CHAPTER 1

INTRODUCTION

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1.1 Background

1.1.1 Historical evidence of the traditional use of medicinal plants

People in all continents have been using infusions of plant extracts for various ailments for centuries. Over decades, traditional medicine has significantly increased as global importance with profound effect in both health and international trade. In both Old and New worlds some of the archaeological records showed that medicinal plants have been used for centuries as remedies for human diseases because of their therapeutic value (Nostro *et al*., 2000). Civilisations of the ancient Chinese, Indians, and North Africans furnished written proof of man's resourcefulness in utilising plants for the treatment of a wide variety of ailments (Phillipson, 2001).

Egyptian pharaohs sent scouts far and wide searching for medicinal plants (Schultes & Von Reis, 1995). In ancient Egypt, garlic was used as part of early diet and it was fed to people involved in heavy labour such as building of the pyramids. Garlic was also used for the treatment of abnormal growths such as abscesses. In ancient Greece, garlic was fed to the soldiers to give them courage during war and and was also given to the athletes before they competed the Olympic games. Garlic was also used to protect the skin against poisons and toxins. Hippocrates, widely regarded as the father of Medicine advocated the use of garlic for various ailments. In ancient Rome, Japan and China, garlic was used for digestion, respiratory ailments,

arthritis, convulsions, animal bites, alleviate depression and for male potency (Rivlin, 2001).

In the middle of nineteen century, not less than 80% of medicines were developed from plants. Aspirin, atropia, artemisinin, atropine, taxol, morphine, reserpine, pilocarpine, quinine, digoxin, ephedrine and quinidine are some of the examples of plant-derived drugs that were initially discovered through the study of traditional remedies and folk knowledge of indigenous people. From the opium poppy (*Paper somniferum*), morphine was isolated and is one of the early molecules that entered into conventional medicine as the best painkiller available to humans. Reserpine isolated from the roots of *Rauwolfia serpentine* made in roads in modern Western medicine in 1949 for its ability to treat hypertension. Later it was established through clinical research that interest in the product gradually diminished when safer antihypertensive drugs were available after the unfavourable side effects of reserpine were determined. The adverse effects were depression and parkinsonism (Gilani and Atta-ur-Rahman, 2005). An active compound from Willow bark, aspirin is considered as one of the most effective analgesic, antipyretic, and anti-inflammatory agents that is usually utilised in modern medicine. Aspirin has been found recently to have antiplatelet/anticoagulant properties. The raw ingredients of a common aspirin tablet have been used as painkiller long before the tablet manufacturing machinery was invented (Gilani and Atta-ur-Rahman, 2005).

Taxol obtained from the bark of *Taxus brevifolia* was reported to have anticancer effect. Although the clinical trials did take place in the early 1980's it was until the 1990's that the semisynthetic derivative of taxol, taxotere were shown to be clinically active against breast and ovarian cancers. From *Artemisia annua*, the compound artemisinin has been shown during clinical trials that it is antimalarial and can be used to treat infections of multidrug resistant strains of *Plasmodium falciparum* (Phillipson, 2001).

Example of the drug developed from medicinal plant that failed in the clinical trial is bruceantin. Bruceantin was first isolated from a tree, *Brucea antidysenterica* (Simaroubaceae), used in Ethiopia for the treatment of cancer exhibited activity in animal models bearing tumours, but no object responses were observed in clinical trials. Further development for the drug was terminated (Gragg and Newman, 2004). The compounds, 'lapachol' isolated from the species *Tabebuia rosea* and *T. serratifolia* showed significant in vivo anti-tumour activity in some early mouse models and was advanced to clinical trials in the 1970s, but they were terminated due to high unacceptable levels of toxicity (Gragg and Newman, 2004).

Europeans discovered a tree called 'Quin-Quin' on the Eastern slope of Andes Mountains in the early 1600s. The Indians used the bark of quin-quin to cure malaria (Kaufman, 1989). Historians have produced evidence that show that people in the past used plants as medicine. From *Cinchona* bark, quinine was used to treat the symptoms of malaria long before the disease was identified (Phillpson, 2001).

The information on medicinal use of plants in Brazil was recorded in the book '*Historia Naturales Brasiliae*' in 1648 by the physician Guilherme Piso (Schultes & von Reis, 1995). In Mexico and Guatemala, many engravings narrate the use of different medicinal or hallucinogenic plants by local people (Schultes & von Reis, 1995). Use of medicinal plants has been stated for centuries as an aphrodisiac, as a tonic to increase mental and physical efficiency, to combat stress and as a cure for many human illnesses (Kaufman, 1989). In Iraq plant materials of a number of species have been found to have some horticultural use but a significant number of plants are also being used in local medicine as well. Chinese ethnopharmacological knowledge dates back several thousand years and some written records date from the beginning of the Christian era (Schultes & von Reis, 1995).

In Latin America 71% of the people in Chile and 40% in Columbia use traditional

medicinal plants. In China 40% of the health care is traditional medicine. In the United Kingdom 40% of allopathic practitioners offer the use of traditional medicine. Forty two percent of the population in the US, 70% in Canada, 48% in Australia, 38% in Belgium have at least used traditional medicine once at some stage (Bussmann and Sharon, 2006).

Many countries in Africa, Asia and Latin America use traditional medicine to help meet some of their primary health care. In industrialized countries, adaptations of traditional medicine are termed "complementary" or "alternative". In China, traditional herb medicine preparations account for 30 % - 50% of the total medical consumption these days. In Ghana, Mali, Nigeria and Zambia, 60% of children with high fever resulting from malaria is treated first with an herbal medicine at home (Kassaye *et al*., 2006).

1.1.2 The use of traditional medicine in South Africa

In Africa a move has begun to record medicinal plants, evaluate them and make valuable products (Neuwinger, 2000). A text has been compiled (Ross, 2003) describing the traditional use, chemical constituents, pharmacological activities and clinical trials of the commonly used medicinal plants found in Africa. Various African communities still use traditional remedies for primary health care (Louw *et al*., 2002). It is through the development of traditional medicine that the therapeutic effect of drugs has been revealed (Hikino, 1991). Despite the important contribution that medicinal plants make to primary health care, Western and traditional medicine systems seldom work together (Taylor & Van Staden, 2001).

In developing world, particularly in Africa up to 80% of the population uses traditional medicine as part of primary health care system (Bussmann and Sharon, 2006). About 4000 species of plants are used as traditional remedies for ailments in southern Africa (Van Wyk

and Gericke, 2000). In South Africa a large section of utilised medicines are still derived from plants and their extracts. Plants and their extracts are sold both in the informal and the commercial sector of the economy. Up to 60% of the South African population consult one of an estimated 200 000 traditional healers in preference to or in addition to Western medical doctors (Van Wyk *et al*., 1997; Taylor and Van Staden, 2001). An estimation of 27 million South Africans use traditional herbal medicine from more than 1020 plant species (Stafford *et al.*, 2005). Although traditional medicine is firmly rooted in the past, it is a dynamic and adaptive indigenous system of medicine.

South African medicinal plants are used for a wide variety of ailments such as diarrhoea, headaches, heart problems, inflammation, prevention of abortion, etc. South Africa's contribution to the world's medicines from plants are, just to name a few, 'Buchu' *(Agathosma betulina*) for the treatment of kidney and urinary tract, stomach problems, rheumatism and wounds, *Aloe ferox* for the treatment of eczema, arthritis, conjunctivitis, hypertension, stress and as a laxative, and 'Devil's claw' (*Harpagophytum procumbens*) for the treatment of rheumatism and arthritis (Van Wyk *et al.,* 1997; Van Wyk and Gericke, 2000). *H. procumbens* has been reported for the treatment in reducing the pain associated with osteoarthritis and other related chronic conditions (McGregor *et al*., 2005). *H. procumbens* and *Sutherlandia frutescens* have been reported to be used in South Africa for the management of pain and inflammation (Kundu *et al*., 2005). The phytochemical screening of *H. procumbens* was found to have iridoid glycosides, acetylated glycosides and terpenoids. The *S. frutescens* has been reported to contain arginine, γ-aminobutyric acid and pinitol (Kundu *et al*., 2005). *S. frutescens* has been reported to have antibacterial activity against *Staphylococcus aureus*, *Enterococcus faecalis* and *Escherichia coli.* The medicinal use of *S. frutescens* for staphylococcal infections, when formulated in an oily base seems to have a rational basis (Katerere and Eloff, 2005). One plant that is indigenous to

South Africa and Namibia that has been shown to have a commercial spin off is *Hoodia* in particular the *H. gordonii species. H. gordonii* belongs to the *Apocynaceae* family. *H. gordonii* has been found to have an appetite suppressant properties (Van Heerden, 2008; Lee and Balick, 2007; Van Heerden *et al*., 2007). *Hoodia* is being used by San people of South Africa as an appetite suppressant. The species has been commercialised as a weight loss product and therefore, it has been widely marketed (Vermaak *et al*., 2010(a)). *H. gordonii* has been marketed as a functional food and products in dosage form that include tablets, capsules, powders, sprays, tea, fruit and chocolate bars (Vermaak *et al*., 2010(b)). Another species of *Hoodia*, *H. pilifera* has also been found to have appetite suppressing activity on rats. It is not surprising that the *Hoodia* species has been patented (Van Heerden *et al*., 2007; Moyer-Henry 2008). The Council for Scientific Research Council (CSIR) patented the compound responsible for suppression from *H. gordonii* and named it P57. Initially CSIR did not give any credit to San people, the owners of indigenous knowledge about *Hoodia* who accused CSIR and its partners of biopiracy. The South African government intervened by offering cash settlement to San people for using their indigenous knowledge. The San people from four countries South Africa, Botswana, Namibia and Angola were offered payment of at least $30 000 during clinical testing of *Hoodia* and 6% of the royalties received on the market (Moyer-Henry, 2008). This clearly shows that the bioactive compounds from plants do play a role in discovering remedies for various ailments.

The following South African plants, *Artemisia afra*, *Pteronia incana* and *Rosmarinus officinalis* were tested against *L. monocytogenes* and other pathogens. The three plants were found to have antilisterial activity (Mangena and Muyina, 1999; Sandasi *et al*., 2010). Another South African medicinal plant, *Agathosma betulina* has been reported to have antilisterial activity (Molla and Viljoen, 2008). *Echinacea angustifolia*, *Thymus vulgaris* and *Mentha*

piperita are some of the South African plants that have been reported to have antilisterial activity. The plants were also found to have inhibitory effect against the formation and development of the listerial biofilm (Sandasi *et al*., 2010).

Listeriosis is reported mainly from industrialized countries with few or no reports from Africa, Asia and South America (Rocourt *et al*., 1990). In South Africa there is a dearth of literature on the epidemiology of the microorganisms. South Africa has one of the highest rates of HIV infection in Africa and the immunocompromised are susceptible to listerial infection. The last official report of listeriosis from South Africa was in 1978 (Jacobs *et al*., 1978). There is limited research on the South African plants that have antilisterial activity. Most of the research is on plants that have antimycobacterium, antimalaria, anti-oxidant and antiHIV activities. Due to the side effects of existing antilisterial drugs it was decided to explore the potential of South African plants for listerial infections in the present study.

1.2 Listeriosis

1.2.1 Epidemiology

Listeria monocytogenes is a food-borne pathogen of public health concern (Schmid *et al*., 2009) which causes serious diseases such as encephalitis sepsis and meningitis, endocarditis, etc in humans. A pregnant woman infected with these bacteria may suffer from flu-like febrile illness and depending on the stage of pregnancy the foetus may be either stillborn (abortion) or born with signs of infection (Goldenberg and Thompson, 2003; de Souza *et al*., 2008). *L. monocytogenes* is widespread in nature and because of its ubiquity, the pathogen is frequently isolated from foodstuff and food related environments (Amalaradjou *et al*., 2009). *L. monocytogenes* is pathogenic not only to humans but also to animals (Hof, 2003(a)). *L.*

monocytogenes is found widely in a variety of ready-to-eat foodstuff (Saunders *et al*., 2005, Schmid *et al*., 2009). People most prone to the disease are pregnant women, newborns, the elderly, and those with Human immuno virus (HIV) and immunocompromised (DiMaio, 2000; Békondi *et al*., 2006). Listeriosis is a severe human infection, characterised by gastro-enteritis, meningitis, encephalitis, septicaemia, spontaneous abortions and deaths (Guzman *et al*., 1995; Goldenberg and Thompson, 2003; de Souza, 2008). Listeriosis is mainly reported from industrialised nations with few or no report from Africa, Asia and South America (Racourt *et al*., 2000). In most industrialised countries listeriosis has been made a reportable disease and these countries have implemented active surveillance of food and food processing plants (Pagotto, *et al*., 2006). Table 1.1 shows gastrointestinal listeriosis outbreak from 1993 to 2001 reported in the industrialised nations.

Table 1.1. Gastrointestinal listeriosis outbreaks, 1993-2001 (Table adapted from Swaminathan and Gerner-Smidt, 2007)

Year	**Location**	**Number of cases**	**Implicated source**
1993	Northern Italy	18	Rice salad
1994	Illinois, USA	44	Chocolate milk
1997	Northern Italy	1566	Cold corn and tuna salad
1998	Finland	N/A	Cold-smoked fish
2000	New Zealand	32	Ready–to-eat meat
2001	California, USA	16	Delicatessen turkey ready-to-eat meat
2001	Sweden	48	Raw milk cheese
2001	Japan	38	Cheese

N/A: number of cases not given.

Symptoms of listerial meningitis usually begins with mild flu, followed by a sudden pulsating fever, pain, stiffness of the neck and back, nausea, vomiting, lethargy, flu, headache, and inflammation (Nester *et al*., 2001; Prescott *et al*., 2005).

Cases of listeriosis have been reported in Canada), the United States, Japan, Germany, Australia, South Africa and in other parts of the world. *L. monocytogenes* is a zoonotic foodborne pathogen that is responsible for 28% of food related deaths in the United States annually (Borucki *et al*., 2004; Amalaradjou *et al.*, 2009). When an outbreak of listeriosis cropped up in California in 1985 (Table1.2), almost every case was traced back to a fresh, soft cheese made with contaminated milk that had not been properly pasteurised. Forty-eight deaths (Table 1.2) were reported of which 30 were among foetuses and newborns (Talaro and Talaro, 1993). Mortality cases of listeriosis in 2 500 cases per year in the US has mortality of approximately 20% (Tominga, 2006). Because of its widespread in nature, *L. monocytogenes* has been frequently isolated from foods and food processing environments. *L. monocytogenes* has been found to be able to adhere all food contact surfaces such as glass, stainless steel and rubber (Amalaradjou *et al*., 2009). The organism is a challenge to food production industry as well as food related environments (Schmid *et al*., 2009). Forty-two cases of listeriosis from human isolates were reported in Maryland and California in 2000 and 2001, out of the 4500 isolates from cases that had occurred throughout the United States (Gray *et al*., 2004).

A listeriosis epidemic in newborns was recorded in Germany in 1949. In eighty-five newborns or stillborn infants, granulomas were detected histopathologically in various organs such as the spleen, brain, lung, liver and skin (Hof, 2003(a)). During the same year, Holland reported 4.3 cases per million inhabitants per year. England and Wales reported 2.6 cases per million inhabitants per year from 2001 to 2004.

Table 1.2 Outbreaks of invasive listeriosis, 1981-2003 (Adapted from Swaminatham and Gerner-Smidt, 2007)

Year	Location	No. of cases	Perinatal cases	No of deaths	Source/implicated vehicle
1981	Nova Scotia, Canada	41	34	18	Coleslaw
1983	Massachusetts, USA	49	7	14	Pasteurized milk
1985	California, USA	142	94	48	Mexican-style cheese
1983-1987	Switzerland	122	65	34	Vacherin Mont d'Or cheese
1987-1989	United Kingdom	366	?	?	Paté
1989-1990	Denmark	26	3	7	Blue mold cheese
1992	France	279	0	85	Pork tongue in jelly
1993	France	38	31	10	Rillets
1998-1999	Multiple states, USA	108	?	14	Hot dogs
1999	Finland	25	0	6	Butter
1999-2000	France	32	9	10	Pork tongue in aspic
2000	Multiple states, USA	30	8	7	Delicatessen turkey ready-to-eat meats
2000	North Carolina, USA	13	11	5	Home-made Mexican – style cheese
2002	Multiple states, USA	54	12	8	Delicatessen turkey
2002	Quebec, Canada	17	3	0	Cheese made from raw milk

? Number not known

Fifty-seven cases of listeriosis occurred in Switzerland during an outbreak, which was caused by the consumption of soft cheese (Bula *et al*., 1995). The mortality was reported to be 32% from the 57 cases. In Japan a nationwide surveillance study of listeriosis was performed and from the data collected between 1980 and 2002, 95 cases were identified (OzFoodNet Working Group, 2003).

In Johannesburg (South Africa) during August 1977 to April 1978, 14 patients were reported to have systematic infections due to *L. monocytogenes* (Jacobs *et al*., 1978). Out of the 14 patients, nine were neonates who had septicaemia and meningitis. The mortality rate was 43% (Jacobs *et al*., 1978). It has been found that 17% of patients admitted to hospital with prosthetic endocarditis (caused by *L. monocytogenes*) die in hospital (Miguel-Yanes *et al*., 2004).

1.2.2 Listeriosis in animals

L. monocytogenes pathogen does not only affect humans but also the animals. According to Jemmi & Stephan (2006) infection in both animals and humans occurs in the following manner, namely, an entry of the pathogen into the host, lysis of phagosomal vacuole, multiplication in the cytosol and finally the direct cell-to cell spread using actin-based motility. Cattle and other ruminants (Figure 1.1) can be infected with *L. monocytogenes* by consuming contaminated plant materials, soil or silage (Mantovani & Russel, 2003). In ruminants, *L. monocytogenes*, primarily causes encephalitis and uterine infections. These infections are characterised by late-term abortions or septicaemia in neonates (Nightingale *et al*., 2004). It is clear from literature (Loeb, 2004; Miyashita *et al*., 2004; Nightingale *et al*., 2004; Evans *et al*., 2004) that listeriosis does affect the animals in the same way as it affects humans. Septicaemia and abortions in a housed flock of sheep have been reported (Low & Renton, 1985). An outbreak

of listeriosis in horses, cattle and other ruminants have been reported (Vandegraaf *et al*., 1981; du Toit, 1977; Loeb, 2004*).* In Germany, Bavarian cattle clinically suspected of bovine spongiform encephalitis were diagnosed with listeriosis (Miyashita *et al*., 2004). Du Toit (1977) reported an outbreak of listeriosis in the Western Cape province of South Africa. Abortion in sheep caused listeric-infection has been reported (Dennis, 1975). The cause of listeriosis in Gippland, Victoria in 1978 was confirmed by the histology of the brain or culture of *L. monocytogenes* from sheep on 21 farms (Vandergraaf *et al.,* 1981). The fatality rate from the affected flocks (0. 2 to 8.0 %) was almost 100 %. An analysis of 42 ruminants with suspected meningo-encephalitis caused by *Listeria* was reported in the Netherlands (Loeb, 2004). Listeriosis may be the cause of eye infections in ruminants and horses (Evans *et al*., 2004).

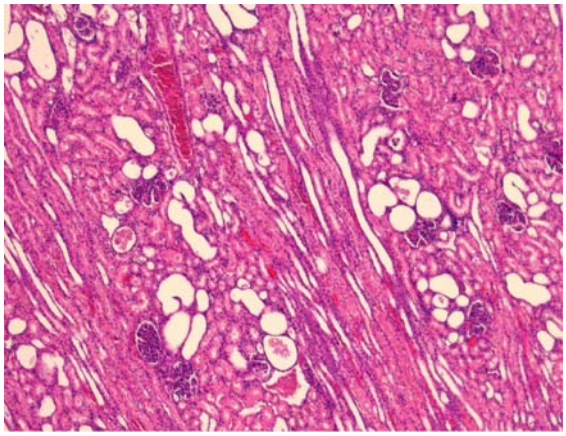

Figure 1.1 Characteristic lesions of acute renal tubular necrosis including renal tubular dilation and flattening of the renal tubular epithelium in sheep caused by *L. monocytogenes* (magnification x 40) - photo William Byrne (Regional Veterinary Laboratories, 2006**)**

1.2.3 Causative agent of listeriosis – *Listeria monocytogenes*

Listeria belonging to the family *Listeriaceae* contains short rods that are aerobic or or facultative, catalase positive, and motile (Prescott *et al*., 2005). The genus comprises six species,

two pathogenic species *L. monocytogens* and *L. ivanovii*, and four non-pathogenic *L. welshimeri*, *L. grayi*, *L. innocua* and *L. seeligeri*. *L. monocytogenes* was first discovered in 1924 by E.G.D Murray when he isolated the gram positive rods from the blood of rabbits in the laboratory. It was called *Bacterium monocytogenes,* the genus name was then changed to *Listeria* by J.H.H. Pirie in 1940 after it was isolated from humans (Hof, 2003 (a)). *L. monocytogenes* is a facultative anaerobic motile non-spore forming species (Prescott *et al.,* 2005). Factors that affecting the pathogenicity of *L. monocytogenes* its capacity for intracellular growth, iron compounds, catalase and superoxide dismutase, surface components, hemolysins – have been proposed over the years, indicating that its virulence is caused by a number of factors (Farber and Peterkin, 1991). It is a ubiquitous opportunistic pathogen causing fatal infections known as listeriosis (Poros-Gluchowska & Markiewicz, 1988; Dussurget *et al*., 2004).

1.2.4 Drug resistance of listeriosis

Listeriosis is treated with a series of different antibiotics (Hof, 2003 (b)). Drugs that are available to treat listeriosis in humans are ampicillin, trimethoprim sulfamethoxazole, erythromycin, vancomycin, fluroquinolones, etc. (Becq-Giraudon and Breux, 1987; Temple and Nahata, 2001; Benes *et al*., 2002; Poros-Gluchowska and Markiewcz, 2003; Gunter and Philipson, 1988; Friedrich *et al.,* 1990).

The antibiotic, 'Meropenem' has been found to be effective in the treatment of meningitis caused by *L. monocytogenes* in animals (Nairn *et al*., 1995). Meropenem is able to penetrate into the cerebrospinal fluid (CSF) to produce effective reduction in a number of pathogens (Nairn *et al.,* 1995). The drugs used for the treatment of listeriosis in animals are coumermycin Hof, 1991) and levofloxacin (Nichterlein *et al.,* 1998(a)). Levofloxacin has been found to be

(effective in the treatment of infections caused by facultative intracellular Gram-positive such as *L. monocytogenes* (Nichterlein *et al*., 1998(a)).

L. monoctogenes has been found to be resistant to some antibiotics (Nichterlein *et al.,* 1998(b); Temple & Nahata, 2000; Hof, 2003 (b); Friedrich *et al*., 1990). In most cases (Cone *et al*., 2003) it has been found that listeriosis treatment needs multidrug therapy. Therefore the combination of two or more antibiotics is needed for treatment (Cone *et al*., 2003; Poros-Gluchowska and Markiewicz, 2003; Temple & Nahata, 2000; Nichterlein *et al*., 1998 (b); Rossi *et al*., 2001; Hof, 2003 (b); Friedrich *et al*., 1990). Therapy with high dose of ampicillin in combination with gentamicin was the preferred treatment in humans (Cone *et al.,* 2003). Most antibiotics are not bactericidal for *L. monocytogenes.* The combination of various drugs may exert a synergistic effect against *L. monocytogenes* (Hof, 2003 (b)). It has been reported that erythromycin is inefficient against *L. monocytogenes* in multi-drug resistant cells. Erythromycin was unable to curb the growth of *L. monocytogenes* in multi-drug resistant human carcinoma cell lines (KBV-1 MDR cells) but restricted the growth of the bacteria in non-resistant human epidermoid carcinoma cell lines (KB3-1 cells) (Nichterlein *et al*., 1995).

Certain host cells may have gained the property of eradicating some bacteria, for example macrolides (family of antibiotics used to treat a wide range of bacterial infections) from intracellular spaces, which might elucidate therapeutic failures of antibiotic therapy in spite of low MICs (Hof *et al*., 1997). The high cost involved in the manufacturing of the synthetic drugs has also made the available drugs used for the treatment of listeriosis expensive. The combination of trimethoprim/sulfamethoxazole is the preferred treatment for listeriosis (Gleckman & Borrego, 1997; Poros-Gluchowska & Markiewcz, 2003). Ampicillin, amoxicillin vancomycin, erythromycin, are also used for the treatment of listeriosis. The side effects of these drugs are quite alarming. Trimethoprim/sulfamethoxazole increases the risk of

hypoglycaemia, bone marrow suppression and increases the anticoagulant effect in patients. Ampicillin increases the frequency of rash, vancomycin results in hypersensitivity reactions (red-neck or red-man syndrome) and amoxicillin causes diarrhoea (Gleckman & Borrego, 1997). In one study it was reported that drug related toxicity was one of the most common causes of death for hospitalized patients (Gleckman & Czachor, 2000). Finding the most effective novel drugs from plants against *L. monocytogenes* could reduce the risk of multidrug resistant species and reduce the treatment costs.

1.3 Scope of the thesis

1.3.1 Antilisterial activity of plant extracts

Medicinal plants such as *Clivia miniata, Artemisia afra, Aloe arborescens, Tulbaghia violacea, Heteromorpha arborescens*, etc are used by South Africans to treat listeriosis related symptoms such as fever, flu, headache, inflammation, heart condition, etc. (Van Wyk *et al*., 1997; Van Wyk & Gericke, 2000). The high cost of synthetic drugs and the problem of multidrug resistance have necessitated the need to explore the potential of South African medicinal plants for antilisterial activity. The extracts from plants are inexpensive and accessible. The antilisterial activity of local medicinal plants that have been used to treat listeriosis symptoms was investigated for this study.

1.3.2 Susceptibility testing (to the plant extracts) of *L. monocytogenes*

The disc diffusion method was used to test antibacterial activity against *L. monocytogenes* in order to select the best extract for further tests as described by Alzoreky & Nakahara (2003).

The disc method is usually used to exhibit growth inhibition of microorganism by plant extracts (Masika and Afolayan, 1998; Rabe and Van Staden, 1998; Pretorius *et al*., 2003). Sterile filter paper impregnated with plant extracts were placed on petri dishes. Bacterial culture was spread on Petri dishes and incubated for 24 hours. Zone of bacterial inhibition was determined. Selected exracts were investigated to determine their minimum inhibitory concentrations (MIC) against *L. monocytogenes* using the microtitre dilution method (Eloff, 1998). In the liquid medium there is both contact of the extract and microorganism hence this is more sensitive and accurate than the disc diffusion. The *p*-iodonitrotetrazolium violet (INT) was used to determine the viability of bacteria. The microtitre method or microboth dilution method by Eloff (1998) has been used extensively by researchers in the determination of the MICs (Stafford *et al*., 2005; McGaw *et al*., 2007; Buwa and Afolayan, 2009; Amoo *et al*., 2009; Mulaudzi *et al*., 2009).

1.3.3 Cytotoxicity assay of plant extracts

Cytotoxicity evaluation of the plant extracts and their active principles are required for their effective therapeutic uses. Cytotoxicity tests are essential to ascertain the intrinsic ability of the extract or compound to cause harm to the cells or cell death as a result of damage to cellular functions (Bouaziz *et al*., 2006). Cytotoxicity tests are also necessary in the development of the potential drug as these tests provides crucial information on *in vitro* testing on parameters such as genotoxicity or programmed cell death (Bouaziz *et al*., 2006).

After establishing the antilisterial activity of 13 plant extracts against *L. monocytogenes*, the next step was to isolate the active compound(s) from one of the most active and least toxic of the plants. Cytotoxicity assay of plant samples were carried out on Vero cell lines with the intention to select a plant for the isolation of the active compound(s) with good antilisterial activity and

low toxicity.

1.3.4 Isolation, purification and identification of the active compound(s) from *Acacia karroo*

The isolation of natural products that have biological activity toward organisms other than the source has several advantages. The first advantage is that pure bioactive compounds can be administered in reproducible, accurate doses, with obvious benefits from an experimental or therapeutic point of view. Secondly, it can lead to the development of analytic assays for particular compounds or classes of compounds. This is necessary, for example in the screening of plants for potential toxicity and for quality control of food for human and animal consumption. Thirdly, it permits the structural determination of bioactive compounds that may enable the production of synthentic material, incorporation of structural modifications, and a rationalization of mechanisms of action. This, in turn, will lead to reduction in the dependency of plants as sources of bioactive compounds and will enable investigations of structure/activity relationships, facilitating the development of new compounds with similar or more desirable bioactivities.

Out of the thirteen plants screened for activity and cytotoxicity it was found that *Acacia karroo* and *Plectranthus ecklonii* exhibited the highest antilisterial activity as compared to the other plants investigated. Our objective was to isolate the bioactive compound(s) from *A. karroo* and *P. ecklonii* and determine the minimum inhibitory concentration (MIC) of the isolated compounds against *L. mononcytogenes*. Through the bioassay guided fractionation of the ethyl acetate extracts of *A. karroo* two bioactive compounds were isolated and identified. The MICs of the isolated compounds were determined against *L. monocytogenes.*

Bacterial biofilms are more resistant to the action of antimicrobial and disinfectant agents (García-Almendárez *et al*., 2007). Briefly, a standardized overnight culture was allowed to develop a biofilm on glass slides that had been previously coated with hundred microlitre of tryptone soya broth (TSB) to provide nutrients for adhering bacteria (Chae and Schraft, 2000). The viability and distribution of listerial cells exposed to crude extracts and pure compounds were investigated using the method described by Kives *et al*. (2005). Biofilms of *Listeria* were prepared by growing *L. monocytogenes* cells on sterile cover slips. The aggregation of the *Listeria* biofilm on exposure to the plant extracts and / or the purified compounds was investigated.

1.3.5 Isolation, purification and identification of the active compound(s) from *Plectranthus ecklonii*

Plectranthus ecklonii Benth. is traditionally used in South Africa for treating stomach-aches, nausea, vomiting and meningitis, all symptoms associated with listeriosis infection. Leaves of the plants are also used for respiratory problems, chest complaints and coughs (TB-related problems) (Lukhoba *et al*., 2003). The pure compounds as well as the crude extract of *P. ecklonii* were tested for activity against *L. monocytogenes*, *Staphylacoccus aureus, Escherichia coli, Mycobacterium tuberculosis,* and *M. smegmatis*. The minimum inhibitory concentration (MIC) and minimum bactericidal activity (MBC) of *P. ecklonii* were determined. Melanin is a key pigment responsible for skin and / or hair colour. Tyrosinase is an enzyme that catalyses the production of melanin. The inhibition of tyrosinase has an effect on the production of melanin. The skin problem such as albinism is a lack of tyrosinase. The antityrosinase activity of the extract of *P. ecklonii* and its isolated compounds were also investigated

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