen treen growing up to 15m and found in the forests from coast to mountains in KwaZulu-Natal and in the Eastern Cape. The main stem is slender with light grey bark. Leaves are opposite, leathery, shiny, dark green. Fruits are green and generally eaten by monkeys, birds and bush pigs (Pooley, 1993). There wasn't any information in the literature on traditional medicinal usage of *C. foveolatus*.

7. *Heteropyxis natalensis*:

Figure 7: *Heteropyxis natalensis* tree growing in the field

Distribution and description:

As seen from the species name, *H. natalensis* originates from the coastal and inland regions in KwaZulu-Natal (Hutchings, 1996). This species is a large, deciduous tree growing up to 10m high. The leaves are narrowly elliptic, arranged spirally and shiny dark green above and paler-green below, slightly hairy to hairless when mature (Palgrave, 1997).

Traditional uses:

Wood is hard and suitable for use as fencing posts and charcoal. Leaves are boiled and drank as a herbal tea. The leaves and roots are used medicinally to treat worms in live stock. Steam from a decoction of the roots is inhaled for treating a bleeding nose. Roots are also used for the treatment of mental disorders (Watt & Breyer-Brandwijk, 1962).

8. *Hibiscus cannabinus*:

Figure 8: *Hibiscus cannabinus* tree growing in the field

Distribution and description:

This plant species is native to Africa and Asia, generally cultivated in India. *H. cannabinus* is a woody to herbaceous annual, mostly unbranched, fast-growing tree with prickly stems, up to 4m tall. The leaves alternate, long-petiolate, shallowly to

deeply parted, with 3–7 toothed lobes. The flowers are solitary, large to 10cm in diameter, short-stalked, axillary, yellow with purple centers. The fruit is a many-seeded, hairy capsule about 1cm long. Roots are a deep-penetrating with deep-seated laterals (Reed, 1976).

Traditional uses and chemistry:

In Africa, the leaves, roots and seeds of this species are used medicinally as a remedy for eye diseases and dysentery. Seeds contain stearic, palmitic, oleic and linoleic acids. Flowers contain a pigment known as canna hibiscetin (Watt & Breyer-Brandwijk, 1962).

9. *Hibiscus sabdariffa*:

Figure 9: *Hibiscus sabdariffa* tree growing in the field

Distribution:

This plant species is found in southern, central and eastern Africa, where it is used for traditional medicinal purposes.

Traditional uses and chemistry:

In East Africa, the leaves are used as an emollient. Leaves are also used in central Africa as a poultice on abscesses and elsewhere in Africa, as as diuretic. Calyces are

used for cough remedies and wound dressings. Infusion of the flowers contain citric, malic, (acting as a diuretic), hibiscic and ascorbic acids, gossipetin and hibiscin (Watt & Breyer-Brandwijk, 1962).

2.3 Microorganisms used in this study

The bacterial and fungal species selected were *Klebsiella pneumoniae* (ATCC 12265), *Bacillus cereus* (ATCC 11778), *Salmonella typhimurium* (ATCC 13311), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 35219), *Staphylococcus aureus* (ATCC 29213), *Candida albicans* (ATCC 10231). These microorganisms were selected based on the fact that they are pathogenic to human life and also on their availability.

2.3.1 Review of microorganisms:

1. *Klebsiella pneumoniae*:

These bacteria live in the gastrointestinal tract, primarily in the large intestines. All such bacteria are important for normal health and functioning of our systems. When bacteria such as *K. pneumoniae* get outside the gut, serious infections can occur. Generally *Klebsiella* infections tend to occur in patients with a weakened immune system, like people with alcoholism, diabetes and chronic lung diseases.

K. pneumoniae causes severe, rapid-onset illness that often causes areas of destruction in the lungs. Symptoms from infected patients include high fever, flu, coughs production of mucous which is often thick and blood-tinged. Most *Klebsiella* bacteria are resistant to commonly used antibiotics, such as penicillin (Brochert, 1999). Usually two or more antibiotics are employed to eliminate *Klebsiella* infections, but these antibiotics may not be effective due to resistance of the bacteria.

2. *Bacillus cereus*:

This bacterium has been recognized as an agent of food poisoning since the early 1950's. Reports have been made in 1972 and 1986, of outbreaks of food-borne diseases associated with *B. cereus*. This bacterium causes two types of food-borne intoxications, one type, characterized by nausea, vomiting and abdominal cramps, has an incubation period of 1 to 6h and resembles *Staphylococcus aureus* food poisoning

in its incubation period. This type is often referred to as the emetic form.

The other type manifests primarily by abdominal cramps and diarrhoea with an incubation time of 8 to 16h and is often referred to as the diarrhoeal form and resembles food poisoning caused by *Clostridium perfringens*. *B. cereus* is occasionally implicated in local infections especially involving the eye. *B. cereus* can cause conjunctivitis, keratitis, iridocyclitis, dacrocystitis, orbital abscess and panophthalmitis (Todar, 2002).

3. *Salmonella typhimurium*:

This bacterium causes typhoid fever. Twelve and a half million people worldwide are affected by this fever. *S. typhimurium* only lives in humans, in their bloodstream and intestinal tract.

Typhoid fever is transmitted through drinking beverages or eating food that has been handled by someone who is shedding *S. typhimurium* or if sewage contaminated with these bacteria gets into the water supply. Typhoid fever can be treated with antibiotics (Anonymous D, 2004).

4. *Pseudomonas aeruginosa*:

This is a gram-negative rod bacterium with more than half of all clinical isolates producing the blue-green pigment, pyocyanin. These bacteria are widespread in nature and inhabit soils, water, plants and animals including humans (Qarah, 2004).

This bacterium has become an important cause of infection, especially to patients with a weakened defense system. It is commonly isolated from patients who have been hospitalized for more than a week and it is a frequent cause of pneumonia and urinary tract infections (Pollack, 2000).

5. *Escherichia coli*:

Although this bacterium lives in human intestines, it is also a source of food-borne illnesses. The infections of this microorganism often lead to bloody diarrhoea, and occasionally to kidney failure. Most illnesses associated with *E. coli* are a result of eating undercooked, contaminated ground beef (Anonymous C, 2004).

6. *Staphylococcus aureus*:

This is a gram-positive spherical bacterium that also causes food poisoning similar to that of *B. cereus*. Some strains of *S. aureus* are capable of producing a highly heat stable protein toxin that causes illness in humans (Anonymous B, 2003). Staphylococcal food poisoning is caused by enterotoxins which some strains of this bacterium produce. The onset of symptoms is usually rapid and in most cases acute, depending on the individual's susceptibility to the toxin in the food ingested, and the general health of the victim. The most common symptoms are nausea, vomiting, abdominal cramps and prostration but in more severe cases, headaches, muscle cramping and transient changes in blood pressure and pulse rate may occur.

7. *Candida albicans*:

This yeast fungus is present on most humans as a harmless commensal organism. The human gastrointestinal tract houses thousands of these yeasts without any ill effects. However *C. albicans* is also a major fungal human pathogen. Infections can be vaginal or oral, which cause a considerable degree of discomfort. In some patients (prematurely born infants, leukemia and burn patients) with a severely compromised defense, this yeast can turn out to be a deadly pathogen causing systemic infections and up to 50% of infected individuals die as a result. Anti-candida drugs are very limited and these can also cause severe side effects (Schmid, 2003).

CHAPTER 3

3. METHODS AND MATERIALS

3.1 Plant, bacterial and fungal material used in the study:

Fresh leaves and stem bark of *P. longifolia* (Bernh.) Engl., *S. birrea* (Hochst.) and leaves of *S. cordatum* (Hochst.) were collected between June and July 2001 from a small woodland between Clermont and Pinetown, and voucher specimens prepared were lodged with the University of KwaZulu-Natal (Westville) Herbarium. Leaves of *A. venenata* (G. Don.), *C. macrocarpa* (Eckl.) A. DC., seeds of *C. foveolatus* (E. Mey.), stem bark of *H. cannabinus* (L.), *H. natalensis* (Harvey) and calyces of *H. sabdariffa* (L.) were donated by Prof. F. O. Shode. Stem bark and leaves were collected and air-dried in the laboratory to minimize alteration (oxidation) of secondary metabolites. Bacterial and fungal microorganisms [*Klebsiella pneumoniae* (ATCC 12265), *Bacillus cereus* (ATCC 11778), *Salmonella typhimurium* (ATCC 13311), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 35219), *Staphylococcus aureus* (ATCC 29213), *Candida albicans* (ATCC 10231)] were donated by the Department of Biotechnology, Durban Institute of Technology. These microorganisms were selected on the basis that they cause infectious diseases. These are reference strains recommended by the National Committee for Clinical Laboratory Standards (NCCLS, 1990).

3.2 Extraction procedure:

The stem bark and leaves were ground to a powder using an electric grinder. The ground plant material was doubly extracted successively in a Soxhlet extractor for 48 to 72 h at a temperature not exceeding the boiling point of the solvent (Lin *et al.*, 1999) using four organic solvents. The stem bark and leaves of *P. longifolia* and *S. birrea* were successively doubly extracted, in order of increasing polarity with hexane (Hex), dichloromethane (DCM), ethylacetate (EtOAc), and methanol (MeOH) respectively. Leaves of *S. cordatum* were doubly extracted with chloroform ($CHCl_3$). Leaves of *A. venenata* were doubly extracted with both EtOAc and MeOH respectively. Leaves of *C. macrocarpa*, seeds of *C. foveolatus*, stem of *H. cannabinus*, calyces of *H. sabdariffa* were all doubly extracted with methanol and the stem of *H. natalensis* was extracted with ethylacetate.

All the solvents used in this study were of analytical (HPLC) grade. The extracts were filtered through Whatman No.1 filter paper to obtain crude, non-concentrated extracts and stored in the refrigerator at 4°C until further bioassay. The extracts were concentrated by rotary-evaporation. Extracts were further dried by leaving them exposed to air in the laboratory to give a somewhat oily product, until further analyses. For anti-bacterial analysis, 0.25g crude extracts were suspended in about one millilitre of the extracting solvent to obtain a watery suspension. Anti-bacterial and phytochemical analyses were undertaken on the extracts. Seventeen extracts, from all the plant species were further diluted to five different concentrations (0.1, 0.25, 0.4, 0.67 and 1.0mg ml^{-1}) with methanol, distilled water and dimethylsulphoxide (DMSO) in the following manner, and these were for the determination of the minimum inhibitory concentration (MIC) of the extracts. Extracts of *A. venenata* leaves, *C. macrocarpa* leaves and stem of *H. natalensis* were all diluted five times with DMSO from 0.1 to 1.0mg ml^{-1}. Water-soluble leaf extracts of *A. venenata*, *C. macrocarpa*, seeds of *C. foveolatus*, stem of *H. cannabinus* and calyces of *H. sabdariffa* were diluted 5 times with distilled water in a similar way. Extracts from *P. longifolia*, *S. birrea* and *S. cordatum* were diluted with methanol. Four of the seventeen extracts were from *P. longifolia*, one from *S. birrea*, two from *S. cordatum*, four from *A. venenata*, two from *C. macrocarpa* and one each from *H. cannabinus*, *H. natalensis*, *H. sabdariffa* and *C. foveolatus*.

3.2.1 Extract dilutions:

All seventeen stock solutions were prepared by dissolving approximately up to 85mg of extract in 100ml of either DMSO. These were the stock solutions. The five-fold dilutions of these stock solutions were prepared as follows: for 0.1mg ml^{-1}, 1ml of the stock solution was diluted to 10ml with DMSO (9ml), 2ml diluted with 8ml DMSO, 3ml diluted with 7ml, 4ml diluted with 6ml DMSO and finally 5ml diluted with 5ml DMSO and the concentrations of these diluted sub-extracts ranged from 0.1 to 1.0mg ml^{-1} and these were tested further for antimicrobial activity.

3.3 Antimicrobial assay:

Bacterial material, consisting of *K. pneumoniae, B. cereus, S. typhimurium, E. coli, S. aureus, P. aeruginosa* was grown on both Mueller-Hinton (MH) and Brain-Heart

Infusion (BHI) agar. The agar was prepared by mixing 47g of powdered agar in the case of MH agar and 50g in the case of BHI agar with one litre distilled water, heated to boiling point with constant agitation, sterilized by autoclaving for 15 minutes at 121°C and allowed to cool to 45-50°C and dispensed to plates. The yeast, *C. albicans*, was grown on Potato Dextrose agar (PDA) which was prepared by mixing 39g of powder with one litre distilled water, heated to boiling, autoclaved for 15 minutes at 121°C, cooled to 45-50°C, mixed well and poured to plates in a laminar flow unit, and left overnight to set. Sterile paper discs, previously soaked for 24h in the extracts (at different concentrations) were placed at the centre of each agar plate. One paper disc was used for stock solution analysis and five sterile discs were used for the five-fold dilutions. Extract-soaked discs were placed on plates using sterilized forceps. Control plates containing only the solvents on paper discs were also prepared. This was done to determine if the solvents had any antimicrobial effect on the growth of microorganisms. All plates were tightly sealed with parafilm and incubated for 24h at 37°C for the bacteria and 48h for the yeast to determine any antibacterial or antifungal activity of the extracts. The antimicrobial tests were then carried out by the well, disc-diffusion and micro-dilution broth methods (Murray *et al.*, 1995). After observations and measurements of diameters of zones of inhibition after 24 and 48 hours respectively, the plates from the stock solution analyses were photographed with a digital camera.

3.4 Chromatographic analyses:

Nuclear Magnetic Resonance (NMR) is used to determine the structure of organic compounds by measuring the magnetic moments of their hydrogen (H) and carbon (C) atoms. In most compounds, H-atoms are attached to different functional groups such as –CHO, -CH2, -NH2, -CHOH etc. and the NMR spectrum provides a record of a number of H-atoms in the different positions. At least 5-10mg of a sample is needed for NMR analysis. However, the advantage of using NMR over the other spectroscopic methods such as mass spectroscopy (MS) is that the sample can be recovered and used for other analyses. In practice, a sample is placed in a sample of an inert deuterated solvent, between the poles of a powerful electromagnet. The protons or C-atoms undergo chemical shifts according to their molecular environment within the bulk molecule, upon being subjected to radio waves. The complexity of

the spectrum is related to the number of different protons and functional groups. Interpretation of the spectrum leads to the determination of the chemical structure.
Mass spectrometry (MS) depends on the production of ions in a gaseous phase from the sample. These ions then separate according to mass:charge ion ratio (m/*e*). The principle of MS is that when an organic molecule is bombarded with electrons of sufficient energy, it looses an electron and so becomea free radical ion. The imparted excess energy accumulates in a particular bond which then cleaves. Different bonds require different energies to break. Each molecule will give rise to unique fragments. The mass spectrometer separates and records positive ions according to their mass:charge ratio (m/*e*) (Chiu and Muddiman, 2001).

All columns viz. *P. longifolia* leaves in ethylacetate, *P. longifolia* leaves in hexane, *S. birrea* leaves in methanol and *S. cordatum* leaves in EtOAc, were prepared using the following procedure: Five grams of the above crude extracts were dissolved in the appropriate solvent, preferably chloroform or methanol, mixed thoroughly with 30g silica gel 60 of particle size 0.063-0.200mm (70-230 mesh) and dissolved in hexane. The mixture was heated until the silica gel was homogeneous with the sample extract after most of the solvent (hexane) has been removed. The solution was poured in the column that had been stoppered with cotton wool and filled with acid-purified sand 0.1-0.3mm (50-150 mesh) at the bottom. The sand was poured followed by the sample mixture.

The solvent system used depended on the crude extract being analyzed but for all columns it was a gradient elution system of 10:0, 9:1, 8:2, 7:3, 6:4, 1:1 and 0:10 (v/v) respectively. For both *S. cordatum* leaves in ethylacetate and *P. longifolia* leaves in ethylactate and in hexane, the solvent system was hexane:ethylacetate (HEX:EA) and for *S. birrea* leaves-in-methanol column the solvent system used was chloroform-methanol ($CHCL_3$:MeOH) from 10:0 to 0:10 (v/v). The column tap was opened and each set of fractions was collected as approximately 25ml in volumetric flasks. All fractions, up to more than 100 obtained from these columns, were spotted on TLC plates against their respective crude extracts (for comparison) and these plates were observed under UV light for UV active compounds. Plates that showed no observable spots under UV light were either sprayed with a chromogenic spray made up of

anisaldehyde and sulphuric acid 1:1 (v/v) or enclosed in a beaker containing iodine crystals to enhance the visibility of the spots and heated in an incubator at 120°C for about five minutes to develop the visibility of the spots.

Fractions that were composed of mixtures were run on smaller columns (burettes) to separate these mixtures. In all the crude extracts tested, more than one column was packed (flash chromatography) but all were packed similar to the first larger column of that crude sample. After this second spotting of the plates, similar spots, i.e. similar compounds having similar R_f values and similar physical properties, were recombined in one volumetric flask, concentrated *in vacuo* using a rotary evaporator, redissolved in either chloroform or methanol, weighed, labelled and left in the laboratory to air dry. The dry, powdery compounds were subjected to more elucidatory techniques, e.g. Infra red (IR), one and two dimensional NMR (1-D NMR or ^{13}C and 2-D NMR or ^{1}H-^{1}H NMR), Correlated Spectroscopy (COSY) and Destortionless Enhancement by Polarization Transfer (DEPT).

Flash chromatography was carried out using 60-230 mesh silica gel. Analytical thin layer chromatography was performed with silica gel 60 F_{254}, 0.25mm precoated TLC plates and visualised using UV_{254} and vanillin and/or anisaldehyde. ^{1}H NMR spectra were recorded at 400 – 500MHz and ^{13}C NMR were recorded at 200MHz on a Varian spectrometer. Elucidations of chemical structures were based on ^{1}H, ^{13}C, DEPT135 and NMR experiments. Chemical shifts are reported in parts per million (ppm) relative to residual solvent peaks. Signals are reported as multiplet (m), singlet (s), doublet (d), triplet (t), quinquet (q), complex (c), broad singlet (br s) and coupling constants are reported in hertz (Hz). Melting points were determined in capillaries and were uncorrected. Optical rotations were obtained using sodium D line at ambient temperature on a Jasco DIP-360 digital polarimeter. All mass and NMR spectral data were obtained from the Department of Chemistry, University of KwaZulu-Natal, Howard College campus.

Table 1: Coding of samples for the antimicrobial bioassay

Number	CODE	DESCRIPTION	REMARK
1	PL/A3	Crude DCM extract of *P. longifolia* leaves	Soluble in DMSO
2	PL/A4	Crude EtOAc extract of *P. longifolia* leaves	Soluble in DMSO
3	PL/A6	Crude methanolic extract of *P. longifolia* leaves	Soluble in water
4	PL/B6	Crude methanolic extract of *P. longifolia* bark	Soluble in water
5	SB/A4	Crude EtOAc extract of *S. birrea* leaves	Soluble in DMSO
6	MG/9/B	Extractive from the chloroform extract of *S. cordatum*	Soluble in DMSO
7	MG/9/C	Extractive from the EtOAc extract of *S. cordatum*	Soluble in DMSO
8	RT/2/C8-9	Extractive from the EtOAc extract of *A. venenata* leaves	Soluble in DMSO
9	RT/1/D2	Crude methanolic extract of *A. venenata* leaves	Soluble in water
10	RT/28/C8	Extractive from the EtOAc extract of *Acokanthera sp.*	Soluble in DMSO
11	RT/20/D	Crude methanolic extract of *Acokanthera sp.* Leaves	Soluble in water
12	RT/25/C10	Extractive from the EtOAc extract of *C. macrocarpa* leaves	Soluble in DMSO
13	RT/21/D	Crude methanolic extract of *C. macrocarpa* leaves	Soluble in water
14	JK/43/F	Extractive from the methanolic extract of *C. faveolatus* seeds	Soluble in water
15	M/8/D1	Crude methanolic extract of *H. cannabinus* stem	Soluble in water
16	DUR/228/B	Crude methanol soluble fraction of alcoholic extract of *H. sabdariffa* calyces	Soluble in water
17	JK/33/9	Extractive from EtOAc extract of *H. natalensis* stem	Soluble in DMSO

All extracts form *P. longifolia*, *S. birrea* and *S. cordatum* were coded according to the plant and organ they came from (see Table 1 above). *P. longifolia* extracts were coded with PL, *S. birrea* and *S. cordatum* were coded as SB and SC respectively.

Plant organs were also coded as follows, leaves were coded by an A and bark with a B. Organic solvents were also given code numbers between 2 and 6 according to the

first and last solvent in the line. Chloroform was coded as 2, dichloromethane 3, ethylacetate 4, hexane 5 and methanol 6. Therefore a SC/B2 extract code is *S. cordatum* bark extract with chloroform. Extracts and/or compounds donated by the chemistry department were already coded as shown in Table 1.

3.5 Statistical analyses:

For the antimicrobial assay, the experiment was repeated five times. Data from each experiment were averaged and this average value was the data point used in the statistical analysis. Data were given as means and analysed by one-way analysis of variance (ANOVA) with Tukey's multiple range comparison test method for comparing means using Instat. A significance level of $p < 0.05$ was adopted for all comparisons.

CHAPTER 4

4. RESULTS

4.1 Antimicrobial analyses

Yields from the double organic extraction using DCM, EtOAc, hexane and MeOH are shown in Table 2. It was observed that, for all four solvents the first extraction yielded more of the material than the second. Methanol was observed to be the best extractant (in terms of combined yield) followed by DCM, hexane and EtOAc.

Table 2: Yield (in grams) of the various extracts from the double extractions and their total combined yield.

Plant part extracted	Yield of the 1st extraction	Yield of the 2nd extraction	Combined yield
Dichloromethane extraction			
PL bark	52.035	0.880	52.915
PL leaves	28.561	9.544	38.105
SB bark	12.640	6.593	19.233
SB leaves	0.909	0.449	1.358
Ethylacetate extraction			
PL bark	2.090	0.791	2.881
PL leaves	6.540	2.367	8.907
SB bark	1.923	0.444	2.367
SB leaves	1.759	0.145	1.904
Hexane extraction			
PL bark	3.480	1.357	4.837
PL leaves	7.561	3.190	10.751
SB bark	1.788	0.548	2.336
SB leaves	7.332	0.998	8.330
Methanol extraction			
PL bark	81.038	25.129	106.167
PL leaves	92.460	15.342	107.802
SB bark	71.359	10.200	81.559
SB leaves	67.137	20.404	87.541

Inhibitory zone diameters and mean diameters from antimicrobial activity of the crude leaf and bark extracts of *S. birrea* and *P. longifolia* extracted with the solvents mentioned above in Table 2 are shown in Table 3 below. DCM and MeOH crude extracts exhibited the highest antimicrobial activity against both *E. coli* and *S. aureus* followed by EtOAc and hexane respectively (Table 3). Crude MeOH and DCM leaf and bark extracts from both *S. birrea* and *P. longifolia* plants exhibited the highest activity. There was no observable activity against *E. coli* by both EtOAc and chloroform extracts of *S. cordatum* but there was activity by both extracts

against *S. aureus*, with EtOAc extracts showing the highest activity among the two. All solvent controls showed no observable activity against all microorganisms tested.

Table 3: Diameter of inhibition zones on the gram-negative *Escherichia coli* and gram-positive *Staphylococcus aureus*.

Extract code	Diameter of activity (mm)					
	E. coli			*S. aureus*		
	Replicate 1	**Replicate 2**	**Mean**	**Replicate 1**	**Replicate 2**	**Mean**
SB/A3	9	9	**9**	11	11	**11**
SB/B3	24	20	**22**	15	16	**16**
PL/A3	12	12	**12**	**30**	**30**	**30**
PL/B3	28	26	**27**	8	10	**9**
SB/A4	20	20	**20**	20	20	**20**
SB/B4	16	18	**17**	25	24	**25**
PL/A4	29	31	30	25	25	**25**
PL/B4	14	16	**15**	16	20	**18**
SB/A5	13	13	**13**	11	15	**13**
SB/B5	18	18	**18**	14	12	**13**
PL/A5	11	11	**11**	12	12	**12**
PL/B5	5	7	**6**	12	12	**12**
SB/A6	15	12	**14**	15	18	**17**
SB/B6	10	10	**10**	15	16	**16**
PL/A6	20	22	**21**	25	26	**26**
PL/B6	22	22	**22**	23	24	**24**
DCM	0	0	**0**	0	0	**0**
EA	0	0	**0**	0	0	**0**
HEX	0	0	**0**	0	0	**0**
MeOH	0	0	**0**	0	0	**0**
$CHCl_3$	0	0	**0**	0	0	**0**
MG9/B	0	0	**0**	9	9	**9**
MG9/C	0	0	**0**	13	15	**14**

The antimicrobial activity of the 16 crude extracts and extractives tested against the 7 microorganisms, in the well/agar diffusion method is shown in Table 4.

Most crude extracts/or individual extractives were active against all or most of the microorganisms tested. There was no observable activity against *B. cereus* by the ethylacetate leaf and methanolic leaf extracts of *P. longifolia*, chloroform extractive of *S. cordatum*, crude methanolic leaf extract of *Acokanthera sp.*, crude methanolic leaf extract of *C. macrocarpa*, methanolic seed extractive of *C. foveolatus*, crude methanolic stem extract of *H. cannabinus* and crude methanolic calyces extract of *H. sabdariffa*. *K. pneumoniae* was resistant against methanolic bark extract of *P. longifolia*, ethylacetate leaf extractive and crude methanolic leaf extract of *A. venenata*, ethylacetate leaf extractive of *Acokanthera sp.*, crude methanolic leaf extract of *C. macrocarpa*, crude methanolic stem extract of *H. cannabinus* and ethylacetate stem extractive of *H. natalensis*. There was also no activity observed by the ethylacetate leaf extractive and crude methanolic leaf extract of *A. venenata*, ethylacetate leaf extractive of *Acokanthera sp.*, ethylacetate leaf extractive of *C. macrocarpa* and crude methanolic calyces extract of *H. sabdariffa* against *S. typhimurium*.

The ethylacetate leaf extractive of *A. venenata*, methanolic leaf extract of *Acokanthera sp.*, crude methanolic leaf extract of *C. macrocarpa*, crude methanolic calyces extract of *H. sabdariffa* and ethylacetate stem extractive of *H. natalensis* were all found to be inactive against *E. coli*. The methanolic bark extract of *P. longifolia*, ethylacetate leaf extractive and crude methanolic leaf extract of *A. venenata*, crude methanolic leaf extract of *Acokanthera sp.*, crude methanolic leaf extract of *C. macrocarpa* and crude methanolic calyces extract of *H. sabdariffa* were inactive against *S. aureus*. *P. aeruginosa* was the most susceptible bacterium and was observed to be resistant only against the crude methanolic stem extract of *H. cannabinus* and ethylacetate stem extractive of *H. natalensis*. The fungus *C. albicans* was susceptible to all 16 plant extracts tested. The dichloromethane leaf extract of *P. longifolia* and chloroform leaf extract of *S. cordatum* were the only two extracts found to be active against all the microorganisms tested.

Table 4: Antimicrobial activity of the 16 extracts against bacterial and fungal species tested based on disc-diffusion method. Activity was expressed as +, high activity as ++, very high activity as +++ and absence of activity as n.a.

Plant extract code	Microorganism	Activity of extracts
PL/A3	*K. pneumoniae*	++
	B. cereus	+
	S. typhimurium	++
	E. coli	+
	S. aureus	+
	P. aeruginosa	++
	C. albicans	++
PL/A4	*K. pneumoniae*	+++
	B. cereus	n.a
	S. typhimurium	+
	E. coli	+
	S. aureus	++
	P. aeruginosa	++
	C. albicans	+
PL/A6	*K. pneumoniae*	++
	B. cereus	n.a
	S. typhimurium	++
	E. coli	++
	S. aureus	++
	P. aeruginosa	++
	C. albicans	++
PL/B6	*K. pneumoniae*	n.a
	B. cereus	+
	S. typhimurium	++
	E. coli	++
	S. aureus	n.a
	P. aeruginosa	++
	C. albicans	++
MG/9/B	*K. pneumoniae*	+++
	B. cereus	n.a
	S. typhimurium	+++
	E. coli	++
	S. aureus	+
	P. aeruginosa	+++
	C. albicans	++
MG/9/C	*K. pneumoniae*	+
	B. cereus	+++
	S. typhimurium	+
	E. coli	+++
	S. aureus	+++
	P. aeruginosa	+++
	C. albicans	++
RT/2/C8-9	*K. pneumoniae*	n.a

	B. cereus	+++
	S. typhimurium	n.a
	E. coli	n.a
	S. aureus	n.a
	P. aeruginosa	+
	C. albicans	+
RT/1/D2	*K. pneumoniae*	n.a
	B. cereus	+
	S. typhimurium	n.a
	E. coli	+
	S. aureus	n.a
	P. aeruginosa	+
	C. albicans	++
RT/28/C8	*K. pneumoniae*	n.a
	B. cereus	+++
	S. typhimurium	n.a
	E. coli	+++
	S. aureus	+
	P. aeruginosa	+
	C. albicans	++
RT/20/D	*K. pneumoniae*	+
	B. cereus	n.a
	S. typhimurium	+
	E. coli	n.a
	S. aureus	n.a
	P. aeruginosa	+++
	C. albicans	++
RT/25/C10	*K. pneumoniae*	+
	B. cereus	+++
	S. typhimurium	n.a
	E. coli	+
	S. aureus	++
	P. aeruginosa	+
	C. albicans	++
RT/21/D	*K. pneumoniae*	n.a
	B. cereus	n.a
	S. typhimurium	++
	E. coli	n.a
	S. aureus	n.a
	P. aeruginosa	+
	C. albicans	++
JK/43/F	*K. pneumoniae*	+
	B. cereus	n.a
	S. typhimurium	+
	E. coli	+
	S. aureus	+
	P. aeruginosa	++
	C. albicans	++

M/8/D1	*K. pneumoniae*	n.a
	B. cereus	n.a
	S. typhimurium	+
	E. coli	+
	S. aureus	+++
	P. aeruginosa	n.a
	C. albicans	++
DUR/228/B	*K. pneumoniae*	++
	B. cereus	n.a
	S. typhimurium	n.a
	E. coli	n.a
	S. aureus	n.a
	P. aeruginosa	+
	C. albicans	+
JK/33/9	*K. pneumoniae*	n.a
	B. cereus	+++
	S. typhimurium	++
	E. coli	n.a
	S. aureus	+
	P. aeruginosa	n.a
	C. albicans	+

Table 5 shows the antimicrobial activity of 17 extracts (each one diluted 5 times) against the 7 microorganisms tested based on the disc-diffusion assay. The results (Table 5) showed that *P. longifolia* leaf extracts were active (depending on the solvent used) against all the 7 microorganisms tested with the exception of the DCM extract against *B. cereus*, *S. aureus* and *P. aeruginosa* and the EtOAc extract against *B. cereus*, *S. typhimurium* and *E. coli*. The methanolic bark extract of *P. longifolia* was only active against *E. coli* and *C. albicans* and active against *S. typhimurium* and *P. aeruginosa* at the highest concentrations but was found to be inactive against *K. pneumoniae*, *B. cereus*, and *S. aureus* and against both *S. typhimurium* and *P. aeruginosa* at the two lowest concentrations (0.1 and 0.25mg ml^{-1}).

The ethylacetate leaf extract of *S. birrea* was only active against *B. cereus*, *S. typhimurium* and *C. albicans* but was not active against *K. pneumoniae*, *E. coli*, *S. aureus* and *P. aeruginosa* at all concentrations tested. The chloroform and ethylacetate leaf extracts of *S. cordatum* were found to be active against all the micro-organisms at all the concentrations, with the highest activity observed at the highest concentration, except for the chloroform leaf extract of *S. cordatum* against *K. pneumoniae* and ethylacetate leaf extract of *S. cordatum* against *S. aureus* at all

concentrations. Ethylacetate leaf extractive of *A. venenata* was found to be only active against both *B. cereus* and *C. albicans*. *K. pneumoniae*, *S. typhimurium*, *E. coli*, *S. aureus*, and *P. aeruginosa* were resistant against this EtOAc leaf extractive of *A. venenata*.

All the microorganisms were resistant against crude methanolic leaf extract of *A. venenata* with the exception of *S. typhimurium* at the lowest concentration. Ethylacetate leaf extractive of *Acokanthera sp.* was highly active against *K. pneumoniae*, *B. cereus* and *E. coli* and also mildly active against *C. albicans* and only active against *P. aeruginosa* at the 3 highest concentrations. Only *S. typhimurium* and *S. aureus* were resistant against this extract. Crude methanolic leaf extract of *Acokanthera sp.* was only active against both *K. pneumoniae* and *S. typhimurium* with all the concentrations showing the same inhibitory effect. Ethylacetate leaf extractive of *C. macrocarpa* was active against *K. pneumoniae*, *B. cereus* and *S. aureus*. It was observed that the lowest concentrations (0.1 and 0.25mg ml^{-1}) of this extract caused the highest inhibition. It was also observed that this extract had a uniform inhibitory effect against *B. cereus* at all concentrations and was highly active against *S. aureus* at the two highest concentrations. Ethylacetate leaf extractive of *C. macrocarpa* was found to be inactive against *S. typhimurium*, *E. coli*, *P. aeruginosa* and *C. albicans*. Crude methanolic leaf extract of *C. macrocarpa* was only active against *S. typhimurium*, *P. aeruginosa* and *C. albicans*, with *P. aeruginosa* showing the highest susceptibility at the highest concentration and this extract was inactive against *K. pneumoniae*, *B. cereus*, *E. coli* and *S. aureus*. Methanolic leaf extractive of *C. foveolatus* was found to be active against *K. pneumoniae*, *S. typhimurium* and *C. albicans* at all concentrations and only active against *S. aureus* and *P. aeruginosa* at the three highest concentrations. This extract was observed to be more active against the fungus than any other microorganism tested in this study.

Crude methanolic stem extract of *H. cannabinus* was highly active against *S. aureus* at the highest concentrations and was uniformly active against *S. typhimurium*, *E. coli* at the two highest concentrations tested but was found to be inactive against *K. pneumoniae*, *B. cereus*, *P. aeruginosa* and *C. albicans*. Crude methanolic calyces extract of *H. sabdariffa* was found similarly active against *K. pneumoniae*, *P.*

aeruginosa, and *C. albicans* and was inactive against *B. cereus*, *E. coli* and *S. aureus* but was found to be active against *S. typhimurium* at 0.1mg ml^{-1} and inactive at 0.67 and 1.0mg ml^{-1} but was found active against this bacterium at the last two highest concentrations. Ethylacetate stem extractive of *H. natalensis* was found to be highly active against *B. cereus* at the last 3 highest concentrations and similarly active against *C. albicans* at all concentrations but was inactive against *K. pneumoniae*, *S. typhimurium*, *E. coli*, *S. aureus* and *P. aeruginosa*.

Table 5: Antimicrobial activity of the five-fold dilutions of the 17 extracts against the bacterial and fungal species tested based on the disc-diffusion method. Activity was expressed as +, high activity as ++, very high activity as +++ and absence of activity as n.a.

Plant extract code	Microorganism	Activity of extracts at each concentration (mg ml^{-1})				
		0.1	0.25	0.4	0.67	1.0
PL/A3	*K. pneumoniae*	+	+	+	+	+
	B. cereus	n.a	n.a	n.a	n.a	n.a
	S. typhimurium	+	+	+	+	++
	E. coli	+	+	+	+	+
	S. aureus	n.a	n.a	n.a	n.a	n.a
	P. aeruginosa	n.a	n.a	n.a	n.a	n.a
	C. albicans	+	+	++	++	+++
PL/A4	*K. pneumoniae*	+	++	+	+	++
	B. cereus	n.a	n.a	n.a	n.a	n.a
	S. typhimurium	n.a	n.a	n.a	n.a	n.a
	E. coli	n.a	n.a	n.a	n.a	n.a
	S. aureus	+	+	+	+	++
	P. aeruginosa	+	+	+	+	+
	C. albicans	+	+	+	+	+
PL/A6	*K. pneumoniae*	+	+	+	+	++
	B. cereus	++	n.a	n.a	++	+
	S. typhimurium	+	+	+	+	+
	E. coli	+	+	+	+	+
	S. aureus	+	+	+	+	+
	P. aeruginosa	+	+	+	+	+
	C. albicans	+	+	+	+	+
PL/B6	*K. pneumoniae*	n.a	n.a	n.a	n.a	n.a
	B. cereus	n.a	n.a	n.a	n.a	n.a
	S. typhimurium	n.a	n.a	+	+	+
	E. coli	+	+	++	++	+++
	S. aureus	n.a	n.a	n.a	n.a	n.a
	P. aeruginosa	n.a	n.a	+	+	++
	C. albicans	+	+	++	++	+++
SB/A4	*K. pneumoniae*	n.a	n.a	n.a	n.a	n.a
	B. cereus	+	+	++	n.a	+
	S. typhimurium	+	+	++	++	++
	E. coli	n.a	n.a	n.a	n.a	n.a
	S. aureus	n.a	n.a	n.a	n.a	n.a
	P. aeruginosa	n.a	n.a	n.a	n.a	n.a
	C. albicans	+	++	++	++	+++
MG/9/B	*K. pneumoniae*	n.a	n.a	n.a	n.a	n.a
	B. cereus	+	++	+	++	++
	S. typhimurium	++	++	++	++	+++
	E. coli	+	+	++	++	++

	S. aureus	+	+	+	+	+
	P. aeruginosa	+	+	++	+++	+++
	C. albicans	+	+	+	++	+++
MG/9/C	*K. pneumoniae*	+	+	+	+	+
	B. cereus	+	++	++	++	+++
	S. typhimurium	+	+	+	++	++
	E. coli	+	++	++	+++	+++
	S. aureus	n.a	n.a	n.a	n.a	n.a
	P. aeruginosa	+	+	++	++	+++
	C. albicans	+	+	+	++	+++
RT/2/C8-9	*K. pneumoniae*	n.a	n.a	n.a	n.a	n.a
	B. cereus	++	++	+++	+++	+++
	S. typhimurium	n.a	n.a	n.a	n.a	n.a
	E. coli	n.a	n.a	n.a	n.a	n.a
	S. aureus	n.a	n.a	n.a	n.a	n.a
	P. aeruginosa	n.a	n.a	n.a	+	+
	C. albicans	+	++	++	+++	+++
RT/1/D2	*K. pneumoniae*	n.a	n.a	n.a	n.a	n.a
	B. cereus	n.a	n.a	n.a	n.a	n.a
	S. typhimurium	+	+	n.a	+	n.a
	E. coli	n.a	n.a	n.a	n.a	n.a
	S. aureus	n.a	n.a	n.a	n.a	n.a
	P. aeruginosa	n.a	n.a	n.a	n.a	n.a
	C. albicans	n.a	n.a	n.a	n.a	n.a
RT/28/C8	*K. pneumoniae*	++	+++	+++	+++	+++
	B. cereus	+	+++	++	++	+
	S. typhimurium	n.a	n.a	n.a	n.a	n.a
	E. coli	+	+	++	++	+++
	S. aureus	n.a	n.a	n.a	n.a	n.a
	P. aeruginosa	n.a	n.a	+	+	+
	C. albicans	+	+	+	+	++
RT/20/D	*K. pneumoniae*	+	+	+	+	+
	B. cereus	n.a	n.a	n.a	n.a	n.a
	S. typhimurium	+	+	+	+	+
	E. coli	n.a	n.a	n.a	n.a	n.a
	S. aureus	n.a	n.a	n.a	n.a	n.a
	P. aeruginosa	n.a	n.a	n.a	n.a	n.a
	C. albicans	n.a	n.a	n.a	n.a	n.a
RT/25/C10	*K. pneumoniae*	+++	++	++	+	+
	B. cereus	++	++	++	++	++
	S. typhimurium	n.a	n.a	n.a	n.a	n.a
	E. coli	n.a	n.a	n.a	n.a	n.a
	S. aureus	+	+	++	++	++
	P. aeruginosa	n.a	n.a	n.a	n.a	n.a
	C. albicans	n.a	n.a	n.a	n.a	n.a
RT/21/D	*K. pneumoniae*	n.a	n.a	n.a	n.a	n.a
	B. cereus	n.a	n.a	n.a	n.a	n.a
	S. typhimurium	+	+	+	+	+

	E. coli	n.a	n.a	n.a	n.a	n.a
	S. aureus	n.a	n.a	n.a	n.a	n.a
	P. aeruginosa	+	+	+	++	++
	C. albicans	+	+	+	+	+
JK/43/F	*K. pneumoniae*	+	++	+	+	+
	B. cereus	n.a	n.a	n.a	n.a	n.a
	S. typhimurium	+	+	+	+	+
	E. coli	n.a	n.a	n.a	n.a	n.a
	S. aureus	n.a	n.a	+	+	+
	P. aeruginosa	n.a	n.a	+	+	+
	C. albicans	+	+	++	++	++
M/8/D1	*K. pneumoniae*	n.a	n.a	n.a	n.a	n.a
	B. cereus	n.a	n.a	n.a	n.a	n.a
	S. typhimurium	+	++	+	+	+
	E. coli	n.a	n.a	n.a	+	+
	S. aureus	+	+	++	++	+++
	P. aeruginosa	n.a	n.a	n.a	n.a	n.a
	C. albicans	n.a	n.a	n.a	n.a	n.a
DUR/228/B	*K. pneumoniae*	+	+	+	+	+
	B. cereus	n.a	n.a	n.a	n.a	n.a
	S. typhimurium	+	n.a	n.a	+	+
	E. coli	n.a	n.a	n.a	n.a	n.a
	S. aureus	n.a	n.a	n.a	n.a	n.a
	P. aeruginosa	+	+	+	+	++
	C. albicans	+	+	+	+	+
JK/33/9	*K. pneumoniae*	n.a	n.a	n.a	n.a	n.a
	B. cereus	+	++	+++	+++	+++
	S. typhimurium	n.a	n.a	n.a	n.a	n.a
	E. coli	n.a	n.a	n.a	n.a	n.a
	S. aureus	n.a	n.a	n.a	n.a	n.a
	P. aeruginosa	n.a	n.a	n.a	n.a	n.a
	C. albicans	+	+	+	+	+

Table 6 shows antimicrobial activity of the isolated compounds and mixtures (each diluted 3 times, but only the means are shown), tested against the 7 microorganisms, based on the disc-diffusion method. Lupane triterpenoids (lupeol, lupulon, lupinine, lupenone and luteolin), isolated from *P. longifolia* leaf extracts (table 6) were found to be active against all the 7 microorganisms at the lowest concentrations (0.1mg ml^{-1}), with the highest activity against *K. pneumoniae*. OA-UA and MM-MC mixtures were both found to be active against all but one microorganism, *S. aureus* (for OA-UA) and *K. pneumoniae* (for MM-MC). Betunilic acid (BA) was found to be equally active against *B. cereus* and *C. albicans*, and showed mild activity against *P. aeruginosa*. BA was inactive against *K. pneumoniae*, *S. typhimurium*, *E. coli* and *S. aureus*. The

S. birrea mixture was found to be markedly active against *C. albicans* and mildly active against *B. cereus* and *S. typhimurium* but inactive against *K. pneumoniae*, *E. coli*, *S. aureus* and *P. aeruginosa*.

Table 6: Mean antimicrobial activity of the isolated compounds against the bacteria and fungal species tested based on the disc-diffusion method. Activity measured between 7 and 14mm diameter was expressed as +, between 15 and 19mm as ++, greater than 20mm diameter was expressed as +++ and no activity as n.a. (n=3)

Compound isolated	Microorganism tested	Activity of the compound
Betulinic acid (BA)	*K. pneumoniae*	n.a
	B. cereus	+++
	S. typhimurium	n.a
	E. coli	n.a
	S. aureus	n.a
	P. aeruginosa	+
	C. albicans	+++
Lupane tripterpenoids	*K. pneumoniae*	++
	B. cereus	+
	S. typhimurium	+
	E. coli	+
	S. aureus	+
	P. aeruginosa	+
	C. albicans	+
UA-OA mixture	*K. pneumoniae*	+
	B. cereus	+++
	S. typhimurium	++
	E. coli	+++
	S. aureus	n.a
	P. aeruginosa	+++
	C. albicans	+++
MM-MC mixture	*K. pneumoniae*	n.a
	B. cereus	++
	S. typhimurium	+++
	E. coli	++
	S. aureus	+

	P. aeruginosa	+++
	C. albicans	+++
S. birrea mixture	*K. pneumoniae*	n.a
	B. cereus	+
	S. typhimurium	++
	E. coli	n.a
	S. aureus	n.a
	P. aeruginosa	n.a
	C. albicans	+++

Table 7 shows MIC values of all the extracts, isolated compounds and mixtures against the 7 microorganisms tested based on the disc-diffusion assay. Most of the extracts tested had the lowest MIC value of 0.1mg ml^{-1} against most of the microorganisms. Exceptions were observed in cases where active concentration was much higher, as in the methanolic bark extract of *P. longifolia* against *S. typhimurium* and *P. aeruginosa* (0.40mg ml^{-1}), ethylacetate leaf extractive of *Acokanthera sp.* against *P. aeruginosa* (0.40mg ml^{-1}), methanolic leaf extractive of *C. foveolatus* against *S. aureus* (0.4mg ml^{-1}), crude methanolic stem extract of *H. cannabinus* against *E. coli* (0.67mg ml^{-1}).

Table 7. Minimum inhibitory concentration of crude extracts and isolated compounds. Activity was expressed as the minimum concentration that inhibited growth of microorganism and n.a for no activity.

Extract/compound	Minimum Inhibitory Concentration of tested micro-organisms (mg ml^{-1})						
	KP	BC	ST	EC	SA	PA	CA
PL/A3	0.1	n.a	0.1	0.1	n.a	n.a	0.1
PL/A4	0.1	n.a	n.a	n.a	0.1	0.1	0.1
PL/A6	0.1	0.1	0.1	0.1	0.1	0.1	0.1
PL/B6	n.a	n.a	0.4	0.1	n.a	0.4	0.1
SB/A4	n.a	0.1	0.1	n.a	n.a	n.a	0.1
MG/9/B	n.a	0.1	0.1	0.1	0.1	0.1	0.1
MG/9/C	0.1	0.1	0.1	0.1	n.a	0.1	0.1
RT/2/C8-9	n.a	0.1	n.a	n.a	n.a	n.a	0.1
RT/1/D2	n.a	n.a	0.1	n.a	n.a	n.a	n.a
RT/28/C8	0.1	0.1	n.a	0.1	n.a	0.4	0.1
RT/20/D	0.1	n.a	0.1	n.a	n.a	n.a	n.a
RT/25/C10	0.1	0.1	n.a	n.a	0.1	n.a	n.a
RT/21/D	n.a	n.a	0.1	n.a	n.a	0.1	0.1
JK/43/F	0.1	n.a	0.1	n.a	0.4	n.a	0.1
M/8/D1	n.a	n.a	0.1	0.67	0.1	n.a	n.a
DUR/228/B	0.1	n.a	0.1	n.a	n.a	0.1	0.1
JK/33/9	n.a	0.1	n.a	n.a	n.a	n.a	0.1

KP (*Klesbsiella pneumoniae*) BC (*Bacillus cereus*) ST (*Salmonella typhimurium*) EC (*Escherichia coli*) SA (*Staphylococcus aureus*) PA (*Pseudomonas aeruginosa*) CA (*Candida albicans*)

Five hundred and ninety five diluted and 102 concentrated organic and aqueous extracts were tested for antimicrobial activity. This activity was quantitatively assessed by the presence or absence of inhibition zones. Zone diameters were measured and minimum inhibitory concentrations (MIC) of diluted extracts and isolated compounds were determined by means of the liquid dilution method.

Overall, the results showed that the extracts from *A.venenata*, *C. macrocarpa*, *C. foveolatus*, *H. cannabinus*, *H. natalensis*, *H. sabdariffa*, *P. longifolia*, *S. birrea* and *S. cordatum* possessed antimicrobial activity. The results also showed that the diluted extracts (0.1mg ml^{-1}) exhibited inhibition against all the microorganisms tested. Although the activity varied against the microorganisms, all diluted extracts showed antimicrobial activity against *B cereus*, *S. typhimurium*, *E. coli* and *C. albicans* (Table 5) and the stock solution extracts (1.0mg ml^{-1}) showed antimicrobial activity against *K. pneumoniae*, *B. cereus*, *S. typhimurium*, *E. coli*, *S. aureus*, *P. aeruginosa* and *C. albicans* (Table 4).

The crude extracts (Table 3) showed activity against both *E. coli* and *S. aureus* with the highest activity exhibited by dichloromethane, ethylacetate, methanolic leaf extracts, methanolic bark extract of *P. longifolia* and ethylacetate leaf extract of *S. birrea*. The results showed that all the concentrated stock solution extracts (0.85mg ml^{-1}) had an inhibitory effect on the growth of the yeast *C. albicans* (Table 4) and all the diluted extracts (0.1mg ml^{-1}) had a similar inhibitory effect (Table 5) except for crude methanolic leaf extract of *A. venenata*, crude methanolic leaf extract of *Acokanthera sp.*, ethylacetate leaf extractive of *C. macrocarpa* and crude methanolic stem extract of *H. cannabinus*. All diluted organic and aqueous extracts also showed activity against the pneumonia bacterium, *K. pneumoniae* with the highest activity exhibited by ethylacetate leaf extractive of *Acokanthera sp.* Extracts that exhibited no activity against *K. pneumoniae* included methanolic bark extract of *P. longifolia*, ethylacetate leaf extract of *S. birrea*, chloroform leaf extract of *S. cordatum*, ethylacetate leaf extractive and crude methanolic leaf extract of *A. venenata*, crude methanolic leaf extract of *C. macrocarpa*, crude methanolic stem extract of *H. cannabinus* and ethylacetate stem extractive of *H. natalensis* (Table 5). Almost 60% of the diluted extracts (Table 5) had no inhibitory effects on the growth of *B. cereus*. The methanolic leaf extract of *P. longifolia*, ethylacetate leaf extract of *S. birrea*, chloroform and ethylacetate leaf extracts of *S. cordatum*, ethylacetate leaf extractive of *A. venenata*, ethylacetate leaf extractive of *Acokanthera sp.*, ethylacetate leaf extractive of *C. macrocarpa* and ethylacetate stem extractive of *H. natalensis* had a marked inhibitory effect with zone diameters of up to 35mm.

The diluted ethylacetate leaf extract of *P. longifolia*, ethylacetate leaf extractive of *A. venenata*, ethylacetate leaf extractive of *Acokanthera sp.*, ethylacetate leaf extractive of *C. macrocarpa* and ethylacetate stem extractive of *H. natalensis*, had no effect on the growth of *S. typhimurium*, the causative agent of typhoid fever. *E. coli* was found to be resistant to 60% of the extracts (ethylacetate leaf extracts of *P. longifolia* and *S. birrea*, ethylacetate leaf extractive of *A. venenata*, crude methanolic leaf extract of *A. venenata*, crude methanolic leaf extract of *Acokanthera sp.*, both the ethylacetate leaf extractive and crude methanolic leaf extract of *C. macrocarpa*, methanolic leaf extractive of *C. foveolatus*, crude methanolic calyces extract of *H. sabdariffa* and ethylacetate stem extractive of *H. natalensis*) and was inhibited at higher concentrations of crude methanolic stem extract of *H. cannabinus*. Also *S. aureus* was resistant to 60% of the diluted extracts (dichloromethane leaf and methanolic bark extracts of *P. longifolia*, ethylacetate leaf extracts of *S. birrea* and *S. aureus*, ethylacetate leaf extractive and crude methanolic leaf extract of *A. venenata*, ethylacetate leaf extractive and the crude methanolic leaf extract of *Acokanthera sp.*, crude methanolic leaf extract of *C. macrocarpa*, crude methanolic calyces extract of *H. sabdariffa* and ethylacetate stem extractive of *H. natalensis*) with crude methanolic stem extract of *H. cannabinus* being the most effective extract against *S. aureus*. *P. aeruginosa* was found to be resistant to extracts (dichloromethane leaf extract of *P. longifolia*, ethylacetate leaf extract of *S. birrea*, both the crude methanolic leaf extract and ethylacetate leaf extractive of *C. macrocarpa*, crude methanolic leaf extract of *Acokanthera sp.*, crude methanolic stem extract of *H. cannabinus* and ethylacetate stem extractive of *H. natalensis*).

The dichloromethane leaf extract of *P. longifolia* showed inhibitory activity against all the microorganisms tested with the lowest activity (10mm diameter) exhibited against *B. cereus*, *E. coli* and *S. aureus* and the highest activity (18mm diameter) against *P. aeruginosa*. The ethylacetate leaf extract of *P. longifolia* was only inactive against *B. cereus* but had an activity against all the other microorganisms with the highest inhibition (22mm diameter) against *K. pneumoniae*. The methanolic leaf extract of *P. longifolia* had no effect against *B. cereus* but was active against all the other microorganisms, with highest activity against *C. albicans*. Methanolic bark extract of *P. longifolia* was found to be active against all microorganisms except for

K. pneumoniae and *S. aureus* and the lowest activity (8mm diameter) was against *B. cereus* and the highest against *E. coli* (17mm diameter). Chloroform and ethylacetate leaf extracts of *S. cordatum* were both highly active against all microorganism with the exception of the ethylacetate extract. The highest activity (20mm diameter) of these 2 extracts was exhibited against *K. pneumoniae*, *S. typhimurium* and *P. aeruginosa*. Ethylacetate leaf extract of *S. cordatum* was found to be active against all 7 microorganisms with highest inhibition (20, 21, 22 and 23mm diameter) observed against *S. aureus*, *B. cereus*, *E. coli* and *P. aeruginosa* respectively. Ethylacetate leaf extractive of *A. venenata* was active against *B. cereus* and *P. aeruginosa* with the highest activity (37mm diameter) exhibited against *B. cereus*. Ethylacetate leaf extractive of *Acokanthera sp.* showed activity against all the other microorganisms, with highest activity (26mm diameter) against *B. cereus* and *E. coli*. Crude methanolic leaf extract of *Acokanthera sp.* was active against *K. pneumoniae*, *S. typhimurium*, *P. aeruginosa* and *C. albicans*, with the yeast being the most susceptible microorganism (16mm diameter). Ethylacetate leaf extractive of *C. macrocarpa* showed high activity (30mm diameter) against *B. cereus* and all others and no activity against *S. typhimurium*. Crude methanolic leaf extract of *C. macrocarpa* exhibited inhibition against *S. typhimurium* (15mm diameter) and *P. aeruginosa* (9mm diameter). Methanolic leaf extractive of *C. foveolatus* was active against all microorganism. Crude methanolic stem extract of *H. cannabinus* was highly active against *S. aureus* (20mm diameter) and least active against *E. coli*. Crude methanolic calyces extract of *H. sabdariffa* was active against *K. pneumoniae*, *P. aeruginosa* and *C. albicans*. Ethylacetate stem extractive of *H. natalensis* had the highest activity (40mm diameter) of all the extracts and this was against *B. cereus*.

4.2 Chemical analyses:

4.2.1 Overview of spectra of all flavanoid compounds isolated

The NMR spectra of these compounds were similar and typical of flavonoids. They possess 2-phenyl chromanone as the parent skeleton. The heterocyclic ring has three carbon resonances, namely the oxymethine (C-2) resonating between 70 – 80ppm, aliphatic methylene (C-3) resonating between 39 – 46ppm and the carbonyl (C-4) resonating between 186 – 198ppm (Agrawal, 1989). C-2 of the molecules is the centre of asymmetry and two forms of each structure are possible. However, most of

the naturally occurring flavanones acquire phenyl substituent at C-2 position in the pseudo-equitorial position. They bear a hydroxyl substituent at C-5 and C-7 positions hence the assignment of the carbon resonances of ring A in case of 5,7-dihydroxyflavanone are of significance importance (Wagner and Bladt, 1976). The carbonyl resonance (C-4) depends on the presence or the absence of the *para*-substituted C-5. In case of 5-unsubstituted flavanones, C-4 resonance absorbs between 189 – 191ppm except for the 7,8,3',4'-tetrahydroxyflavanone, where it resonates appreciably at low field position δ194 . In 5-hydroxylated flavanones, C-4 absorbs at deshielded position 195 – 197ppm because of hydrogen bonding (Agrawal, 1989). The chemical shift of C-3 is independent of the substituents in the aromatic rings and should there be a shift it will be upfield. One to 2 ppm in 5-hydroxylated flavanones compared to the 5-unsubstituted. All the six carbons of ring A in flavanones do not superimpose with each other hence give rise to six signals unless there is symmetry.

4.2.2 Isolation of oleanolic and ursolic acids and methyl maslinate and methyl corosolate

The *S. cordatum* leaf ethylacetate extract, subjected to column chromatography over silica gel in a gradient elution using HEX:EA (10:0 to 0:10 v/v) yielded 7 fractions, SN2/A1 to A7. Spectroscopic analysis of fractions SN2/A2, A3 and A4 revealed mixtures of oleanolic (3-hydroxylean-12-en-28-oic acid) (OA) and ursolic (3β-hydroxyurs-12-en-28-oic acid) (UA) acids (Fig. 22 & 23). Spectroscopic (NMR and ^{13}C) analysis of fractions SN2/A5, A6 and A7 showed that they were mixtures of methyl maslinate (2α, 3β-dihydroxyolean-12-en-28-oic acid methyl ester) (MM) (Fig. 24a) and methyl corosolate (MC) (Fig. 24b).

4.2.3 Isolation of lupane derivatives

The *P. longifolia* leaf ethylacetate and hexane extracts were combined because of their similar composition. Column chromatography of the combined extracts over silica gel in a gradient elution using HEX:EA (10:0 to 4:6 v/v) resulted in 11 fractions, SN4/A1 to A11. Fractions A1 and A3 were sufficiently pure for further characterization. Fraction A2 was a mixture of A1 and A3. Fractions A10 and A11 were not subjected to further analysis because they were not pure. ^{1}H-$^{1}HNMR$

spectra of A1 and A3 showed that they are terpenes of similar structure. Further structural elucidation of these fractions could not be continued due to technical operational problems. Fraction SN4/A5 afforded a pure, brownish, semi-oil compound, an alkaloid triterpene that was acetylated and produced a white powder and identified as lupeol (lup-20(29)-en-3β-ol) (Fig. 21). Fractions SN4/A6 to SN5/A9 were identified as luteolin [(2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4H-1-benzopyran-4-one), 3',4',5,7-tetrahydroxyflavone or 5,7,3',4'-tetrahydroxyflavone] (Fig. 29), lupinine (octahydro-2H-quinolizine-1-methanol) (Fig. 30), lupulon (3,5-dihydroxy-2,6,6-tris(3-methyl-2-butenyl)-4-(3-methyl-1-oxobutyl)-2,4-yclohexadien-1-one) or (3,5-dihdroxy-4-isovaleryl-2,6,6-tris(3-methyl-2-butenyl)-2,4-cyclohexadien-1-one) (Fig. 31) and lupenone [lup - 20 (29) - en - 3 - one or lup - 20 (30) - en - 3 – one] respectively.

4.2.3.1 Isolation of lupeol

The finely ground leaf material of *P. longifolia*, collected in the field in Durban, South Africa, was extracted in ethylacetate and purified by flash chromatography to give a white powder, Rf 0.63; mp 213-217°C (Connolly *et al.*, 1991); $[\alpha]^{20}_{D}$ + 20.1 9 (*c* 1.2, $CHCl_3$). ^{1}H and ^{13}C spectral data of lupeol were in agreement with those published in the literature (Setzer & Setzer, 2003). High Resolution Electron Impact Mass Spectroscopy (HREI-MS) *m/z* 426.72 $[M]^{+}$ (calculated for $C_{30}H_{50}O$, 426.3864).

4.2.4 Isolation of oily mixtures from *S. birrea*

S. birrea leaf methanol extract was also subjected to column chromatography over silica gel in a gradient elution using $CHCL_3$:MeOH (10:0 to 0:10 v/v) resulted in four fractions, SN3/A1 to A4. TLC analysis of all the fractions showed that they were mixture of oils. The purification of fractions SN3/A1 to A4 remains a future research objective.

4.2.5 Isolation of betulinic acid

H. natalensis stem ethylacetate extractive was subjected to spectroscopic analysis (^{1}HNMR and DEPT) and was identified as betulinic acid (3β-hydroxy-20(29)-lupaene-28-oic acid) (BA) (Fig. 28). The ground powder of *H. natalensis* was extracted with ethylacetate and purified by flash chromatography to give a white

powder, Rf 0.17; mp 250-252°C (Connolly *et al.*, 1991); $[\alpha]^{20}_{D}$ + 19.1 (*c* 0.67, C_5H_5N). ^{1}H and ^{13}C spectral data of betulinic acid were in agreement with those published in the literature (Tinto *et al.*, 1992). HREI-MS *m/z* 459.70 $[M]^{+}$ (calculated for $C_{30}H_{38}O_3$, 459.6657).

4.3 Statistical analyses:

The statistical analysis of the results is presented (Figures 10 to 20) showing that all dilutions were either extremely significant, very significant or significant ($P<0.05$). The statistical analyses showed that as the concentration of the extracts or extractives increased the zone of inhibition diameter also increased. Increase in concentration of *P. longifolia* bark in methanol against *E. coli* (Fig. 10), against *C. albicans* (Fig. 11) showed the bacteria are more inhibited with increase concentration, with the first and the second concentrations being not significantly different but both are significantly different to the third, fourth and the fifth. Again the third and the fourth dilutions are not significantly different but are different compared to the first two and the highest (fifth) dilution in both figures 10 and 11. *S. birrea* leaves in ethylacetate showed inhibitory activity against *C. albicans* (Fig. 12), with the first dilution being significantly different from all the other four. Dilutions two and three were not significantly different to each other but were different to the fourth and the fith dilutions, which in turn were not significantly different to each other.

S. cordatum leaves in ethylacetate against *E. coli* (Fig. 13) showed significant difference between the first and the third dilution but with the second dilution being not different from the fisr and the third. The forth and the fifth dilutions were not significantly different to each other but were significantly different to all the other three, viz dilutions one, two and three. *Acokanthera venenata* leaves in ethylacetate against *C. albicans* (Fig. 14) had dilution one significantly different from all the other four dilutions, with two and three showing no significant difference but were different to the fourth and the fifth which in turn were significantly different from each other. *Acokanthera sp.* leaves in ethylacetate against *E. coli* (Fig. 15) showed significant difference between dilution one against all the other three, with the second dilution being not significantly different from the first and the third, and the third dilution was in turn not significantly different from the fourth but was different to the fifth.

The fouth dilution was also found not significantly different to the fifth dilution, confirming increased inhibition with an increase in concentration. *Acokanthera sp.* leaves in ethylacetate against *C. albicans* (Fig. 16) showed significant difference between the first, fourth and fifth dilutions, with the first, second and third dilutions showing no significant difference among each other, and the third and fourth dilutions showed no significant difference but the fifth dilution was significantly different to all the other four dilutions. Fig. 17 shows *C. macrocarpa* leaves in ethylacetate against *K. pneumoniae*, with the lowest (first) concentration exhibiting the highest activity. Only the third dilution was significantly different to all the other dilutions, with the first and the second dilutions showing no difference and the fourth and the fifth dilutions were also not significantly different to each other. *C. foveolatus* seeds in methanol against *K. pneumoniae* (Fig. 18) showed significant difference between the first, third and fourth dilutions against the second and fifth, which in turn were not significantly different from each other, i.e the first, third and fourth were not different and the second and fifth dilutions were also not different from each other. Fig. 19 shows *H. sabdariffa* calyces in methanol against *P. aeruginosa*, with the first dilution being significantly different from the other four, but dilutions two, three and four were not significantly different from each other with the fourth dilutions also not different from the fifth dilution. Fig. 20, *H. natalensis* stem in ethylacetate against *B. cereus* with all the five dilutions being observed significantly different from each other, i.e dilution one different from two and two from three and three from four and four from five.

Few results were not significant (data not shown), where the increase in concentration of extracts, e.g. *H. sabdariffa* calyces showed a decrease in the inhibition of *K. pneumoniae* and where it was found that increasing the concentration, e.g. of *P. longifolia* leaves in both DCM and MeOH respectively, did not increase its inhibitory effect against *E. coli*. Overall, the results showed an increase in the diameters of zones of inhibition with every increase in concentration of extracts. This was also observed in the active stock solutions where the diameter of the highly concentrated extracts equals or exceed the diameter of the stock solution.

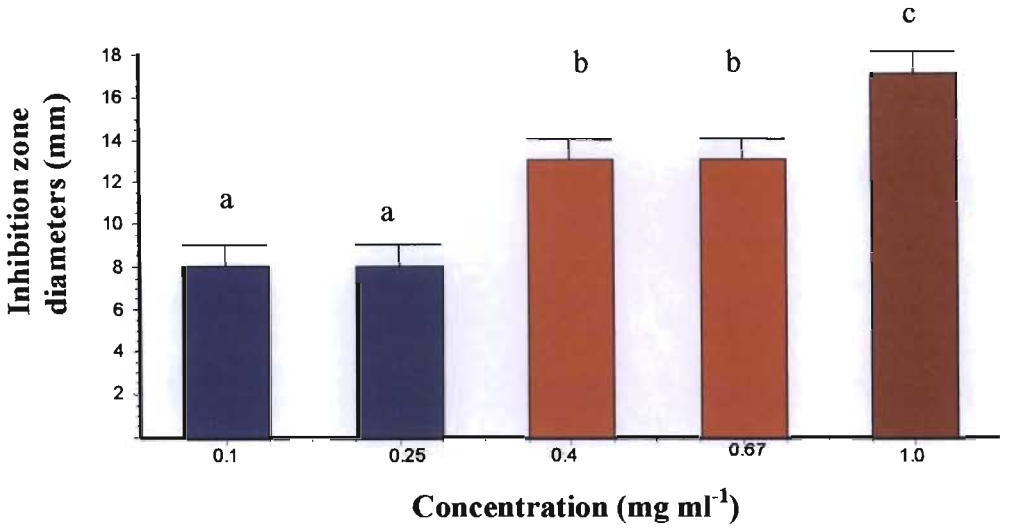


Figure 10: Effect of *Protorhus longifolia* bark in methanol (PL/A6) extract on growth of *Escherichia coli*. Means with different letters are significantly different at $p<0.05$ using Tukey's multiple range comparison test. (n=5)

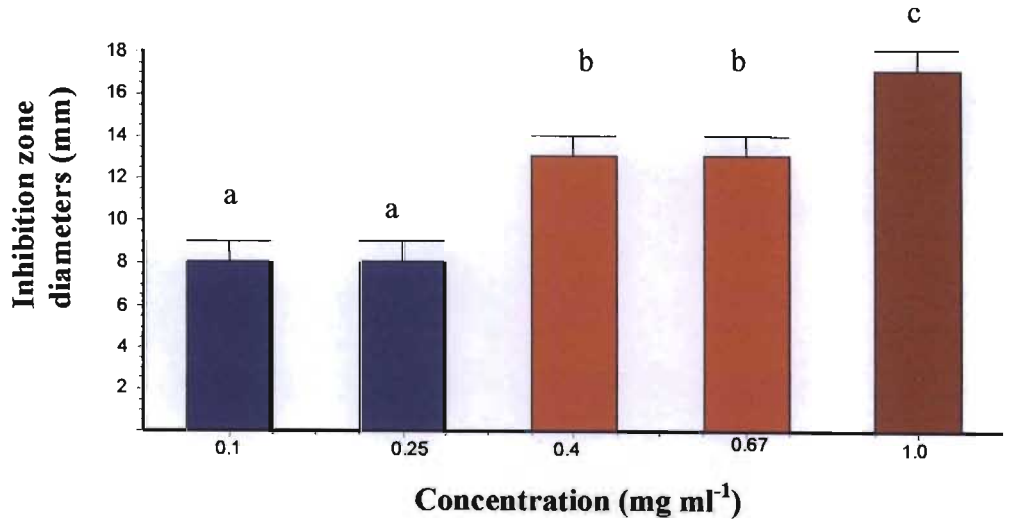


Figure 11: Effect of *Protorhus longifolia* bark in methanol extract on growth of *Candida albicans*. Means with different letters are significantly different at $p<0.05$ using Tukey's multiple range comparison test. (n=5)

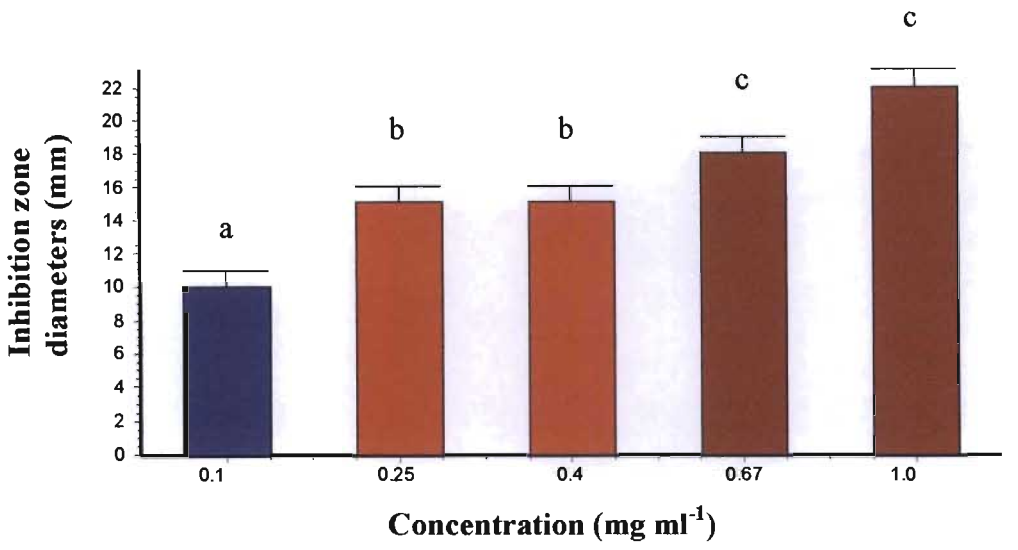


Figure 12: Effect of *Sclerocarya birrea* leaves in ethylacetate extract on growth of *Candida albicans*. Means with different letters are significantly different at $p<0.05$ using Tukey's multiple range comparison test. (n=5)

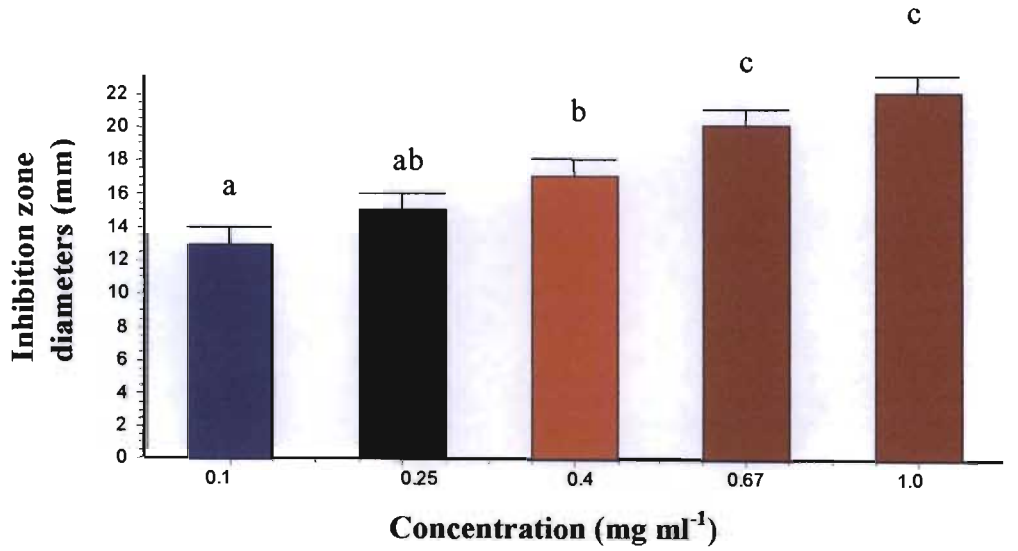


Figure 13: Effect of *Syzygium cordatum* leaves in ethylacetate extract on growth of *Escherichia coli*. Means with different letters are significantly different at $p<0.05$ using Tukey's multiple range comparison test. (n=5)

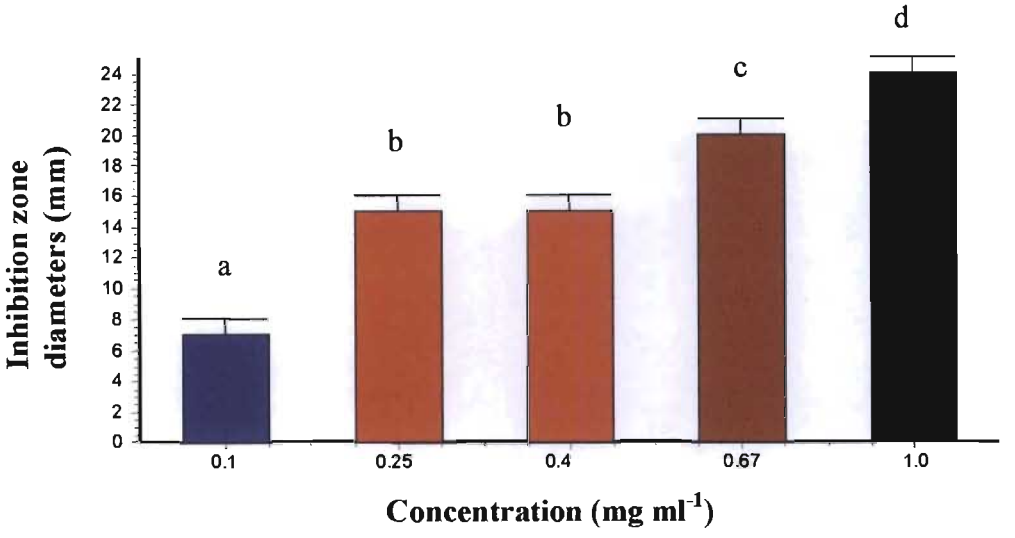


Figure 14: Effect of *Acokanthera venenata* leaves in ethylacetate extract on growth of *Candida albicans*. Means with different letters are significantly different at $p<0.05$ using Tukey's multiple range comparison test. (n=5)

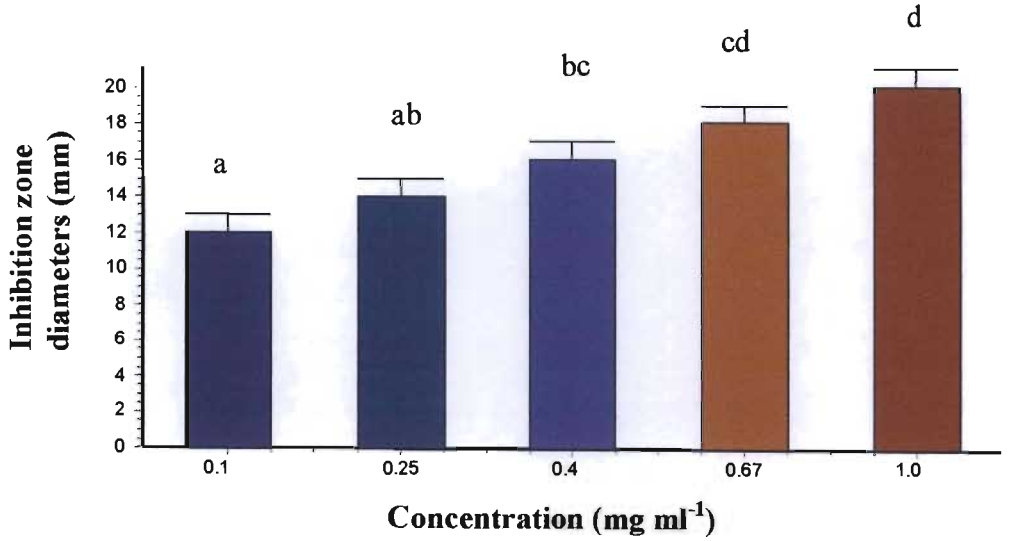


Figure 15: Effect of *Acokanthera sp.* leaves in ethylacetate extract on growth of *Escherichia coli*. Means with different letters are significantly different at $p<0.05$ using Tukey's multiple range comparison test. (n=5)

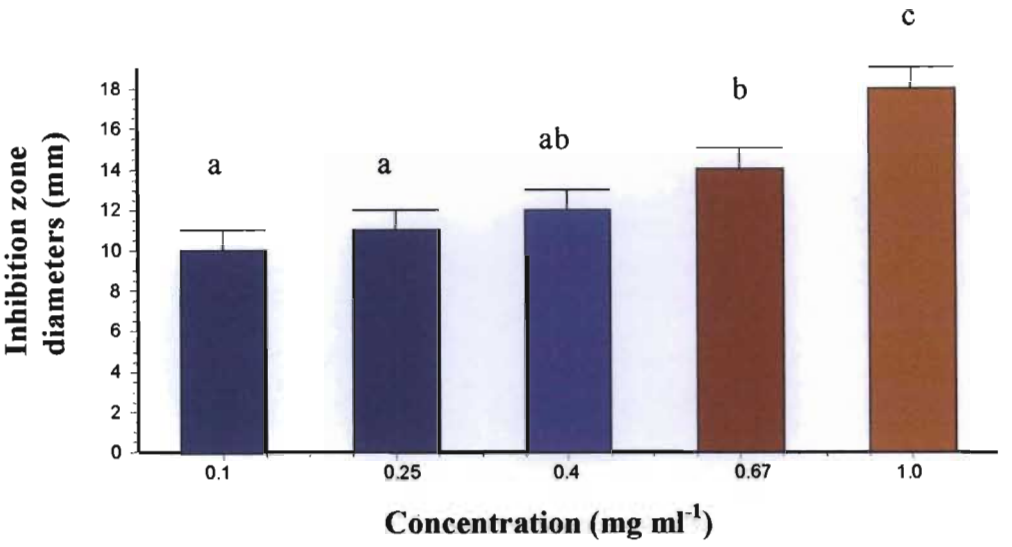


Figure 16: Effect of *Acokanthera sp.* leaves in ethylacetate extract on growth of *Candida albicans*. Means with different letters are significantly different at $p<0.05$ using Tukey's multiple range comparison test. (n=5)

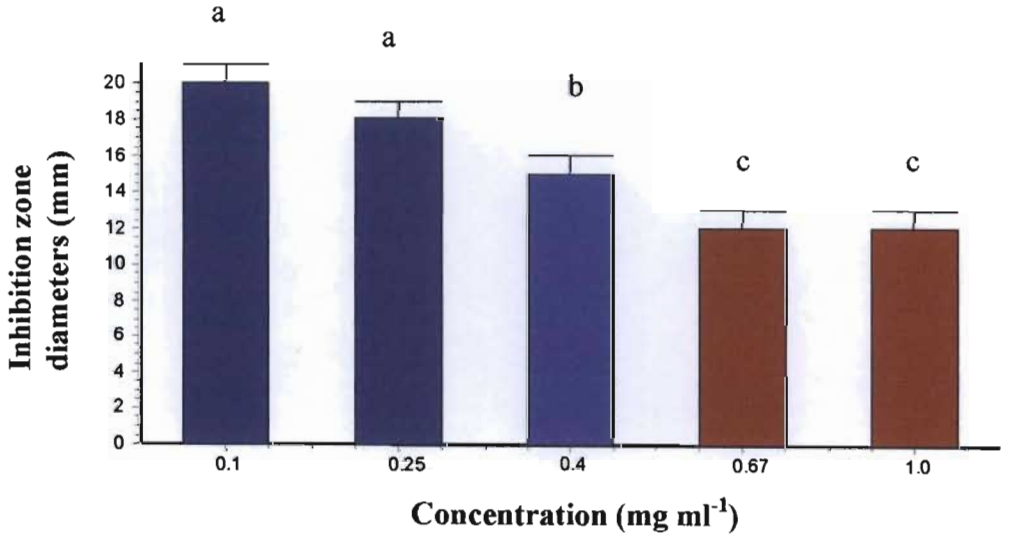


Figure 17: Effect of *Carissa macrocarpa* leaves in ethylacetate extract on growth of *Klebsiella pneumoniae*. Means with different letters are significantly different at $p<0.05$ using Tukey's multiple range comparison test. (n=5)

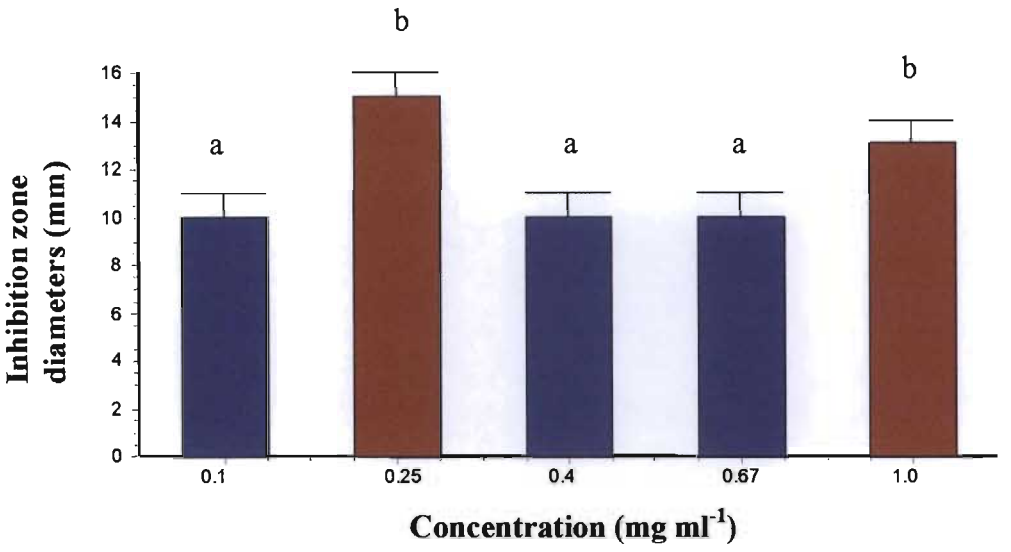


Figure 18: Effect of *Chiononthus foveolatus* seeds in methanol extract on growth of *Klebsiella pneumoniae*. Means with different letters are significantly different at $p<0.05$ using Tukey's multiple range comparison test. (n=5)

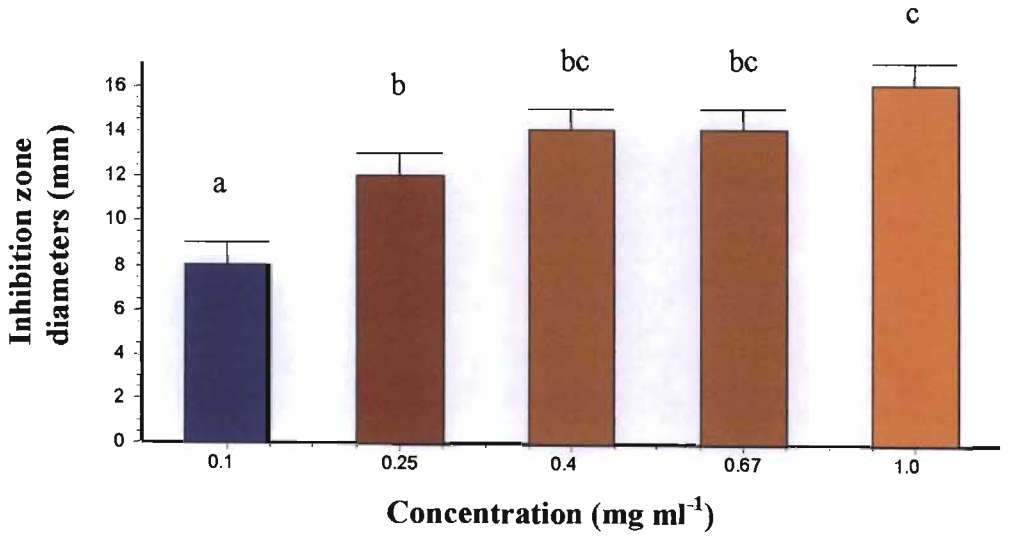


Figure 19: Effect of *Hibiscus sabdariffa* calyces in methanol extract on growth of *Pseudomonas aeruginosa*. Means with different letters are significantly different at $p<0.05$ using Tukey's multiple range comparison test. (n=5)

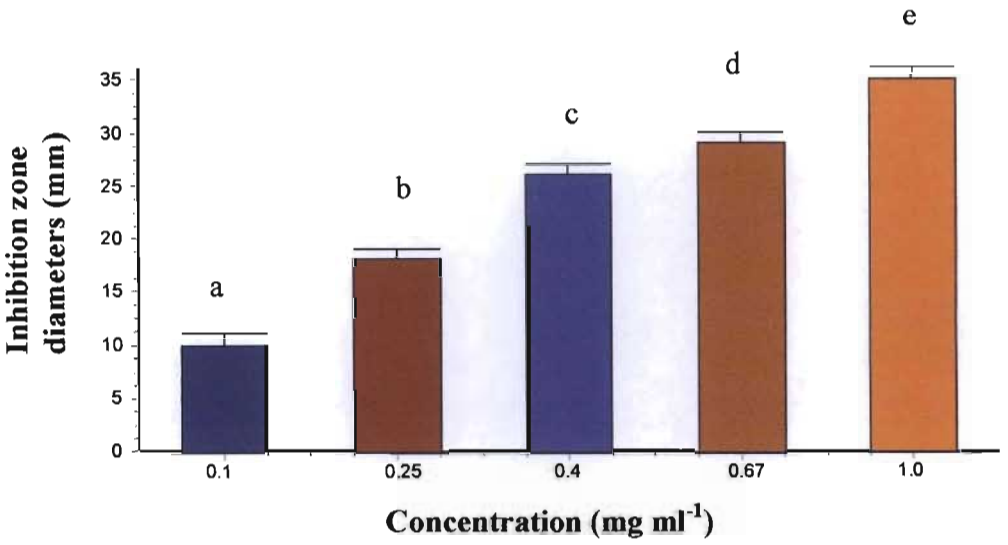


Figure 20: Effect of *Hibiscus natalensis* stem in ethylacetate extract on growth of *Bacillus cereus*. Means with different letters are significantly different at $p<0.05$ using Tukey's multiple range comparison test. (n=5)

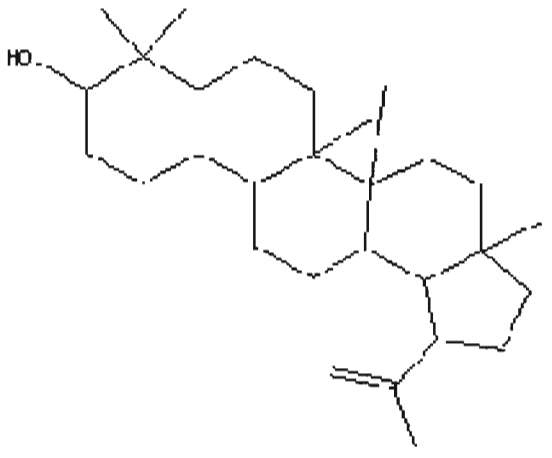


Figure 21: Chemical structure of lupeol from the leaves of *Protorhus longifolia*

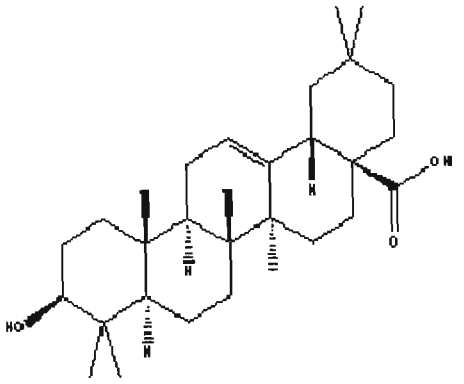


Figure 22: Chemical structure of oleanolic acid from the leaves of *Syzygium cordatum*

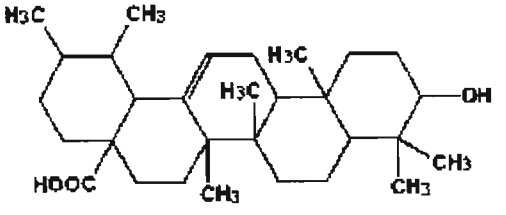
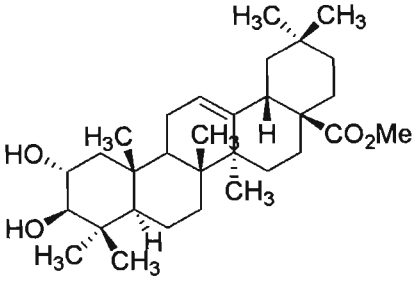
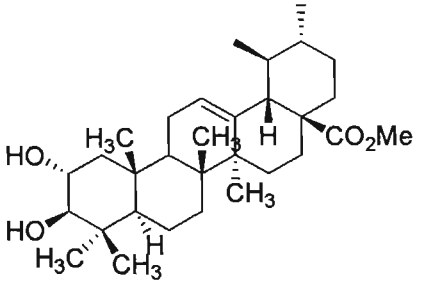


Figure 23: Chemical structure of ursolic acid from the leaves of *Syzygium cordatum*



Methyl maslinate



Methyl corosolate

Figure 24: Chemical structures of methyl maslinate (a) and methyl corosolate (b) from the leaves of *Syzygium cordatum*

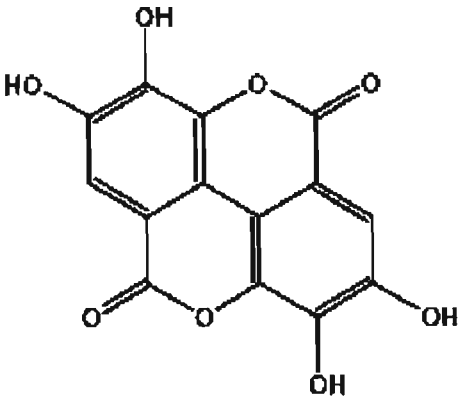


Figure 25: Chemical structure of ellagic acid from the leaves of *Syzygium cordatum*

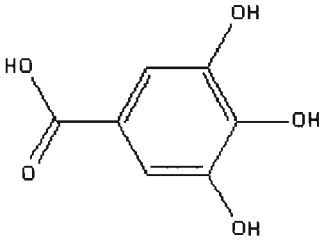


Figure 26: Chemical structure of gallic acid from the leaves of *Syzygium cordatum*

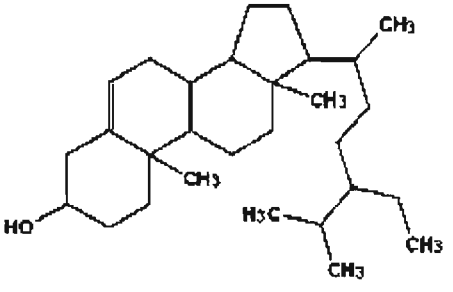


Figure 27: Chemical structure of β-sitosterol from the leaves of *Syzygium cordatum*

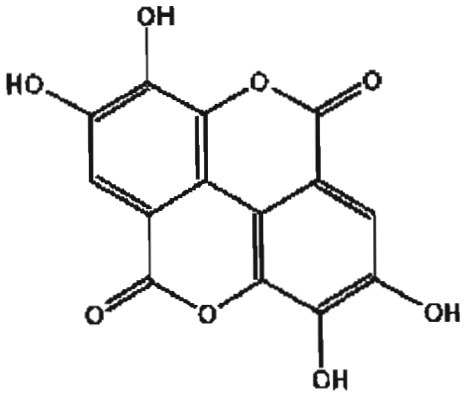


Figure 25: Chemical structure of ellagic acid from the leaves of *Syzygium cordatum*

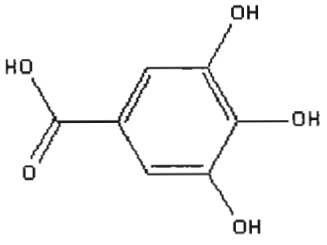


Figure 26: Chemical structure of gallic acid from the leaves of *Syzygium cordatum*

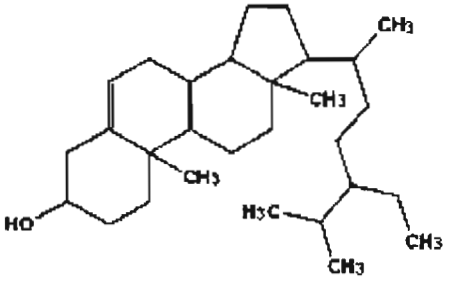


Figure 27: Chemical structure of β-sitosterol from the leaves of *Syzygium cordatum*

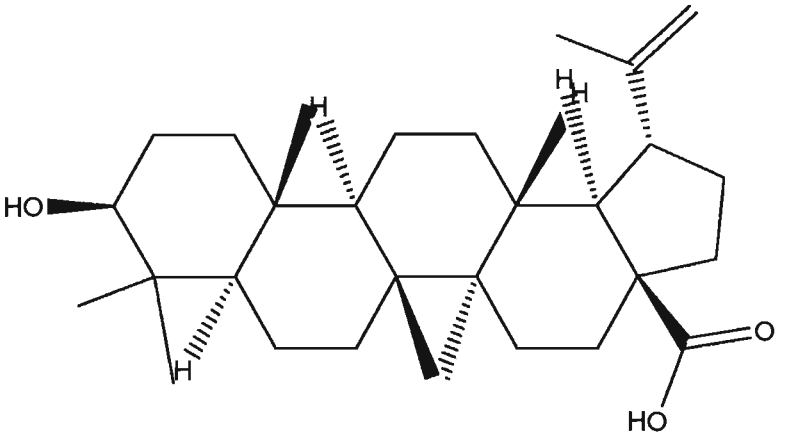


Figure 28: Chemical structure of betulinic acid from both the leaves of *Protorhus longifolia* and stem bark of *Hibiscus natalensis*

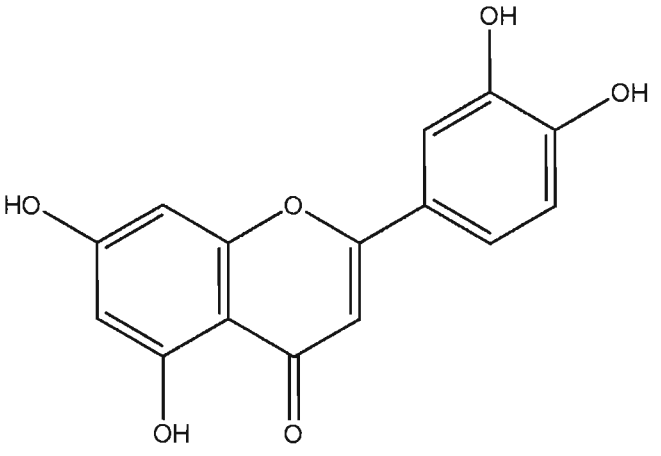


Figure 29: Chemical structure of luteolin from the leaves of *Protorhus longifolia*

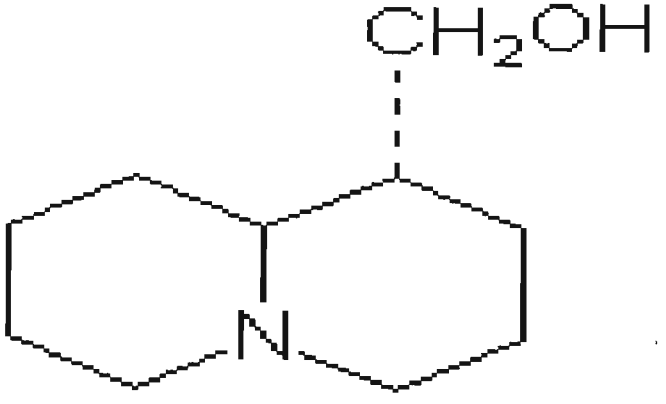


Figure 30: Chemical structure of lupinine from the leaves of *Protorhus longifolia*

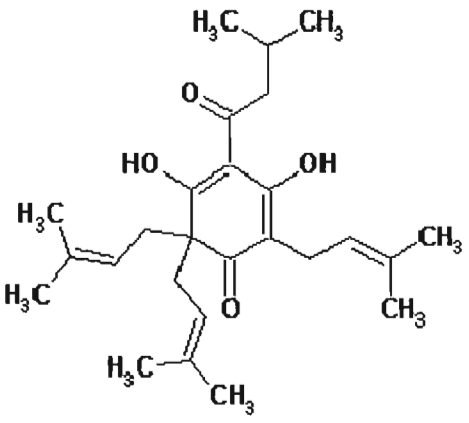


Figure 31: Chemical structure of lupulon from leaves of *Protorhus longifolia*

CHAPTER 5

5. DISCUSSION

5.1 Antimicrobial analyses:

The results showed that all of the species tested possessed antimicrobial activity and may be useful as a source of the active ingredients. Thirteen extracts active against *C. albicans*, dichloromethane and ethylacetate leaf extracts, both methanolic leaf and bark extracts of *P. longifolia*, ethylacetate leaf extract of *S. birrea*, chloroform and ethylacetate leaf extracts of *S. cordatum*, ethylacetate leaf extractive of *A. venenata*, ethylacetate leaf extractive of *Acokanthera sp.*, crude methanolic leaf extract of *C. macrocarpa*, methanolic leaf extractive of *C. foveolatus*, crude methanolic calyces extract of *H. sabdariffa* and ethylacetate stem extractive of *H. natalensis* showed potential in the treatment of diseases such as thrush, candidiasis as well as others known to be caused by *C. albicans*.

The extracts active against *S. typhimurium*, dichloromethane leaf extract and both methanolic leaf and bark extracts of *P. longifolia*, ethylacetate leaf extract of *S. birrea*, chloroform and ethylacetate leaf extracts of *S. cordatum*, crude methanolic leaf extract of *A. venenata*, crude methanolic leaf extract of *Acokanthera sp.*, crude methanolic leaf extract of *C. macrocarpa*, methanolic leaf extractive of *C. foveolatus*, crude methanolic stem extract of *H. cannabinus* and crude methanolic calyces extract of *H. sabdariffa* showed promise in the development of drugs in the treatment of typhoid fever. Extracts active against *E. coli*, dichloromethane leaf extract and both methanolic leaf and bark extracts of *P. longifolia*, chloroform and ethylacetate leaf extracts of *S. cordatum*, ethylacetate leaf extractive of *Acokanthera sp.*, and ethylacetate leaf extractive of *C. macrocarpa* would probably be promising sources for diseases caused by *E. coli* such as those associated with food poisoning.

About 40% of extracts including ethylacetate and methanolic leaf extracts of *P. longifolia*, chloroform leaf extract of *S. cordatum*, ethylacetate leaf extractive of *C. macrocarpa*, methanolic leaf extractive of *C. foveolatus* and crude methanolic stem extract of *H. cannabinus* showed activity against *S. aureus* and a promise in the treatment of such diseases. Ethylacetate and methanolic leaf extracts and methanolic bark extract of *P. longifolia*, chloroform and ethylacetate leaf extracts of *S. cordatum*,

ethylacetate leaf extractive of *A. venenata*, ethylacetate leaf extractive of *Acokanthera sp.*, crude methanolic leaf extract of *C. macrocarpa*, methanolic leaf extractive of *C. foveolatus*, crude methanolic calyces extract of *H. sabdariffa* showed potential in treatment of diseases associated with *P. aeruginosa*. The differences in susceptibility among microorganisms against antimicrobials in plant extracts may be due to differences in cell wall composition and/or inheritance genes (including those of resistance) on plasmids that could easily be transferred among bacterial microorganisms (Fewell & Roddick, 1993). Both the chloroform and ethylacetate leaf extracts of *S. cordatum* were active against all the bacterial and fungal strains tested, with both extracts showing high activity against *K. pneumoniae*, *S. typhimurium*, *P. aeruginosa*, *B. cereus*, *E. coli* and *S. aureus* but the chloroform leaf extract of *S. cordatum* was found inactive against *B. cereus*. Bacterial inhibition shown in Table 4 for the concentrated stock solutions showed very high inhibition and deserve further investigation in the development of novel drugs in treating diseases caused by microorganisms.

Most traditional medical practitioners use water to make decoctions to treat patients and to isolate the active compounds from medicinal plants. Water extractions may lead to difficulties in the extraction of non-polar active compounds. Successful isolation of compounds from plant material largely depends on the type of solvent(s) used in the extraction process. MeOH and hexane, in this study were the best extractants, extracting a greater quantity of compounds than DCM and EtOAc. This does not imply that methanolic and hexane extracts are the only active extracts and/or compounds isolated. Off all extracts tested, EtOAc were most active. This may be because EtOAc also extracts organic compounds that are useful medicinally, like the triterpenoids, flavonoids, alkaloids and phenolic compounds.

Medicinal plants offer, to traditional and medical health care practitioners, a rich source from which novel antibacterial and antifungal chemotherapeutic agents may be obtained (Rates, 2001). Thus preliminary results from previous studies (Rates, 2001) and from this present study, therefore not only confirm the justifiable use of some of the medicinal plants against these micro-organisms in the traditional healthcare system but also reflects the hope for the development of effective chemotherapeutic

agents in the future. Geyid *et al.* (2005) indicated that the more diverse the chemical make-up of a given species, the more diverse the microorganism it acts upon. These workers substantiated this in their earlier studies by showing that 20 species which accumulated about 2-5 compounds, inhibited three or more organisms compared to 19 species with 2-3 compounds that inhibited growth of only one type of organism. Explanation to this would be that the activity of the species is possibly largely due to synergism (Geyid *et al.*, 2005). The importance of fungicidal and bactericidal activity investigation cannot be over emphasized in view of the fact that microbial infections are a major source of mortality throughout the world (Abebe *et al.*, 2003). The activity of some plant extracts on different organisms explains their broad spectrum nature, while most of the plant extracts are found to have effects on a single organism may be due to their narrow spectrum of activity. This difference in activity would appear to be directly related to the qualitative and/or quantitative diversity of compounds being investigated (Geyid *et al.*, 2005).

5.2 Chemical analyses

This study has identified the most active substances in the extracts of *P. longifolia* leaves and most of these compounds were found to be flavonoids/or phenolic compounds. These compounds (flavonoids & phenolics), are known to be widely distributed in the plant kingdom and form part of the human diet. Recent interest in these compounds has been stimulated by their potential in health benefits arising from their antioxidant activities (Croft, 1998).

The active compounds from the EtOAc extract with the molecular formula $C_{30}H_{50}O$ (molecular weight 426.72g mol^{-1}) was assigned to lupeol (lup-20(29)-en-3β-ol) (Fig. 21), an alkaloid, by combined carbon (^{13}C) and proton (^{1}H-^{1}H) NMR spectroscopy. This triterpene has previously showed marked antimicrobial activity with other terpenoids such as lupenone [lup - 20 (29) - en - 3 - one or lup - 20 (30) - en - 3 – one] and has been active against gram-positive bacteria (Anonymous A, 2003) and shows promise as a drug for numerous illnesses of bacterial origin. Lupeol, a triterpene flavonoid, is the principal constituent of common fruit plants such as olive, mango, figs and medicinal herbs that have previously been used to to treat skin ailments. Lupeol has also been reported to possess a wide range of medicinal properties including strong antioxidant, antimutagenic, anti-inflammatory and

antiarthriatic effects (Mukhtar *et al.*, 2004).

Lupeol topical application (1-2mg/mouse) has recently showed to possess antitumour-promoting effects in mouse skin tumourigenesis model by significantly inhibiting (3.2nmol/mouse) 12-O-tetradecanoyl-phorbol-13-acetate (TPA)-induced conventional markers and other novel markers of skin tumor promotion, in a time- and dose-dependent manner, against TPA-mediated increase in skin edema and hyperplasia, epidermal ornithine decarboxylase (OCD) activity and protein expression of OCD, cyclo-oxygenase-2 and nitric oxide synthase. It was also showed that lupeol treatment to mouse skin inhibited TPA-induced activation of P13K, phosphorylation of Akt at Thr^{308}, activation of NF-αB and IKKα and degradation and phosphorylation of IαBα. The animals pretreated with lupeol showed significantly reduced tumour incidence, lower tumour body burden and a significant delay in the latency period for tumour apperance (Mukhtar *et al.*, 2004).

Lupeol acetate from a methanolic root extract of Indian medicinal plant *Hemidesmus indicus* have shown to neutralize venom induced action of *Daboia russellii* and *Naja kaouthia* on experimental animals. This salt could significantly neutralize lethality, haemorrhage, defibrinogenation, edema PLA_2 activity induced by *Daboia russellii* venom. It also neutralized *Naja kaouthia* venom induced lethality, cardiotoxocity, neurotoxicity and respiratory changes in experimental animals. Lupeol acetate potentiated the protection by snake venom antiserum action against *Daboia russellii* venom induced lethality in male albino mice. Venom induced changes in lipid peroxidation and superoxide dismutase activity was antagonised by lupeol acetate (Chatterjee *et al.*, 2006).

Lupinine (Fig. 30), also an alkaloid (octahydro-2H-quinolizine-1-methanol) with molecular formula $C_{10}H_{19}NO$ and molecular weight 169.27g mol^{-1}, was also isolated. Lupinine has been found to be active against gram-positive bacteria (Jul *et al.*, 2003) and preliminary results of this study showed that this compound could be useful in the treatment of illnesses caused by food poisoning from bacteria such as *B. cereus* and *E. coli*. Lupinine has previously been identified from the essential oil of the leaves of *Calycotome villosa* which was observed to be very active against several gram negative and positive bacteria, including *S. aureus*, *Bacillus lentus*, *E. coli*, *P*

aeruginosa, *K. pneumoniae*, *Providencia rettgeri* and *Morganella morganii* and was found inactive against fungi (Loy *et al.*, 2001).

Lupulon (3,5-dihydroxy-2,6,6-tris(3-methyl-2-butenyl)-4-(3-methyl-1-oxobutyl)-2,4-yclohexadien-1-one) or (3,5-dihdroxy-4-isovaleryl-2,6,6-tris(3-methyl-2-butenyl)-2,4-cyclohexadien-1-one) (Fig. 31), a sesquiterpenoid of molecular formula $C_{26}H_{38}O_4$ and molecular weight 414.56g mol^{-1}, was also isolated and identified. Lupulon has previously tested positive for the inhibitory effect against the TB bacterium *Mycobacterium tuberculosis* (Pauli *et al.*, 2005). The synergism of these flavonoids in this study certainly showed that these compounds possess potential in the treatment of diseases such as TB.

Luteolin [(2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4H-1-benzopyran-4-one), 3',4',5,7-tetrahydroxyflavone or 5,7,3',4'-tetrahydroxyflavone] (Fig. 29) of molecular formula $C_{15}H_{10}O_6$ and molecular weight 286.23gmol^{-1}, a triterpene was identified. Luteolin, a flavonoid, was previously shown to possess antimicrobial (Cushnie & Lamb, 2005) and antioxidant (Ahmad *et al.*, 2006) properties. Luteolin, as observed in this study is known to be active against gram-positive bacteria (Delaquis *et al.*, 2002; Yu *et al.*, 2004). These compounds have not been previously described in the chemical literature as active components in the leaves of *P. longifolia*. Luteolin, isolated from rooibos tea extract has also previously showed to have protective effects against 2-acetamidofluorene (2-AAF) and aflatoxin B_1 (AFB_1) in a *Salmonella typhimurium* microsome assay for antimutagenicity using tester strains TA98 and TA100 (Snijman *et al.*, 2007)

Spectral data obtained also confirmed the presence of traces of other monoterpenes and sesquiterpenes in the leaves of *P. longifolia*. These spectral data from this current study, which suggested that these were lupane triterpenoids such as lupeol, lupinine, lupulon and luteolin or very closely related compounds. All the plant species investigated in this study tested positive for the presence of flavonoids. The anitmicrobial activity of these medicinal plants suggests that the synergy of alkaloids is more effective against gram-negative bacteria than positive ones. Prenylated flavonoids are more hydrophobic than the unprenylated ones and easily penetrate the cell membrane (Chi *et al.*, 2001). Gram-negative membranes are more susceptible to

these flavonoids (prenylated) than gram-positive ones (Chi *et al.*, 2001).

Both the $CHCl_3$ and EtOAc leaf extracts of *S. cordatum* inhibited growth of only the gram-positive *S. aureus*. This suggests that both these extracts may possess similar compounds (e.g. BA) working similarly.

The greater resistance of gram-negative bacteria in general, to plant extracts has been well documented (Paz *et al.*, 1995; Vlietinck *et al.*, 1995 and Kudi *et al.*, 1999) and partly supported by this study. These observations are likely to be as a result of differences in the cell wall structure between gram-positive and gram-negative bacteria. The outer membrane of gram-negative bacteria is thicker and acts as a barrier to many external environmental substances, including conventional and synthetic antibiotics (Tortora *et al.*, 2001). The medicinal plants investigated in the current study also tested positive for alkaloids, steroid alkaloids and alkaloid derivatives which were shown to have a wide spectrum of biological activities, including antimicrobial effects (Fewell and Roddick, 1993). Phenolic compounds and phenolic derivatives interact with the membrane structure of microorganisms, causing membrane rupture which leads to leakage of the cytosol and cell death (Tortora *et al.*, 2001).

Leaves of *Syzygium sp.* have previously been found to contain β-sitosterol (3β)-stigmast-5-en-3-ol) of molecular formula $C_{29}H_{50}O$ and molecular weight 414.69g mol^{-1} (Fig. 27) and was detected in traces in this study, arjunolic acid (olean-12-en-28-oic acid) (AA) and OA (3-hydroxylean-12-en-28-oic acid) with molecular formula $C_{30}H_{48}O_3$ and molecular weight 456.71g mol^{-1} (Fig. 22) (Shode *et al.*, 2001). A compound of interesting bioactivity, a pentacyclic triterpene, UA (3β-hydroxyurs-12-en-28-oic acid) of molecular formula $C_{30}H_{48}O_3$ and molecular weight 456.68g mol^{-1} (Fig. 23) (*Shode et al.*, 2001), an isomer of OA, has showed antitumor and antimicrobial properties against mouse skin (Simon *et al.*, 1992) and has also been reported to be cytotoxic against tumor cells (Yamagishi *et al.*, 1988).

UA has also been reported to be active against *Leishmania amazonensis* (Phillipson and Wright, 1997). In the present study, UA was also isolated and identified in a mixture with OA and the activity demonstrated here could be the result of some synergism between both UA and OA with other minor compounds like MM (2α, 3β-

dihydroxyolean-12-en-28-oic acid methyl ester) (Fig. 24a) and MC (Fig. 24b). Synergy of UA, OA, MM, MC has been shown to be effective against mild diabetes mellitus or glucose tolerance impairment (Musabayane *et al.*, 2004).

In this study, crude methanolic extract and EtOAc extractive from the leaves of *A. venenata* showed some degree of inhibition against *B. cereus* and *C. albicans* but was found to be inactive against all the other microorganisms tested. These observations suggest that *A. venenata* leaves lack antimicrobial agents active against these microorganisms. This study also showed another unspecified *Acokanthera* species' crude methanolic extract and EtOAc extractive having a growth inhibitory activity.

The EtOAc leaf extractive was active against all microorganisms tested, with the highest activity exhibited against both *B. cereus* and *E. coli*. These observations suggest that *Acokanthera sp.* contain compounds active against these microorganisms. These compounds were easily extracted with EtOAc, the most effective extractant after methanol. The whole plant of *C. macrocarpa* is known to be very toxic against humans and animals (van Wyk and van Wyk, 1997) but the results of this study showed that the compounds had low MIC/MBC values that could not be regarded as cytotoxic against the microorganisms tested. These values compare to standard antibiotic MIC/MBC of clinical standards. The crude methanolic stem bark extract of *H. cannabinus* was found to be highly active against *S. aureus* and mild activity was also observed against both *S. typhimurium* and *E. coli*.

This showed the high susceptibility of gram-positive bacteria against antimicrobials of plant origin. *H. cannabinus* is likely to possess membrane-destroying compounds, probably phenolic and phenolic derivatives found commonly in medicinal plant extracts that are active on gram-positive bacteria (Tortora *et al.*, 2001). An ethylacetate stem bark extractive of *H. natalensis* (identified as BA) (Fig. 28) showed high activity against *B. cereus* and *S. typhimurium* which are gram-positive and gram-negative bacteria respectively, and a mild activity against *S. aureus* and *C. albicans*. BA (3β-hydroxy-20(29)-lupaene-28-oic acid) of molecular formula $C_{30}H_{48}O_3$ and molecular weight 459.70g mol^{-1}, is a pentacyclic triterpene, normally derived from betulin found abundant in the outer bark of white birch trees (*Betula alba*).

BA has previously been found to selectively kill human melanoma cells while leaving

healthy ones alive. The cytotoxic potential of BA has been tested using three human melanoma cell lines (Pisha *et al.*, 1995). The growth of all of the cell lines was inhibited significantly with BA treatment. The effectiveness of BA against melanoma cancer cells was also tested using athymic (nude) mice (Pisha *et al.*, 1995).

BA seemed to effectively inhibit the growth of tumors in mice with no sign of drug toxicity side effects, such as weight loss. BA seemed to work by inducing apoptosis (voluntary cell death) in cancer cells. Because of specificity for melanoma cells, BA seems to be a more promising anti-cancer substance than drugs like taxol.

BA has previously been found to have anti-HIV activity (Fujioka *et al.*, 1994) in that it retards the progression of HIV-1 infection, which eventually leads to AIDS (Puniani, 2003), by preventing the formation of syncytia (cellular aggregates) (Pisha *et al.*, 1995). In addition, BA has various other medicinal properties including antibacterial (also seen in this study), antiinflammatory (Safayhi & Sailer, 1997) and antimalarial (Steele *et al.*, 1999) and inhibits the growth of both *S. aureus* and *E. coli* (Pisha *et al.*, 1995). BA was shown to possess anti-anxiety and pesticidal properties (Puniani, 2003). BA is not very poisonous and relatively inexpensive, and is found in most medicinal plants and found abundant from the bark of white birch trees in the form of betulin, and in the leaves of *H. natalensis* and traces of BA were suspected also in the leaves of *P. longifolia*.

The weak hydrosolubility of BA hampers its clinical development as an anticancer agent. To circumvent this and improve its pharmacological activity, a sugar moiety is added to betulin, the precursor in the synthesis of BA (Pichette *et al.*, 2006). This compound (BA) and its sugar esters, in purified form would be promising in human diseases research, for a wide range of diseases. With the exception of the leaf extract in EtOAc of *C. macrocarpa* against *K. pneumoniae*, effects of all extracts were greater at the highest concentration tested. This study is the first to demonstrate, *in vitro*, the bioactivity of organic and aqueous extracts from the plant species studied in this work.

This work is also the first to isolate and identify compounds from leaves and bark of *P. longifolia*. Results obtained justify the wide usage of these medicinal plants in traditional medicinal practices.

5.3 General discussion:

Scientific information that is documented can be used to enhance the overall knowledge of plant species and their role in alternative medicine. Nice (2002) has advised patients and healthcare professionals to approach herbal medication use cautiously. Scientific validation is essential in all herbal medicines, to provide more confidence to the claims made by the public and traditional healers. The agar diffusion and disc diffusion methods, employed in this current study were reported to be ideal for both water-soluble and organic solvent-soluble antimicrobial compounds (van der Berghe *et al.*, 1991).

Inactivity of an isolated compound or a crude extract could be attributable to a number of factors except that it cannot inhibit growth of a microorganism tested. Such factors include the ability of the compound to diffuse in the agar. In comparison to the above techniques (disc-diffusion and agar-diffusion), other techniques provide at best, information on antimicrobial activity. These techniques however, do not give any quantitative information about the active components. By separating bioactive extracts on thin layer chromatography, information about the composition of a mixture is easily obtained (Springfield *et al.*, 2003).

Infectious diseases of microbial origin, such as those caused by *S. aureus*, *E. coli*, *B. cereus*, *S. typhimurium*, *K. pneumoniae*, *P. aeruginosa*, *C. albicans* etc, constitute the major cause of morbidity and/or mortality in third world countries, most of which are in Africa (Kloos and Zein, 1993). One of the major problems faced by medical researchers is the resistivity of microorganisms to antimicrobial compounds. Nowadays, there are a very few antibiotics to which these microorganisms have not developed resistance and this is due to the indiscriminate use of antimicrobial drugs, increasing clinical problems in the treatment of such infectious diseases. The situation is further compounded by lack of patient compliance and by the cost of the antibiotics. To circumvent this, one approach is to screen local medicinal plants for possible antimicrobial properties.

According to the liquid dilution screening method for antimicrobial activity of higher plants reported by van den Berge and Vlietinck (1991), a prominent antibacterial

effect worthy of further investigation should be obtained at dilutions as low as $1/32^{th}$. An inhibition shown only at half dilutions is less promising for further investigation. In this current study, antimicrobial activity was obtained at dilutions as low as $1/20^{th}$. All crude extracts and isolated compounds were found to be active against microorganisms tested and could be useful in the development of antibiotics.

Orthodox researchers and most traditional healers use water as an extracting solvent. However, water does not extract non-polar active components. This is easily overcome by using organic solvents. In this study MeOH and DCM were the best extractants.

CHAPTER 6

6. CONCLUSION

The main aims of this study were to (i) test for antimicrobial activity of crude extracts of the leaves and bark of *P. longifolia*, *S. birrea*, *S. cordatum*, *A. venenata*, *C. macrocarpa*, *H. cannabinus*, *H. natalensis*, calyces of *H. sabdariffa* and seeds of *C. foveolatus*; (ii) isolate, purify and identify some of the major compounds from the leaves and stem bark of these medicinal plants listed above and (iii) test the isolated compounds and mixtures for possible antimicrobial activities. MeOH was found to be the best extractant used but EtOAc extracts were found to be highly active against the microorganisms tested. Fractionation of these EtOAc and other extracts led to the isolation of antimicrobial compounds through column chromatography over silica gel. Chemical and physical properties of these compounds (and mixtures) were determined.

All plants and plant parts investigated in this current study were known to be used in traditional medicinal practices, especially in developing countries. The plant extracts used exhibited significant ($P<0.01$) antibacterial and antifungal properties against *K. pneumoniae*, *B. cereus*, *S. typhimurium*, *S. aureus*, *E. coli*, *P. aeruginosa* and *C. albicans*. The results obtained suggest that these species show promise as a source of plant material for the development of new drugs to treat diseases associated with the microorganisms studied. All plant parts were extracted with different solvents of different polarities.

S. cordatum leaves and stem bark showed the presence of OA (3-hydroxylean-12-en-28-oic acid), UA (3β-hydroxyurs-12-en-28-oic acid), MM (2α, 3β-dihydroxyolean-12-en-28-oic acid methyl ester) and MC. In addition, acids identified in these extracts included gallic (p-methoxybenzoic acid or 4-methoxybenzoic acid), ellagic (benzoaric acid) and arjunolic (olean-12-en-28-oic acid). β-sitosterol (3β)-stigmast-5-en-3-ol) was one of the compounds isolated in the leaves and bark of *S. cordatum*.

P. longifolia leaves and stem bark showed the presence of alkaloids, mainly lupeol (lup-20(29)-en-3β-ol) and lupane triterpenoids, including lupulon (3,5-dihydroxy-2,6,6-tris(3-methyl-2-butenyl)-4-(3-methyl-1-oxobutyl)-2,4-yclohexadien-1-one) or

(3,5-dihdroxy-4-isovaleryl-2,6,6-tris(3-methyl-2-butenyl)-2,4-cyclohexadien-1-one), lupenone [lup - 20 (29) - en - 3 - one or lup - 20 (30) - en - 3 – one] lupinine (octahydro-2H-quinolizine-1-methanol) and luteolin [(2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4H-1-benzopyran-4-one), or 3',4',5,7-tetrahydroxyflavone or 5,7,3',4'-tetrahydroxyflavone], BA (3β-hydroxy-20(29)-lupaene-28-oic acid), sesquiterpenes and terpenoid mixtures.

H. natalensis stem bark revealed the presence of BA (3β-hydroxy-20(29)-lupaene-28-oic acid), a pentacyclic triterpene, normally derived from betulin, found abundant in the outer bark of white birch trees (*Betula alba*). Additional compounds in the extracts still need to be characterized and identified and further work needs to be done on the compounds that were not isolated and identified. This study has shown that the species investigated show promise as a source of several antibacterial and antifungal compounds.

Additional studies need to be undertaken to further characterise and purify, more biologically active plants found in these and other traditionally used medicinal plants. This study attempted, for the first time to isolate and characterise active compounds found in *P. longifolia*. This study also identified bioactive compounds from *S. cordatum* and *H. natalensis* but could not separate the mixtures from *S. birrea* due to solvent complications. The preliminary results of this study justifies the usage of these plant, studied herein and their parts/organs in herbal medicine.

CHAPTER 7

7. REFERENCES

ABEBE, D, DEBELLA, A AND URGA, K. 2003. **Medicinal Plants and Other Useful Plants of Ethiopia**. Camerapix Publishers International, Singapore, pp 54–61.

AGRAWAL, P. K., 1989. NMR spectroscopy in the structural elucidation of oligosaccharides and glycosides. Carbon – 13 NMR of flavonoids. **Studies in Organic Chemistry 39**.

AHMED, F, SADHU, S. K., OHTSUKI, T AND ISHIBASHI, M. 2006. Flavonoids from *Sonneratia caseolaris*. **Journal of Natural Medicines 60** 3.

AMPOFO, O. 1977. Plants that heal. **World Heath**, pp 26-30.

ANONYMOUS A. 2003. Foodborne pathogenic micro-organisms and national toxicology handbook. www.vm.cfsan.fda.gov/~mow/chap3.htm.

ANONYMOUS B. 2003. http://www.edpsciences.org/articles/apido/pdf/2003/03.

ANONYMOUS C. 2004. *E. coli* 0157:H7. www.cdc.gov/ncidod/dbmd/diseaseinfo/escherichiacoli_g.htm.

ANONYMOUS D. 2004. Typhoid fever. www.cdc.gov/ncidod/dbmd/diseaseinfo/typhoidfever_g.htm.

BRANDWIJK, M. G. 1928. Physiological activity in extracts of *Albizia* species. **Journal of Pharmacology 120** 172, 192, 212.

BROCHERT, A. 1999. ***Klebsiella*: One potentially nasty bacteria**. www.personalmd.com/news/*Klebsiella*_102299.5html.

BRUNETON, J. 1995. **Pharmacognosy, phytochemistry, medicinal plants**. Intercept, Hampshire.

BRYANT, A. T. 1966. Zulu medicine and medicine men. **Annuals of the Natal Museum 2** 1.

BUSSON, F. 1965. **Plantes alimentaires de l' Ouest Africain: Etude botanique, biologique et chimique**. Marseille: Ministere de la Cooperation.

CARLISLE, C. H. & LADD, M. F. C. 1966. The crystal structure of euphenyl iodoacetate. **Acta Crystallographica 21**, pp 689-693.

CHATTERJEE, I., CHAKRAVATRY, A. K. AND GOMES, A. 2006. *Daboia russellii* and *Naja kaouthia* venom netralization by lupeol acetate isolated from the root extract of Indian sarsaparilla *Hemidesmus indicus* R. Br. **Journal of Ethnopharmacology 106** 1, pp 38-43.

CHI, Y. S., CHEON, B. S. AND KIM, H. P. 2001. Effect of wogonin, a plant flavone from *Scutellaria radix*, on the suppression of cyclooxygenase-2 and the induction of inducible nitric oxide synthase in lipopolysaccharide-treated RAW 264.7 cell. **Biochemical Pharmacology 61**, pp 1195–1203.

CHIU, C. M. AND MUDDIMAN, D. C. 2001. **What is mass spectrometry**. American Society for Mass Spectrometry: Education. www.asms.org.

CONNOLLY, J. D. AND HILL, R. A. 1991. **Dictionary of triterpenoids, di and higher terpenoids**. Vol 2, Chapman and Hall, Cambridge, p 1460.

CRAGG, G. M. AND NEWMANN, D. J. 1997. Natural products in drug discovery and development. **Journal of Natural Products 60**, pp 52-60.

CROFT, K., D. 1998. The Chemistry and Biological Effects of Flavonoids and Phenolic Acids. **Annals of the Newy York Academy of Science 854**: pp 435- 442.

CUNNINGHAM, A. B. 1988. An Investigation of the Herbal Medicine trade in KwaZulu/Natal. **Investigative Report 29**. Institute of Natural Resources, Scottsville, South Africa.

CUNNINGHAM, A. B. 1988. An Investigation of the Herbal Medicine trade in KwaZulu/Natal. **Investigative Report 29**. Institute of Natural Resources, University of Natal, Pietermaritzburg.

CUSHNIE, T. P. T., AND LAMB, A. J. 2005. Errata for "Antimicrobial activity of flavonoids". **International Journal of Antimicrobial Agents 26** pp 343-356.

DELAQUIS, P. J., STANICH, K., GIRARD, B. AND MAZZA, G. 2002. Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus essential oils. **International Journal of Food Microbiology 74**, pp 101–109.

FERREIRA, F. H. Personal Communication to John Mitchel Watt.

FEWELL, A. M. AND RODDICK, J. G. 1993. Interactive antifungal activity of the glycoalkaloids, solanine, and chaconine. **Phytochemistry 33**, pp 323–328.

FOX, F. W. *et al.* 1938. Food From the Veld: Edible Wild Plants of Southern Africa. **South African Journal of Medical Science 3** 7.

FUJIOKA, T., KASHIWADA, Y., KILKUSKIE, R. E., COSENTINO, L. M., BALLAS, L. M., JIANG, J. B. JANZEN, W. P., CHEN, I-S AND LEE, K. H. 1994. Anti-AIDS agents, 11. Betulinic acid and platanic acid as anti-HIV principles from *Syzygium claviflorum* and the anti-HIV of structurally related triterpenoids. **Journal of Natural Products 57**, 2: pp 243-247.

GALVEZ, J., ZARZUELO, A., CRESPO, M. E., UTRILLA, M. P., JIMINEZ, J., SPIESSENS, C. AND DE WITTE, P. 1991. Antidiarhoeic activity of *Sclerocarya birrea* bark and its active tannin constituent in rats. **Phytotherapy Research 5**, pp 276-278.

GALVEZ, J. CRESPO, M. E. AND ZARZUELO, A. 1993. Pharmacological activity of a procyanidin isolated from *Sclerocarya birrea* bark: Antidiarrhoeal activity and effects on isolated guinea pig ileum. **Phytotherapy Research** 7, pp 25-28.

GELFAND, M., MAVI, S., DRUMMOND, R B. AND NDEMERA, B. 1985. **The Traditional medical practitioner in Zimbabwe.** Mambo Press, Gweru.

GERSTNER, J. 1938. A preliminary checklist of Zulu names of plants with short notes. **Bantu Studies 12** (3), pp 215-236, (4) pp 321-342.

GERSTNER, J. 1939. A preliminary checklist of Zulu names of plants with short notes. **Bantu Studies 13** (1), pp 49-64, (2) pp 131-149, (4) pp 307-326.

GERSTNER, J. 1941. A preliminary checklist of Zulu names of plants with short notes. **Bantu Studies 15** (3), pp 277-301, (4) pp 369-383.

GEYID, A., ABEBE, D., DEBELLA, A., MAKONNEN, Z., ABERRA, F., TEKA, F., KEBEDE, T., BIZA, T., BISRAT, H. M. AND MULUGETA, G. 2005. Screening of some medicinal plants of Ethiopia for their antimicrobial properties and chemical profiles. **Journal of Ethnopharmacology 97**, pp 421-427.

GOOD, C. M. 1987. **Ethnomedicinal systems in Africa: Patterns of Traditional Medicine in Rural and Urban Kenya**. The Guilford Press, New York & London.

HEIM DE BALSAC, F. *et al.* 1928. Notes sur la présence et la répartition de quelques grands mammifères dans le sud-Oranais et le Maroc oriental. **Revue française de Mammologie 1**: pp 83-92.

HENRY, C. M., 2000. Ten decades of drug discovery. http://pubs.acs.org/journals/pharmacenth10.html. **American Chemical Society**.

HUSSEIN, H. S. N. AND DEENI, Y. Y. 1991. Plants in Kano ethnomedicine: screening for antimicrobial activity and alkaloids. **International Journal of Pharmacognosy 29**, pp 51-56.

HUTCHINGS, A. 1989. A survey and analysis of traditional medicinal plants as used by the Zulu, Xhosa and Sotho. **Bothalia 19**: pp 111-123.

HUTCHINGS, A. AND VAN STADEN, J. 1994. Plants used for stress related ailments in traditional Zulu, Xhosa and Sotho medicine: part 1 plants used for headaches. **Journal of Ethnopharmacology 43**, pp 89-124.

HUTCHINGS, A. 1996. **Zulu Medicinal Plants**. Natal University Press, Pietermaritzburg.

HUTCHINGS, A., SCOTT, A. H., LEWIS, G. AND CUNNINGHAM, A. B. 1996. **Zulu Medicinal Plants: An Inventory**. University of Natal Press, Scottsville, South Africa, pp 195-196.

IWU, M. M. 1993. **Handbook of African Medicinal Plants**. CRC Press, Florida.

JAGER, A. K., HUTCHINGS, A. AND VAN STADEN, J. 1996. Screening of Zulu medicinal plants for prostaglandin-synthesis inhibitors. **Journal of Ethnopharmacology 52**, pp 95-100.

JAIN, S. R. AND SHARMA, S. N. 1967. Hypoglycaemic drugs of Indian origin. **Planta Medica 15**, pp 439-442.

JUL, L. B., FLENGMARK, P., GYLLING, M. AND ITENOV, K. 2003. Lupin seed (*Lupinus albus* and *Lupinus luteus*) as a protein source for fermentation use. **Industrial Crops and Products 18** (3): pp 199-211.

KAUFMAN, P. B., CSEKE, L. J., WARBER, S., DUKE, J. A., BRIELMANN, H. L. 1998. **Natural Products from Plants**. CRC Press, Washington, D. C.

KERHARO, J. AND ADAM, J. G. 1974. **La Pharmacopée Sénégalaise Traditionnelle : Plantes médicinales et toxiques**. Ed. Vigot & Frères, Paris, p 1011.

KINGHORN, A. D. AND BALANDRIN, M. F. 1993. Human medicinal agents from plants. **American Chemistry Society Symposium Series 534**, American Chemical Society, Washington.

KLOOS, H. AND ZEIN, A. H. 1993. **The Ecology of Health and Diseases in Ethiopia**. West View Press Inc., Washington DC.

KOKWARO, J. O. 1976. **Medicinal plants of East Africa**. East African Literature Bureau, Nairobi, p 384.

KOSKINEN, A. 1995. **Asymmetric Synthesis of Natural Products**. John Wiley & Sons, Chichester.

KUDI, A. C., UMOH, J. U., EDUVIE, L. O., AND GEFU, J. 1999. Screening of some Nigerian medicinal plants for antibacterial activity. **Journal of Ethnopharmacology 67**, pp 225–228.

LESNEY, M. S. 2000. Ten decades of drug discovery. http://pubs.acs.org/journal/pharmacenth1.html. **American Chemical Society**.

LIN, J., OPOKU, A., GEHEEB-KELLER, M., HUTCHINGS, A., TERBLANCHE, S., JAGER, A. AND VAN STADEN, J. 1999. Preliminary screening of some traditional Zulu medicinal plants for anti-inflamatory anti- microbial activities. **Journal of Ethnopharmacology 68**, pp 267-274.

LOCHER, C., BURCH, M., MOWER, H., BERESTECKY, J., DAVIS, H., VAN POEL, VAN DEN BERGUE, D. AND VLIETINCK, A. 1995. Anti-microbial activity and anti-complement activity of extracts obtained from selected Hawaiian medicinal plants. **Journal of Ethnopharmacology 49**, pp 23-32.

LOY, G., COTTIGLIA, F., GARAU, D., DEIDDA, D., POMPEI, R. AND BONSIGNORE, L. 2001. Chemical composition and cytotoxic and antimicrobial activity of *Calycotome villosa* (Poiret) Link Leaves. **IL Farmaco 56**, pp 433-436.

MABOGO, D. E. N. 1990. **The Ethnobotany of the Vha-Venda**. Unpublished Master of Science Thesis, University of Pretoria.

MANDER, M., MANDER, J., CROUCH, N., MCKEAN, S. AND NICHOLS, G. 1995. **Catchment action: Growing and knowing muthi plants**. ShareNet Booklet, Institute of Natural Resources, Scottsville, South Africa.

MARLOTH, R. 1913. **The Chemistry of South African plants and plant products.** Press Ad. Cape Chemistry Society. 30/5/13.

MERCK 1989. **The Merck Index**. 11th edition. Merck, Rahway.

MITSCHER, L. A., DRAKE, S., GOLLAPUDI, S. R. AND OKWATE, S. K. 1987. A modern look at folkloric use of anti-infective agents. **Journal of National Products 50**, pp 1025-1039.

MUKHTAR, H., SALEEM, M., AFAQ, F., AND ADHAMI, M. V. 2004. Lupeol modulates NF-αB and P13K/Akt pathways and inhibits skin cancer in CD-1 mice. **Oncogene 23** 30, pp 5203-5214.

MURRAY, P. R., BARON, E. J., PFALLER, M. A., TENOVER, F. C. AND YOLKE, R. H. 1995. **Manual of Clinical Microbiology**, vol. 6 ASM, Washington, DC.

MUSABAYANE, C. T. *et al.* 2004. Effects of *Syzygium cordatum* leaf extract on plasma glucose and hepatic glycogen in streptozotocin-induced diabetes in rats. **Endocrine Abstracts 8**, p 27.

NATIONAL COMMITTEE FOR CLINICAL LABORATORY STANDARDS. 1990. **Performance standards for antimicrobial disc susceptibility test**. 4th ed, Approved standards. NCCLS document M2-A4, Villanora, Pa.

NTULI, S. 2000. **Exploitation and the utilisation of the tree bark of selected medicinal plants in the Inner West City Council of KwaZulu-Natal**. Unpublished Honours Thesis, University of KwaZulu-Natal.

PALMER, E. AND PITMAN, N. 1972b. **Trees of Southern Africa**, vol 3. A. A. Balkema, Cape Town.

PALGRAVE, K. C. 1997. **Trees of Southern Africa**, C. Struik Publishers: Cape Town.

PAULI, G. F., CASE, R. J., INUI, T., CHO, Y. W. S., FISCHER, N. H. AND FRANZBLAU, S. G. 2005. New perspectives on natural products in TB drug research. **Life Science 78** (5), pp 485-494.

PAZ, E. A., CERDEIRAS, M. P., J. FERNANDEZ, J., FERREIRA, F., MOYNA, P., SOUBES, M., VAZQUEZ, A., VERO, S. AND ZUNINO, L. 1995. Screening of Uruguayan medicinal plants for antimicrobial activity. **Journal of Ethnopharmacology 45**, pp 67–70.

PHILLIPSON, J. D. AND WRIGHT, C. W. 1997. Plants with antiprotozoal activity. In: W.C. Evans, Editor, **Trease and Evans Pharmacognosy** (14th ed.), WB Saunders, London, pp 426–433, Chapter 27.

PICHETTE, A., GAUTHIER, C., LEGAULT, J., LEBRUN, M. AND DUFOUR, P. 2006. Glycosidation of lupane-type triterpenoids as potent *in vitro* cytotoxic agents. **Bioorganic and Medicinal Chemistry 14**, 19: pp 6713-6725.

PISHA, E *et al.* 1995. Discovery of betulinic acid as a selective inhibitor of human melanoma that functions by induction of apoptosis. **Natural Medicine 1** (10), pp 1046–1051.

POLLACK, M. 2000. *Pseudomonas aeruginosa*. In: Mandell, G. L., Bennett, T. E., Dolin, R. eds. **Principles and Practices of Infectious Diseases**, 5th ed. New York, NY: Churchill Livingstone: pp 2310-27.

POOLEY, E. 1993. **The complete field guide to trees of Natal, Zululand and Transkei**. Natal Flora Publications Trust.

PRETORIUS, V., ROHWER, E., RAPP, A., HOLTZHAUSEN, L C. AND MANDERY, H. 1985. Volatile flavour components of marula *Sclerocarya birrea* sbsp *caffra* juice. **Z Lebensm-Unters-Forsch 181**, pp 458-461, from **Biosis BA81**: 71862.

PUJOL, J. 1990. **The Herbalist Handbook: Africal Flora, Medicinal Plants**. NaturAfrica, Durban.

PUNIANI, E. 2003. **Novel Natural Products Based Anti-Anxiety Therapy and Natural Insecticides**. www.tongahighschool.com/thesis/eva_phd_thesis.pdf. Tonga High School Ex-Students' Website Publications. Tonga, New Zealand.

QAHAR, S. 2004. *Pseudomonas aeruginosa* infections. www.emedicine.com/med/topic1943.htm.

QUIN, P. J. 1954. **Ph. D. Thesis.** Wits University Press, University of Witwatersrand.

RASOANAIVO, P., PETITJEAN, A., RATSIMAMANGA-URVEG, S. AND RAKOTO-RATSIMAMANGA, A. 1992. Medicinal plants used to treat malaria in Madagascar. **Journal Ethnopharmacology 37**, pp 117-128.

RATES, S. M. K. 2001. Plants as source of drugs, **Toxicon 39**, pp 603–613.

REED, C. F. 1976. **Information summaries on 1000 economic plants**. Typescripts submitted to the USDA.

ROBBERS, J. E., SPEEDIE, M. K. AND TYLER, V. E. 1996. **Pharmacognosy and Pharmacobiotechnology**. William & Wilkins Publishers, Pennsylvania.

SAFAYHI, H. AND SAILER, E-R. 1997. Antiinflammatory actions of pentacyclic triterpens. **Planta Medica 63**, 6: pp 487-493.

SCHMID, J. 2003. **Research Interests**. www.imbs.massey.ac.nz/Staff/schmid_research.htm.

SEPAHA, G. J. AND BHOSE, S. N. 1956 Clinical observations on the antidiabetic properties of *Pterocarpus marsupium* and *Eugenia jambolata* Lam. **Journal of Indian Medical Association 27**, p 388.

SHODE, F. O., SOMOVA, L. I., MOODLEY, K. AND GOVENDER, Y. 2001. Cardiovascular and diuretic activity of kaurene derivatives of *Xylopia aethiopica* and *Alpedia amatymbica*. **Journal of Ethnopharmacology 77**, pp 165-174.

SIMON, A., DELAGE, C., SAUX, M., CHULIA, A. J., NAJID, A AND RIGAUD, M. 1992. Structure of ursolic acid ethanol solvate. **Acta Crystallographica Section C 48**, pp 726–728.

SIMONSEN, J. AND ROSS, W. C. J. 1957. The crystal structure of euphenyl iodoacetate. **The Terpenes**. Cambridge University Press Vol. IV, London.

SNIJMAN, P., SWANEVELDER, S., JOUBERT, E., GREEN, I. AND GELDERBLOM, W. C. A. 2007. The antimutagenic activity of the major flavonoids of rooibos (*Aspalathus linearis*). **Mutation Research**. Submitted for publication.

SPENCER, C. F. *et al.* 1947. Survey of plants for antimalarial activity. **Lloydia 10**, pp 145-74.

SPRINGFIELD, E. P., AMABEOKU, G., WEITZ, F., MABUSELA, W. AND JOHNSON, Q. 2003. An assessment of two *Carpobrotus* species extracts as potential antimicrobial agents. **Phytomedicine 10**, pp 434–439.

STEELE, J. C. P., WARHURST, D. C., KIRBY, G. C. AND SIMMONDS, M. S. J. 1999. *In vitro* and *in vivo* evaluation of betulinic acid as an antimalarial. **Phytotherapy Research 13**, 2: pp 115-119.

TAKECHI, M. AND TANAKA, Y. 1981. Purification and characterization of antiviral substances from the bud of *Syzygium aromatica*. **Planta Medica 42**, pp 69-74.

TINTO, W. F., BLAIR, L. C. AND ALLI, A. 1992. Lupane triterpenoids in *Salacia cordata*. **Journal of Natural Products Lloydia 55**, 3: pp 395-398.

TODAR, K. 2002. **Online textbook of Bacteriology**. www.textbookofbacteriology.net/*B.cereus*.html.

TORTORA, G. J., FUNKE, B. R. AND CASE, C. L. 2001. **In Microbiology: An Introduction**, Benjamin Cummings, San Francisco.

VAN DEN BERGHE, D. A. AND VLIETINCK, A. J. 1991. **Screening methods for antibacterial and antiviral agents from higher plants**. In: DEY P. M., HARBONE J. B., HOSTETTMAN, K. (eds): **Methods in Plant Biochemistry**. Vol. 6. Assay for Bioactivity. Academic Press, London: pp 47-69.

VAN WYK, P. 1972. **Trees of the Kruger National Park**. Vol. 1. Purnell Publishers, Cape Town, JHB and London.

VAN WYK, P. 1974. **Trees of the Kruger National Park**. Vol. 2. Purnell Publishers, Cape Town, JHB and London.

VAN WYK, B. AND VAN WYK, P. 1997. **Field Guide to Trees of South Africa** Struik Publishers: Cape Town.

VAN WYK, B-E, VAN OUDTSHOORN, B. AND GERICKE, N. 1997. **Medicinal Plants of South Africa.** Briza Publications, Pretoria.

VLIETINCK, A. J., VAN HOOF, L., TOTTÉ, J., LASURE, A., VAN DEN BERGHE, D., RWANGABO P. C. AND MVUKIYUMWAMI, J. 1995. Screening of hundred Rwandese medicinal plants for antimicrobial and antiviral properties. **Journal of Ethonopharmacology 46**, pp 31-47.

WAGNER, H. AND BLADT, S. 1996. **Plant drug analysis**. A thin layer chromatograph Atlas. Springer-Verlag Berlin. Heidelburg, New York.

WATT, J. AND BREYER-BRANDWIJK, B. 1962. **Medicinal and Poisonous Plants of Southern and Eastern Africa (2nd ed.)**. E. and S. Livingstone Ltd., London, pp 202-237.

WILLIAMS, V. 1996. The Witswatersrand muthi trade. **Veld and Flora 82**, pp 12-14.

WORLD HEALTH ORGANISATION. 2001. **Legislation and Statistics of Traditional Medicine & Complementary/Alternative Medicine**: A world Review. Geneva.

WWW.FIP.ORG/NICE2002

www.wikipedia.com

YAMAGISHI, T. ZHANG, D., CHANG, J., MCPHAIL, D., MCPHAIL, A. T. AND LEE, K. 1988. The cytotoxic principles of *Hyptis capitata* and the structure of the new Triterpenes hyptatica acid-A and -B. **Phytochemistry 27**, pp 3213–3216.

YU, J., LEI, J., YU, H., CAI, X. AND ZOU, G. 2004. Chemical composition and antimicrobial activity of the essential oil of *Scutellaria barbata*. **Phytochemistry 65** (7), pp 881-884.