

CHAPTER 9

GENERAL DISCUSSION AND CONCLUSIONS

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Although eye infections are usually considered to be self-limiting, if left untreated they may develop into more serious, sight-threatening conditions (Papa *et al.*, 2002). According to the WHO, there are an estimated 37 million people worldwide, who have been blinded by infections and a further 277 million with severe visual impairment (Kevin and Allen, 2003). In South Africa, many people still use phytomedicine as an alternative or to supplement modern western drugs for the treatment of diseases, including eye infections (Van Wyk *et al.* 1997). Plant products are perceived to be healthier than manufactured medicines (Gesler, 1992), primarily because of the general belief that herbal drugs are without any side effects in addition to being cheap and locally available (Gupta and Raina, 1998). Reports of adverse effects of conventional medications are found in the lay press at a much higher rate than reports of herbal toxicities, in part because mechanisms to track adverse effects exist for conventional medicines whereas such data for self treatment is harder to ascertain. Even physicians often dismiss herbs as harmless placebos (Winslow and Kroll, 1998).

The main objective of this study was to validate the ethnomedical uses of *Hippobromus pauciflorus* for the treatment of eye infections by investigating its antimicrobial, antiviral, anti-inflammatory, analgesic and antipyretic properties, as well as to perform a toxicological evaluation and isolation of bio-active compounds from the plant. The results of this study were expected to provide valuable information on the medicinal uses of this plant to the medical practitioners, traditional healers and the communities which use the plant for medicinal purposes.

Ethnobotanical information on plants used for the treatment of eye infections

Information on medicinal plants, used by the people of the Eastern Cape Province for the treatment of eye infections, was collected through questionnaires which were administered to herbalists, traditional healers and rural dwellers. The information collected revealed 12 plant

species, which belonged to nine families, of which Sapindaceae was the most prominent. The leaves were reported to be the most used part of the plants, constituting 75% of herbal preparations. This was followed by bark and roots which together constituted 25%. Generally, the plant parts were crushed and the sap squeezed directly into the infected eyes. In a few cases, the material was mixed with water and boiled; after cooling, the extract was applied to the infected eyes until the patient experienced recovery. *Hippobromus pauciflorus* was the most commonly used plant species for the treatment of eye infections in the Province and was chosen for further study.

Antibacterial and antifungal activity of *H. pauciflorus*

The leaves, bark and roots of *Hippobromus pauciflorus* were investigated for *in vitro* antimicrobial activity against 10 bacterial and four fungal species. The acetone extracts from the bark and roots were active against Gram-positive and Gram-negative bacteria with the MIC ranging between 0.1 and 10.0 mg/ml, whereas the acetone extract of the leaves inhibited the growth of Gram-positive bacterial strains at 1.0 to 5.0 mg/ml. The water extracts of the leaf showed activity against Gram-positive bacteria at 1.0 to 5.0 mg/ml. The methanol extracts of the three plant parts were the most active and showed activity against all the bacterial isolates with MIC values ranging between 0.5 to 10 mg/ml. The antibacterial potency of *H. pauciflorus* against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Bacillus cereus* is noteworthy, because all these bacteria have been implicated in eye infections (Levison and Jawetz, 1992; Goldstein *et al.*, 1999; Kowalski *et al.*, 2001; Cuong and Micheal, 2002; Marangon *et al.*, 2004; Morrissey *et al.*, 2004). The methanol extracts of the leaves and bark were particularly inhibitory to the growth of the fungi with inhibition percentages ranging from 78.70 to 100% on *Aspergillus niger* and *Penicillium notatum* at 10 mg/ml. The acetone extracts of the leaves and bark were active

against *A. niger* (51.76%) and *P. notatum* (77.22%). The water extract of the stem bark significantly inhibited the growth of *P. notatum* (81.02%). The results therefore, validated the use of this plant for the treatment of eye infections.

Anti-inflammatory, analgesic and antipyretic activities of the aqueous extract

The aqueous extract of *Hippobromus pauciflorus* leaves at 50, 100 and 200 mg/kg body weight doses were evaluated for anti-inflammatory, analgesic and antipyretic activities using male Wistar rats as a model. The extract at all the doses used significantly inhibited both the carrageenan- and histamine-induced inflammation in a manner that was not dose dependent. The carrageenan-induced paw oedema model in rats is known to be sensitive to cyclooxygenase (COX) inhibitors and has been used to evaluate the effect of non-steroidal anti-inflammatory agents (Rao *et al.*, 2005). The extract reduced the formalin-induced pain licking as well as prolonged the reaction time in the tail flick-induced pain. The formalin test is considered a suitable model for chronic pain (Dubuisson and Dennis, 1977). The 50 and 100 mg/kg body weight doses of the extract reduced the brewer's yeast provoked elevated body temperature in rats after 60 min more than that of 200 mg/kg body weight manifested from 30 min. The reduction in the brewer's yeast induced fever by the extract in this study suggests some influence on the prostaglandin biosynthesis since it is believed to be a regulator of body temperature (Dascombe, 1985). The results suggest a potential benefit of *H. pauciflorus* leaves in treating conditions associated with inflammation, pain and fever. These properties might be adduced to the presence of the phytoconstituents.

Possible toxicologic effect of aqueous extract of *Hippobromus pauciflorus* leaves in male Wistar rats

The effects of the administration of aqueous extract of *H. pauciflorus* leaves at 50, 100 and 200 mg/kg body weight doses for 14 days on some biochemical parameters of male Wistar rats were investigated. The extract at all the doses did not significantly alter the levels of white blood cells, red blood cells, mean corpuscular volume, platelets, neutrophils, monocytes, lymphocytes and large unstained cells. While the levels of haemoglobin, packed cell volume and basophils increased at specific doses, those of mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration and eosinophils were decreased. Assessment of haematological parameters in rats can be used to determine the extent of deleterious effect of a plant extract on the blood (Yakubu *et al.*, 2007). Again the extract did not significantly alter the computed liver and kidney body weight ratios, sodium, chloride and total protein, though the levels of potassium, inorganic phosphorus, globulin, urea, total and conjugated bilirubin were increased at certain doses. The biochemical indices monitored in the serum such as the electrolytes and other secretory substances of the liver and kidney are sometimes used as 'markers' for assessing the functional capacities of the organs (Yakubu *et al.*, 2003). The absence of significant effect of the extract on the liver and kidney body weight ratios suggests that the extract did not cause swelling, atrophy or hypertrophy of the organs (Amresh *et al.*, 2007). In contrast, the level of albumin and creatinine also decreased at specific doses. While the activities of alkaline phosphatase, gamma glutamyl transferase and alanine aminotransaminase remained unaltered in the serum, that of aspartate aminotransaminase increased only at the dose of 200 mg/kg body weight. The atherogenic index as well as the concentrations of cholesterol, high and low-density lipoprotein cholesterol in the serum of the animals was not altered. However, the extract increased the concentration of triacylglycerol. The results suggest that the extract has mild and dose

specific haemato-, hepato- and nephrotoxic effects and thus may not be completely safe for oral administration.

Antiviral activity of aqueous extract of *H. pauciflorus*

The aqueous extract from *H. pauciflorus* was evaluated for antiviral activity against herpes simplex virus type 1(HSV-1) and coxsakie B virus type 6 *in vitro*. No antiviral activity was noted at concentrations ranging from 3.90 µg/ml to 15.60 µg/ml. The plant extract showed partial antiviral activity against HSV-1 at concentration of 31.25 µg/ml 24 h post infection (p.i.) as suggested by the delay in the appearance of a CPE. However, after 48 h p.i. no antiviral activity was noted. Cytotoxicity was evaluated by MTT assay in Vero cells. At concentrations from 165 to 270 µg/ml significant antiviral activity against HSV-1 as well as toxicity was observed. The extract exhibited no antiviral activity against Coxsakie virus B6. This study suggests that the traditional uses of *H. pauciflorus* against viral infections are questionable.

Bioactive compounds from *H. pauciflorus*

Bioactivity- guided fractionation of the leaves of *H. pauciflorus* yielded three compounds; epicatechin, β-sitosterol and lupeol. It is reported in the literature that these compounds exhibit varying degrees of antimicrobial, anti-inflammatory, analgesic and antipyretic activity (Salvador *et al.*, 2004; Saleem *et al.*, 2008). Epicatechin is reported to have greater antibacterial activity against the Gram-negative bacteria, *E. coli* and *P. aeruginosa*, than against the Gram-positive bacteria *B. subtilis* and *S. aureus* (Masika and Afolayan, 2004). Antimicrobial, anti-inflammatory, analgesic and anti-pyretic activity of β-sitosterol have been reported previously (Gupta *et al.*, 1980; Salvador *et al.*, 2004). Geetha and Varalakshmi (2001) reported that lupeol reduced paw swelling by 39% in an adjuvant arthritis rat model.

The antimicrobial, anti-inflammatory, analgesic and anti-pyretic activity of these compounds, has justified the ethnobotanical uses of *H. pauciflorus* in the treatment of eye infections.

Foliar micromorphology of *H. pauciflorus*

Trichome appendages arise from a series of anticlinal and periclinal divisions of epidermal cells to form specialized structures that function as glandular or nonglandular trichomes. The structure and distribution of foliar appendages on the leaves of *Hippobromus pauciflorus* was examined by scanning electron microscopy. The leaves were characterized by anisocytic stomata which were found only on the abaxial surface. The leaves have long unicellular nonglandular trichomes which were distributed over the mid rib and edges of the adaxial and abaxial surfaces. Crystal deposits were also observed on the surface of the leaves. Energy dispersive X-ray spectroscopy of the crystals showed predominant mineral components of Na, Al, Si, K and Ca. Trichomes have been reported to secrete to the surface ions such as Na and Cl (salt glands), Ca, Cd, Zn, Mn, Ni, Pb, S, Si and others (Salt *et al.*, 1995; Choir *et al.*, 2001). Because the trichomes observed in the *H. pauciflorus* were nonglandular, it is postulated that the compounds responsible for the bioactivity are produced in other tissues of the leaf rather than the trichomes.

Conclusions

An estimated three million people in South Africa are currently using indigenous, herbal medicine for primary health care problems (Van Wyk and Gericke, 2000). The country's vast variety of indigenous flora (Van Wyk *et al.*, 1997) indicates a high potential for the discovery of novel bioactive compounds (Duncan *et al.*, 1999). The following are concluded from this research work:

- The strong antimicrobial activity exhibited by *H. pauciflorus* against the eye infection causing organisms makes it a candidate for bioprospecting for antibiotic drugs.
- The significant reduction as well as inhibitory effect of the extract on the carrageenan-induced oedema paw volume in male Wistar rats is an indication of the anti-inflammatory potential of the plant. Triterpenes, flavonoids and steroids found in this plant could be responsible for the observed anti-inflammatory, analgesic and antipyretic activities.
- The extract from the leaves of *H. pauciflorus* has brought about selective alterations in the biochemical parameters of male Wistar rats investigated. It is likely that the extract has mild and dose specific haemato-, hepato- and nephrotoxic effects and thus may not be completely safe for oral administration.
- The aqueous extract of the plant showed slight antiviral activity against HSV-1. Hence the use of the plant against the antiviral infections is questionable.
- Three known compounds β -sitosterol, epicatechin and lupeol were isolated through bioactivity- guided fractionation of the extracts of the leaves. The antimicrobial, anti-inflammatory, analgesic and anti-pyretic activities observed in this study could be attributed to the presence of these compounds.

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