Chapter 1 – Introduction

"Medicinal plants are the backbone of traditional medicine." (Farnsworth, 1994)

In his struggle against disease, man has used plants as medicinal agents. The discovery and application of antibiotics against infectious disease led to the development of the pharmaceutical industry in the second half of the last century that has done much to combat disease in man. The pharmaceutical industry has contributed to significantly reducing mortality and increasing life expectancy, but the affordability and availability of medicine in developing countries still remains a challenge. Despite the advances in synthesising new pharmaceuticals, many products sold by drug companies are still derived from plants. Most herbal medicines of current interest originate from the ancient civilizations of Africa, the Asian subcontinent, and North, Central and South America (Phillipson, 2001).

Despite the recent interest in molecular modelling and other synthetic chemistry techniques by pharmaceutical companies and funding organizations, natural products, and particularly medicinal plants, remain an important source of new drug leads (Newman *et al*., 2000; Newman *et al*., 2003; Butler, 2004).

Due to floristic biodiversity, South Africa is considered to be a botanical "hotspot" with 22,000 plant species occurring within its boundaries. This represents 10% of the world's total flora occurring on less than 1% of the total terrestrial surface. Indigenous medicinal plants are used by more than 60% of South Africans for their health care needs or cultural practices. Approximately 3,000 species are used by an estimated 200,000 indigenous traditional healers (van Wyk *et al.*, 1997).

1.1 *Mentha longifolia* – an overview

Mentha longifolia (Lamiaceae), also known as wild mint, is widely distributed throughout southern Africa, occurring in most parts of South Africa (Figure 1.1), as well as in parts of Botswana, Namibia, and Zimbabwe. In South Africa, three different subspecies of *Mentha longifolia* are recognised. *M. longifolia* subsp. *wissii* (Launert) Codd (Cape velvet mint) is known from only two localities, Brandberg (Namibia) and near Garies in Namaqualand. The long thin, grey-green leaves are known to be unpleasantly aromatic. *Mentha longifolia* subsp. *capensis* (Thunb.) Briq. is the most widespread taxon in South Africa and usually has a strong peppermint scent. *Mentha longifolia* subsp. *polyadena* Briq. (spearmint) has a disjunct distribution occurring in Gauteng, Swaziland, northern KwaZulu-Natal, eastern Free State and northern Lesotho and the southern Cape. It is also found between Humansdorp and the Swartberg. The work reported here is restricted to the latter taxon. The plant is a perennial herb common in wet places, with creeping rhizomes below the ground and erect flowering stems of up to 0.8 metres in height. All parts are highly aromatic with a typical mint smell. The leaves appear opposite each other in pairs along the stems, which are square in cross-section. Small white or pale purple flowers are borne in elongated clusters (Figure 1.2) on the tips of the stems (Codd, 1985). The parts of the plant that are mostly used are the leaves, but sometimes the stems and rhizomes are used in traditional potions (van Wyk *et al.*, 1997).

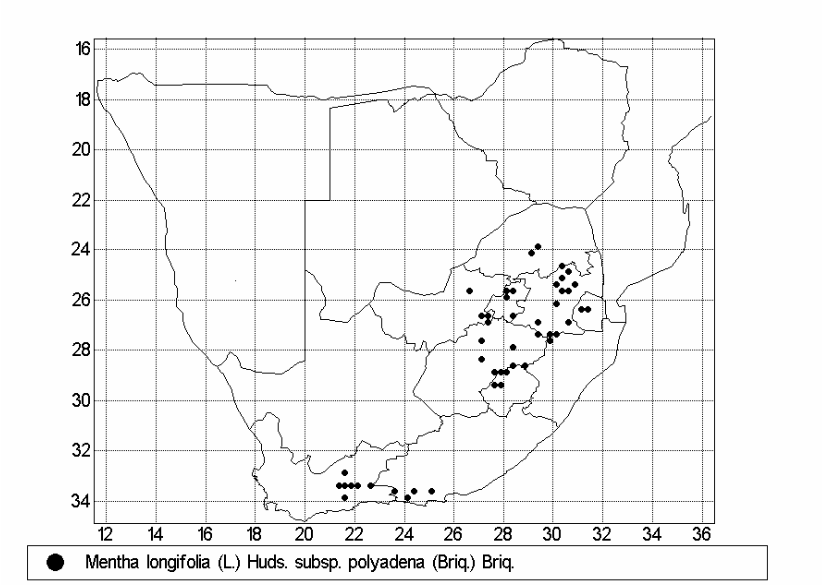

Figure 1.1 Geographical distribution of *Mentha longifolia* subspecies *polyadena* in South Africa (obtained and included with permission from SANBI).

Figure 1.2 *Mentha longifolia* subspecies *polyadena* during the flowering season. (all photos unless otherwise stated were supplied by AM Viljoen)

Harley and Brighton (1977) estimated there to be approximately 25 species of the genus *Mentha* and rather fewer hybrids. The yield and composition of the essential oils differ for each species or subspecies and may be of diagnostic value. Additionally, environmental influences such as annual variations or different cultivation conditions are responsible for compositional changes of the essential oils. To demonstrate the environmental influence on the composition of the essential oils of the mints, researchers cultured some of the plants outdoors and others were cultivated in sterile culture medium under ultraviolet (UV)-lighting. It was shown that there were dramatic variations in the essential oil composition of the plants grown under the two different conditions (Rosch *et al*., 2000).

The fragrant oils of the genus *Mentha*, normally contain complex mixtures of monoterpenes, such as carvone, menthol or menthone, with lower amounts of sesquiterpenes (Rosch *et al*., 2000). These compounds are produced and accumulated in highly specialized structures called glandular trichomes (Figure 1.3 and 1.4). The secreted monoterpenes are stored in the subcuticular space outside the secretory cells. Before such substances can be analysed, they have to be liberated from the matrix of these secretory cells (Wise *et al*., 1999).

Medicinally, milk or water decoctions of wild mint are mainly used for coughs, colds, asthma and other bronchial ailments by the Xhosa (Figure 1.5 A) (Watt and Breyer-Brandwijk, 1962). Decoctions have also been used to treat headaches, fevers, indigestion, flatulence, hysteria, painful menstruation, delayed pregnancy and urinary tract infections (van Wyk *et al.*, 1997).

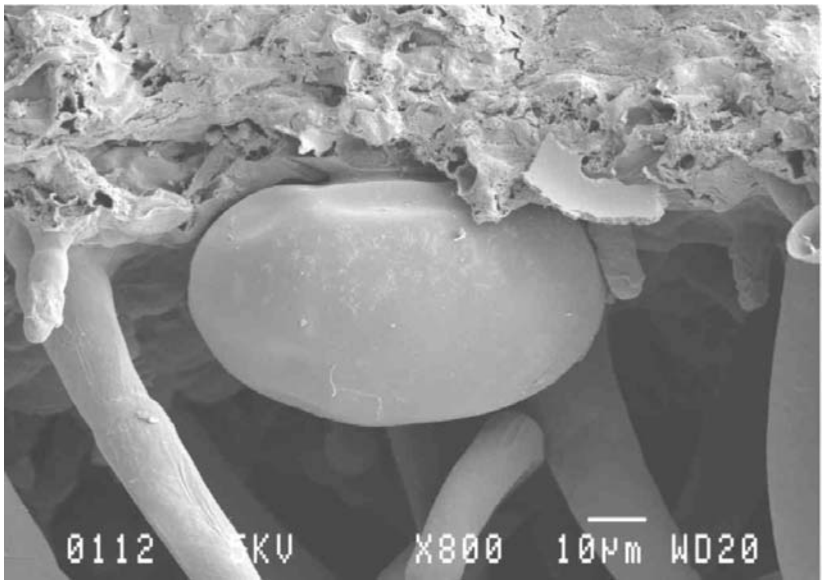

Figure 1.3 Scanning electron micrograph of the leaf surface of *Mentha longifolia* subspecies *polyadena* showing one of many oil glands.

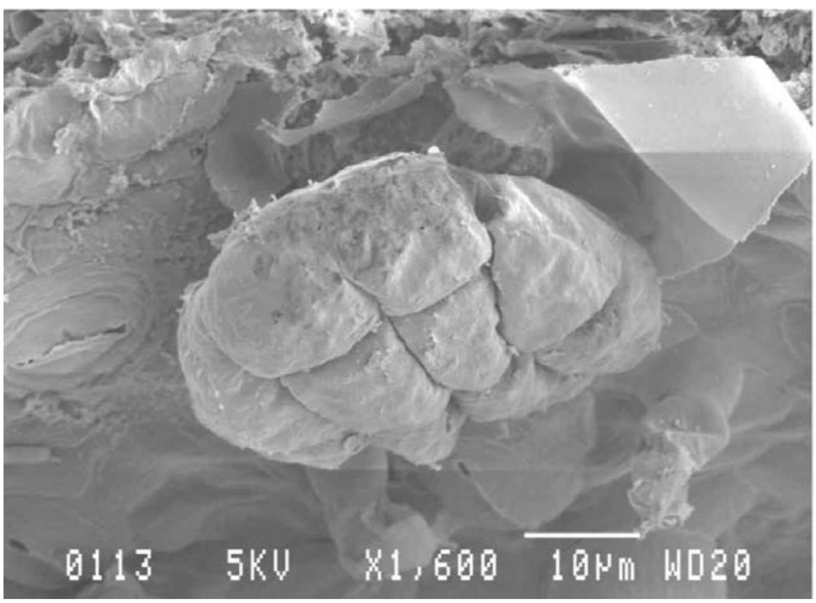

Figure 1.4 Scanning electron micrograph of the abaxial leaf surface of *Mentha longifolia* subspecies *polyadena* showing one of many multi-cellular oil glands after removal of the outer cuticle layer.

(A)

(B)

Figure 1.5 (A) name plate describing some of the medicinal uses of *Mentha longifolia* for the treatment of respiratory ailments; (B) name plate describing the use of *Mentha longifolia* in the management of headaches and colds (Kirstenbosch Botanical Garden, Cape Town).

The plant is reported to possess diaphoretic properties and has mild spasmolytic action on the smooth muscle of the digestive tract hence useful for cramp-like complaints of the gastro-intestinal tract, gall bladder and the biliary tract (van Wyk *et al.*, 1997). The volatile oil is likely to be partly responsible for the decongestant, antispasmodic and antimicrobial effects. Externally it has been used to treat wounds and swollen glands (Batten and Bokelmann, 1966). The method of preparation and dosage include drinking infusions of the leaves, roots or stems and the administration as enemas against incipient colds (Watt and Breyer-Brandwijk, 1962). Medicinal usage is reported to produce marked diuretic effects (Batten and Bokelmann, 1966). The Sotho sometimes plug the nostrils with crushed leaves and bind a cloth around the nose and head for relief of headaches and colds (Figure 1.5 B). Plants are also placed under the bedding to facilitate breathing in patients with respiratory ailments or those suffering from bleeding problems (Batten and Bokelmann, 1966). Leaves are sometimes used in decoctions known as *inembe,* which are taken regularly during pregnancy to facilitate labour (Veale *et al*., 1992).

The active constituents in the volatile oils of *Mentha* species include numerous monoterpenoids such as carvone, limonene, menthone and menthol (Bruneton, 1995). The composition of oils obtained from plants growing in different localities is known to vary considerably, but no information appears to be available on South African species. Table 1.1 shows the variation in the major components present in the essential oils of *Mentha longifolia* growing in various countries.

Piperitenone oxide has been identified as the major compound in three previous studies (Venskutonis, 1996; Ghoulami *et al*., 2001; Mastelic and Jerkovic, 2002;). Carvone and limonene are common components in the oils of plants collected in Croatia, Iran and

Sudan (Mastelic and Jerkovic, 2002; Younis and Beshir, 2004; Zeinali *et al*., 2005). The plant analysed from Greece contained carvone as one of its major components (Kokkini *et al*., 1995). In the same study it was reported that plants collected from one of the three collection sites also had a high content of 1,8-cineole (13.4%) whilst plants collected from the second of the three collection sites had a high content of *trans*-dihydrocarvone (32.9%). 1,8–Cineole is a common component in the essential oil of plants collected from Lithuania, Greece and Iran (Kokkini *et al*., 1995; Venskutonis, 1996; Monfared *et al*., 2002). East Serbia produced the only chemotype rich in both thymol and *para*-cymene (Mimica-Dukic *et al*., 1993). It must be noted however that the *M. longifolia* from Sudan (Younis and Beshir, 2004) was a different subspecies and analysis of the oil revealed high levels of carvone (67.3%). Morocco produced the only chemotype rich in both piperitenone oxide and piperitone (Ghoulami *et al*., 2001). The proportion of oxide is known to vary with age of the plant (Hutchings *et al*., 1996). The Russian chemotype (Bui and Nikolaev, 1975) is characterised by high levels of (+)-linalool (88.6%).

According to some reports, the differences in the quality of mint oils could be attributed to the environmental factors influencing their biosynthesis (Franz *et al*., 1984; Brun *et al*., 1990; Voirin *et al*., 1990). Environmental factors, such as day and night temperatures, photoperiod, and light intensity are known to affect the composition of peppermint oil (Burbott and Loomis, 1967; Clark and Menary, 1979). The oil composition of young leaves grown under long day conditions changed from menthofuran to menthone and menthol (Voirin *et al*., 1990). Menthol and menthofuran content were greatly affected by changes in the growing season (Court *et al*., 1993).

The species also produces hesperidin and several other non-volatile bioflavonoids which are thought to improve capillary function. These compounds have been used to relieve capillary impairment and venous insufficiency of the lower limbs (Martindale, 1993).

Table 1.1 The quantities (%) of the major components found in the essential oils of *Mentha longifolia* growing in different parts of the world.

Compound	Rus (1)*	Kaz (2)*	E.Ser (3)*	Mor (4)*	Cro (5)*	Sud (6)*	Lith (7)*	Gre (8)*	Ira (9,10)*	Yug (11)*	S.Af	Bots
Piperitenone oxide		.		25.0	28.9		44.2				65.7	14.6
Piperitone oxide				24.0								
Piperitone										38.8		
Carvone					33.5	67.3		58.0-66.3	61.8 (9)			
Limonene					10.3	13.5			19.8 (9)			
Menthone		18.6								11.2		
1,8-Cineole							15.1	13.4	18.3 (10)			
trans-dihydrocarvone								32.9				
Thymol			13.3									
para-Cymene			14.1									
(+)-Linalool	88.6											
Caryophyllene	16.5											
Menthol		47.1										
cis-Carveol									53.8-78.2 (10)			
Menthofuran											51.4 - 61.6	
cis-piperitone epoxide											14.7	35.7

Key: Rus – Russia; Kaz – Kazhakstan; E. Ser – East Serbia; Mor – Morocco; Cro – Croatia; Sud – Sudan (*M. longifolia* (L) Huds ssp. Schimperi); Lith – Lithuania; Gre – Greece; Ira – Iran; Yug – Yugoslavia; S. Af – South Africa; Bots – Botswana.
* - references : (1) Bui and Nikolaev, (1975); (2) Sharipova *et al*., (1983); (3) Mimica-Dukic *et al*., (1993); (4) Ghoulami *et al*., (2001); (5) Mastelic and Jerkovic, (2002); (6) Younis and Beshir, (2004); (7) Venskutonis, (1996); (8) Kokkini *et al*., (1995); (9) Zeinali *et al*., (2005); (10) Monfared *et al*., (2002); (11) Mimica-Dukic *et al.*, (2003).

1.2 The antimicrobial activity of volatile oils

Volatile oils of many plants are known to have antimicrobial activity (Dorman and Deans, 2000; Inouye *et al*., 2001). This activity could act as a chemical defence against plant pathogenic diseases. Pathogens can readily penetrate at wound sites caused, for example, by herbivores. Aromatic leaves which are bruised, result in rupturing of the glands causing the oil to flow over the 'wound'. The existence, therefore, of antimicrobial activity in the oil, would be of considerable benefit to the plant. It is suggested that a complex oil presents a greater barrier to pathogen adaptation than would a more simple mixture of monoterpenes. Deans and Ritchie (1987) examined 50 plant volatile oils for their antibacterial properties against 25 genera of bacteria and found that volatile oils exhibited various reductions in growth of micro-organisms, depending on the oil concentration and chemical composition. In addition, enantiomeric composition of various monoterpenes in different species can further complicate the biological activity of a given oil (Ravid *et al*., 1987).

1.3 Mode of action of essential oils

A survey on the uses of fragrances and essential oils as medicaments was published by Buchbauer and Jirovetz (1994). The report stated that volatile oils, either inhaled or applied to the skin, act by means of their lipophilic fraction reacting with the lipid parts of the cell membranes, and as a result, modify the activity of the calcium ion channels. At certain levels of dosage, the volatile oils saturate the membranes and show effects similar to those of local anaesthetics. They can interact with the cell membranes by means of their physicochemical properties and molecular shapes, and can influence their enzymes, carriers, ion channels and receptors. Various studies concerning the physiological effects of essential oil components on humans have been described. These include brain stimulation, anxiety-relieving sedation and antidepressant activities, as well as increasing the cerebral blood flow. The fragrance compounds are absorbed by inhalation and are able to cross the blood-brain barrier and interact with receptors in the central nervous system (Buchbauer and Jirovetz, 1994).

The aims of the present study are to document the chemo-geographical variation and antimicrobial activities of both volatile and non-volatile compounds of natural populations of *Mentha longifolia* subspecies *polyadena*. The study also aims to determine any correlation between the chemistry and antimicrobial activity.

1.4 Study Objectives

(i) To document the chemo-geographical variations of volatile essential oils and non-volatile compounds of natural plant populations of *Mentha longifolia* subspecies *polyadena*.

(ii) To document the antimicrobial activities of this widely used medicinal plant. This information will provide a scientific rationale for the use of *Mentha longifolia* subspecies *polyadena* in traditional medicine.

(iii) To determine any correlation between the antimicrobial activity and the chemistry of *Mentha longifolia* subspecies *polyadena*.