Combretum microphyllum

CHAPTER 1

LITERATURE BACKGROUND

1.1 MOTIVATION

The increasing use of antibiotics and misuse by over prescribing and/or insufficient patient compliance, have led to the development of bacteria resistant to antibiotics. This resistance may be unavoidable (Spratt, 1994).

Berkowitz (1995) indicates that this resistance is a result of the rate at which bacteria multiply and disperse, as well as the ease with which their genetic material can change, thus inactivating the curative or remedial treatment, or changing the target area. Berkowitz (1995) and Leggiadro (1995) warn that we may reach a post-antibiotic era where antibiotics are no longer effective and they emphasize the need for the development of new antimicrobial compounds to control resistant organisms. Berkelman *et al.* (1994) also stress the importance of finding new antibiotics because more and more resistant pathogenic organisms are identified.

Many plants contain antibacterial compounds. Some of these compounds may be useful in controlling resistant bacteria. Several scientists are therefore investigating plants as alternative sources of antibacterial compounds. In 1967, 58 % of all antibiotics were produced by Actinomycetes, 18 % by other fungi, 12% by higher plants, 9 % by bacteria and the remaining 3 % by algae, lichens and animals (Edwards, 1980).

The pharmaceutical industry has a renewed interest in the development of chemicals of medicinal value from natural products, as combinational

chemistry did not deliver the expected yield of lead compounds. As the interest in natural products is increasing, many laboratories are focussing on this type of research. Screening and analysis of plants for pharmacological activity are carried out on a routine basis by teams of scientists in different disciplines at universities and pharmaceutical companies (Alexander *et al.*, 1992).

Plants in southern Africa can be of great value as a source of medicinal components. South Africa has c. 10 % of the plant diversity of flowering plants in the world. With the diminishing of species diversity, the international community is becoming increasingly aware of the potential medicinal value of chemical compounds in plants. Alexander *et al.* (1992) warn against the isolation of research units in South Africa. South African universities should establish natural product research units to prevent the bulk of the natural products of South African plants to be investigated and exploited overseas. The rights of the country of origin concerning the economic advantages involved with the development of secondary chemical compounds from plants have recently been acknowledged. The development of this valuable resource in southern Africa will become increasingly important in the future (Eloff, 1998a).

There is consequently value in investigating southern African plants for antimicrobial activity.

1.2 SELECTION OF PLANT TO INVESTIGATE

1.2.1 Introduction

According to ethnobotanical literature, the genus *Combretum* is used widely and for a variety of conditions. Members of the genus have, inter alia, the following biological activities: anti-inflammatory, diuretic, hypotensive, antibiotic, antifungal, molluscicidal, antimicrobial, as well as being poisonous, having an

antihepatitic B virus effect, and the inhibition of gastric ulcers (Hutchings *et al.,* 1996).

Several members of the Combretaceae have been used for treating bacterial diseases in southern Africa (Watt and Breyer-Brandwijk, 1962). Traditional healers in southern and central Africa have used certain *Combretum* species for the treatment of a wide range of disorders (Rogers and Verotta, 1996). Leaves and bark are used extensively by traditional healers, but the winged fruits, which are produced in great abundance, are never used in medicine, nor eaten by wild animals because they are apparently poisonous. A characteristic symptom of this type of poisoning is violent and continuous hiccupping, hence the common name of 'hiccup nut' for *C. bracteosum.* It is suggested that the toxins are common to the fruit of all *Combretum* species. Isolation of these toxins has not yet been accomplished (Panzini *et al.,* 1993).

Carr and Rogers (1986) showed that there are differences in the chemical composition between *Combretum* species. They developed a TLC method to identify *Combretum* species. This method is useful because of the presence of abundant polar compounds in the genus. In our laboratory we have subsequently developed several TLC systems that indicate the extreme complexity of Combretaceae extracts.

1.2.2 Combretaceae

Combretaceae, with some 19 genera and 600 species, is a family of the tropics and subtropics. In South Africa its members are widespread, other than in the extreme southern and south western areas. They rival the *Acacias* in the frequency of their occurrence.

The Combretaceae is one of the prominent plant families in southern Africa. Members of the family include as attractive trees and creepers. Easily observed features of the Combretaceae such as leaves, stems, flowers and fruits

are useful in field identification. The leaves are entire, alternate or opposite, and without stipules. The main stem produces whorls of horizontal lateral branches, which in turn are made up of a succession of branchlet units with the tips turned up with a cluster of leaves at its apex. Flowers are usually clustered in axillary heads or spikes, and fruits are characteristically 4-winged (Van Wyk and Van Wyk, 1997).

Carr (1988) has worked on Combretaceae in southern Africa. He has made a substantial contribution to the indigenous flora of the subcontinent with information on propagation, descriptions of each species including synonyms, common names, distribution, habitat, bark foliage, inflorescence, fruit and also keys to identification.

Essential characteristics of Combretaceae (Carr, 1988) are summarized in **Table 1.1.**

Table 1.1 Characteristics of Combretaceae.

Plant type	**Trees, shrubs, climbers, sometimes spinose mangrove**
Inflorescences	Axillary, terminal, spicate (sometimes subcapitate) or paniculte.
Flowers	Sessile, or pedicellate, bisexual, or bisexual and male on the same inflorescence, receptacle usually in 2 parts, the lower containing the ovary, the upper terminating in 4 or 5 sepals.
Petals	Number 4 or 5, or absent.
Stamens	Number 8 or 10, but 4 with *Meiostemon.* Style centrally situated on disc and free, except with *Quisqualis* where the lower portion is attached to the calyx tube.
Fruit	A stipiate or sessile samara with single (surrounding) wing, 4 or 5 or more wings, or a nut.
Seedling cotyledons	Mostly 2, but sometimes 3 or 4, petiolate (sometimes connate) or sessile, arising above or below ground.

Table 1.2 The subgeneric classification of species of the genus *Combretum* occurring in southern Africa.

***Combretum* Loefl**		
Subgenus *Combretum*		**Subgenus *Cacoucia***
Section *Hypocrateropsis*		Section *Lasiopetala*
• *C. celastroides*	•	• *C. obovatum*
• *C. imberbe*		
• *C. padoides*		
Section *Combretastrum*		
• *C. umbricola*		
Section *Angustimarginata*		Section *Conniventia*
• *C. caffrum*	•	• *C. microphyllum*
• *C. erythrophyllum*	•	• *C. paniculatum*
• *C. kraussii*	•	• *C. platypetalum*
• *C. vendae*		
• *C. woodii*		
Section *Macrostigmatea*		Section *Oxystachya*
• *C. engleri*	•	• *C. oxystachyum*
• *C. kirkii*		
• *C. sp. nov.*		
Section *Metallicum*		Section *Megalantherum*
• *C. collinum*	•	• *C. wattii*
Section *Glabripetala*		Section *Poivrea*
• *C. fragrans*	•	• *C. bracteosum*
Section *Spathulipetala*	•	• *C. mossambicense*
• *C. zeyheri*		
Section *Ciliatipetala*		
• *C. albopunctatum*		
• *C. apiculatum*		
• *C. edwardsii*		
• *C. moggii*		
• *C. molle*		
• *C. petrophilum*		
• *C. psidioides*		
Section *Fusca*		
• *C. coriifolium*		
Section *Breviramea*		
• *C. hereroense*		
Section *Elaeagnoida*		
• *C. elaeagnoides*		

The Combretaceae family in southern Africa is divided into six genera: *Combretum, Lumnitzera, Meiostemon, Quisqualis, Pteleopsis* and *Terminalia*. Of the six genera in southern Africa, *Combretum* Loefl and *Terminalia* L. are the most important.

The subgenera and sections of the genus *Combretum* are presented in **Table 1.2** (Carr, 1988).

1.2.3 The genus *Combretum*

The taxonomy of *Combretum* is complex. Flowering plants are grouped into families and families are divided up into genera, which may also have subgenera. Each genus is split into species and some species may have two or more subspecies or varieties (Carr, 1988).

The genus *Combretum* Loefl has two subgenera, these being subgenus *Combretum* and subgenus *Cacoucia*. Subgenus *Combretum* is divided into 11 sections and subgenus *Cacoucia* is divided into 5 sections. The genus *Terminalia* L. is divided into 3 sections only, *Abbreviatae*, *Psidioides* and *Platycarpae* (Carr, 1988).

The genus *Combretum* comprises some 180 species of trees, shrubs and climbers of which c. 33 species occur in southern Africa. It is the largest genus in the Combretaceae.

In the subgenus *Combretum*, trichomes present are scales with or without hairs, but in the subgenus *Cacoucia* the trichomes are stalked glands, accompanied or not by hairs. Carr (1988) argues that characteristics of the trichomes provide an additional aid to identification as even fragmentary specimens of *Combretum* can usually be assigned at least to sectional level under a microscope.

Of the six genera in southern Africa, *Combretum*, being the focus of this study can be identified according to the following essential characteristics (Carr, 1988).

Table 1.3 Characteristics of *Combretum*.

Leaves	Usually opposite or verticillate and having scales or stalked glands.
Inflorescences	Axillary or terminal, spicate or paniculate.
Flowers	Sessile, 4- to 5-merous, bisexual, the receptacle having 2 parts, lower and upper.
Petals	Number: 4 or 5.
Stamens	Number: 8 or 10.
Fruit	With one exception, a 4- or 5-winged samara.

1.2.4 Previous studies

Only about 25 of the *Combretum* species have hitherto been subjected to even a superficial scientific study. The following classes of compounds were found in the genus: alkaloids, tannins, flavonoids, amino acids, substituted phenanthrenes from various heartwoods, a series of unique stilbenes and their glucosides and macrocyclic lactones known as 'combretastatins', 'triterpenoid acids' and their saponins, mainly of the cycloartane and oleanane types (Rogers and Verotta, 1996).

Combretum species occurring in southern Africa are used for many medicinal purposes. These include treating abdominal disorders, abdominal pains, bilharziasis, chest coughs, colds, conjunctivitis, diarrhoea, dysmenorrhoea, earache, fattening babies, fever, headache, hookworm, infertility in women, leprosy, pneumonia, scorpion bite, snake bite, swelling caused by mumps, syphilis, toothache and general weakness (Gelfand, *et al*, 1985; Hutchings, *et al*, 1996; Kokwaro, *et al*, 1976 and Watt, *et al*, 1962).

Breytenbach and Malan (1989) isolated three antimicrobial compounds from *C. zeyheri* and Alexander, *et al.* (1992) found antimicrobial activity in six species of *Combretum.*

Eloff (1998a; 1999) investigated leaf material from 27 southern African members of the Combretaceae. Based on the minimal inhibitory concentration (MIC) values of extracts and the total content of each plant, the species with the highest antibacterial activity (for the four bacteria used) are *C. molle, C. petrophilum, C. moggii, C. erythrophyllum, C. padoides, C. paniculatum, C. mossambicense, C. nelsonii, C. apiculatum,* subspecies *apiculatum, P. myrtifolia* and *C. woodii* (Eloff, 1999).

All the members of Combretaceae examined contain antibacterial compounds. Martini and Eloff (1998) found at least 14 antibacterial compounds in *C. erythrophyllum* and some of these had activities higher than chloramphenicol and ampisillin.

1.2.5 Why *C. microphyllum* was selected

I could not find any publication or report on the antibacterial activity of *C. microphyllum. C. microphyllum* possesses different chemical and antibacterial compounds from other *Combretum* species as well as a relatively high antibacterial activity (unpublished results).

C. microphyllum falls in another section (*Conniventia*) of the subgenus *Cacoucia*.

Other students in our laboratory are investigating species from other sections in an effort to provide chemotaxonomic evidence for the sectional classification and to confirm the occurrence of different antibacterial compounds in the different species.

1.2.6 *Combretum microphyllum*

C. microphyllum falls under the subgenus *Cacoucia* which is divided into five sections and *C. microphyllum* forms part of the section *Conniventia*. A short overview of *Combretum microphyllum* from Carr, 1988 and Van Wyk and Van Wyk, 1997 is given.

- **Description**

Features of *C. microphyllum* are typically those of the family Combretaceae. Stems have petiole bases, which often persist as spines. *C. microphyllum* possesses bark with rather rectangular flakes, lifting, light to medium grey; climbers can reach 10 m, stems twine, spines spread to 25 mm and is found in woodland, savannah, and riverine at low altitudes up to 1500 m. Leaves are ovate, oblong-elliptic to almost circular. The average sizes of leaves are 65 mm (lamina length) x 41 mm (lamina width) x 12 mm (petiole length). Principal lateral veins are yellowish varying from 4 to 6 pairs. Basal portions of petioles are thickened and often persist after leaves fall to form blunt spines. They are produced in terminal or axillary panicles, usually before the new leaves.

Flowers are showy and crimson-red. *C. microphyllum* flowers from September to October with an abundance of scarlet blossoms [**Fig. 1.1**] (Joffe, 1993).

C. microphyllum produces 4-winged fruit. The average sizes of fruit are up to 25 mm (length over wing/s) x 23 mm (width over wing/s) x 5 mm (stipe length), averaging respectively 19 mm x 19 mm x 3 mm. They are green tinged with red or pink when young, drying to pale yellowish brown [**Fig. 1.2**] (Van Wyk and Van Wyk, 1997).

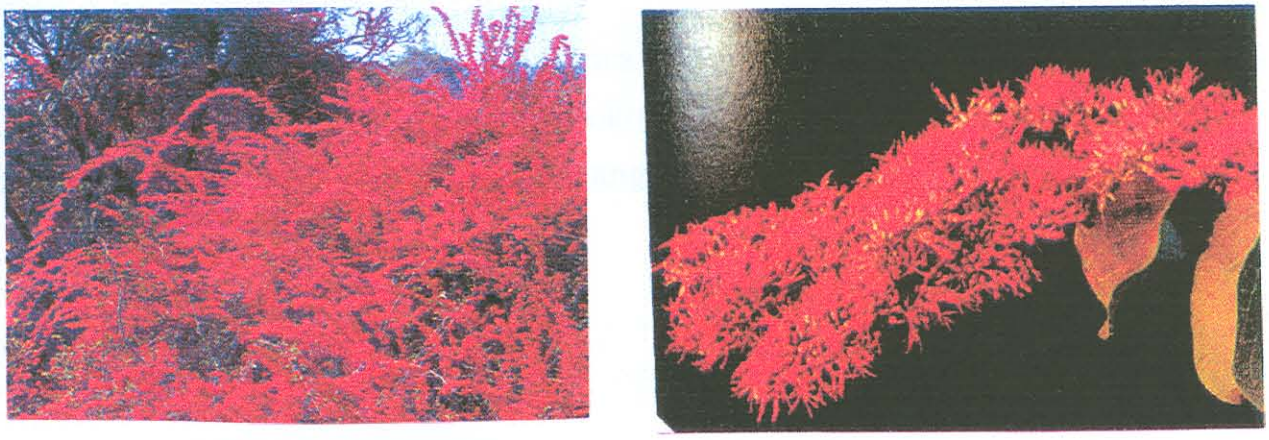

Figure 1.1 ***Combretum microphyllum:*** **Flowers.**

Figure 1.2 ***Combretum microphyllum:*** **Fruit at an early and late stage.**

- **Synonyms**

Synonyms are *Combretum lomuense* and *Combretum paniculatum*, subspecies *microphyllum*.

- **Common names**

Combretum microphyllum in South Africa and Zimbabwe is commonly known as 'flame creeper' ('vlamklimop'), 'mukopokopo', 'burning bush combretum', 'mupfurura', 'bambagwenya' (or 'bambangwenya') and 'muzutsu'.

- **Distribution**

In southern Africa *C. microphyllum* grows in the north eastern parts of South Africa, the Swaziland region and scattered in the northern parts of Kwazulu-Natal, in Mozambique, and in Zimbabwe, mostly in the eastern highlands [**Fig. 1.3**] (Van Wyk and Van Wyk, 1997).

The species is mainly confined to the lowveld in Mpumulanga, extending from north of the Soutpansberg over to the east and down to the extreme south. Isolated occurrences of *Combretum microphyllum* are noted elsewhere on the Tambotie river near Ellisras and at Doorndraai Dam Nature Reserve. In Kwazulu Natal it is mainly confined to the north-eastern part to the east of the Lebombo range and reaching as far south as the Mkuze valley. There is also a record from the Kranskop area.

Although there are no records of occurrences in Botswana it seems likely that it occurs in the north western area. Occurrences are further recorded in Swaziland and in all 5 geographical divisions of Zimbabwe. The species is also found in Zambia, Mozambique, Malawi and Tanzania.

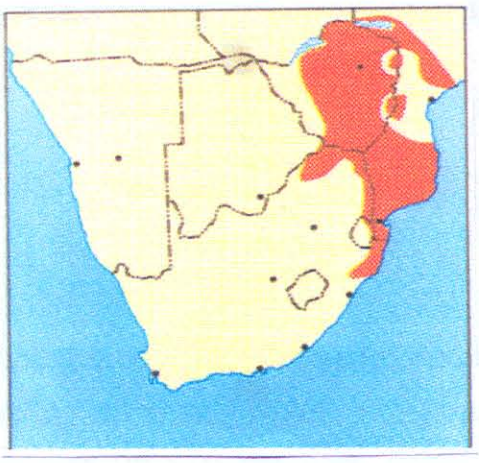

Figure 1.3 Distribution map of *C. microphyllum*.

- **Habitat**

C. microphyllum is a robust climber, sometimes a scrambling shrub or small tree, growing in bushveld and forest and often along rivers. The habitat of this species is in the tropical and warm regions of Africa, in the savanna and forests of warmer areas and woodland in the hotter areas. It flourishes especially in thickly forested regions along water sources at low to medium altitudes, and occasionally up to 1 500 meter in tropical parts (Carr, 1988).

- **General**

The species is usually a several-stemmed deciduous liane, which may grow into a dense dome-shaped mass 2 - 3 m high without supporting vegetation. It spreads to a width of 12 m or more with sturdy, almost straight, unsupported stems of up to 3,5 m extending upwards seeking support. Irvine (Carr, 1988) reports that single-stemmed specimens in the presence of supporting large trees have stems up to 240 mm diameter at breast height. Two adjacent stems will coil around each other to form a right-hand helix. Stems, which are supported by nearby vegetation, will climb up over the crowns of these, thus providing floral displays up to 15 m or more above the ground. The species, when in full flower with vast

expanses of brilliant scarlet provides a powerful show which last for 3 weeks or more.

- **Bark**

Bark on the main stem near the base may have a crocodile skin pattern with light to medium grey flakes curling up at the edges. Stems are about 50 mm in diameter and are fairly smooth. Current growth stems are green and puberulous, even pubescent at the terminals and thus grey.

- **Foliage**

A typical season's extension comprises a substantial straight stem reaching lengths of several metres with a few other long stems emerging from the basal portion. Leaf size varies as already indicated. Emerging leaves and exposed portions of their petioles are initially purple-tinged while venation on the upper side is a lighter green and slightly recessed.

- **Spines**

Most stems are armed with blunted, slightly recurved spines, occurring singly or in pairs.

- **Inflorescence**

The species is in full flower during September or October, rarely in November and sometimes as early as the end of August. The brilliant showy display of non-scented flowers, make it a useful horticultural subject in warm climates.

- **Fruit**

Fruit is a 4- (or sometimes 5-) winged samara with a broadly elliptic to subcircular outline. The base is rounded and usually broadly notched, as is the apex.

- **Propagation and cultivation**

The fruit matures rapidly and can be ripe by mid December. To hasten the process seeds should be removed from the fruit for sowing. Soaking beforehand will lead to earlier emergence of seedlings, usually from 10 to 21 days after sowing. Having established its head well above the ground *C. microphyllum* will withstand a fair amount of cold as well as resisting drought as little or no artificial watering of the established plant is necessary.

1.3. AIM OF THE STUDY

The aim of this study is to investigate the antibacterial compounds by following a bio-assay guided approach.

Different extractants will be used on dried, powdered leaves of *C. microphyllum* to determine:

- If there is any specificity in extracting the antibacterial compounds,
- Which extractant extracts the highest antibacterial activity,
- Which extractant extracts the most antibacterial compounds.

I will attempt to isolate one or more antibacterial compounds by:

- Simplifying the extracts through several group separation techniques and following the fractionation quantitatively and qualitatively,

- Applying relevant column chromatographic techniques.