CHAPTER 2

BOTANICAL AND PHYTOCHEMICAL OVERVIEW

2.1. Taxonomy and habitat

Combretaceae consists of 18 genera distributed mainly in Africa and Asia. The largest genus wolrdwide is *Combretum* with about 370 species and *Terminalia* is second largest with about 200 species (Lawrence, 1951). The species of the genus *Combretum* and *Terminalia* are widely used for medicinal purposes and occur in most parts of Africa (Rogers and Verotta, 1996). The other genera are small and include: *Calopyxes, Carnocarpis, Quisqualis, Buchenavia* and *Pteleopsis* (Rogers and Verotta, 1996). This study will focus on *C. apiculatum.* It is divided into, *Combretum apiculatum* Sond subspecies *apiculatum* Exell and *Combretum apiculatum* Sond subsp. *leutweinii* (Schinz) Exell. (Fig.2.1). Some plant taxonomists doubt the validity of *Combretum apiculatum* subsp. *leutweinii*, which is extremely hairy and only occurs in Namibia. In this report *Combretum apiculatum* will refer to subsp. *apiculatum* Exell as distinguished by Carr, (1998).

Table 2.1 The subgeneric classification of the genus *Combretum* in South Africa according to Carr 1988

Combretum Loefl	
Subgenus *Combretum*	**Subgenus *Cacoucia***
Section *Hypocrateropsis*	**Section *Lasiopetala***
C. celastroides	*C. obovatum*
C. imberbe	Section Conniventia
C. padoides	*C. microphyllum*
Section *Combretastrum*	*C. paniculatum*
C. umbricola	*C. platypetalum*
Section *Angustimarginata*	Section *Oxystachya*
C. caffrum	*C. oxystachytum*
C. erythrophyllum	**Section *Megalantherum***
C. kraussili	*C. wattii*
C. vendae	**Section *Poivrea***
C. woodii	*C. bracteosum*
Section *Macrostigmatea*	*C. mossambicense*
C. zeyheri	
Section *Ciliatipetala*	
C. albopunctatum	
C. apiculatum	
C. edwardsii	
C. moggii	
C. molle	
C. petrophilum	
C. psidioxides	
Section *Fusca*	
C. coriifolium	
Section *Breviramea*	
C. hereroense	
Section *Elaeagnoida*	
C. elaeagnoides	

Common names of *C. apiculatum* include, Bush willow (English), Rooiblaar, Rooiboswilg (Afrikaans) *Mohwelere* (Northern Sotho) and *Umbondwe* or *Umbondwe omnyama* (Zulu), (Hutchings, 1996). It occurs as a tree, shrub or climber. Generally, the tree is small about 4 – 6 m tall or even 10 m. Main branches commence 1 – 3 m above the ground, mainly bushy and shrub-like. The tree is deciduous and provides good shade when in leaf (Carr, 1998). The leaves are simple, sessile or petiolate, opposite, alternate verticulate, whorled, without stipules, with margins, endumentum comprising of hairs,

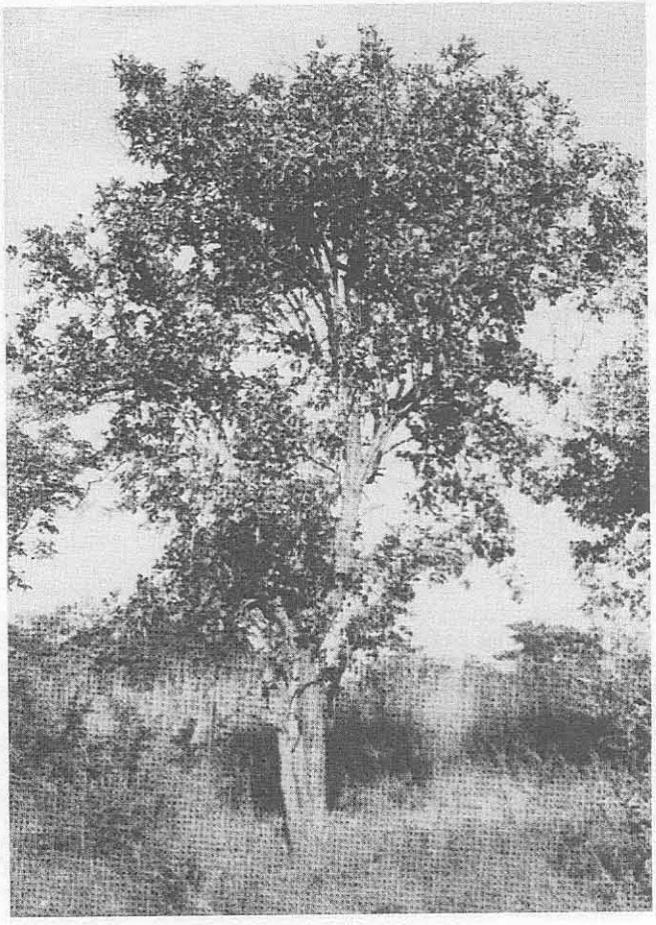
stalked glands and scales. The leaves are oblong and broadly obviate spaced in an opposite arrangement on the branch. They may have hairs on both sides. The flowers are sessile or pedicillate. Seeding cotyledons mostly two but sometimes three or four petriplate or sessile, arising above or below the ground (Carr, 1998). Flowers are present for only a short period along the annual cycle; they are yellow to creamy green with reddish brown winged fruits when matured. Fruit serves as best aid for plant identification because they occur for a long period in contrast to the leaves and flowers. Bark found on the main stem, black and comparatively smooth. On young trees, it is light brown in colour particularly in the highest rainfall areas.

C. apiculatum occurs in various savannah situations under medium rainfall to semi-arid conditions. Found on soils varying from dark and heavy to Kalahari sands, mainly at low altitudes but up to 1400 m in the Northern parts of South Africa (formerly known as Transvaal) and 1500 m in Zimbabwe. It is well distributed in Southern Africa, [fig. 2.1].

2.2. Ethnomedicinal use of *Combretum* species

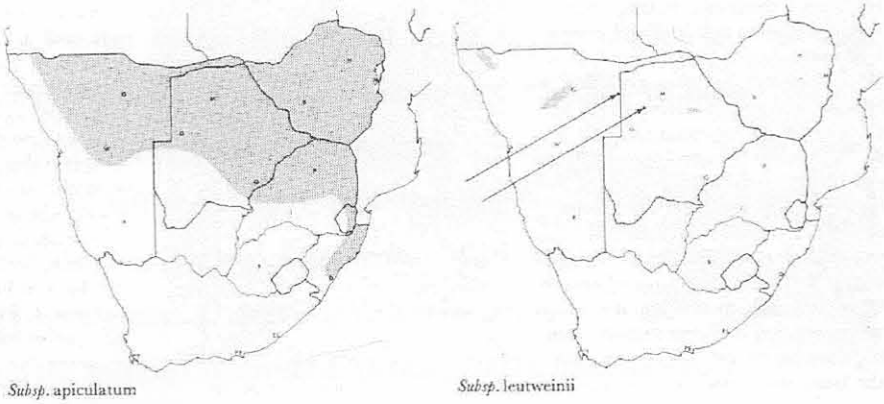
Three antimicrobial agents were found in *Combretum zeyheri*, and six more species of *Combretum* have been found to contain antimicrobial agents. (Breytenbach and Malan, 1989, Alexander *et al.*, 1992). The 27 members of Combretaceae assayed had antimicrobial activity against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa and Enterococcus faecalis* (Eloff, 1999b). *C. erythrophyllum* was found to contain at least fourteen antimicrobial agents with some having an activity superior to that of chloramphenicol and ampicillin (Martini and Eloff, 1998). Several members of the Combretaceae also had anti-inflammatory activity (Eloff at el 2000) as well as anthelmintic activity (McGaw *et al.*, 2001).

Combretum apiculatum Sond.



Habit: subsp. apiculatum, *Selous, Zimbabwe.*

Synonyms
These are as listed in *Flora Zambesiaca:*
Combretum apiculatum Sond. forma *sulphureum* Heurck & Müll. Arg.
Combretum apiculatum Sond. forma *viscosum* Heurck & Müll. Arg.
Combretum apiculatum Sond. var. *parvifolium* Bak. f.
Combretum glutinosum Wood
Combretum apiculatum Sond. var. *sulphureum* (Heurck & Müll. Arg.) Duemmer
Combretum apiculatum Sond. var. *viscosum* (Heurck & Müll. Arg.) Duemmer
Combretum apiculatum Sond. subsp. *boreale* Exell



Subsp. apiculatum *Subsp.* leutweinii

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Figure 2.1 Distribution of the two subspecies of *Combretum apiculatum* (Carr, 1998)

The West African drug, "kinkeliba" isolated from the leaves of *C. micranthum* was found to have diuretic activity, antimicrobial activity against both Gram-positive and Gram-negative pathogens. It is used in Nigeria, Sudan and Senegal for the treatment of biliary fever, colic and vomiting, (Paris, 1942). Species of Combretaceae have been used by traditional healers throughout Africa for the treatment of various diseases e.g. pneumonia, syphilis, leprosy, wound healing, colds, chest cough, diarrhea, conjunctivitis and mumps (Hutchings *et al.* 1996) [Table 1.2]. *C. erythrophyllum* has been used by the communities of Venda of Northern South Africa for the treatment of worms, infertility and wound healing (Mabogo, 1990). And also was used as a purgative by the Zulu communities (Watt and Breyer-Brandwijk, 1962).

Table 2.2. Some of the medicinal uses of some species of Combretaceae (Rogers and Verotta, 1996)

Combretum species	**Uses**
C. apiculatum	snake bite, diarrhea, conjunctivitis, abdominal disorders
C. fragrans	chest cough, syphilis
C. erythrophyllum	fattening, tonic of dogs
C. molle	helmintics, leprosy, headaches, fever
C. hereroense	bilharziasis, headaches, infertility
C. imberbe	coughs, colds, diarrhea
C. zeyheri	toothache, cough, eye lotion, abdominal disorders.

Approximately 100 Combretaceae species occur in Africa; only about 25 species have been subjected to any scientific studies (Rogers and Verotta 1994). It is evident that this family represents an important class of potential sources of medicine. Amongst others, *C. apiculatum* has been found to have antibacterial activity although the active compounds have not been isolated yet (Eloff *et al.* 2000). This study is intends to follow up on this previous work. Other students in our laboratory have isolated antibacterial compounds from the section Angustimarginata: *C. erythrophyllum* (Martini, 1998), *C. woodii* (Famakin, 2002); from the section Conniventia: (Kotze, 2002); from the section Hypocrateropsis (Angeh, 2003). This study is the first on the section Ciliatipetala in our laboratory.

2.3. Phytochemical overview

The following classes of compounds were among those found in *Combretum* species: tannins, amino acids, phenanthrenes, stilbenoids, triterpenoids, and flavonoids (Rogers and Verotta, 1996). Some of the metabolites show cytotoxic, molluscicidal, anti-HIV and general antimicrobial and anti-inflammatory activity. Among the known angiogenesis inhibitors are flavonoids and triterpenoids, (Paper, 1998).
A brief overview of compounds isolated from Combretaceae to date, will be discussed.

2.3.1. Tannins

Tannins are water-soluble polyphenols with an ability to precipitate proteins such as gelatin from solution (Scalbert, 1991). This astringent property is the reason for their past and present use in the tanning of animal skins. Tannins are toxic towards microorganisms. Tannins play a role in food science, wood science, soil science, plant pathology, therapeutics and human and animal nutrition (Scalbert, 1991).

The action of tannins on bacteria appears to be similar to that of related synthetic phenolics such as diphenyl and diphenylalkane compounds, which have found wide application as disinfectants (Scalbert, 1991). The obvious interactions of tannins with enzymes or substrates may have led to a failure to examine other possible mechanisms involved in their biological activity. Their mode of action probably depends on the individual microorganism. Despite the antimicrobial properties of tannins, many microorganisms can grow and develop on tannin-rich materials.

Tannins are found in a variety of woody and herbaceous plants. They can be found in high quantities in any part of plants. They are classified in two groups according to their structures, hydrolysable and condensed. Hydrolysables are esters of phenolic acids and a polyol which is usually glucose (Scalbert, 1991). When dissolved in hot water they form colloidal dispersions, appearing as yellow-brown amorphous substances. They are astringent and have the ability to tan hide (Farnsworth 1966).

Condensed tannins are polymers of phenolic compounds related to the flavonoids and are similar to the hydrolysable tannins. Their toxicity towards filamentous fungi and bacteria were not found to differ significantly (Scalbert, 1991). In general *Combretum* and *Terminalia* species are the source of tannins, [fig 2.3].

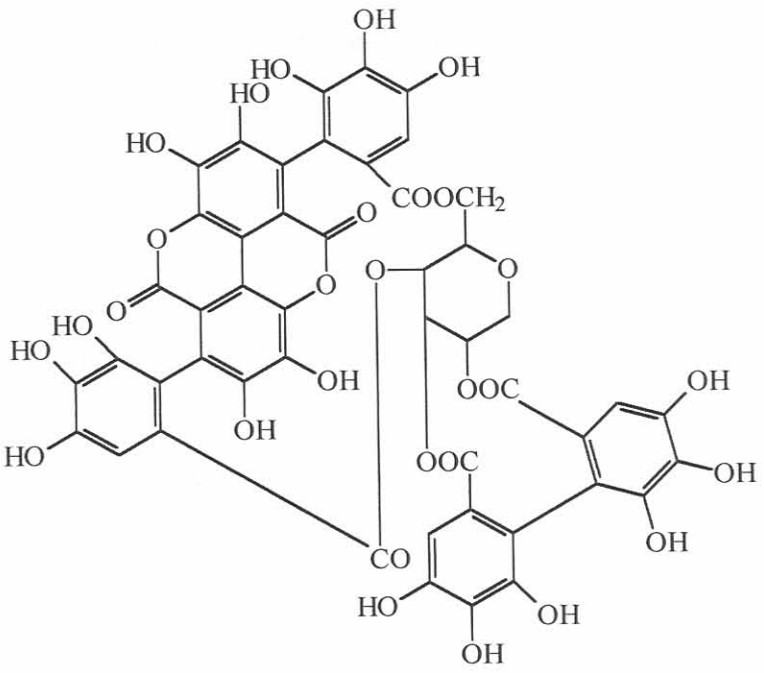

Fig.2.2. 2,3-(S)-Hexahydroxydiphenol-4,6-(S,S)-gallagylglucose (puncalagin) from *Terminalia oblongata* (Scalbert, 1991)

2.3.2. Amino acids and other nitrogen compounds

Choline, betaine and combretins A and B are nitrogenous compounds responsible for the activity of the drug "Kinkaleba" extracted from the leaves of *C. micranthum*. The amino acids N-methyl-L-tyrosine, 3-amino-methyl-2-phenylalanine was isolated from the fruits of *C. zeyheri*. The amino acids have been attributed to fungal intrusion. (Mwauluka et al., 1975; Panzini et al., 1993; Perosa, 1992).

2.3.3. Phenanthrenes

Seventeen substituted phenanthrenes and about 10 dihydrophenanthrenes have been isolated from the heartwood of *C. molle, C. psidioides,* and *C. hereroense*, (Letcher et al 1971 and 1973). Dihydrophenanthrenes, isolated from *C. caffrum*, showed reasonable antileukemic activity and inhibition of penicillin in antifungal tests, (Malan and Swinny, 1993).

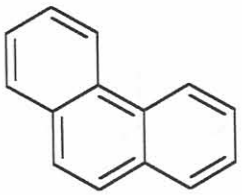

Fig.2.3. Phenanthrene ring

2.3.4. Stilbenoids

The stilbenoids isolated from *Combretum* species are known as combretastatins, (Pettit et al, 1998; Malan and Swinny, 1993). The seed of *Combretum kraussi* and *Combretum hereroense* showed the presence of combretastatins A and B as well as their glycosides. Interest in the family of Combretaceae has increased as a result of the therapeutic potential of the combretastatins, which is being clinically evaluated, (Pettit et al 1995). Combretastatins have cytotoxic activity, inhibit tubulin polymerisation and are antimitotic agents. Stilbenes have been also found in *C. caffrum, C. bracteosum* and *C. zeyheri* (Famakin, 2002).

2.3.5. Terpenoids

A variety of acidic triterpenoids and their glycosides have been isolated from the scale-like trichomes of the leaf surface of *C. molle* and *C. imberbe* [Fig. 2.5]. Many *Combretum* species also contain these substances. Isolated triterpenoids from *Combretum*

species fall under two groups, namely cycloartanes and oleananes (Pegel and Rogers 1976, 1985). They have demonstrated molluscicidal activity (Rogers 1995), exhibit good activity *in vitro* against *Listeria* species and also *Mycobacterium fortuitum* (Katerere 2001).

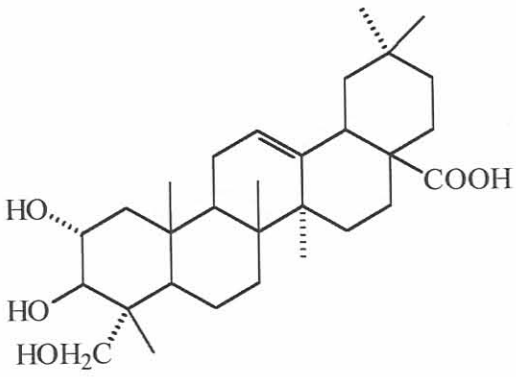

Fig 2.4. Pentacyclic terpenoid from *C. molle* (Saleh, 1994)

2.3.6. Flavonoids

In the broad sense of the word, flavonoids are virtually universal plant pigments (Farnsworth 1966). Almost always water soluble, they are responsible for the colour of flowers, fruits and sometimes leaves. A number of specific colour reactions for various types of flavonoids have been reported, colours ranging from orange to red (flavones), red to crimson (flavanols) crimson to magenta (flavanones) and occasionally to green or blue (Farnsworth, 1966). Flavonoids are universally present in the leaf cuticle and epidermal cells where they ensure tissue protection against the damaging effects of UV radiation. All flavonoids have a common biosynthetic origin and therefore possess the same structural frame, namely the 2-phenylchromane skeleton [fig 2.6.].
Flavones and flavonols represent the two largest classes of flavonoids. They occur in the plant either in the glycosidic form (in leaf, stem, flower and fruit) or in the free aglycone form (in root, bark, heartwood or external leaf surface). Common flavones such as apigenin, luteolin and chrysoeriol are encountered in the African flora. (Saleh, 1994).

Flavonoids isolated from *C. miranthum* leaves have shown antimicrobial activity against both Gram-positive and Gram-negative microorganisms, (Rogers and Verotta, 1996).

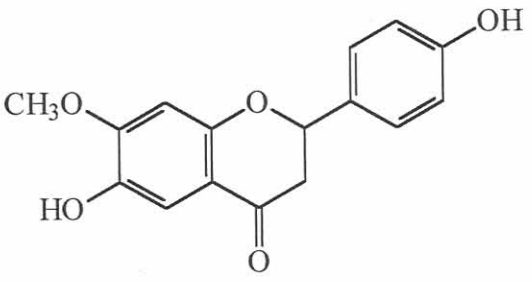

Fig.2.5. Structure of a flavone from *Terminalia arjuna* (Saleh, 1994)

2.4. Aims and objectives of this study

Previous studies (Alexander et al, 1992; Eloff, 1999) have indicated antibacterial activity in *Combretum apiculatum* leaf extracts. This is a follow up bioassay-guided study. *C. apiculatum* may contain antimicrobial compounds, that may be useful in anti-infective therapy. Furthermore studies in our laboratory have shown that other members of the same section of the genus contain several flavonoids and stilbenes with substantial antibacterial activity. A preliminary bioautographic experiment indicates that the antibacterial compounds of *C. apiculatum* differ from the other members of the Angustimarginata (Eloff, unpublished data).

This study aims to isolate and characterize antibacterial compounds from *C. apiculatum*. This will be attempted by:

- Determining the best extractant for antibacterial compounds present in *C. apiculatum*.
- Using different techniques to isolate antibacterial compounds by bioassay guided fractionation.

- Determining the chemical structure of isolated compounds.
- Determining antibacterial activity of isolated compounds.