CHAPTER 7

GENERAL DISCUSSION AND CONCLUSIONS

GENERAL DISCUSSION

Constipation has been a subject of multiple definitions and interpretations; it is usually defined as the irregular and infrequent evacuation of the bowels with straining to defeacate as the main symptom (Camilleri, 2005; Wald, 2008). It affects the old and young as well as the rich and the poor of both developing and developed countries. Constipation can be very unpleasant, with its accompany symptoms which can cause crises and considerable impact on life and health (Annels, and Koch 2002). It has been a health problem risk factor throughout history, increasing with the growth of civilization and industrialization (Whorton, 2000). It occurs in 2- 28% of the world population, most especially among women (Higgins and Johanson, 2004; Fleming and Williams, 2010). In South Africa, 29% of the population, especially the elderly people is at greater risk of constipation (Meiring and Joubert, 1998; Mugie et al., 2011).

The use of both prescribed and non-prescribed over-the-counter (OTC) laxatives is common for the treatment of constipation. Unfortunately however, these drugs often show some undesirable side effects which may have substantial impact on morbidity and quality of life (Drossman et al., 1993; De Lillo and Rose, 2000; Erasto et al., 2005).

A medicinal plant is one in which one or more of its organ contains substances which are precursors for the synthesis of chemo pharmacy (Okigbo et al., 2009). It has been identified as the basis of traditional medicine, which forms part of the culture of human existence in many African countries especially in South Africa (Van Staden, 2008). The use of medicinal plants to treat various ailments including constipation is very common among the people of the Eastern Cape Province of South Africa, particularly in the Nkonkobe Municipality. These are *Xhosa*-speaking people, majority of who are poor income earners and rural farmers with low socio-economic status. The herbal medicines have, for years, proved to be highly effective and safer

than synthetic medicines, simply because they are of natural origin (Ashafa et al., 2010). These reasons, together with the persistence of chronic side effects of some synthetic drugs, have led to the growth of interest on the use of herbal medicines for the treatment of various ailments including constipation especially in the developed countries (Erasto 2006). Thus, valuable information on traditional pharmacopoeias needs proper documentation to avoid diminishing knowledge on medicinal plant usage. This work has focused on the use of plant (*Aloe ferox*) in the treatment of constipation.

Ethnobotanical survey of plants used for the treatment of constipation

The result of the ethnobotanical survey carried out in the Nkonkobe Municipality at the beginning of this project revealed 10 plant species belonging to 8 families that are frequently used for the treatment of constipation. Out of these, *A. ferox* was chosen for further study because it had the highest frequency of citation by the local community. The leaves were reported to be the most used part of the plants, constituting 60% of its preparations. This was followed by the root (30%), rhizome (10%), bark (10%) and the bulb (10%). The commonest method of *A. ferox* preparation was decoction which is orally administered two or three times per day for a short period of time depending on the age of the patient but usually not more than two weeks, or as soon as the condition disappears. Proper documentation of this knowledge and experience of the traditional herbalist was the focus of this part of the project in order to avert the loss of important information considering the rate of biodiversity depletion through deforestation and overexploitation of most medicinal plants (Appidi, 2009).

Laxative effects of the aqueous whole leaf extract of A. ferox

Constipation is not only discomforting but also a risk factor of colorectal cancer. It can also cause abdominal distension, vomiting, restlessness, gut obstruction and perforation, and may be associated with aspiration or fatal pulmonary embolism (Mostafa et al., 2003; Chappell et al., 2008). Many species of *Senna* and *Aloe* have been used traditionally for the treatment of constipation. *A. ferox* is traditionally believed to have a laxative property for the treatment of constipation (Watt and Breyer-Brandjik, 1963). The aqueous extract of *A. ferox* in this study has clearly demonstrated the laxative property of the plant.

Loperamide used as a constipation inducer inhibits intestinal water secretion (Hughes et al., 1984) and colonic peristalsis (Sohji et al., 1978). This inhibition extends fecal evacuation time and delays intestinal luminal transit (Yamada and Onoda 1993). It thus, might have reduced the number, weight and water content of fecal pellets as shown in this study. The finding is similar to that of Shimotoyodome et al. (2000). Although, the loperamide did not affect feeding, it was believed to have an effect on the water intake of the constipated animals which probably accounted for the reduction in the water content of the fecal pellets.

Furthermore, the administration of aqueous extract of *A. ferox* to the constipated Wistar rats was effective in curing the constipation by influencing increased defecation frequency, fecal volume and motility of the colon. The extract possibly exerts its action by disturbing the equilibrium between the absorption of water from the intestinal lumen via the active sodium transport (Ishii et al., 1990). In addition, the secretion of water into the lumen by prostaglandin-dependent mechanism probably produced a stimulatory activity (Collier et al., 1976; Capasso. et al., 1983). The weight gained in the untreated constipated rats may be due to the accumulation of fecal pellets in their bodies. This clearly indicates that the plant extract increased intestinal

secretion and motility in the constipated rats. Similar observation was reported by Niwa et al. (2002) where dietary fiber was used for the treatment of morphine-induced constipation in rats. Carmine was used to measure the colonic movement and provided quantitative information about colonic transit. This enables the identification and characterization of transit abnormalities and allows assessment of the severity of the problem as well as the response to therapy (Degan, 2007). The transit process of the entire gastrointestinal tract helps to reflect the overall gastrointestinal motor activity important in measuring colonic transit time, which is also useful in detecting constipation, abdominal bloating and refractory irritable bowel syndrome.

The extract increased intestinal motility by enhancing colonic peristalsis in the rats. The possible mechanism of the extract in this process could enhance the release of fluid and increase intestinal secretion. The laxative effect of the extract could also be attributed to changes in the intestinal motility, which produced an increase in intestinal transit and colonic movement (Capasso and Gaginella, 1997). The extract treatment effect in this study was dose dependent and the highest dosage actually compared favourably well with senokot, a standard laxative drug. This indicates that the herb was effective in ameliorating bowel obstruction, thereby enhancing easy movement in the intestine.

Effect of A. ferox aqueous leaf extract on biochemical parameters in loperamide-induced constipated rats

Despite the widespread use of herbal medicine, few studies have been undertaken to ascertain the safety or toxicity of the herbal remedies. The effects of the aqueous whole leaf extracts of *A. ferox* at the dosages of 50, 100 and 200 mg/kg body weight for 7 days on some biochemical parameters were investigated. The non-significant difference in the organ-body weight ratio observed in the treated constipated animals compared to the control implied that the

extract did not cause any inflammation in both the liver and the kidney (Schmidt et al., 2007). This is similar to the findings of Amresh et al. (2008) in which the administration of *Cissampelos pareira* did not produce any effect on organ-body weight ratio. The liver indices such as albumin and bilirubin which were good indicators of the functional capacity of the liver were also not affected, indicating the normal functioning of the liver was not impaired. Furthermore, alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT) used to assess the integrity of plasma membrane, endoplasmic reticulum and glutathione metabolism in the liver (Kaplan and Pesce, 1996; Shahjahan et al., 2004) and aspartate aminotransferase (AST) and alanine aminotransferase (ALT), used for assessing the functional capacities of the kidney (Yakubu et al., 2003; Sunmonu and Oloyede, 2006) were also not affected.

Increasing ALP activity in the serum of the untreated constipated rats observed in this study may be an indication of damage to the plasma membrane leading to a compromise of membrane integrity (Yakubu et al., 2003). The non-significant difference in GGT activity suggests that glutathione metabolism may not be affected by the extract. Also, the observed elevation of the transferases (AST and ALT) in the untreated constipated rat suggests possible damage to the hepatocytes arising from the change in membrane permeability (Latha et al., 1998). This was however lowered by the oral administration of the aqueous extract of *A. ferox* in the constipated animals. Haematological parameters of the constipated rats compared well with the control showing no significant alteration in the parameters. This was an indication that the extract has no adverse effect on the blood system. RBC, Hb, PCV, MCH, MCHC, MCV, RCDW, WBC, neutrophils, monocytes, basophils, eosinophils, LUC, lymphocytes and platelets determined were also not altered except in the untreated constipated animals which exhibited a

rise in the lymphocyte counts. The administration of *A. ferox* extract was however effective in reverting the level back to near normal.

Inorganic electrolytes occur in large quantities in both extracellular and intracellular fluids (Yakubu et al. 2003). They comprise the most single important factor in the transfer and movement of electrolytes between the extracellular and intracellular compartments (Zilva et al., 1991). The absence of significant effects of *A. ferox* on calcium and potassium ions may be an indication that these ion-dependent processes were not adversely affected. This might be an indication that the herb did not affect the haematological parameters or alter normal body homeostasis at the doses investigated. This may be indicative of non-toxic and protective nature of the extract thereby showing that the *A. ferox* may be relatively safe as an oral remedy.

Phytochemical and antioxidant activities of A. ferox

Phytochemicals like tannins, phenols, alkaloids and saponins are believed to be responsible for the laxative effect of medicinal plants (Longanga-Otshidi et al., 2000). The laxative activity of the leaf extracts of *A. ferox* observed in this study may be attributed to the phytochemical constituents with laxative properties. The leaf extracts prepared in ethanol and acetone showed higher level of phenol, but moderate phenolic content was exhibited by the aqueous and methanol extracts. Phenols contain hydroxyl groups which make them to possess strong and direct antioxidant properties (Dasgupta and De 2004; Dogan et al., 2010). The level of flavonoids was generally low in all the solvent extracts, but appreciably high in methanol extract. Proanthocyanidin content was relatively high in acetone extract as compared with other solvent extracts used in this study. This observation agreed with the report of Loots et al. (2007),

who found strong antioxidant activity of the leaf gel extract of *A. ferox* to be due to high concentration of phenolic compounds.

Tannins are believed to be responsible for the much acclaimed medicinal value of plants such as analgesic, bactericidal, antimalarial and antiseptic (Madhavi and Salunkhe, 1995). The tannin level in all the solvent extracts was low and not significantly different from each other, whereas the concentration of alkaloids in the acetone, ethanol and methanol extracts was significantly high. The data obtained from this study on saponins content of *A. ferox* was high in the ethanolic extract, while other solvents extracts were moderate.

The plant extracts exhibited strong antioxidant activity as evidenced in their ability to scavenge DPPH, ABTS hydrogen peroxide and nitric oxide radicals as well as reducing power and lipid peroxidation (Chapter 3). Their strong antioxidant activity by reducing Fe^{3+} to Fe^{2+} showed the reductive capabilities when compared with ascorbic acid and BHT. The scavenging of DPPH and ABTS radicals by the extracts of *A. ferox* was concentration dependent. Furthermore, the scavenging activity of $ABTS^{\cdot+}$ by the plant extracts was found to be higher than that of DPPH radical. The difference in solubility and radical reactivity of the various testing systems are believed to be responsible for the higher scavenging activity of ABTS. The plant extracts displayed moderate activity in competing with oxygen that reacts with nitric oxide thereby inhibiting the generation of anions, which may be associated with several diseases (Oyaizu, 1986; Ross, 1990; Liu and Henkel, 2002). The extract was able to scavenge hydrogen peroxide in a concentration dependent manner, which may be due to their high phenolic contents. The extract donates electron to H_2O_2 and thus reduce it to water. Lipid peroxidation assay indicated by the high absorbance values on the extract showed weak antioxidant activity.

Generally, the plant extracts showed concentration dependent activities against free radicals, which particularly may be useful in treating radical related pathological damages (Veerapur et al., 2009).

Characterization of the laxative component in A. ferox

In the approach to isolate the active laxative compound in *A. ferox*, a series of fractionating and isolating procedures was employed. The plant materials was first extracted with methanol and fractionated before being subjected to column chromatography. During these processes, the chemical composition and qualitative laxative activity was determined by TLC on a continuous basis in order to search for single compound and to confirm the laxative activity of the compound. As a result of this, the blue and yellow fluorescence colours identified under UV were believed to be pure compounds, and were subjected to IR and NMR analysis. The FT-IR and ^{1}H-NMR spectra of the isolates revealed the presence of OH and C=O functional groups which might be responsible for the biological activities recorded by this plant (Dosumu et al., 2010). Further attempt to characterize the compounds to their molecular level was not successful. The various spectra obtained from the IR and NMR analyses are attached herewith as appendixes I-L.

Conclusion

This study clearly demonstrated the importance of validating the ethnomedicinal uses of *Aloe ferox* as a basis for the continual use and integration into the modern medical system. The laxative activity of the aqueous extract of *A. ferox* observed may be attributed to the phytochemicals like tannins, flavonoids, flavonols, alkaloids, phenols and saponins. The onset of

constipation and inhibition of intestinal motility caused by loperamide was suppressed and attenuated by the aqueous whole leaf extract of *A. ferox*. This indicated the laxative potentials of the extracts in increasing intestinal motility and enhancing colonic peristalsis, thereby causing easy movement in the intestine. The extracts at therapeutic doses used in this study were not toxic since they did not alter the normal functions of the organs and the haematological parameters, as well as the normal body homeostasis. This may be an indication of non-toxic nature of the extracts, thereby showing that the plant may be relatively safe for oral remedy. The results also showed that the whole leaf of *A. ferox* extracts possess potent antioxidant properties and could serve as a radical inhibitor and scavenger to prevent progression of diseases. Although, conclusive elucidation of the laxative compound(s) of the plant was not achieved due to sensitivity and instability of the laxative compounds in the whole leaf extract of *A. ferox*, the presence of OH group has greatly enhanced its biological activities such as laxative. The evaluation of *A. ferox* for the treatment of constipation has provided information on the active components of the plant. These findings have given scientific support to the folkloric use of *Aloe ferox* in the Eastern Cape Province of South Africa as a laxative agent.

References

Amresh, G.R., Singh, P.N., Rao, C.V., 2008. Toxicological screening of traditional medicine Laghupatha (*Cissampelos pareira*) in experimental animals. Journal of Ethnopharmacology 116, 454-460.

Annels, M., Koch, T., 2002. Older people seeking solution to constipation: the laxative mire. Journal of Clinical Nursing 11, 603-612.

Appidi, J.R., 2010. Evaluation of Antidiarrhoeal and Toxicological properties of *Hermannia incana* Cav. A South African Medicinal plant. PhD thesis, pp. 1-108

Ashafa, A.O.T., Sunmonu, T.O., Afolayan, A.J., 2010.Toxicological evaluation of aqueous leaf and berry extracts of *Phytolacca dioica* L. in male Wistar rats. Food and Chemical Toxicology 48, 1886-1889.

Camilleri, M., 2005. Etiology and pathophysiology of irritable bowel syndrome and clinical constipation. Advance Study in Medicine 5, 955-964.

Capasso, F., Gaginella, T.S., 1997. *Laxatives, a Practical Guide*. Milan Springer Italia. Pp 85

Capasso, F., Mascolo, N., Autore, G., Duraccio, M.R., 1983. Effect of indomethacin on aloin and 1, 8 dioxianthraquinone-induced productions of prostaglandins in rat isolated colon. Prostaglandins 26, 557–562.

Chappell, D., Rehm M., Conzen P., 2008. Opoid-induced constipation in intensive care patients: relief in sight? Critical care, 124: 161.

Collier, H.O.J., McDonalds-Gibson, W.J., Sated, S.A., 1976. Stimulation of prostaglandin biosynthesis by drugs: effects *in-vitro* of some drugs affecting gut function. Brazilian Journal of Pharmacology 58, 193–198.

Dasgupta, N., De, B., 2004. Antioxidant activity of *Piper betel* L. leaf extract *in-vitro*. Food

Chemistry 88, 219-224.

De Lillo, A., Rose, S., 2000. Functional bowel disorder in the gastric patient: constipation fecal impaction and fecal incontinence. The America Journal of Gastroenterology 95, 901-905

Degan, L., 2007. Gastrointestinal motility-physiology and methods of measurement. Therapeutic Umschau, 64, 195-199.

Dogan, S., Diken, M.E., Dogan M., 2010.Antioxidant, phenolic and protein contents of some medicinal plants. Journal of Medicinal plant Research 4, 2566-2573.

Dosumu, O.O., Idowu, P.A., Onacha, P.A., Ekundayo, O., 2010. Isolation of 3-(4-hydroxyphenyl) methylpropenoate and bioactivity evaluation of *Gomphrena celosioides.* EXCLI Journal, 9; 173-180.

Drossman A., Li, Z., Andruzzi, E., Temple, R.D., Talley, N.J., Thompson, W.G., 1993. U.S house-holder survey of functional gastrointestinal disorders. Prevalence, sociodemography and health impact. Digestive Disease and Science 38, 1569–1580.

Erasto, P., Adebola P.O., Grierison D.S., Afolayan A.J 2005. An ethnobotanical study of plants used for the treatment of diabetes in Eastern Cape Province, South Africa. African Journal of Biotechnology 4, 1458-1460.

Erasto, P., 2006. Phytomedical investigation of *Vernonia amygdalina*: a medicinal plant use for the treatment of diabetes. PhD Thesis, pp.1-136.

Fleming, V., Williams, E.W., 2010. A review of laxative therapies for treatment of chronic constipation in older adults. The American Journal of Geriatrics Pharmacotherapy 8, 514-550.

Higgins, P.D., Johanson, J.F., 2004. Epidemiology of constipation in North America: a systematic review. American Journal of Gastroenterology 99, 750-759.

Hughes, S., Higgs, N.B., Turnberg., L.A., 1984. Loperamide has antisecretory activity in the human jejunum *in-vivo*. Gut 25, 931 –935.

Ishii, Y., Tanizawa, H., Takino, Y., 1990. Studies of aloe: III. Mechanism of cathartic effect. Chem Pharm Bull, 38, 197–200.

Kaplan, L.A., Pesce A.J., 1996. Clinical Chemistry. Theory, Analysis and Correction. 3rd Edition. A Harcourt Health Science Company, Mosby, London, 8, 140-152.

Latha, R.M., Geentha, T., Varalakshmi, P., 1998. Effect of *Vernonia cinerea* Less. Flower extract in adjuvant-induced arthritis. General Pharmacology 31, 601–606.

Liu, J., Henkel, T., 2002. Traditional Chineese medicine (TCM): Are polyphenols and saponins the key ingredients triggering biological activities? Current Medical Chemistry 9, 1483 1485.

Longanga-Otshudi, A., Vercruysee, A., Foriers, A., 2000. Contribution to the ethnobotanical, phytochemical and pharmacological studies of traditionally used medicinal plants in the treatment of dysentery and diarrhoea in Lomela area, Democratic republic of Congo (DRC), Journal of Ethnopharmacology 71, 411-423.

Loots, D.T., Van Der Westhuizen, F.H., Botes, L., 2007. *Aloe ferox* leaf gel phytochemical content, antioxidant capacity, and possible health benefits. Journal of Agricultural Food Chemistry 55, 6891–6896.

Madhavi, D.L., Salunkhe, D.K., 1995. Toxicological aspects of food antioxidants in Madhavi, DL. Deshpande, SS and Salunkhe, DK. Food antioxidants. Dekker , New York, pp 267.

Meiring, P.J., Joubert, G., 1998: Constipation in elderly patients attending a polyclinic. South African Medicine Journal 88, 888-890.

Mostafa, S.M., Bhandari, S., Ritchie, G., Gratton, N., Wenstone, R., 2003. Constipation and its

implications in the critically ill patient: a national survey of United Kingdom intensive care units. British Journal of Anaesthesia 91, 815-819.

Mugie, S.M., Benninga, M.A., Di Lorenzo, C., 2011. Epidemiology of constipation in children and adult: a systematic review. Best Practice Research Clinical Gastroenterology 25, 3 18.

Niwa, T., Nakao, M., Hoshi, S., Yamada, K., Inagaki, K., Nishida, M., Nabeshima, T., 2002. Effect of dietary fiber on morphine-induced constipation in rats. Bioscience Biotechnology Biochemistry 66, 1233-1240.

Okigbo, R. N., Anuagasi, C.L., Amadi J.E., 2009. Advances in selected medicinal and aromatic plants indigenous to Africa. Journal of Medicinal Plants Research 3, 86-95.

Oyaizu, M., 1986. Studies on products of browning reactions prepared from glucose amine. Japan Journal of Nutrition 44, 307-315.

Petticrew, M., Watt, I., Sheldon, T., 1997. Systematic review of the effectiveness of laxatives in the elderly. Health Tech Assess 1, 1-52.

Ross, R., 1990.The pathogenesis of atherosclerosis: A perspective for the 1990. Nature 362, 801 803.

Schmidt, B.M., Ilic, N., Poulev, A., Raskin, I., 2007. Toxicological evaluation of a chicory root extract. Food Chemistry Toxicology 45, 1131-1139.

Shahjahan, M., Sabitha, K.E, Mallika J., Shyamala-Devi, C.S., 2004. Effect of *Solanum trilobatum* against carbon tetrachloride induced hepatic damage in albino rats. Indian Journal of Medicinal Research 120, 194-198.

Shimotoyodome, A., Meguro, S., Hase, T., Tokimitsu, I., Satake, T., 2000. Decreased colonic mucus in rats with Loperamide-induced constipation. Complementary Biochemistry

Physiology 126, 203-211.

Sohji, Y., Kawashima, K., Shimizu, M., 1978. Pharmacological studies of loperamide, an anti diarrhoeal agent. Folia Pharmacology Japan 74, 155–163.

Sunmonu, T.O., Oloyede, O.B., 2006. Nephrotoxicity of crude oil contaminated catfish (*Clarias gariepinus*) on albino rats. Nigerian Journal of Biochemistry and Molecular Biology 26, 25 34.

Van Staden, J., 2008. Ethnobotany in South Africa. Journal of Ethnopharmacology 119, 329-330.

Veerapur, V.P., Prabhakar, K.R., Parihar, V.P., Kandadi, M.R., Ramakrishana, S., Mishra, B., Satish Rao, B.S., Srinivasan, K.K., Priyadarsini, K.I., Unnikrishnan, M.K., 2009. *Ficus racemosa* Stem Bark Extract: A potent antioxidant and a probable natural radioprotector**.** Evidence-Based Complementary and Alternative Medicine 6, 317-324.

Wald, A., Scarpignato, C., Muller-Lissner, S., Kamms, M.A., Hinkel, U., Helfrich, I., Schuijt, C., Mandel, K.G., 2008. A multinational survey of prevalence and patterns of laxative use among adults with self-defined constipation. Alimentary Pharmacology & Therapeutics 28, 917-930.

Watt, C., Breyer-Brandjik, M.G., 1963. The medicinal and poisonous plants of Southern and Eastern Africa. Livingstone, Edinburgh, London, Great Britain, pp. 449-455.

Whorton, J., 2000. Civilisation and the colon: constipation as the "disease of diseases". British Medical Journal, 321, 1586-1589.

Yakubu, MT., Bilbis, L.S., Lawal, M., Akanji, M.A., 2003. Effect of repeated administration of sildenafil citrate on selected enzyme activities of liver and kidney of male albino rats. Nigeria Journal of Pure Applied Science 18, 1395–1400.

Yamada, K., Onoda, Y., 1993. Comparison of the effects of T-1815, yohimbine and naloxone on

mouse colonic propulsion. Journal of Smooth Muscle Research 29, 47-53.

Zilva, J.F., Panmall, P.R., Mayne, P.D., 1991. Clinical chemistry in diagnosis and treatment, 5th ed. England Clays Ltd., St. Ives, Plc, England, pp. 32.