Chapter 1

Introduction and aim of study

1.1 Antibiotics and antibacterial resistance

The discovery of antibiotics must certainly be one of the most important events in the history of medicine. Although the discovery of antibiotics only occurred within the lifespan of modern man, there is anthropological evidence that Nubian man more than a thousand years ago used tetracycline. The survival of the Nubian nation up to the 14th century is partially ascribed to the use of antibiotics such as tetracycline (Levy, 1984).

The development of anti-infective therapy occurred in three phases. The first was from the early 1600's to the 1900's and involved the use of *Cinchona* bark for the treatment of malaria. Quinine was isolated as the active ingredient in the 1820's. The 1900's marked the development of the synthetic era and saw the advent of dyes to treat bacterial infections, the first being pyocyanase, a blue pigment produced by *Pseudomonas aeruginosa* that reduced the growth of other bacteria *in vitro*. However, when used *in vivo*, it proved to be toxic and unstable (Edwards, 1980).

The most important event in the history of antibiotics was the discovery of penicillin by Alexander Fleming in 1929. The real potential for its use was only recognized with the

advent of the Second World War, which speeded up the commercial use of the product in the treatment of septic wounds. The subsequent wide distribution of the drug in over-the-counter preparations and even in cosmetic use led to the rapid development of widespread bacterial resistance (Levy, 1984).

Finding healing power in plants is an ancient idea. There is evidence that Neanderthal man 60,000 years ago used plants such as hollyhock for medicinal purposes. Since the advent of antibiotics in the 1950's however, the use of plant materials as antimicrobials has been virtually non-existent in First World countries (Cowan, 1999).

It has become apparent in recent times that increasing numbers of pathogenic bacteria are becoming resistant to currently available antibiotics (Berkowitz, 1995). This resistance results from the rate at which bacteria multiply and the ease with which they can change their genetic material or acquire new genes. Resistance to penicillin in some strains of staphylococci was recognised almost immediately after the discovery of the drug by Alexander Fleming (Anon, 1939). Today, resistance occurs in as many as 80% of all strains of *Staphylococcus aureus*.

A difference of opinion exists over the actual degree of antibiotic resistance. This can be ascribed to a general lack of national or international monitoring systems for antibiotic susceptibility testing conforming to a single set of standard operating procedures with appropriate quality controls. Confusion also exists around the terminology amongst those who report the results of the susceptibility testing (Walker and Thornsberry, 1998).

The basis of resistance can be classified as follows:

- Inherent (natural) resistance: bacteria may be inherently resistant to antibiotics. For instance, a Streptomycete may have a gene responsible for resistance to an antibiotic. In a further example, Gram-negative bacteria have an outer membrane structure that establishes a permeability barrier against certain antibiotics.
- Acquired resistance: bacteria can develop resistance to antibiotics by changing the bacterial genome. Acquired resistance is driven by two genetic processes in bacteria, mutation and selection, sometimes referred to as

  i) Vertical evolution, which is strictly a matter of Darwinian evolution driven by principles of natural selection: a spontaneous mutation in the bacterial chromosome imparts resistance to a member of the bacterial population.

  ii) Horizontal evolution, which is the acquisition of genes for resistance from another organism. Some bacteria develop genetic resistance through a process of mutation and natural selection and then donate these genes to some other bacterium through one of several processes for genetic exchange that exist in bacteria.

Bacteria are able to exchange genes in nature by three processes:

- Conjugation - involves cell-to-cell contact as DNA crosses a sex pilus from donor to recipient.
- Transduction - a virus transfers the genes between mating bacteria.
- Transformation - DNA is acquired directly from the environment, having been released from another cell.

Genetic recombination can follow the transfer of DNA from one cell to another, leading to emergence of a new genotype (recombinant). It is common for the DNA to be transferred as plasmids between mating bacteria. Since bacteria usually carry their genes for drug resistance on plasmids (called resistance transfer factors or RTF's), they are able to spread drug resistance to other strains and species during genetic exchange processes.

The combined effects of fast growth, high concentration of cells, genetic processes of mutation and selection and the ability to exchange genes, account for the extraordinary rates of adaptation and evolution that can be observed in bacteria. For these reasons, bacterial adaptation (resistance) to the antibiotic environment occurs very rapidly in evolutionary time; bacteria evolve fast!

1.2 Background to Phytomedicine

1.2.1 Ethnopharmacological overview

1.2.1.1 Introduction

With the present renewed interest in natural medicine, pharmaceutical companies are increasing their commitment in this field. In the USA and Europe, in particular, widespread screening and analysis of plants for biological activity is carried out with plants mainly sourced from Africa (Alexander *et al.,* 1992). Plants are used for many different ailments as described by Leder (1997)(Table 1.1)

Table 1.1. List of ailments that can be treated with some medicinal plants and the plant parts used (Leder, 1997)

Ailment	Medicinal Plant	Plant part used
Backache	*Pleiotaxis antunesii*	leaves
Birth, heavy bleeding while giving	*Peltophorum africanum*	roots
Birth, induce	*Dichapetalum rhodesicum*	roots
Bruise	*Psydrax livida*	roots
	Ricinus communis	
Burns	*Ochna pulchra*	bark
Chest pain	*Ancylanthos bainesii*	roots
Colds	*Becium filamentosum*	whole herb
	Hibiscus sabdariffa	calyx
	Tephrosia cephalanta var decumbens	roots
Contraceptive	*Dichapetalum rhodesicum*	roots
	Grewia falcistipula	roots
	Melhania burchellii	roots
Cough	*Ancylanthos bainesii*	roots
	Combretum zeyheri	leaves
	Guibourtia coleosperma	young leaves
	Peltophorum africanum	roots
	Pterocarpus angolensis	red plant sap
	Rhus tenuinervis	roots
	Strychnos pungens	leaves
	Tephrosia lupinifolia	roots
	Tephrosia cf. Oxygona	roots
	Tephrosia cf. Purpurea	roots
	Terminalia sericea	leaves
	Ziziphus mucronata subsp mucronata	roots
Diarrhoea	*Combretum platypetalum subsp. baumii*	roots
	Dialium englerianum	inner bark
	Dichapetalum cymosum	roots
Ailment	**Medicinal Plant**	**Plant part used**

	Ozoroa insignis subsp. Latifolia	roots
	Schinziophyton rautanenii	bark
	Terminalia sericea	roots, leaves
	Ziziphus mucronata subsp mucronata	roots
Eyes, sore	*Grewia avellana*	roots
Ears, suppurating	*Sansevieria pearsonii*	sap, rhizome
Fever	*Becium filamentosum*	whole herb
	Psydrax livida	roots
	Tephrosia lupinifolia	roots
Headache	*Ancylanthos bainesii*	roots
Infertility	*Clerodendron dekindtii*	roots
	Combretum albopunctatum	roots
Liver problems	*Dichapetalum cymosum*	roots
	Peltophorum africanum	bark
Malaria	*Combretum psidioides*	roots
Mental disorientation	*Otoptera burchellii*	roots
Sickness during Pregnancy	*Peltophorum africanum*	roots
	Schinziophyton rautanenii	bark
Sinusitis	*Otoptera burchellii*	roots
Skin inflammations	*Lonchocarpus nelsii subsp. Nelsii*	frass of larvae
Spleen complaints	*Solanum delagoense*	roots, sap of fruits
Stomach pain	*Burkea africana*	roots
	Combretum zeyheri	roots
	Grewia avellana	roots
	Schinziophyton rautanenii	bark
	Strychnos cocculoides	bark
	Terminalia sericea	roots
Stomach ulcers	*Ziziphus mucronata subsp. Mucronata*	roots
Tuberculosis	*Acacia erioloba*	roots
	Peltophorum africanum	roots
	Ziziphus mucronata subsp. Mucronata	roots
Tooth ache	*Acacia erioloba*	roots
	Annona stenophylla subsp. Nana	roots
	Burkea africana	roots
	Psydrax livida	roots
	Securidaca longepedunculata	roots
Urine, blood in the	*Pleiotaxis antunesii*	leaves
Vomiting	*Ziziphus mucronata subsp. Mucronata*	roots
Vomiting, induce	*Strychnos cocculoides*	unripe fruits
Wounds	*Psydrax livida*	roots
	Strychnos cocculoides	leaves
	Terminalia sericea	roots
Plants used as general Treatments and Tonics:		
For babies	*Baphia massaiensis subsp. Obovata var. obovata*	roots
	Ochna pulchra	young leaves
	Ozoroa paniculosa var. paniculosa	roots
For infants	*Bauhinia urbaniana*	roots
	Dichapetalum rhodesicum	roots
	Hoffmannseggia burchellii	roots
For adults	*Bauhinia urbaniana*	roots
	Combretum psidioides	roots
	Lapeirousia otaviensis	Tuber

South African scientists have also started focussing attention on this important indigenous resource, both in the authentication of traditional medicinal plant use, and in the discovery of compounds with therapeutic/clinical potential. Several members of the Combretaceae have been used for treating bacterial diseases in southern Africa. A number of studies have been conducted on plants of the Combretaceae to verify antibacterial and other biological activities (Eloff, 1999b; Martini and Eloff, 2000, Katerere, 2001,Martini *et al*, 2004).

1.2.1.2 Overview of research on methodology and antibacterial activity

Although most of the work done to date was on *Combretum* species, an outline of the in Combretaceae research activities in the Phytomedicine Programme, University of Pretoria, is given to provide background information to the selection of the topic and methodology employed in this PhD project.

i) Selection of plants to investigate

An analysis was made of approaches to be followed in selecting plants for research and gene banking. Plants used as phytomedicines in Africa were also analyzed, and of these, the Combretaceae constituted a major group (Eloff 1999a).

ii) Selection of optimal extraction procedure

Several extractants were tested and evaluated on many different parameters and acetone was found to be the best extractant (Eloff, 1998b).

iii). Selection of best purification procedures

The solvent-solvent fractionation procedure used by the USA National Cancer Institute was tested and refined, and several TLC separation procedures were developed (Eloff, 1998b).

iv) Development of a novel way to determine antibacterial activity

It could be shown that the traditional agar diffusion assays for determining activity of plant extracts were not reliable. A new serial dilution microplate assay using INT was developed (Eloff, 1998c).

v) Antibacterial activity of *Combretum erythrophyllum*

Using the techniques developed above, it was found that *Combretum erythrophyllum* contains at least 14 antibacterial compounds (Martini and Eloff, 1998). Extracts had MIC values as low as 50 µg/ml.

vi) Antibacterial activity and stability of members of the Combretaceae

Acetone leaf extracts of 27 species of *Combretum, Terminalia, Pteleopsis* and *Quisqualis* had antibacterial activity ranging from 0.1 – 6 mg/ml (Eloff, 1999b). Storing extracts for 6 weeks at room temperature did not affect the MIC values.

vii) Stability of antibacterial activity in *C. erythrophyllum*

Leaves of *C. erythrophyllum* collected from the same area and stored in herbaria for up to 92 years did not lose any antibacterial activity (Eloff, 1999c)

viii) A proposal for expressing antibacterial activity

MIC values do not give sufficient indication of the activity present in a plant. A proposal was made that "total activity" should be determined by dividing the quantity in mg extracted from 1 g of plant material by the MIC in mg/ml. The resultant value in ml/g gives the highest volume to which a plant extract can be diluted and still inhibit the growth of the test organism (Eloff, 2000).

ix) Other biological activities of *Combretum* species

Breytenbach and Malan (1989) isolated three antimicrobial compounds from *C. zeyheri* and Alexander *et al.* (1992), found antimicrobial activity in 6 species of *Combretum*. The anti-inflammatory, anthelminthic and antischistosomal activity of 20 *Combretum* species was determined. There was very little antischistosomial activity, low to medium anthelminthic activity and medium to strong anti-inflammatory activity in extracts of the different species (McGaw *et al.* 2001).

x) The stability and relationship between antibacterial and anti-inflammatory activity of southern African *Combretum* species

Both antibacterial and anti-inflammatory activities were stable in stored extracts and there was a reasonable correlation between antibacterial and anti-inflammatory activity,

indicating that similar compounds may be responsible for the biological activities (Eloff *et al,* 2001).

xi) Extraction of antibacterial compounds from *Combretum microphyllum*

Several extractants were tested to determine if any extractant selectively extracted antibacterial compounds. The three most promising extractants were di-isopropyl ether, ethanol, ethyl ether, acetone and ethyl acetate. The activity towards Gram-negative and Gram-positive bacteria was similar (Kotze and Eloff, 2002).

xii) Isolation of antibacterial compounds from *C. erythrophyllum*

For her PhD study Nataly Martini isolated and characterized seven antibacterial compounds from *C. erythrophyllum*. Four were flavanols: kaempferol, rhamnocitrin, rhamnazin, quercitin 5, 3'-dimethylether and three were flavones: apigenin, genkwanin and 5-hydroxy-7, 4'-dimethoxyflavone(Martini et al 2004 a). All test compounds had good activity against *Vibrio cholerae* and *E. faecalis*, with MIC values in the range of 25-50 µg/ml. Rhamnocitrin and quercetin-5, 3'-dimethylether also showed good activity (25 µg/ml) against *Micrococcus luteus* and *Shigella sonei.* Toxicity testing of the isolated compounds showed little or no toxicity towards human lymphocytes with the exception of 5-hydroxy-7, 4'-dimethoxyflavone. This compound is potentially toxic to human cells and exhibited the poorest antioxidant activity. Both rhamnocitrin and rhamnazin exhibited strong antioxidant activity with potential anti-inflammatory activity. Although these flavonoids are known, this was the first report of biological activity relating to some of these compounds (Martini, 2000, Martini *et a*l 2004 b).

(xiii) Isolation of antibacterial compounds from *C. woodii*

For his M.Sc study James Famakin isolated the stilbene 2', 3', 4-trihydroxyl, 3, 5, 4'-trimethoxybibenzyl (combretastatin B5) from the leaves of *C. woodii*. It showed significant activity against *S. aureus* with an MIC of 16 µg/ml, some activity against *P. aeruginosa* (MIC of 125 µg/ml) and *E. faecalis* (MIC of 125 µg/ml), and slight activity against *E. coli*. This is the first report of the antimicrobial activity of combretastatin B5 (Famakin, 2002).

xiv) Isolation of antibacterial compounds from *C. apiculatum*

For his M.Sc study Andrew Serage elucidated the structures of two flavanones (alpinetin and pinocembrin) and one chalcone (flavokawain) from the leaves of *C. apiculatum* subsp. *apiculatum*. All the compounds had substantial activity against the bacterial pathogens tested (Serage, 2003).

More students are working on isolating antibacterial, antioxidant and antifungal compounds from other *Combretum* species. Jerry Angeh has isolated and elucidated seven compounds including two new compounds from *C. imberbe* and *C. padoides* as part of his ongoiing PhD study. The promising preliminary results on *Terminalia* species motivated this study. The techniques used are described in previous publications and theses.

1.2.1.3 *Terminalia* species and antimicrobial activity

Some *Terminalia* and *Combretum* species are used for a variety of complaints, ranging from the treatment of infertility and sexual impotence to the management of stomach disorders, including diarrhoea and helminthiasis, to haematuria (Gelfand *et al.,* 1985, Table 1.1). The genus *Terminalia* contains about 250 species, of which only a handful have been investigated phytochemically and/or for pharmacological efficacy (Katerere, 2001). Table 1.2 describes the traditional medical uses of some *Terminalia* species in southern Africa.

Table 1.2. Some uses of *Terminalia* spp. in southern African traditional medicine as reported in the PhD thesis of Katerere, 2001.

PLANT SPECIES	MEDICINAL USES
Terminalia brachystema	Bile emesis, Constipation, Haematuria
Terminalia sericea	Abscesses and wounds, Abdominal pain, Anti-emetic, Anthelmintic, Bilharziasis, Diarrhoea, Dilatation of birth canal, Depressed fontanelle in children, Epistaxis, General malaise, Gonorrhoea, Infertility, Prevention of abortion, Sore throat, Tonic
Terminalia mollis	Bile emesis

Sato *et al.* (1997) investigated the antimicrobial activity of six East African medicinal plants including *Terminalia spinosa* against 105 strains of bacteria from seven genera. The study showed that *Terminalia spinosa* possesses limited antibacterial activity (MIC

< 8 mg/ml) against *Staphylococcus aureus, Enterococci spp., Pseudomonas aeruginosa* and species of the Enterobacteriaceae.

Methicillin-resistant strains of *S. aureus* (MRSA) are resistant to many aminoglycoside antibiotics, and essentially to all beta-lactam antibiotics. Ethanol extracts of the fruiting bodies of *Terminalia chebula* contained two compounds with antimicrobial activity against MRSA (Sato *et al.,* 1997).

The bark of *Terminalia alata* is active against eight out of eleven selected bacterial and fungal species, as well as against fungal spores (Taylor *et al.,* 1996). The ethanolic extracts of twelve plants selected through an ethnomedical survey (the roots of the plants are used to treat venereal diseases) in Guinea-Bissau were investigated for their *in vivo* antibacterial properties against ten bacterial species and *Candida albicans* using agar diffusion and dilution methods. *Terminalia macroptera* extracts showed activity against the bacteria as well as some activity against *C. albicans.* An interesting profile of activity against most of the enteropathogenic microorganisms was also shown (Taylor *et al.,* 1996).

The activity of a crude extract formulation of *Terminalia chebula* and other plants was evaluated in experimental amoebic liver abscess in golden hamsters (Sohni and Bhatt, 1996). The formulation had a maximum cure rate of 73% at a dose of 800 mg/kg/day, showing a reduction in the average degree of infection (ADI) of 1.3 compared to 4.2 for the sham-treated controls (Sohni and Bhatt, 1996). In immunomodulation studies,

humoral immunity was enhanced as evidenced by the haemagglutination titre. The T-cell counts remained unaffected in the animals treated with the formulation but cell-mediated immune response was stimulated as observed in the leukocyte migration inhibition tests (Sohni and Bhatt, 1996). Gallic acid, chebulagic acid and chebulinic acid isolated from *Terminalia chebula* were found to have moderate activity against cultured tumor cells *in vitro* (Lee *et al.,* 1995). Gallic acid and its ethyl derivative are also responsible for the potent antimicrobial activity which this plant exhibits even against methicillin-resistant *Staphylococcus aureus* (Sato *et al.,* 1997).

A bioactivity-guided fractionation of an extract of *Terminalia bellerica* fruit rind led to the isolation of two new lignans named termilignan (1) and thannilignan (2), together with 7-hydroxy-3', 4'-(methylenedioxy) flavan (3) and anolignan B (4). All four compounds possessed demonstrable anti-HIV-1, anti-malarial and anti-fungal activity *in vitro.*

In another study by Sohni *et al.* (1995) on the crude drug formulation of plants including *Terminalia chebula,* the dried and pulverized plants were extracted with ethanol together and individually. The *in vitro* amoebicidal activity was studied to determine the minimum inhibitory concentration (MIC) values of all the constituent extracts as well as the whole formulation. The formulation had a MIC of 1000 μg/ml as compared to the 10 μg/ml of the reference compound metronidazole. In experimental caecal amoebiasis in rats the formulation had a curative rate of 89% with the average degree of infection (ADI) reduced to 0.4 in a group dosed with 500 mg/kg/day compared with ADI of 3.8 for the sham-treated control rats. Metronidazole had a cure rate of 89% (ADI = 0.4).

Extracts of *T. bellerica*, *T. chebula*, *T. horrida* and 38 other plants were screened for their inhibitory effects on Human Immunodeficiency Virus-1 transcriptase (El-Mekkawy *et al.,* 1995). The extracts showed significant inhibitory activity with IC_{50} values less than or equivalent to 50 µg/ml. Extracts of *Terminalia spinosa* and other plants showed activity against various *Candida* species at MIF's (minimum fungicidal concentrations) of 0.06 to > 8 mg/ml and MIC's of 0.006 to 8 mg/ml. A methanol extract of the dried fruit of *Terminalia pallida* was evaluated and found to have a broad spectrum of antimicrobial activity (Gupta *et al.,* 2001).

Terminalia arjuna is widely used in India by Ayurverdic doctors for managing cardiovascular disorders. The tannins isolated from the plant were found to exert a cholinergic hypotensive effect in rats (Tanaka *et al.,* 1986). The compounds 3β-hydroxyolean-12-ene and related oleane-type triterpenoids were isolated from the leaves (Chauhan *et al.,* 1997). The bark powder apparently has hypolipidaemic effects (Khanna *et al.,* 1996) and Petit and colleagues (1998) found the bark constituents to be gallic acid, ethyl gallate and luteolin, which were found to be inhibitors of cancer cell growth.

Young branches of *Terminalia spinosa* have been found to have anti-fungal activity (Fabry *et al.,* 1997), as well as activity against *Helicobacter pylori* and *Candida* species. *Terminalia macroptera* root extracts showed some activity against *Candida albicans* and enteropathogenic bacteria, with the best results obtained against clinical strains of

Shigella dysenteriae and *Vibrio cholera* (Silva *et al.,* 1996). Ellagitannins were found to be the main constituents in the active fractions. In general, biological activity arising from condensed tannins is not reproducible *in vivo* but some ellagitannins can enter the bloodstream and have shown biological activity which may have potential for use in chemotherapy (Nash, 2000). Tannins isolated from the methanol extract of *Terminalia citrina* have been tested for antimicrobial activity (Burpadaja and Bunchoo, 1995). *Terminalia avicennoides* has been found to possess activity against *Vibrio cholera* (Alkinside and Olukoya, 1995).

Plants of the genus *Terminalia* are used in African traditional medicine for a variety of conditions that may be of infective nature e.g. bilious vomiting, diarrhoea, billharzia, sore throat, gonorrhoea, hepatitis and malaria. The use of many of the traditional medicinal plants discussed in this section has in many cases been scientifically validated for infectious ailments. The antimicrobial action of plant extracts, including anti-vibrio and anti-amoebic activity, has been demonstrated in various studies (Eloff, 1999b; Sato *et al.,* 1997; Silva *et al.,* 1996).

1.2.2 Botanical overview

This section deals with the botanical background of the plants investigated in this study. The Combretaceae family belongs to the order Myrtales and is divided into two sub-families: Combretoidaea and Strephonematoidaea. Combretoidaea, which is of interest in this study, is divided into three sub-tribes, Combretinae, Pteleosidinaea and Terminaliinae (which consists of 8 genera).

The African Combretaceae comprise 19 genera but the two largest, *Combretum* and *Terminalia*, occur in most parts of Africa where they are often numerically the dominant group (Carr, 1988). They may be climbers, shrubs or trees, and are readily characterized by fruits with wing-shaped appendages. Although traditional healers throughout Africa have used species of the Combretaceae for the treatment of a wide range of disorders, only about 25 of the approximately 99 African species of Combretum have been subjected to any form of scientific study (Rogers and Verotta, 1988).

According to Lawrence (1951), the Combretaceae family has, within its 18 genera, some 500 species distributed throughout the tropics and subtropics. Carr (1988) classified the *Terminalia* species occurring in southern Africa into three sections based on external morphology as shown in Table 1.3. Thirty-three species of the Combretaceae occur in southern Africa including eleven *Terminalia* species (Carr, 1988). In this study, three species (representing each section) were initially chosen for extractant suitability studies. Subsequently, seven species were selected for preliminary antibacterial activity investigation and these are described in detail below.

1.2.2.1 Terminalia sericea

Common names: Vaalboom, (Afrikaans) Silver cluster-leaf, (English) Mokonona (Shona)

SA National tree number; 551

Distribution: Most widely distributed of all the *Terminalia* species in southern Africa. Found in the northern parts of Gauteng, mainly in the Limpopo province and northern

parts of the Northern Cape in South Africa, as well as the whole of Zimbabwe and Botswana and western Namibia.

Table 1.3. The three sections of *Terminalia* (Carr, 1988) with the species represented in each section

***Section Abbreviate** Exell*
Terminalia prunoides Exell
Terminalia randii Bak. F.
Terminalia stuhlmanii Engl.
***Section Psidioides** Exell*
Terminalia brachystema Welw. Ex Hierr
Terminalia sericea Burch. Ex DC
Terminalia trichopoda Diels
***Section Platycarpae** Eng. & Diels emend* Exell
Terminalia gazenzis Bak. f.
Terminalia phanerophlebia Eng. & Diels
Terminalia mollis Laws
Terminalia sambesiaca Eng. & Diels
Terminalia stenostachya Eng. & Diels

Habitat: Restricted to sand or sandy soils at altitudes ranging from sea level to 1600 m. It occurs in open and tree savanna, woodland situations and as a riverine growth in

semi-desert areas.

General: Deciduous species, often seen as a shrub or sapling 2-4 m high. The usual maximum height is 7–8 m but it can reach heights of 12 m. Photographs of a *T. sericea* tree are shown in Figure 1.1.

Bark: Dark to medium grey with pronounced fissuring.

Foliage: Leaves are clustered on whorls of up to 10, have a long even taper to the base and appear blue-grey with silvery tinges. The underside of the leaf has a raised main vein with a pale brownish-green colour.

Fruits: Ripens in June and is a single winged samara, elliptic in outline with a constricted taper to the base and a slight attenuation near the apex, which is emarginate.

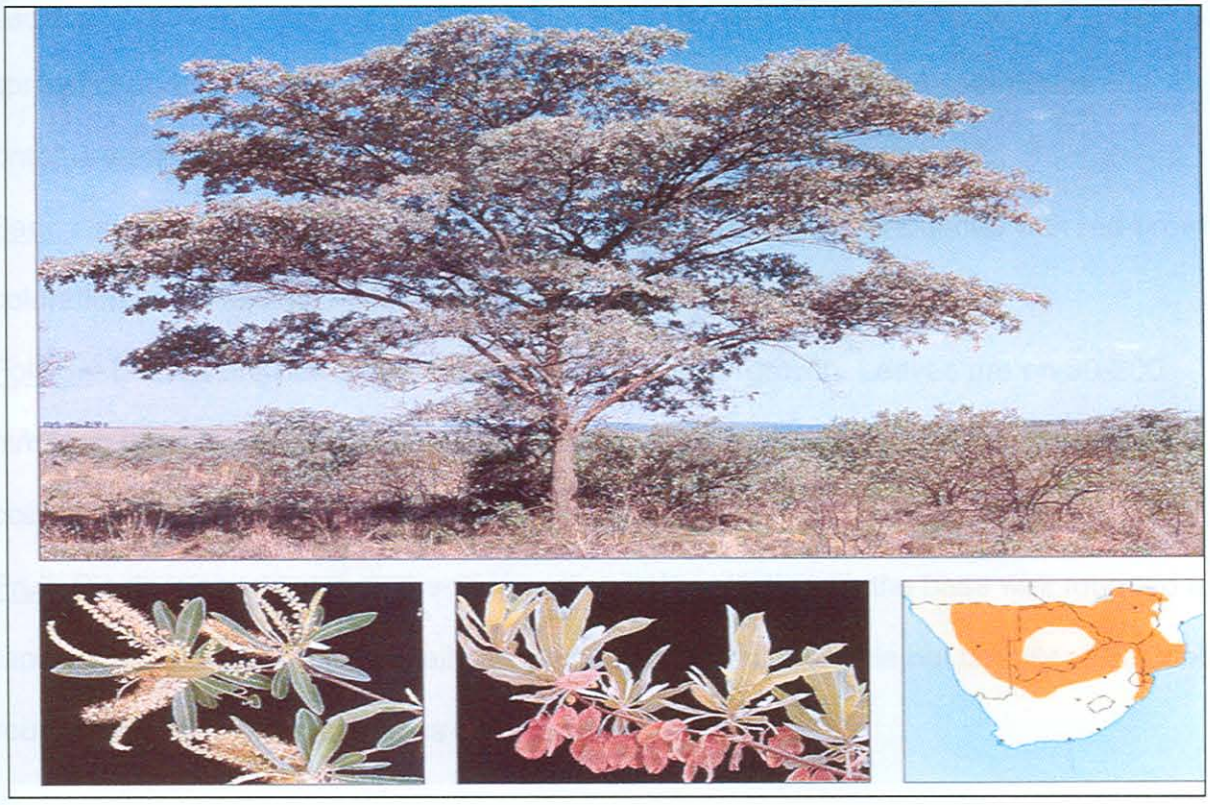

Fig. 1.1. A *Terminalia sericea* tree, with close-up view of foliage and flowers van Wyk *et al* 2002

1.2.2.2 Terminalia brachystema

Common names: Bastervaalboom (Afrikaans), Bastard Clusterleaf (English), Muogonono (Shona).

SA National tree number: 548

Distribution: Sporadic occurrences in the central part of the Limpopo province with the main distribution along the Caprivi-strip and the area adjacent the Zambezi river.

Habitat: Savannah and woodland areas, on the Kalahari and other sand at altitudes of 800-1200 m.

General: Shrub or tree usually up to 4–5 m but in favourable conditions up to 7 m with a spread of up to 9 m. The species has been split by Wickens into the subspecies *brachystema* and *sessiliflora* (not occurring in the southern African area).

Bark: Dark to medium grey or grey-brown. Fissures are fairly pronounced with red-brown coloration in the hollows.

Foliage: Leaves are borne only on the last 12 months' growth. Leaves are on 30-200 mm long laterals on which there can be one or more whorls having up to eight leaves. It could have sessile or petiolate leaves.

Fruit: Single winged samara, the wing outline being elliptic with the base well rounded to tapered and the apex occasionally acuminate and even attenuate but usually rather well rounded and in most such cases emarginate.

1.2.2.3 Terminalia gazenzis

Common name: Fringe-leafed Terminalia (English).

SA National tree number (Zimbabwe) 786

Distribution: Central-southern Zimbabwe and odd clusters in other parts of Zimbabwe, Malawi and Mozambique.

Habitat: Found in savanna, woodland and forest margins at medium altitudes of up to 1200 m.

General: It is a tree of up to 10–20 m, wide spreading with a flattish to deeply rounded crown.

Bark: Usually single stemmed with a dark brown bark, somewhat fissured near the base but further up a light brown to dark biscuit colour.

Foliage: Four to five or even up to twelve leaves per whorl. The lamina is obovate with a gradual taper to the base and the apex is rounded. It usually has an inconspicuous apicula but is sometimes emarginate.

Fruit: Single winged samara with an outline that is elliptic to oblong elliptic, often with appreciable irregularities.

1.2.2 4 Terminalia mollis

Common name: Large-leafed Terminalia (English).

National tree number: (Zimbabwe) 787

Distribution: Various geographical areas in Zimbabwe, as well as in Tanzania, Zaïre, Angola, Kenya and West Africa.

Habitat: Found in savanna or woodland situations in association with mopane trees.

General: A robust-looking tree, usually up to 12 m with a well-rounded or rather flatly rounded crown which can exceed the height.

Bark: Medium grey, coarsely longitudinally fissured with light grey coloration in the fissures.

Foliage: Leaves are borne on the previous year's growth as well as on current extensions and are orientated 360 degrees around the stems in groups, to form in effect a whorl. Leaves are petiolate and broadly elliptic, tapering or rounding at the base with the apex rounded to bluntly acuminate and often with a smaller apicula.

Fruit: Single-winged and elliptic in outline though there can be considerable irregularities in the wing margins. Fruit is borne in large numbers and, being at the terminals, is prominently displayed.

1.2.2.5 Terminalia phanerophlebia

Common names: Lebombo cluster leaf (English), Lebombo trosblaar (Afrikaans).

SA National tree number: 549

Distribution: In the southern parts of the Kruger National Park and the northern parts of Kwa Zulu-Natal in the Republic of South Africa as well as parts of Swaziland.

Habitat: Found in mixed savanna, along watercourses and on slab rock hillslopes at altitudes usually not exceeding 700 m but sometimes as high as 1200 m.

General: A shrub of up to 1.5-2 m, but sometimes as high as 6 m. May be single-stemmed but are often multiple-stemmed. Irregular crown, which may exceed the height.

Bark: Darkish grey with pronounced, wavy, longitudinal fissures, which may result in an elongated diamond pattern.

Foliage: Leaves are in whorls of up to 20 where there are no extensions. The lamina, sometimes broadly elliptic, is usually more obovate with a rounded to straight to attenuate taper to the base where it may also be decurrent along the petiole.

Fruit: Single-winged samara, elliptic to broadly elliptic in outline.

1.2.2.6 Terminalia prunoides

Common names: Lowveld cluster-leaf (English), Sterkbos (Afrikaans), Bakone (Shona).

SA National tree number: 550

Distribution: Right across the width of southern Africa - from the northern parts of Namibia through the northern parts of Botswana, including the Caprivi Strip down to the northern parts of South Africa.

Habitat: Found in many different types of woodland, tree savanna and scrub savanna at medium to low altitudes.

General: Seen most frequently as a many-stemmed, rather rounded shrub, 3-5 m in height with a spread of up to 7 m, but may be a single-stemmed tree of 15 m in height.

Bark: Light grey with a shallow fissure and a gingery coloration at the base of the fissure. Some criss-crossing of the fissures tend to form an elongated diamond pattern.

Foliage: Leaves are produced in whorls of 6-8. Leaves are petiolate, the lamina being elliptic to obovate with a straight to rounded taper to the base and the apex bluntly acuminate and slight attenuate, to rounded and sometimes emarginate.

Fruit: A samara with a single rounded wing, obovate in outline with wing margins tapering to the base and the apex bluntly acuminate to rounded, often with slight attenuation and then, as a rule, emarginate.

1.2.2.7 Terminalia sambesiaca

Common names: River Terminalia (English).

National tree number: (Zimbabwe): 790

Distribution: Northern parts of Zimbabwe around the Kariba region bordering Zambia.

Habitat: Found in riverine fringing forests and rocky hill slopes at altitudes from 800-1250 m.

General: Usually a single-stemmed tree of up to 20 m in height.

Bark: Smooth, somewhat fissured, grey, overmarked with longitudinally orientated, light red-brown, slightly raised ridges.

Foliage: Leaves are clustered in whorls of 3-6. The lamina is obovate to elliptic, either tapering to or rounding at the base and acuminate or rounded at the apex, which almost invariably has a pronounced apicula.

Fruit: Hang in bunches from old inflorescence branches. Has a single surrounding wing elliptic in outline with a blunt taper to the base, the apex rounded and usually emarginate.

All the above information was derived from Carr (1988)

1.2.3 Phytochemical overview

This section deals with the chemistry of biologically active compounds isolated from *Terminalia* species in general, and from the species specifically under investigation, by previous researchers. A range of phytochemical compounds has been isolated from *Terminalia* and *Combretum* species (e.g. combretastatins from *Combretum* species and flavonoids and terpenoids from *Terminalia* species). The main biologically active compounds will be described under the headings that follow.

1.2.3.1 Terpenoids

Plant fragrances are carried in the so-called *quinta essentia* or essential oil fraction (Cowan, 1999). These oils are secondary metabolites and are highly enriched in compounds based on an isoprene structure, called terpenes. Terpenes have a general chemical structure of $C_{10}H_{16}$. They are classified as monoterpenes, diterpenes, triterpenes and tetraterpenes (C_{10}, C_{20}, C_{30} and C_{40}), as well as hemiterpenes (C_5) and sesquiterpenes C_{15}). When the compounds contain additional elements, usually oxygen, they are termed terpenoids (Cowan, 1999).

Terpenoids are synthesized from acetate units and as such, share their origin with fatty acids. They differ from fatty acids in that they contain extensive branching and are cyclized. Examples of common terpenoids are menthol and camphor (monoterpenes). Terpenoids are active against bacteria and fungi (Taylor *et al.,* 1996). The mechanism of action of terpenes is not fully understood but is thought to involve membrane disruption of the lipophilic compounds. A rich variety of triterpenoid acids have been isolated from

C. molle and *C. imberbe* (Rogers and Verotta, 1996). Katerere (2001) isolated oleanane compounds from *T. stuhlmani* that are glycosides of imberbic acid, originally isolated from *C. imberbe.* These compounds establish a chemotaxonomic link between the two genera. An example of a terpenoid is shown in Figure 1.2.

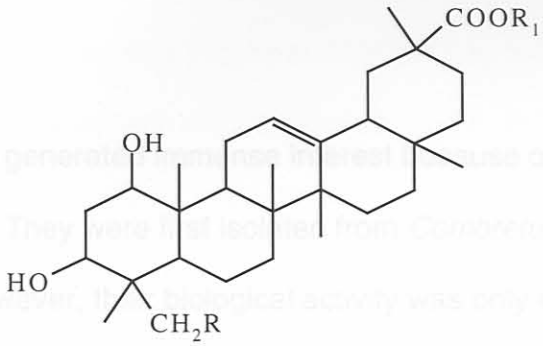

Fig. 1.2. Structure of a terpenoid - an example of a 1,3-hydroxylated pentacyclic triterpenoid

1.2.3.2 Tannins

Tannins are polyphenolic compounds of high molecular weight. They have the ability to precipitate proteins (Katerere, 2001), and they may also form complexes with starch, cellulose and minerals. They are synthethised via the shikimate pathway, which is the same pathway that results in the formation of isoflavones, coumarins, stilbenoids and other phenolic metabolites.

Gallic acid and its derivatives are common constituents of the Combretaceae. A number of elaborate tannins have been isolated mainly from *Terminalia* species, e.g. the

diphenoyl-gallagylglucose isolated from *T. oblongata,* and ellagitannin from the leaf of *T. calamansanai*, as well as derivatives from *T. catappa* and *T. chebula* (Tanaka *et al.,* 1986) These compounds have been shown to have anti-tumour activity in numerous studies (Pettit *et al.,* 1996).

1.2.3.3 Stilbenoids

These compounds have generated immense interest because of their biological potency and structural simplicity. They were first isolated from *Combretum* species by Letcher and Nhamo in 1971. However, their biological activity was only discovered by Pettit and colleagues in the early 1980's who isolated stilbenes from a methylene chloride-methanol extract of the leaves, fruit and stemwood of *C. caffrum* (Cape bush willow tree) (Pettit *et al.,* 1982) They named these compounds "combretastatins", and they have since been isolated from *C. kraussi, C. molle, C. psidiodes*, *C. apiculatum* and *C. woodii* (Malan and Swinny, 1993; Schwikkard *et al.,* 2000; Katerere, 2001; Famakin, 2001). They have been designated A, B and D according to their chemical structures. Combretastatins A and B are almost identical but differ in that the former has an ethylene bridge joining the two benzyl groups and is chemically identified as a stilbene. The former, meanwhile, has an ethane-type bond structure and is closer to the dihydrostilbenes.

1.2.3.4 Flavonoids

Flavonoids are conjugated aromatic phenols which may occur in glycosidic combinations or as aglycones (Harborne, 1973). At least nine classes of flavonoids are recognised, all of which are biosynthetically related and originate from the amino acid, phenylalanine. They are one of the most diverse and widespread groups of natural constituents, and are of great interest to phytochemists (Agrawal, 1989). They may represent an interesting new therapeutic approach with low toxicity and a wide variety of chemical structures. Flavonoids are also widely used taxonomic markers. The flavonoid, luteolin, has been isolated from *Terminalia arjuna* and has shown to be active against murine P388 lymphocytic leukemia and human cancer cell line as well as inhibiting the growth of *Neisseria gonorrhoea* (Pettit *et al.,* 1996). Luteolin has been shown to be both an antitumour promotor and a mutagen.

1.3 Aim of the study

The hypothesis is that *Terminalia* species occurring in South Africa contain useful antibacterial compounds because in a preliminary investigation *T. sericea* possessed the third highest total activity of 27 Combretaceae species examined (Eloff, 1999b).

It is the aim of this study therefore

(1) to investigate the presence of antibacterial compounds in the leaves of different *Terminalia* species found in southern Africa and to determine the antibacterial activity using *in vitro* methods.

(2) to isolate and identify one or more of the antibacterial compounds, and

(3) to determine if a preparation of the isolated compound can be used to treat animals infected with a bacterial pathogen.