CHAPTER 1

GENERAL INTRODUCTION

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Phytomedicine

Human beings have been using natural products of animal, plant and microbial sources for thousands of years either in the pure forms or as crude extracts for the treatment of various diseases (Parekh and Chanda, 2007). Indigenous plants have been the traditional source of raw materials for the manufacture of medicines (Gupta, 1994). The focus on plant research has increased all over the world and a large body of evidence has been collected to show the immense potential of medicinal plants used in various traditional systems. Such scientific studies have led to the isolation of chemical substances with therapeutic properties and many of the isolates have found use as modern drugs while others have served as substrates for the synthesis of drugs (Nadro et al., 2006). In fact, modern pharmaceuticals still contain, at least, 25% drugs derived from plants (Olaleye et al., 2006).

In South Africa, like many other African countries, medicinal plants and herbal formulations play important roles in the daily health care of the people. The country is rich in floral biodiversity, which has provided herbal health practitioners and traditional healers with an immense pool of ‘natural pharmacy’ from which ingredients are selected for the preparation of herbal medicines (Erasto, 2006). Usually, the use of traditional medicines is more prevalent in the regions where western medicines are inaccessible because of the high cost of the latter. Among the most used medicinal plants in South Africa are the members of the genus *Pelargonium.*

The Use of *Pelargonium* species in medicine

The genus *Pelargonium* (Geraniaceae) comprises approximately 270 distinct species of perennial small shrubs of which about 80% occur in southern Africa with the centre of diversity in the Cape Province (Van der Walt and Vorster, 1988), while others occur in Australia, New Zealand and the Far East (Mativandlela et al., 2006). The commonly used *Pelargonium* species in South Africa are *P. luridum* Andr., *P. antidysentericum* (Eckl. & Zeyh.) Kostel., *P. rapaceum* (L.) L'Hér., *P. reniforme* Curt., *P. sidoides* DC. and *P. triste* (L.) L'Hér (Van Wyk et al., 1997). *Pelargonium species* are widely used by traditional healers in areas of southern Africa for the treatment of diarrhea, dysentery, fever, respiratory tract infections, liver complaints and wounds. Infusions and decoctions of the tubers are commonly taken, while a traditional method of using *Pelargonium* roots is to boil the tuber in milk. Also, the roots may be directly chewed or powdered and mixed with food (Latté and Kolodziej, 2004). According to World Health Organization, ethanolic extracts of the roots of two species of *Pelargonium* have been used in Germany since the 1980s as herbal medicine. Since 1983, this ethanolic extract has been marketed in Germany under the name Umckaloabo which is used for acute and chronic infections, especially those of the respiratory tract and ear, nose and throat infections (De Boer et al., 2002)

Alcohol consumption in South Africa

Studies show that South Africa ranks among the world's highest levels of alcohol consumption per drinker: 16.6 liters of pure alcohol a year (Rehm et al., 2003; Parry, 2005). In the 1998 South African Demographic and Health Survey (SADHS), 44.7% of men and 16.9% of women were reported as alcohol consumers. Among them, risky drinking (defined as having five or more

drinks a day for men and three or more for women) was relatively rare on weekdays, but rose significantly on weekends, with around a third of both male and female drinkers reporting risky levels of intake (Parry et al., 2005). Usually, South African drinkers consume various forms of alcoholic beverages which include wine, beer, champagne, whisky, vodka and brandy. These beverages contain different levels of ethanol as the major ingredient from about 5% in beer to 50% in vodka and whisky (Alibaba, 2009). The high rate of alcohol consumption in South Africa accounts for the prevalence of alcoholic liver disease and fetal alcohol syndrome (FAS). FAS is a pattern of anomalies and developmental deficits found in children exposed to large amounts of alcohol in the prenatal period. Children with FAS have a characteristic pattern of facial and body dysmorphology, delayed physical growth and development, and specific mental and behavioral deficits (Stratton et al., 1996). In a first active case ascertainment study in South Africa, the rate of FAS among first grade children was 40.5-46.4 per 1000 (May et al., 2000). In a second similar study, it was even higher two years later at 65.2-74.2 per 1000 (Viljoen et al., 2005). Both these rates of prevalence are the highest ever reported in the world (May et al., 2007).

Alcoholic Liver Disease

Long-term heavy consumption of alcohol plays a major role in the development of alcohol-related liver damage (Maher, 1997; Neuman, 2003). The close relation between ethanol and liver damage is mainly due to the fact that about 80% of ingested alcohol is metabolized in the liver. Ethanol is metabolized into cytotoxic acetaldehyde by alcohol dehydrogenase enzyme in the liver, which in turn, is oxidized to acetate by aldehyde oxidase giving rise to reactive oxygen species (ROS) (Tuma and Cassey, 2003). According to Wu and Cederbaum (2003), one factor that plays a central role in the aetiopathology of alcohol-induced liver disease and which has been the focus of much research is the excessive generation of ROS. Excessive alcohol

consumption not only enhances ROS generation, but also depletes antioxidants, thus, creating a state of oxidative stress.

Pathogens such as bacteria and fungi have also been implicated in alcoholic-liver disease. Some of the bacteria implicated in liver diseases are *Staphylococci sp., Bacillus sp., Pseudomonas aeruginosa* and *Klebsiella pneumonia* (Jones et al., 1967; Wyke et al., 1982). One central component in the complex network of processes leading to the development of alcoholic liver disease is the activation of kupffer cells in the liver by a substance called endotoxin, which is released by bacteria living in the intestine (Wheeler, 2003). Alcohol abuse can lead to increased endotoxin levels in the blood and liver. When activated, kupffer cells produce signaling molecules (cytokines) that promote inflammatory reactions as well as ROS, which can damage liver cells. Fungal infection has been identified as an important cause of morbidity and mortality in patients with acute liver failure. Like bacteria, fungi produce mycotoxins that cause mycotoxicoses. The most widespread and dangerous of these are the aflatoxins produced by the mould *Aspergillus flavus*. Aflatoxins pose a serious threat to humans because the mould grows on poorly stored grains. When eaten, the toxin is stored in the liver where it can eventually cause hepatitis and liver cancer (Charlotte, 2005).

Despite the great progress made in scientific research in the past two decades, the development of suitable medications for the treatment of alcohol-induced health injury remains a challenge (Das et al., 2005). Treatment strategies for alcoholic liver disease e.g. liver transplantation are expensive and often beyond the reach of the common man (Faremi et al., 2008). A phytotherapeutic approach to modern drug development can provide many invaluable drugs from medicinal plants (Saravanan et al., 2006). Traditional medicine, which involves the direct and

physical use of medicinal plants, has potentiated the quest for herbs as a therapeutic approach in the treatment of alcoholic liver disease.

The choice of *Pelargonium reniforme* for this study

Pelargonium reniforme Curtis is an attractive erect shrublet of up to 100 cm in height with kidney-shaped leaves and pink flowers (Figure 1). It is indigenous to the Eastern Cape Province of South Africa and occurs mainly in the coastal regions (Latté and Kolodziej 2004; Kolodziej, 2007). It is widely used by the traditional healers in southern Africa for the treatment of diarrhea, dysentery, fever, respiratory tract infections, liver complaints and wounds (Watt and Breyer-Brandwijk, 1962). Infusions and decoctions of the fleshly tubers are commonly taken, while a traditional method of using the roots is to boil the tuber in milk. Also, the roots may be directly chewed or powdered and mixed with food (Latté and Kolodziej 2004). Verbal ethnomedical information obtained from the indigenous people of the Eastern Cape indicates that the plant is also used for the treatment of liver damage including that caused by alcohol. Hence, the plant was chosen for this study.

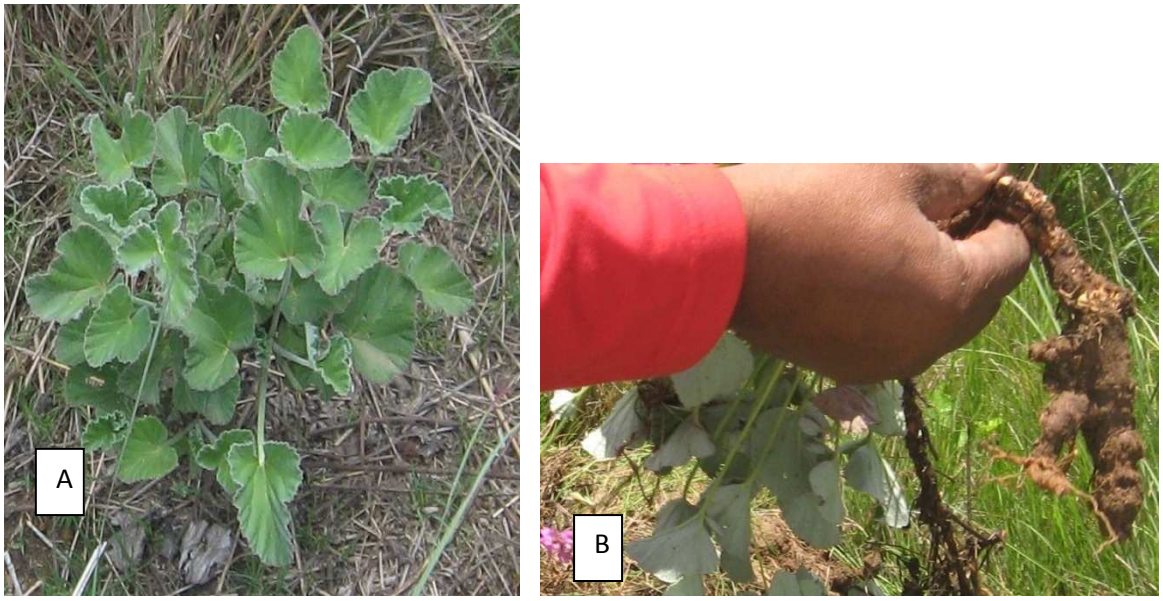

Figure 1: *Pelargonium reniforme* Curtis: A; in the natural vegetation, B; tuberous roots of the herb.

Objectives of this study

The Eastern Cape Province of South Africa is well known for its richness in plant species (Phillipson, 1987). The indigenous people in the province, including the Nkonkobe Municipality, have a long history of using medicinal plants for the treatment of various diseases such as liver diseases. However, the number of plant species used for the treatment of liver damage is limited and the plants are regarded as precious and highly valued (Erasto, 2006). Considering the rate at which the vegetation is getting depleted in this part of the world, there is a need to document the precious knowledge on these plants as well as the experience of the traditional healers and herbalists. One of the objectives of this study was therefore, to carry out the ethnobotanical study of medicinal plants used for the treatment of alcohol-induced liver damage, using questionnaires and general conversions with traditional healers and herbalists, as well as rural dwellers. Ten plant species were identified as the most used species for the treatment of liver damage. Their local and scientific names as well as the various methods of preparation and administration were documented. During the survey and also from the information gathered from the literature, *Pelargonium reniforme* was prominently mentioned as a species used generally for the treatment of liver damage. This plant was therefore chosen for further study.

Alcohol toxicity is one of the world's major health problems as many people are affected due to several fatal diseases caused by alcohol (Singha et al., 2007). The liver is one of the major alcoholic target organs known to be severely damaged due to chronic alcohol intake (Kundu et al., 2008). Alcohol abuse can elicit disturbances in the delicate balance between the pro- and antioxidant system of the organism, therefore leading to oxidative stress (Nordmann et al., 1992). To counteract these oxidants, cells have several enzymatic antioxidants and non-enzymatic antioxidants, but their levels are altered in alcoholics (Saravanan and Nalini, 2007). *Pelargonium*

reniforme roots are used as a remedy in this Province for the treatment of liver damage (Watt and Breyer-Brandwijk, 1962). Although the root of this plant is used in the treatment of liver disorders by several ethnic groups, there is paucity of scientific evidence in the literature regarding its usage in liver disorders. Another objective of this project, therefore, was to evaluate the *in vitro* antioxidant activity as well as the protective and curative effects of *P. reniforme* extracts against alcohol-induced damage using rats as a model.

Some pathogenic bacteria and fungi have been implicated in alcoholic liver disease. Despite the widespread use of broad spectrum antibiotics, bacterial infection is responsible for up to a quarter of the deaths of patients with liver disease (Wyke, 1987). Hence, another objective of this project was to evaluate the antimicrobial activity of the plant in order to validate its use in liver damage.

The medicinally active ingredients for liver disease were found in the bitter tasting roots of the plants (Helmstadter, 1996). This has resulted in the collection of only the roots of the species for local uses and export trade. This is a destructive method of plant harvesting. It reduces the opportunity for natural rejuvenation and could negatively affect plant demography. A number of strategies to solve the problem of overharvesting have been suggested among which is the use of leaves and stems as alternatives to tubers and roots for medicinal purposes (Lewu et al., 2006).

The comparison of the activity of the leaves and roots was aimed at determining the differences and similarities in the pharmacological actions of the aerial and underground parts of the plant. This may encourage the use of an alternative (aerial) part of the herb rather than the roots.

Previous phytochemical screenings have shown that the aerial parts of *P. reniforme* contains benzoic and cinnamic acid derivatives and also flavonoids and tannins which are its principal

phenolic contents (Kolodziej, 2007). The occurrence of tannins may explain the traditional use of the aerial parts as wound healing agent, which may be attributed, at least in part, to their astringent action. A similar rational explanation based on the presence of tannins may be provided for its use in traditional medicine for the treatment of gastrointestinal disorders such as diarrhea. The root extracts have been shown to have antibacterial, antifungal and antitubercular activity and this may justify its use by the people of South Africa in the treatment of coughs and tuberculosis (Mativandlela et al., 2006). The boiled leaves of this herb are used to protect wounds against maggots (Smith, 1895). In animals, the plant is used to prevent purging in horses and also to treat liver complaints in sheep and calves (Batten and Bokelman, 1966).

Despite the usage of this plant in folk medicine over ages, there is paucity of information on its possible toxicity. Another objective of this work was to assess *P. reniforme* for possible toxic effects by using haematology, serum chemistry and organ-body weight ratios as indices of toxicity.

The structure of the dissertation

This dissertation consists of contributions in the form of an accepted article and manuscripts submitted for publication. The dissertation is structured as follows: The ethnobotanical survey of medicinal plants with hepatoprotective effect against alcohol-induced liver damage in Nkonkobe municipality, is presented in chapter 2. Chapter 3 presents the effects of *P. reniforme* on alcohol-induced liver damage and oxidative stress, while chapter 4 deals with the antibacterial, antifungal and antioxidant activity of the roots and leaves of the plant. The safety evaluation of the extract from the roots of *P. reniforme* in male Wistar rats are reported in chapter 5. The general

discussion and conclusion from this study is presented in chapter 6, as an attempt to consolidate the results obtained from the study of this valuable medicinal plant.

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