Chapter 1

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1.1 What is diarrhoea?

Diarrhoea is the passage of stools more than three times in an hour period (WHO, 1998). It occurs due to an imbalance in the absorption and secretory mechanisms in the intestinal mucosa (Yegnanarayan & Shtotri, 1982), which results in an increase in fluid and electrolyte loss into the gut lumen, leading to the production of unformed, liquid faeces (Jawetz et al., 1989). However, malnourished individuals' diarrohea can lead to severe dehydration and can become life-threatening if not treated (Alam and Ashraf, 2003). Three major diarrhoea syndromes exist: they are acute watery diarrhoea, which results in varying degrees of dehydration; persistent diarrhoea, which lasts 14 days or longer, manifested by malabsorption, nutrient losses, and wasting; and bloody diarrhoea, which is a sign of the intestinal damage caused by an infectious agent, drugs, poisons (including bacterial toxins) or acute inflammatory reactions (Keusch, 2006). All three are physiologically different and require specific management. Diarrhoea is one of the leading causes of mortality and morbidity in developing countries especially in children under five years. It is most commonly caused by gastrointestinal infections, which kill around 1.8 million people globally each year (WHO, 2008).

1.2 Diarrhoea as a global burden

The early estimation of the global burden of childhood mortality and morbidity became available in the early 1980's. Diarrhoeal illnesses accounted for about 4.6 million deaths from around 1 billion episodes of diarrhoea every year in children younger than five years (Snyder and Merson, 1982; Thapar and Sanderson, 2004).

Despite advances in the understanding of the causes, treatment and prevention of diarrhoeal diseases, an estimated 2.2 million people died from diarrhoea this year (2009) (http://rehydrate.org/diarrhoea). In South Africa, with the mixture of developed and developing regions, 9.7 million people do not have access to adequate water supply and 16 million lacks proper sanitation services (Kahinda et al., 2007). 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 while the annual public and private direct health care cost incurred due to diarrhoea alone is $ 4.3 million (Pegram et al., 1998). Diarrhea is associated with more general illness such as non-cramping abdominal pain, fever, weight loss, etc. for the 1.1 billion people who lack access to improved water supplies (WHO, 2000) and many more with contaminated water. It also causes rapid depletion of water and sodium which are necessary for life. If the water and salts are not replaced fast, the body starts to dry up or gets dehydrated. If more than 10% of the body's fluid is lost, death occurs (http://rehydrate.org/diarrhoea).

1.3 Causative organisms

Diarrhoea commonly results from gastroenteritis caused by viral infections, protozoa or bacterial toxins (Wilson, 2005). It has been estimated that approximately 30 – 70% of diarrhoeal diseases are due to bacteria, of which the most frequently detected enteric pathogens are non-invasive, enterotoxigenic *Escherichia coli* (ETEC) (Wiedermann and Kollaritsch, 2006). Diarrhoea occurs when these organism disrupt intestinal function, causing malabsorption or diarrhoea by microbial attachment and localized effacement of the epithelium, production of toxins and direct epithelial cell invasion (Guerrant et al., 1999). The major causative organisms of diarrhoea are

bacteria *Escherichia coli, Staphylococcus aureus, Shigella flexneri, Salmonella typhi, Vibrio cholerae* and *Campylobacter jejuni*; fungus *Candida albicans;* viruses such as rotavirus, astrovirus, adenovirus, and calicivirus; and protozoa such as *Giardia lamblia, Entamoeba histolytica,* and *Cryptosporidium parvum* (Brijesh et al., 2006; Bhattacharya, 2000; Martines et al., 1993; Guerrant and Bobak, 1991).

1.4 Western medicine for diarrhoea treatment.

Inappropriate drug use has been identified to be a serious problem in the control of diarrhoeal diseases. The control of diarrhoeal diseases programme has come far since its establishment as a special programme of the World Health Organization (WHO) more than 15 years ago. Antidiarrhoeal drugs are never indicated and their use can even be hazardous. According to WHO (1998), delaying adequate rehydration hampers recovery and may result in death from dehydration, particularly in children. Oral rehydration therapy has contributed greatly to the reduction of diarrhoeal mortality rates in children and the elderly. However, the attack rate of the disease has remained unchanged and this treatment often fails in the high stool output state (Brijesh et al., 2006). During the past three decades there have been major improvements in the treatment of infectious diarrhoea, with a number of new antibiotics which pharmacological industries have produced. However, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to acquire resistance to drugs (Cohen, 1992; Nascimento et al., 2000). Vaccines have been playing an important role in preventing acute bloody diarrhoea and are considered as the most feasible approach to treat diarrhoea. Various attempts for the development of vaccines against diarrhoea causative organisms have been made (Brijesh et al., 2006; Cohen et al., 2000; Ghose, 1996; Klee et al., 1997;

Martines et al., 1993). However, in developing countries the response to these vaccines is less probably due to their high cost. The WHO review concluded that any antidiarrhoeal drug should be considered unsafe for children. Thus there is a need for the development of safe and cost effective alternative drugs and medicinal plants may serve to fulfill this need.

1.5 Phytomedicine for diarrhoea treatment.

Medicinal plants have been used as traditional treatments for numerous human diseases for thousands of years in developing countries; the majority of people almost exclusively use traditional medicines in treating all sorts of diseases including diarrhoea. The use of herbal drugs in the treatment of various infections is a common practice in South Africa. An estimated three million people in South Africa currently use indigenous traditional plant medicine for primary health care purposes (Van Wyk and Gericke, 2000), hence a range of medicinal plants with antimicrobial properties has been widely used by the traditional healers in the Eastern Cape. The people of this province have a long history of traditional medicine usage for the treatment of various infections, diseases and ailments (Van Wyk et al., 1997). Numerous studies have validated the traditional use of antidiarrhoeal medicinal plants by investigating the biological activity of extracts of such plants, which have antispasmodic effects, delay intestinal transit, suppress gut motility, stimulate water adsorption or reduce electrolyte secretion (Palombo, 2006). All these properties of medicinal plants were found to be due to the presence of tannins, alkaloids, saponins, flavonoids, steroids and/or terpenoids (Havagiray et al., 2004). The antidiarrhoeal activities of flavonoids have been ascribed to their ability to inhibit intestinal motility and hydroelectrolytic secretions which are known to be altered in diarrhoeic conditions (Venkatesan et al.,

2005). Tannins and tannic acid present in antidiarrhoeal plants denature proteins in the intestinal mucosa by forming protein tannates which make the intestinal mucosa more resistant to chemical alteration and reduce secretion (Havagiray et al., 2004). All these evidences explain the importance of plants in diarrhoeal management in modern day healthcare.

1.6 The choice of *Hermannia incana* Cav. for this study

At the beginning of this study, an ethnobotanical survey of the plants used by herbalists, traditional healers and rural dwellers in the Eastern Cape for the treatment of diarrhoea was carried out. The results of the survey showed a total of 17 plants from 14 families that are used in this area. During the survey, *Hermannia incana* Cav. (figure 1) was the most frequently mentioned plant by the members of the community, hence the species was chosen for further studies.

Hermannia incana Cav. (Sterculiaceae) is known as Mavulakuvaliwe (Xhosa) or sweet yellow bells (English). It is a sparsely hairy prostrate herb with yellow flowers, found in grassland and marshes of the Eastern Cape Province of South Africa (Appidi et al., 2008a). *Hermannia* is a genus of small shrubs, ranging from upright to sprawling prostrate shrublets. They are characterized by the presence of minute glandular or star-like hairs on the leaves and stems, the stems often have a dark gray bark. Leaves are alternate and entire, lobed or incised. Flowers consist of five petals which are slightly or very strongly spirally twisted into an upended rose (Le Roux, 2005).

Figure 1: *Hermannia incana*. A; in its natural habitat around UFH, Alice and B; in the green house of the Botany Department, UFH.

H. incana is used as an emetic and the leaf sap extracted in cold water is used to treat stomach-ache and diarrhoea. Decoctions of the whole plant are taken to soothe coughs (Appidi et al., 2008b). However, despite the acclaimed folkloric use of *Hermannia incana* as an antidiarrhoeal agent, there is dearth of scientific evidence to substantiate such claim. The aim of this study therefore, was to evaluate the antidiarrhoeal and toxicological properties of the *Hermannia incana* with a view to validating its acclaimed use by the traditional medicine practitioners of Eastern Cape.

1.7 The aims and objectives of this study

1.7.1 *Ethnobotanical study of plants used for the treatment of diarrhoea*

Medicinal plants are an important aspect of the daily lives of many people and an important part of the South African cultural heritage (Van Wyk et al., 1997). They have been used for the treatment of many ailments including diarrhoea for years in the Eastern Cape Province. However, the knowledge and experience of the traditional health practitioners have not been documented in scientific literature. Information on herbal medicine in this part of the world has been dominated by oral tradition (Van Wyk et al., 1997). 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). It is necessary to make an effort to avoid erosion of this knowledge in this province by conserving the information on their useful plants. One of the objectives of this study was to document information gathered from traditional healers and elder rural dwellers on the plants used in the province for the treatment of diarrhoea.

1.7.2 *Antibacterial and antifungal activities of the crude extracts*

Bacteria and fungi are the major causative organisms of diarrhoea (Bhattacharya, 2000; Martines et al., 1993; Guerrant and Bobak, 1991). Plant extracts have long been used for the treatment of many ailments including diarrhoea, stomach-ache (Appidi et al., 2008b). Some other Species of the Sterculiaceae family (*Cola greenwayi* Brenan, *Cola natalensis* Oliv., *Dombeya burgessiae* Gerr. ex Harv., *Dombeya cymosa* Harv., and *Hermannia depressa* N.E.Br), are also used to treat diarrhea in KwaZulu-Natal, were screened for antibacterial activity have shown moderate activity against some diarrhoea causing organisms (Reid et al., 2005). No studies relating to the antimicrobial activities of this species have previously been reported. Therefore another objective of this study was to investigate the antimicrobial activity of *H. incana* by preliminary bioassay screening of its extracts against 10 bacterial (including diarrhoea causative organisms) and four fungal strains. The selected bacterial strains consisted of five Gram-positive (*Staphylococcus aereus, Staphylococcus epidermidis, Bacillus cereus, Micrococcus kristinae* and *Streptococcus faecalis*) and five Gram-negative strains (*Escherichia coli, Pseudomonas aeruginosa, Shigella flexneri, Klebsiella pneumoniae* and *Vibrio cholerae*), while the fungal species were *Aspergillus flavus*, *Aspergillus niger*, *Mucor hiemalis* and *Candida albicans*.

1.7.3 *In vivo antidiarrhoeal activity of the crude extracts*

In vivo animal-based studies have been taking place for bioactivity and its effects on animal immune systems including intestinal function by using traditional medicinal plants (Palombo, 2006). To determine the antidiarrhoeal activity of an extract,

diarrhoea is induced by an agent such as castor oil, arachidonic acid, prostaglandin E_2 or magnesium sulphate. Each of these agents has different mechanisms to induce diarrhoea. No clinical studies are available to support the claim that *Hermannia incana* is used as an antidiarrhoeal agent. Another aim of this study therefore, was to evaluate the antidiarrhoeal activity of the aqueous extracts of *Hermannia incana* with a view to validating its acclaimed use by the traditional medicine practitioners of Eastern Cape.

1.7.4 *Toxicological evaluation of the crude extracts*

Several studies revealed that some phytochemicals such as tannins and flavonoids are responsible for antidiarrhoeal activity of plants by increasing colonic water and electrolyte reabsorption while alkaloids and terpenes inhibit intestinal motility (Havagiray et al., 2004). As some of these active ingredients are also potentially toxic and may cause side effects, there is therefore a need to evaluate the safety of plant preparations. A few clinical trials have evaluated the safety and tolerability of traditional and herbal medicine preparations used to treat diarrhoea all over the world and generally indicate that minimal side effects are observed (Palombo, 2006). One of the objectives of this project therefore, was to evaluate the toxicity of the aqueous extract of *H. incana* leaves.

1.7.5 *Foliar micro-morphology*

Leaves of many plants are densely covered with glandular and non-glandular trichomes, which originate from epidermal cells (Werker, 2000), and are a natural feature of most Sterculiaceae species (Watson and Dallwitz, 1992). Plant species that contain glandular trichomes generally produce relatively large amounts of bioactive

compounds which include highly concentrated secondary metabolites with biological activity of interest to pesticide, pharmaceutical, flavour and fragrance industries (Duke, 1994). No information is available on the morphology and ultrastructure of the leaf appendages of *H. incana.* Therefore another objective of this study was to investigate the structure and distribution of different trichome types observed on the leaves of this plant, with a view to relating this to its antidiarrhoea property.

1.7.6 *Bioactive guided isolation and identification of compounds.*

The natural products derived from medicinal plants have proven to be an abundant source of biologically active compounds, many of which have been the basis for the development of new lead chemicals for pharmaceuticals (Palombo, 2006). With respect to infectious diseases, the increasing resistance of many common pathogens to currently used therapeutic agents, such as antibiotics and antivirals, has led to renewed interest in the discovery of novel bioactive compounds from traditional medicinal plants. Considering the ethnobotanical information on the antidiarrhoeal property of *H. incana*, it became essential to isolate and identify the active compound(s) in this plant and to examine the antimicrobial activity of some of the pure compounds against diarrhoea causative organisms.

1.8 The structure of the thesis

This thesis consists of chapters in the form of reprints of published articles, accepted articles and articles under review in various journals. The thesis is structured as follows: the ethnobotanical study of plants used for the treatment of diarrhoea in the Eastern Cape is described in Chapter 2. Chapter 3 reports on antimicrobial activity of *H. incana*. Antidiarrhoeal activity of *H. incana* crude extracts with the results is

presented in Chapter 4, while Chapter 5 describes the toxicity evaluation of the crude extracts of the leaves. Chapter 6 presents the foliar micro-morphology of *H. incana* and the isolation of bioactive compounds is presented in Chapter 7. Chapter 8 deals with the general discussion and conclusions of the entire study, in an attempt to present a coherent picture of the results.

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