Chapter 8

General discussion and Conclusions

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Diarrhoeal diseases remain a leading cause of preventable death, especially among children under five years old in developing countries. Diarrhoea is caused by infectious organisms, including viruses, bacteria and protozoa and these are transmitted from the stool of one individual to others (Wilson, 2005). Diarrhoeal disease affects rich and poor, old and young, and those in developed and developing countries alike, yet a strong relationship exists between poverty, an unhygienic environment, and the number and severity of diarrheal episodes especially for children under the age of five (Keusch, 2006). In South Africa, it is estimated that about 1.5 million cases of diarrhoea in children under the age of five are reported annually (DWAF, 2001), and about 43,000 South Africans die every year from diarrhoeal disease.

The people of the Eastern Cape province have a long history of traditional medicine usage for the treatment of various infections, diseases and ailments (Van Wyk et al., 1997), including diarrhoea. Despite this wealth of natural pharmaceuticals, only a small proportion of the plants have been scientifically investigated (Coetzee, 2000). Considering the current rate of deforestation with the concurrent loss of biodiversity, there is an urgent need for accurate documentation of the knowledge and experience of the traditional herbalists (Kambizi and Afolayan, 2001), and need to evaluate the efficacy and safety of their remedies as well.

Ethnobotanical information

An ethnobotanical survey was conducted at beginning of the study, revealing 17 plant species belonging to 14 families that were frequently used for the treatment of diarrhoea by the people of the Eastern Cape Province, South Africa. During this

survey some of the plants implicated in the treatment of diarrhoea were obtained directly from the healers and herbalists, while others were collected during walks through the forest accompanied by traditional healers and rural dwellers. Different parts of plants were used by the local traditional healers. Among the different parts, roots were most frequently used, followed by the bark, leaves and bulbs. The observed methods of preparation involved the use of only a single plant part but more than one method of preparation. Decoctions and infusions were the main methods of preparation. The data also showed that majority of the remedies were taken orally. The dosage depended on the age of the patient and was administered orally until the patient was healed. Of these plants, *Hermannia incana* was frequently mentioned and highly recommended by both the traditional healers and rural dwellers. The choice of *Hermannia incana* for further study was thus based on the ethnomedical information from indigenous peoples of South Africa who have been using the plant for years against diarrhoea.

Antimicrobial activity

It has been estimated that approximately 30 – 70% of diarrhoeal diseases are due to bacteria (Wiedermann and Kollaritsch, 2006). The methanol extracts of leaves and roots of *H. incana* inhibited the growth of both the Gram-positive and Gram-negative bacteria at MIC values ranging between 0.5 and 7.0 mg/ml. There was, however, more inhibition of Gram-positive strains than Gram-negative strains. The acetone extracts of the leaves and roots showed moderate activity at concentrations between 5.0 and 10 mg/ml, while there was no activity against *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The water extract of leaves and roots showed activity against Gram-positive bacteria at concentrations between 5.0 and 10.0 mg/ml. The methanol

extracts of this plant thus showed appreciable activity against all 10 organisms. The antibacterial potency of *H. incana* against *Echerichia coli*, *Shigella flexneri, Bacillus cereus, Staphylococcus aureus,* and *Vibrio cholerae* is noteworthy, because all these bacteria have been implicated as causal agents of diarrohea (Brijesh et al., 2006; Bhattacharya, 2000; Martines et al., 1993; Guerrant and Bobak, 1991)..

The majority of the extracts (71.22%) showed antimycotic activity against the test organisms at concentrations of 10 mg/ml or lower. The methanol, acetone, and water extracts of both leaf and root inhibited the growth of *Aspergillus flavus, Aspergillus niger,* and *Mucor hiemalis* with inhibitory percentage ranging from 54.31 to 96.67%. Both organic and aqueous extracts of the leaf and roots of *H. incana* have indicated varied levels of antibacterial and antifungal activity. The traditional use of *H. incana* extracts to treat diarrhoea, stomachache, and other infections has been supported by laboratory results from this study

Antidiarrhoeal activities and Phytochemical screening

Phytochemical screening of the extract of *Hermannia incana* revealed the presence of alkaloids, tannins, saponins, phenolics, triterpenes, flavonoids, cardiac glycosides, cardenolides and dienolides while anthraquinones and steroids were not detected.

In the castor oil-induced diarrhoea experiment on Wistar rats, the aqueous extract of *Hermannia incana* significantly prolonged the time of diarrhoeal induction in a dose dependent manner. The frequency of stooling (number of wet faeces and total number of faeces) as well as fresh weight and water content of the faeces decreased significantly. There was more reduction in these parameters at the 600 mg/kg body weight dose when compared with loperamide. There was also increase in the percentage inhibition of defecation. However, the highest dose (600 mg/kg body

weight) produced inhibition of defecation that compared favourably with the loperamide.

The masses and volumes of the intestinal fluid significantly decreased in dose dependent manner. Similarly, the inhibition of the intestinal content of the animals increased in dose dependent manner. However, the 600 mg/ kg body weight dose of the plant extract produced the highest percentage inhibition of intestinal content among the various groups.

Compared with the distilled water control, the extract reduced the distance moved by the charcoal meal. Whereas the 400 mg/kg body weight resulted in charcoal meal transit time that was similar to the reference drug, atropine sulphate, the 600 mg/kg body weight dose of the extract produced the least transit time.

The antidiarrhoeal activities of medicinal plants have been attributed to the presence of bioactive agents such as tannins, alkaloids, saponins, flavonoids, steroids and terpenoids (Havagiray et al., 2004). While the flavonoids are known to inhibit intestinal motility and hydroelectrolytic secretion (Venkatesan et al., 2005), tannins denature proteins in the intestinal mucosa by forming protein tannates which make intestinal mucosa more resistant to chemical alteration and reduce secretion (Havagiray et al., 2004). Therefore, the antidiarrhoeal activity of *Hermannia incana* leaves observed in this study may be attributed to the presence of tannins, flavonoids, alkaloids and saponins in the aqueous extract.

Toxicological studies

The effects of the administration of aqueous extract of *Hermannia incana* leaves at 200, 400 and 600 mg/kg body weight doeses for 14 days on some hematological biochemical parameters of male wistar rats were investigated. In this study, the levels

of RBC, Hb, PCV, MCV, MCH, MCHC, RCDW, WBC, neutrophils, monocytes and basophils at all the doses were not altered whereas those of LUC, platelets, lymphocytes and eosinophils were affected at specific doses. This may suggest a mild effect on the haematological indices as well as dose and parameter specific of the extract on the blood indices (Schmidt et al., 2006). The biochemical indices of liver and kidney damage monitored in the serum in this study are useful markers for assessing the functional capacities of the organs. Biochemical indices of organ function, if altered, will impair the normal functioning of the organs. Therefore, the absence of significant effects on the liver and kidney indices by the extract of *H. incana* leaves is an indication that the normal functioning of these organs were not affected. It further indicates that the normal functioning of the nephron at the tubular and globular levels was not altered. The available evidence in this study suggests that the extract of *H. incana* leaf is mild, parameter and dose specific.

The foliar micro-morphology

Scientific interest in plant trichomes is based on their functional importance as well as on the economic usefulness of some trichome-produced products (Valkama et al., 2003). Glandular trichomes, which secret lipophilic substances (terpenes, lipids, waxes and flavonoid aglycones) may provide chemical and physiochemical protection against various types of herbivores and pathogens by entrapping, deterring and poisoning (Wanger, 1991). Terpenes and flavonoids are reported to have anti diarrhoeal activity (Palombo, 2006). The structure and distribution of foliar appendages on the leaves of *Hermannia incana* were examined by scanning electron microscopy. Both glandular and non-glandular trichomes were observed. Based on the observation from this study, it is hypothesized that the bioactive therapeutic

compounds secreted by *Hermannia incana* may be produced in the glandular trichomes. Energy dispersive X-ray spectroscopy-SEM of foliar crystals have been reported to be predominantly composed of Al, Ca, K, Na, Ti and Si. Glandular trichomes have been reported to secrete to the surface of the leaves, 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). The glandular trichomes found on the surfaces of this herb could be responsible for either the production or storage of the therapeutic compounds present in this plant.

Isolation and purification of bioactive compounds

Two flavonoids, epicatechin and 3, 5, 7, 2' tetra-hydroxy flavone-3- O-β-D-glucopyranoside were isolated from the leaves of the plant through bioactive guided fractionation. Both these compounds showed appreciable antimicrobial activity against all screened microorganisms (*Echerichia coli, Shigella flexneri, Bacillus cereus* and *Staphylococcus aureus*). The antibacterial potency of these two compounds against *Echerichia coli, Shigella flexneri, Bacillus cereus* and *Staphylococcus aureus,* is noteworthy, because all these bacteria have been implicated as causal agents of diarrhoea (Anne & Geboes, 2002; Krause et al., 2001; McGaw et al., 2000). Recent studies in the chemistry, biochemistry and pharmacy supports the view that flavonoids play a vital biological role, including as an antidiarrhoeal agent. Epicatechin inhibit the development of fluids that result in diarrhoea by targeting the intestinal cystic fibrosis transmembrane conductance regulator Cl^- transport and inhibiting cAMP-stimulated Cl^- secretion in the intestine (Schuier et al., 2005).

Conclusion

Modern scientific evaluation of plants and herbs is mainly concerned with validating the traditional use of plants and identifying the active components of extracts and preparations. As a result, continued examination of traditional plant medicines is required to establish the scientific basis for activity, efficacy and safety (Palombo, 2006). The antidiarrhoeal activity of *Hermannia incana* leaves observed in this study may be attributed to the presence of tannins, flavonoids, alkaloids and saponins in the aqueous extract. The prolonged onset of diarrhoea, inhibition of castor oil-induced enteropooling and the suppressed propulsive movement observed in this study are indications of antidiarrhoeal potential of *Hermannia incana* leaf extract. The antibacterial activity of the crude extracts and the pure compounds epicatechin and 3, 5, 7, 2' tetra-hydroxy flavone-3- O-β-D-glucopyranoside of the plant against diarrhoea causative organism (*Echerichia coli, Shigella flexneri, Bacillus cereus, Staphylococcus aureus,* and *Vibrio cholerae*) also supports the antidiarrhoeal properties of *H. incana*. In toxicological evaluation, the extract at all the doses was not toxicologically significant on all the parameters. At low dosage the plant did not alter the normal functions (haematological parameters, lipid profile, liver and kidney functions) of the animal. These findings lend support to the fokloric use of *Hermannia incana* in the Eastern Cape of South Africa as an antidiarrhoeal agent.

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