CHAPTER 1

INTRODUCTION

1.1 Background

McCorkle (1982) defined ethnoveterinary medicine as the folk or traditional beliefs, knowledge, skills and practices relating to health-care of animals. Although health-care of animals has evolved wherever and whenever people and animals co-exist, ethnoveterinary research has a more recent history than other branches of indigenous knowledge systems; only in the mid 1970's did significant systematic scientific investigation begin on this rich body of knowledge (Schwabe and Kuojok 1981; McCorkle, 1982). Today ethnoveterinary medicine, a systemic study of community animal healthcare and its development applications (Schillhorn van Veen, 2001), is a growing field that brings together a synergistic mix of researchers and practitioners.

The World Health Assembly adopted a resolution urging interested governments to give adequate importance to the utilisation of their traditional systems of medicine as suits their national health systems (Akarele, 1983). Resource-poor people in many countries of the developing world can not afford modern pharmaceutically-derived medicines. As increased food, and income from livestock products hold the greatest promise for increased human well-being throughout the developing world, where herd and kraal animals are raised by the poor (WILRTC, 1978), so research results should be useful for hands-on livestock development and extension with a goal of increased human rather than animal well-being. The process to improve plant and animal health-care and management systems ultimately aim at improvement of human health (McCorkle, 1989).

Most of the art, skills and expertise of traditional healers or practitioners have been passed on from generation to generation almost solely by word of mouth (McCorkle and Mathias – Mundy, 1992; Hirt and M'Pia , 1995). Currently such knowledge is maintained within the older members of the community. Due to migrations, regional conflicts, urbanisation and technological transformation in developing countries, such knowledge may disappear (Hirt and M'Pia, 1995). The risk is that the vast knowledge, skills and expertise gathered over centuries may be lost if not documented. Hence it is crucial to identify and make use of the tropical plant *materia medica*, as ethnic groups disappear and their knowledge disappears with them (Goodland, 1981; Farnsworth and Soejarto, 1985).

A great number of plant-derived therapeutic agents have been discovered following leads provided by indigenous knowledge systems (Farnsworth *et al*., 1985; Farnsworth and Soejarto, 1991; Cox, 1994). Often the process begins as folk knowledge of activity of the plant. The traditional healer uses the plant therapeutically. The healer communicates knowledge of the healing potency to a researcher. The researcher in turn collects and identifies the plant. Making crude extracts follows this. The extracts are tested through bioassays. Bioassay-guided fractionation leads to isolation of an active ingredient or compound. The structure of the compound is determined. In a way, the compound is either used in its native form as a source of direct therapeutic agent, serves as raw material for complex synthetic compounds, or as a taxonomic marker in the search for new compounds. The biblical phraseology 'seek and ye shall find' should be the operative principle (Cox, 1994) as we continue to search and study medicinal plants. The ability to isolate compounds depends on ability to screen (Farnsworth, 1994; Houghton and Raman, 1998). Many modern drugs have been derived directly from plants or these plant derivatives have acted as templates for synthesis of modern pharmaceuticals (Balick, 1994; Soejarto, 1996). Tropical rainforests still present a great storehouse of medical genetic resources that may yield important drugs to treat a number of diseases for which cures are not available (Farnsworth and Soejarto, 1991; Akarele *et al*., 1991), or are too expensive to develop. However, as many of these forests and plants are disappearing at a very terrific rate, conservation measures need to be in place (Cunningham, 1991; Eloff, 1998 a).

Use of medicinal plants in treating diseases is an ancient tradition that has existed with human habitations. Many rural communities in the developing world today, especially where modern drugs are unaffordable or inaccessible, use traditional medicines (Cunningham, 1991; Cunningham and Zondi, 1991). Developed countries have become increasingly interested in traditional or 'alternative' medicine of animals and humans alike (Schillhorn van Veen, 2001). Already, at least 25% of all prescriptions dispensed contain substances of plant origin and, there is a renewed interest in the use of crude plant extract materials for medicament (Farnsworth, 1977). As the focus of medicine shifts from treatment of manifest disease to prevention, herbal medicine (with its four pillars of phytochemistry, phytopharmacy, phytopharmacology and phytotherapy) is coming into focus, being a renaissance of age-old human tradition (Weiss and Fintelmann, 2000). The 'Green' movement in Western society has changed attitudes in the general population who conceive naturally derived substances and extracts as being inherently safer and more desirable than synthetic chemical products, with the net effect of increase in sales of herbal preparations (Houghton and Raman, 1998; Capasso *et al*., 2000).

Despite extensive use of plants as medicines, herbal preparations are not always safe (Capasso *et al*., 2000). For their natural defence against pathogens, and possibly against ingestion by man and animals,

plants ordinarily produce many metabolic chemicals such as saponins, tannins, pyrrolizidine alkaloids, cyanogenetic glycosides. These chemicals render the plants poisonous or toxic when consumed, and there are reports of toxicity and poisoning from medicinal plants usage (Stewart *et al*., 1999; Taylor *et al*., 2003; Fennell *et al*., 2004). As ethnomedicine does not follow western paradigms of scientific proof of efficacy, most medical and veterinary professionals distrust the use of herbs, and know little about them (Sofowora, 1982; Thompson, 1997). There is need therefore, for scientific validation of efficacy and safety of herbal medicines before acceptance and worldwide use.

The isolation and characterisation of bioactive compounds from *P. africanum*, therefore is a step in this direction. The present work was in particular prompted by reports of the use of the *P. africanum* by the Setswana-speaking people, who use root and bark concoctions for treating diarrhoea and boosting resistance to disease in cattle (van der Merwe, 2000), and by Manana (2003), who collected the plant from medicinal plant market vendors in Pretoria, and established antibacterial activity in its bark extracts.

1.2 Hypotheses

- Leaf, root and bark extracts of *P. africanum* have bioactive compounds
- *P. africanum* extracts are safe and have potential value in veterinary medicine

1.3 Aim

To evaluate the potential of *Peltophorum africanum* extracts and isolated compounds in ethnoveterinary medicine

1.4 Objectives

To: -

- Determine the best extractant of bioactive compounds from the leaves, bark and root of *Peltophorum africanum*, and screen for antibacterial and antioxidant activity
- Evaluate the extracts for their activity on *Haemonchus contortus* and *Trichostrongylus colubriformis in vitro.*
- Determine the efficacy of the extracts on *Haemonchus contortus* and *Trichostrongylus colubriformis* in sheep
- Isolate and characterise pure compound(s) from extracts

- Evaluate the safety of the extracts for possibly clinical application
- Quantify the antioxidant activity of the extracts, in view of potential role in neurodegenerative disorders

CHAPTER 2

LITERATURE REVIEW

2.1 The antibacterial agents

Although Fleming discovered penicillin in 1929, a major step in antibacterial era begun in 1935 with the discovery of prontosil, the first sulphonamide. Penicillin proved a better antibacterial drug in 1940s when it was used successfully against gonorrhoea, scarlet fever, pneumonia and meningitis (Edwards, 1980). The impact of antimicrobial chemotherapy is so great that it is unlikely that anybody will live out his or her life without receiving some form of antimicrobial drug therapy. In the USA, antimicrobial drugs make up to 33% of the pharmacopoeia (USA Pharmacopoeia, 1990). However, there has been widespread misuse of antibiotics, which in turn, has created much resistance. Bizimenyera (1986), while working on chicks and hatcheries in Kenya found some strains of bacteria resistant to as many as six common antibiotics. The development of bacterial resistance against antibiotics is moving ahead of the antibiotics turnover by pharmaceutical industry triggering fears that we may enter a post-antibiotic era (Berkowitz, 1995; Leggiadro, 1995). Efforts to search for new antibacterial compounds from plants have thus been intensified (Vlietinck *et al*., 1995; Eloff, 1998b, c; Martini and Eloff, 1998; Eloff, 1999a; Eloff, 2000; Katerere *et al*., 2003).

2.2 Antioxidants

The increased incidences of nervous and stress-related diseases may be attributed to a pro-oxidative environment caused by among other things, cigarette smoking, alcohol abuse, air pollutants, ionizing radiation, inflammation, or inadequate or inappropriate nutrition. In a meeting point of scientists, industrial researchers, research users, organizations and associations and others working in and/or affected by research and technology, it was estimated that more than two million new cases of degenerative diseases may be detected every year (Nair *et al*., 2003). Due to prevalence, morbidity, and mortality of the neurodegenerative diseases, they present a significant medical, social and financial burden on the society. There is thus an impelling need for scientists in preventive medicine to work on the prevention of diseases, particularly the chronic degenerative forms without a specific cure. Oxidative stress has been referred to as a double edged sword, for whereas transient levels may activate defense mechanisms in the cell, it often induces some enzymes like cyclooxygenase-2 (COX-2), lipoxygenase (LOX) and inducible nitric acid synthase (iNOS) that generate intermediaries that damage cellular macromolecules including DNA .The damage is made on proteins, lipids, and nucleic acids signaling cascades leading to disruption of ion

homeostasis and modification of the genetic apparatus, with consequence of apoptotic cell death (Sun and Chen, 1998; Singh *et al*., 2004). This oxidative stress is implicated inter alia in the cause of carcinogenetic, inflammatory, infectious, cardiovascular and neurological diseases in man and animals (Nair *et al*., 2003). The brain is, in particular, very sensitive to oxidation stress possibly because of its high lipid content, high aerobic metabolic activity and low catalase activity (Halliwell and Gutteridge, 1985; Cao *et al*., 1988; Floyd and Carney J M, 1992; Gilgun-Sherki *et al*., 2001).

Oxidative stress is the result of an imbalance in the pro-oxidant /antioxidant homeostasis leading to the generation of excess reactive oxygen species (ROS), a collective name that includes superoxide (O_2), hydrogen peroxide (H_2O_2), hydroxyl (HO), peroxyl (ROO), alkoxy (RO), and nitric oxide (NO) in relation to oxygen as it gets reduced to water (Barnham *et al*., 2004; Singh *et al*., 2004). All aerobic organisms are susceptible to oxidative stress because ROS are produced by mitochondria during the respiration processes (Chance *et al*., 1979). Under normal conditions, the body is equipped with defense mechanisms that scavenge ROS and protect the cells from oxidative damage. Oxidative stress occurs when the detoxifying enzyme processes are overwhelmed, saturated, faulty, or are under conditions of low dietary antioxidant intake, inflammation, aging or exposure to environmental factors such as irradiation or tobacco smoke.

Antioxidants (AOX) are substances that inhibit or delay oxidation of a substrate while present in minute amounts (Halliwell and Gutteridge, 1990; Maxwell, 1995). In nature, AOX are grouped as endogenous or exogenous. The endogenous group includes enzymes (and trace elements as part-of) like superoxidase dismutase (zinc, manganese, and copper), glutathione peroxide (selenium) and catalase, and proteins like albumin, transferrin, ceruloplasmin, metalliothionein and haptoglobin. The most important exogenous AOX are dietary phytochemicals (such as polyphenols, quinines, flavonoids, catechins, coumarins, terpenoids) and the smaller molecules like ascorbic acid (Vitamin C), alpha-tocopherol (Vitamin E) and beta-carotene vitamin-E, carotenoids, and supplements (Nair *et al*., 2003). The antioxidant processes occur in cytosol, mitochondria or in plasma (Larson 1988; Namiki *et al*, 1993; Berger, 2005). Though their mode of action is not yet completely elucidated and though clinical trials involving them are still relatively scarce, AOX offer a promising approach in the control or slowing down progression of neurodegenerative disorders such as Alzheimer's, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis and ischaemic and haemorrhagic stroke (Maxwell, 1995; Floyd, 1999; Mattson, 2000; Moosmann and Behl, 2002; Nair *et al*., 2003; Berger, 2005). Plant derived antioxidants offer prospects in this regard. To write off antioxidants as potentially harmful, is ultimately keeping a powerful weapon out of the therapeutic arsenal.

Strategies aimed at limiting ROS oxidative stress damage, may slow the progression of neurodegenerative diseases (Halliwell, 2001; Singh *et al*., 2004). Since endogenous AOX defences are not always completely effective, and since exposure to damaging environmental factors is increasing, exogenous AOX will find more of a role in diminishing the cumulative effects of oxidative damage (Gilgun-Sherki *et al*., 2001). Many plants nevertheless have been scientifically proved to be effective in control of acute and chronic nervous disorders. As herbal extracts are a complex mixture of compounds, the active molecules, the mode of action, bioavailability and pharmacokinetics, and toxicity issues become difficult to evaluate. Plant-derived AOX are regarded as effective in controlling the effects of oxidative damage, and hence have had influence in what people eat and drink (Viana *et al*., 1996; Sun *et al*., 2002; Pinder and Sandler, 2004).

2.3 Anthelmintic activity

In the tropics and sub-tropics, helminthosis remains one of the most prevalent and economically important parasitoses of livestock (Perry and Randolph, 1999). Gastrointestinal nematodes are a major constraint to economic productivity of livestock as they constitute the chief parasitoses responsible for disease-related production losses arising from stock mortality, severe weight loss and poor production, especially in small ruminants (Chiejina, 2001; Perry *et al*., 2002).

Control of gastrointestinal nematode infections of small ruminants is almost exclusively by use of proprietary anthelmintics. However, in the extreme situations of subsistence farming where anthelmintics are either unavailable or unaffordable, massive mortalities of young stock are tragically commonplace in tropical Africa and Asia (Anon, 1992; Griggs, 1996). Alternatively, misuse and or widespread intensive use of sometimes poor quality pharmaceutically derived anthelmintics has led to development of high level multiple anthelmintic resistance that may lead to failure of control of worm parasites in ruminants (Prichard, 1990; Maciel *et al*., 1996; Monteiro *et al*., 1998; Wolstenholme *et al*., 2004; Jabbar *et al*., 2006). These constraints have made the reliance on pharmaceutically derived anthelmintics difficult in the management of GI parasitic infections in livestock, necessitating novel alternative methods of helminth control. Hence pastoralists and smallholder farmers have continued with the ethnoveterinary practices of using indigenous plant preparations as livestock dewormers (Danø and Bøgh, 1999). The use of traditional remedies may present a cheaper, sustainable alternative if the plant compounds were effectively validated. In addition, there are concerns about the detrimental effects of the chemical anthelmintics on the environment (Cox, 1999) and consumer concerns over potential drug residues in animal products (Knox, 2000).

In recent times, there has been an increasing interest in ethnomedical and ethnoveterinary practices across the world, especially as it relates to the use of medicinal plants in treating various ailments. Use of indigenous plant preparations as livestock dewormers is gaining ground as one of the alternative and sustainable methods readily adaptable to rural farming communities (Hammond *et al*., 1997; Danø and Bøgh, 1999). Important opportunities exist through research on traditional use of herbal medicine, since 80% of people in developing countries rely on phytomedicine for primary healthcare in both humans and animals (Plotkin, 1992; McCorkle *et al*., 1996). Attempts to dismiss the validity of medical knowledge gained through centuries of practical experience is regarded as unfair, for if a drug has been used for ages, repeatedly asked for by patients and prescribed by doctors, one must assume that it is effective, even without double–blind studies (Weiss and Fintelmann, 2000).

2.4 *Peltophorum africanum*

2.4.1 General aspects

Peltophorum africanum Sond (weeping wattle; African wattle; mosetha; huilboom) is a member of the Fabaceae. It is a deciduous tree that grows up to 15 m high with a wide canopy, and is widespread in sub-Saharan Africa and other tropical regions (Venter and Venter, 2002; Palgrave, 2002). The bark in old trees is grey brown with longitudinal fissures or grooves. The common name 'weeping wattle' is due to sap-sucking insects (*Ptyelus grossus*) that attach to the branches, and some of the sap drips down wetting the ground. The stem and leaves bear no thorns. The leaves are acacia-like, silver grey and covered with fine hairs, and are bi-pinnate up to the tip. The flowers have yellow petals, and the fruits are flat ellipsoidal pods tapering to both ends. Livestock eat young leaves and pods.

2.4.2 Ethnomedical and ethnoveterinary use

P. africanum is a unique plant in that it is traditionally used to treat, *inter alia*, diarrhoea, dysentery, helminthosis and promotion of well-being and resistance to diseases in man and animals (Watt and Breyer-Brandwijk, 1962; Van der Merwe, 2000; Van Wyk and Gericke, 2000). Pastoralists have traditionally used it in combination with other plants in animal healthcare practices (Cunningham and Zondi, 1991). The root is one of the ingredients in the 'Kgatla doctors' mixture to promote fertility and the well-being of cattle, the prepared medicine being known as 'leswalo' (Watt and Breyer-Brandwijk, 1962). An infusion of the root bark is also used as a tonic for general improvement and resistance to disease in cattle (van der Merwe, 2000). A decoction of the mixture of *P. africanum* and *Sclerocarya birrea* is used to treat diarrhoea and dysentery

in cattle (Watt and Breyer-Brandwijk, 1962; van der Merwe, 2000). From the foregoing, the plant may have antimicrobial, antiparasitic and antioxidant compounds.

2.4.3 Phytochemistry

Several authors have investigated the phytohemistry of *P. africanum*. Evans *et al*., 1985 isolated a sulphate ester of trans-4-hydroxypipecolic acid in the seeds. Several condensed flavanoids, a novel cyanomaclurin analogue (Bam *et al*., 1988), profisetinidin-type 4-arylflavan-3-ols and related δ-lactones (Bam *et al*., 1990) were found in the heartwood. Mebe and Makuhunga (1992) isolated new compounds (bergenin, norbergenin, and 11-0 - (E)-p-coumaroylbergenin) from an ethanol extract of the bark. Many flavonoids and coumarins are known antioxidants (Das and Ramanathan, 1992; Paya *et al*., 1992; Haragushi, 2001). Some antioxidants from plants have been shown to have antimicrobial activity (Miski *et al*., 1983; Haragushi *et al*., 1998). However, there are only few reports of detailed studies on the bioactivity of the compounds from roots, leaves and bark of *P. africanum*.

2.4.4 Biological activity

Mlambo and Munjeri (1988) showed that leaf extracts acted on beta-adrenergic receptors in the rabbit jejunum to reduce its contractions (an effect blocked by propranolol) and concluded that the plant was a potential source of pharmacological agents. Mølgaard *et al*., 2001 demonstrated activity for extracts of the leaf and stem against the cestode, *Hymenolepis dimunita*. Leaf extracts have been shown to have antibacterial activity when tested against some nasocomial pathogens (Manana, 2003). *In vitro* antibacterial (Obi *et al*., 2003; Samie *et al*., 2005) activities, and inhibitory properties against the human immunodeficiency virus (HIV) type 1 reverse transcriptase and integrase (Bessong *et al*., 2005) of the leaf, bark and root extracts of *P. africanum* have been reported. Nevertheless, much work remains in the study and characterization of bioactive compounds extracted from *P. africanum*, and their clinical evaluation *in vivo*.