Chapter 1

GENERAL INTRODUCTION

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Phytomedicine

Medicinal plants have been used as traditional treatment for numerous human diseases for thousands of years in many parts of the world. In rural areas of developing countries, herbal materials continue to be used as the primary source of medicines (Chitme *et al*., 2003). About 80% of the people in developing countries use traditional medicines for their primary health care (Kim, 2005). At least 122 compounds, 80% of which were used for the same or related ethnomedical purposes, have been derived from 94 species of plants. There are approximately 500 000 plant species occurring worldwide (Palombo, 2006), but only 1% has been phytochemically investigated. There is great potential for discovering novel bioactive compounds from the rest of the plant kingdom. However, according to the United Nations Environment Programme World Conservation Monitoring Centre, current extinctions rates of plants means that the world is losing one major drug every two years (Groombridge and Jenkins, 2002).

Africa, and especially southern Africa, has a rich diversity of plants. According to the African Plant Checklist and Database Project, statistics show that about 25% of the total number of higher plants in the world is found in Africa south of the Sahara (Klopper *et al*., 2006). South Africa boasts a unique and diverse botanical heritage with over 30,000 plant species of which 3000 species are used therapeutically (Van Wyk *et al*., 1997). It is estimated that 27 million South Africans depend on traditional herbal medicines from as many as 1020 plant species (Dauskardt, 1990; Meyer *et al*., 1996; Williams, 1996; Mander, 1998; Fennell *et al*., 2004). The use of herbal remedies in traditional medicine is an integral part of South African cultural life (Cunningham, 1993; Brandt *et al*., 1995; Mander, 1998).

A large number of reviews deal with South African plants used to treat specific ailments and the potential of their phytochemistry to assist in the development of new drugs.

Of particular interest are the articles pertaining to anti-microbial studies (Van Vuuren, 2008; More *et al*., 2008; Tshikalange *et al*., 2008; McGaw *et al*., 2008; Braithwaite *et al*., 2008).

Infectious diseases constitute one of the main problems that modern medicine has faced over the last 30 years. Prominent among these diseases are eye infections which are caused by exposure to bacterial, fungal, viral and other microbial agents. Symptoms of eye infections include redness, swelling of the eyes, itching, increased tear production and photophobia. The discovery of antibiotics has eradicated the infections that once ravaged the humankind, but their indiscriminate use has led to the development of multidrug-resistant pathogens. For example, about 90–95% of *Staphylococcus aureus* strains worldwide are resistant to penicillin (Casal *et al*., 2005) and in most of the Asian countries, 70–80% of the same strains are methicillin resistant (Chambers, 2001).

In addition, certain antibiotics have undesirable side effects while the emergence of previously uncommon infections is also a serious medical problem (Marchese and Shito, 2001). Over 75 % of the antibacterials in clinical use are of natural origin and most of them are obtained from fungal sources (Newman *et al*., 2003). With respect to infectious diseases, the increasing resistance in many common pathogens to currently used therapeutic agents, such as antibiotics and antivirals, has led to renewed interest in the discovery of novel anti-infective compounds.

Eye infections: The eye has several natural mechanisms to defend itself against infection or trauma. For example, tears keep the eye lubricated and physically clear away foreign particles such as dust or microorganisms (Mitchell, 1995). In addition, the tears contain several substances (e.g. lysozymes and interferon) that protect against infection. The eyelids and eye lashes protect the ocular surface from the environment and help maintain the moist surface of the eye. However, occasionally these defense mechanisms may be disrupted, resulting in ocular inflammation (Mitchell, 1995). Eye infections are caused by exposure to bacterial, fungal, viral and other microbial agents, and are commonly and frequently reported in the Eastern Cape Province of South Africa. Plant materials are still being prescribed by traditional healers and herbalists for the treatment of these infections.

Bacterial eye infections: caused by *Streptococcus pneumonia, Haemophilus influenzae, Staphylococcus aureus, Echerichia coli, Pseudomonas aeruginosa, Staphylococcus epidermidis, Bacillus cereus, Chlamydia trachomatis* and *Neisseria gonorrhoea.* (Everett *et al*., 1995; Starr *et al*., 2000; Cuong and Michael, 2002; Hirotoshi *et al*., 2006). The most common causative agents for external ocular infections are *S. aureus* and *S. epidermidis* (Everett *et al*., 1995; Starr *et al*., 2000). Trachoma is caused by *C. trachomatis*. It is the world's leading infectious cause of blindness and the leading cause of ocular morbidity (Taylor and Taylor, 1999). According to WHO, there are 146 million people worldwide with trachoma. Symptoms of bacterial eye infections include burning; irritation, tearing and usually a mucopurulent or purulent discharge. Eyelids may be stuck together, particularly in the mornings. Although bacterial eye infections are usually considered to be self-limiting (Papa *et al*., 2002), if left untreated they may develop into a more serious, sight-threatening conditions.

Fungal eye infections: commonly caused by *Fusarium solani, Fusarium oxysporum, Aspergillus niger, Aspergillus flavus, Candida albicans* and *Penicillium notatum* (Denning, 1998; Gutleb *et al*., 2002; Morgan *et al*., 2005; Hedayati *et al*., 2007). These infections are difficult to treat and can cause blindness (Fabiana *et al*., 2004). Symptoms include redness, blurring vision and photophobia. Amphotericin B and Natamycin are of topical ocular use as treatment for fungal eye infections (Marr *et al*., 2002; Cesaro *et al*., 2004; Fabiana *et al*., 2004).

Viral eye infections: caused by *Herpes simplex virus-1, Adenovirus* and *Coxsackie virus* (Foulis *et al*., 1990; Liesegang, 2001; Kojaoghlanian *et al*., 2003). HSV-1 ocular infection is the leading cause of blindness in developed countries (Liesegang, 2001). Over 95% of ocular herpes infections are caused by HSV-1 (Pavan-Langston, 2000). To date, 51 human adenovirus serotypes have been described, grouped into six species (A–F). In particular, species D infects the eyes (Kojaoghlanian *et al*., 2003). Viral eye infections are highly contagious and are spread by contact, usually with objects which have come into contact with the infected person's eye secretions. For example, the virus can be transmitted when infected persons touch their eyes and then touché another surface (eg, door handle) or share an object that has touched their eyes (e.g. a towel or pillow case). The antiviral agents Famciclovir, Valacyclovir and Acyclovir can be used to shorten the course and decrease the severity of the infections. (Centers for Disease Control and Prevention, 2002).

Choice of *Hippobromus pauciflorus* for this study

From an ethnobotanical survey of the plants used by herbalists, traditional healers and rural dwellers in the Eastern Cape for the treatment of eye infections, a total of 12 plants were identified (Pendota *et al*., 2008). During the survey, *Hippobromus pauciflorus* was the most frequently mentioned plant.

Hippobromus pauciflorus (L.f) Radlk belongs to the family Sapindaceae and is commonly known as Ulathile in Xhosa. It is a multi-branched resinous tree that grows up to 5 m in height (Fig 1). The leaves are alternate, compound, paripinnate, with 3-6 pairs of leaflets; flowers are creamy-white, yellowish or reddish, about 4 cm in diameter. The fruit is almost spherical, about 10 mm in diameter, fleshy, black when mature and not edible. In southern Africa, It is widely distributed in riverine thickets, along stream banks and at the margins of evergreen forests. It grows in areas with high rainfall of more than 700 mm per year and at altitudes from 275 to 1780 m (Grierson and Afolayan, 1999). It has been recorded that the local traditional healers use the leaves for the treatment of various diseases in humans and domestic animals (Masika and Afolayan 2003; Clarkson *et al*., 2004; Pendota *et al*., 2008). During the ethnobotanical survey the author interacted with traditional healers in Eastern Cape Province, who claimed to have treated several eye infections using fresh leaves of the plant which are directly squeezed into infected eyes. In a few cases, the material was boiled in water. After cooling, the extract was then applied to the infected eyes until the patient experienced recovery.

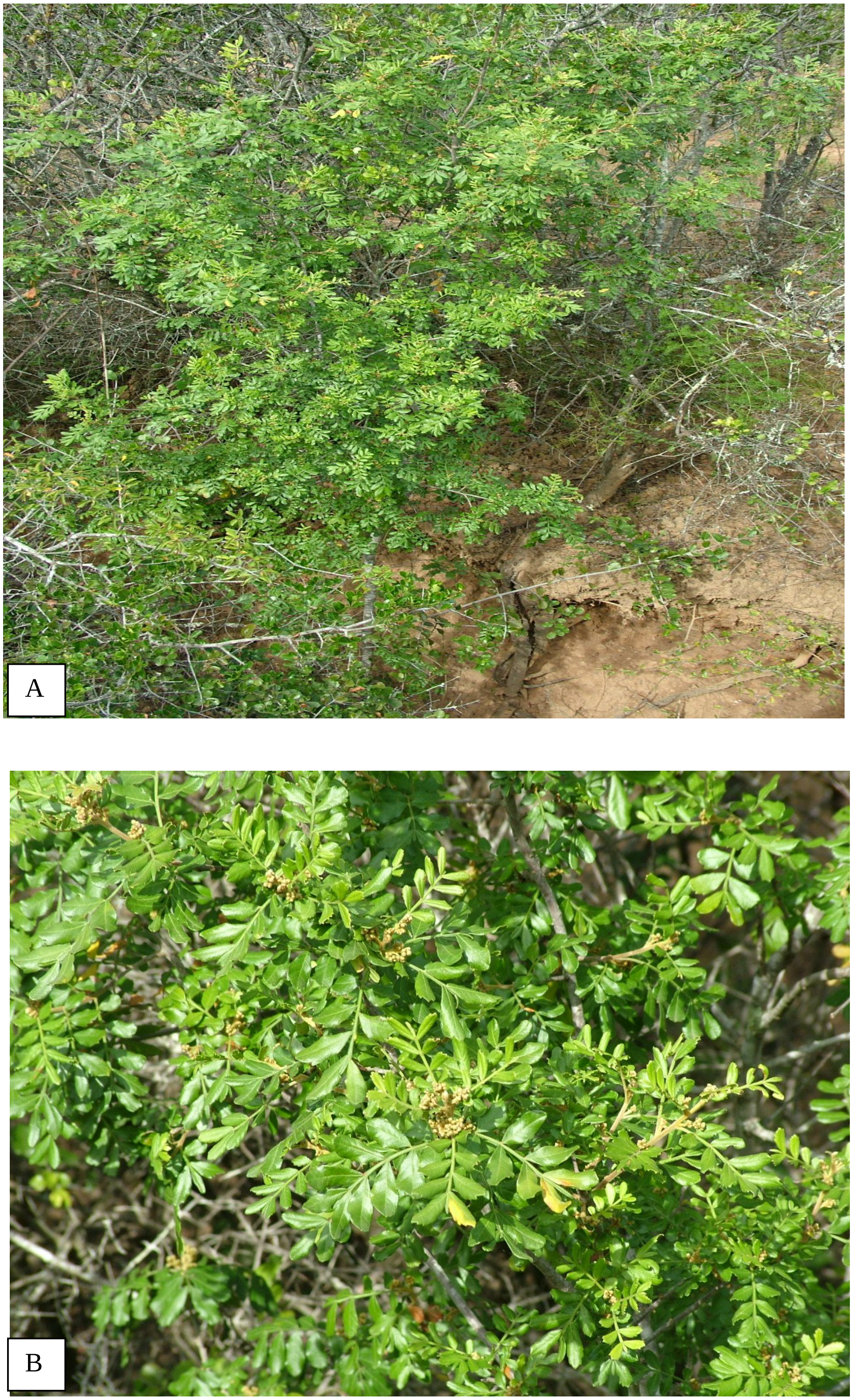

Fig 1: *Hippobromus pauciflorus* (from Sikhusthwana village) A; in its natural vegetation B: at closer range.

Objectives of this study

The overall objective of this project was to validate the use of *Hippobromus pauciflorus* for the treatment of eye infections in the Eastern Cape. The specific objectives were as follows:

A: According to Grierson and Afolayan (1999), information on traditional herbal practice in the Eastern Cape Province of South Africa is passed from one generation to the other through oral tradition. Considering the rapid rate of deforestation and loss of biodiversity, there is a need for accurate scientific documentation of the knowledge and experience of these herbalists. Therefore, an objective of this study was to investigate the use of traditional plants for the treatment of eye infections and to document their local and scientific names as well as the various methods of preparation and administration. A total of 12 plant species were identified as the most used species in the province for the treatment of eye infections. *Hippobromus pauciflorus* was repeatedly mentioned as the prominent plant used for the treatment of eye infections and was therefore chosen for further study.

B: It is a well known fact that natural products from microorganisms have been the source of antibiotics, but with the increasing acceptance of herbal medicine as an alternative form of healthcare, the screening of plant extracts for antimicrobial activity has shown that higher plants represent a potential source of new anti-infective agents (Poole, 2001; Salvat *et al*., 2001; Arias *et al*., 2004). The antimicrobial activity of *H. pauciflorus* has not been reported in the literature, but plants of the Sapindaceae family generally contain saponins, flavonoids, phenolic acids and tannins (proanthocyanidins) which have antibacterial and antimycotic properties (Umadevi and Daniel 1991; Sathiamoorthy *et al*., 2006; Yadava and Jitendra, 2008). Another objective of this study was to investigate the antimicrobial activity of *H.*

pauciflorus by preliminary bioassay screening of its extracts against 10 bacterial and four fungal strains. The selected bacterial strains consisted of five Gram-positive bacteria; *Staphylococcus aereus*, *Staphylococcus epidermidus, Bacillus cereus, Micrococcus kristinae,* and *Streptococccus faecalis* and five Gram-negative bacteria; *Escherichia coli, Pseudomonas aeruginosa, Shigella flexneri, Klebsella pneumoniae* and *Serratia marcescens*, while the fungal species were *Aspergillus niger, Aspergillus flavus, Penicilium notatum* and *Candida albicans.* Among these organisms *S. aereus, S. epidermidis, P. aeruginosa, E. coli, S. marcescens, A. niger, A. flavus* and *C. albicans* have been implicated on many occasions as causal agents of eye infections (Cuong and Michael, 2002; Hirotoshi *et al.*, 2006; Fabiana *et al*., 2004)

C: Inflammatory response is a defense mechanism evoked by body tissues in response to microbial infection and chemical injury that results in cell injury or death (Obyrne *et al*., 2000; Obyrne and Dalgleish, 2001; Charles *et al*., 2004). Plants contain a wide variety of natural compounds that inhibit the molecular target and thus have the potential to inhibit or reduce the inflammatory process (Iwalewa *et al*., 2007). Plants of the Sapindaceae family generally contain saponins, flavonoids, phenolic acids, and tannins (proanthocyanidins) (Umadevi and Daniel, 1991; Van Heerden *et al*., 2000; Abourashed *et al*., 2006). Saponin, particularly the hederagenin type is known to have anti-inflammatory and antinociceptive activities (Takagi *et al*., 1980). One major symptom of eye infections is the inflammation. Prior to this study, the anti-inflammatory, analgesic and antipyretic activity of the aqueous extract of *H. pauciflorus* has not been reported in the literature. In the present investigation, aqueous extracts of this plant were screened *in vivo* for anti-inflammatory, analgesic and antipyretic activity. This was carried out in order to validate the use of the plant for the treatment of eye inflammation.

D: The use of plants for healing purposes is becoming increasingly popular as they are believed to be beneficial and free of side effects (Leonardo *et al*., 2000). However, the rationale for the utilisation of medicinal plants has rested largely on long-term clinical experience with little or no scientific data on their efficacy and safety (Zhu *et al.*, 2002). One of the objectives of this project, therefore, was to carry out an *in vivo* toxicological evaluation of the aqueous extract of *H. pauciflorus* leaves on haematological parameters, liver and kidney function indices and serum lipid profile in male Wistar rats. Assessment of haematological parameters in rats are used to determine the extent of deleterious effect of a plant extract on the blood (Yakubu *et al*., 2007). While the liver and kidney can be used as 'markers' for assessing the functional capacities of the organs (Yakubu *et al*., 2003).

E: Because the plant kingdom is rich in bioactive compounds, plant extracts and their natural derivatives can be considered as potential sources of new drugs active against viral infections (Cox and Balick, 1994). Herpes simplex virus type 1 (HSV-1), a double stranded DNA containing virus, is a member of the family herpesviridae. It is the leading cause of unilateral infectious corneal blindness worldwide (Liesegang, 2001). Over 95% of ocular herpes infections are caused by HSV-1 (Pavan-Langston, 2001). Coxsackie viruses (CVs) are important human pathogens, causing a remarkable variety of diseases, from minor common colds to fatal myocarditis (Foulis *et al*., 1990). The antiviral activity of *H. pauciflorus* has not been reported in the literature and thus an objective of this study was to investigate the antiviral activity of the plant against *in vitro* Herpes simplex virus (HSV-1) and Coxsakie virus B6.

F: After the study of ethnomedical properties of *H. pauciflorus* on eye infections and with the antimicrobial activities of its crude extracts, it became essential to isolate and identify the active compound(s) of this plant and to examine the antimicrobial properties of the pure compounds. From the ethyl acetate fraction, two bio-active compounds were isolated (epicatechin and β-sitosterol) and from the n- hexane fraction one bio-active compound (lupeol) was isolated from the leaves of the plant through bioactivity-guided fractionation performed on TLC plates using *Bacillus subtilis*.

G: Trichomes are found in most plants. They can comprise either single or several cells and can be secretory, glandular or nonglandular (Johnson, 1975; Esau, 1977). Trichomes can be found in many different species of plants and often from glands that secrete phytochemical compounds (e.g. organic acids, polysaccharides, terpenes or salt) as well as secondary compounds such as those produced in trichome exudates (e.g. terpenoids, flavonoids and phenylpropanoids) (Duke *et al*., 2000). No information is available on the morphology and ultrastructure of the leaf appendages of *H. pauciflorus* yet, the objective of this study was therefore, to investigate the structure and distribution of different trichome types observed on the leaves of this plant, with the view to relating this to its antimicrobial property.

The structure of the thesis

This thesis consists of chapters in the form of reprints of published articles and articles under review in various journals. The thesis is structured as follows: An ethnobotanical study of medicinal plants used for the treatment of eye infections in the Eastern Cape is described in Chapter 2. Chapter 3 reports on antimicrobial activity of *Hippobromus pauciflorus*. Chapter 4 describes the *in vivo* anti-inflammatory, analgesic and antipyretic activities of the aqueous extract of *H. pauciflorus* leaves. In chapter 5 the effect of the administration of aqueous extract of the plant leaves in male Wistar rats is presented. In chapter 6, antiviral effects of the aqueous extracts against Herpes simplex virus (HSV-1) and Coxsakie B virus type 6 in cell culture are reported. Chapter 7 presents the isolation of the bioactive compounds from the *H. pauciflorus*. Chapter 8 describes the foliar micro-morphology of the species. Finally, Chapter 9 is the general discussion and conclusions of the entire study, in an attempt to present a coherent picture of the results.

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