ANTIBACTERIAL ACTIVITIES OF SELECTED MEDICINAL PLANTS USED TO TREAT SEXUALLY TRANSMITTED INFECTIONS IN BLOUBERG AREA, LIMPOPO PROVINCE.

BY

MONGALO NKOANA ISHMAEL

STUDENT NUMBER: 200814906

A dissertation submitted in fulfilment of the requirement for the Degree of Masters of Science in the Department of Botany, Faculty of Science and Agriculture, University of Zululand, KwaDlangezwa, South Africa

SUPERVISOR: PROF. A. M ZOBOLO

CO-SUPERVISOR: PROF. A. R OPOKU

FEBRUARY 2013

DEDICATION

This work is dedicated to the late Mrs Sepoleya Phyllis Mongalo-Sekgaphola, You have been a pillar of strength during difficult times from my birth until today. You are the best and I will always cherish the moment we shared.

ACKNOWLEDGEMENTS

I would like to pass my deepest gratitude and acknowledge the following people who diligently contributed to the success of this research work:

My parents Mphasha Samson and Mmamoyahabo Ramokone Mongalo for growing me up with a lot of love and showing me direction in my entire life.

Prof. AM Zobolo and Prof. AR Opoku for their guidance and support through my entire learning time, shaping me up.

MR Moraka Mokgehle who introduced me to a pool of Traditional healers who then contributed towards the indigenous identification of plants.

My brothers, Ngoako and Matome Mongalo, who physically assisted me in the collection of plant specimen in the field. National Biodiversity Institute (NBI) in Pretoria for identification of the plants.

Miss Isabel Rawlins for editing this research work

My family members (BoMmamarao ka moka), especially Bonekalisiwe, Thuto, Mphile, Koko Ramokone, Rakgolo Mphasha and Friends for their encouragement

DECLARATION

This study represents the original work by the author. Where use was made of the work of others, it has been duly acknowledged in the text. I declare the above statement to be true. Moreover, the experimental work described in this dissertation was conducted in the Department of Biochemistry and Microbiology and the Department of Botany, Faculty of Science and Agriculture, University of Zululand.

NKOANA ISHMAEL MONGALO

Prof. AM ZOBOLO

Prof. A.R. OPOKU

ABSTRACT

Thirteen traditional healers were interviewed using a structured questionnaire on the use of medicinal plants in the treatment of sexually transmitted infections within Blouberg area, Limpopo Province- South Africa. Eleven medicinal plants from eight families have been documented and sixty-two plant extracts were prepared and tested for antibacterial activity against 23 human pathogenic bacterial strains using disc diffusion method. The largest zone of inhibition of 15.7 mm was exhibited by methanol extract of *Elephantorrhiza burkei* root at 5mg/ml against *Klebsiella spp* and was comparable to 16.3 mm exhibited by streptomycin at 10 µg per disc. Extracts showing activity were further tested for minimal inhibitory concentrations (MIC) using micro-dilution assay. Lowest MIC of 0.2 mg/ml was exhibited by a number of extracts against variety of selected strains, including acetone extract of *Peltophorum africanum* against *Escherichia coli*.

Methanol extracts of selected plants were further screened for antioxidant properties against both 2, 2-azinobis-3-ethylbenzithiazoline-6-sulfonic acid (ABTS) and 2, 2-diphenyl-1-picryl-hydrazyl (DPPH). *Elehantorrhiza burkei* extract exhibited lowest IC_{50} of 0.10 mg/100 ml against DPPH, while *Jatropha zeyheri* extract exhibited 0.80 mg/100 ml against ABTS. Preliminary phytochemical tests revealed the presence of flavonoids (82 %), saponins (73 %), tannins (73 %), cardiac glycosides (55 %), alkaloids (55%) and anthraquinones (9%). This work validates the use of these medicinal plants in treatment of sexually transmitted

infections (STIs). Phytochemicals detected may well explain the biological activity reported.

List of Abbreviations used

ABTS-2, 2-azinobis-3-ethylbenzithiazoline-6-sulfonic acid)

AIDS- Acquired Immunodeficiency syndrome

DPPH- 2, 2-diphenyl-1-picryl-hydrazyl.

HIV-Human Immunodeficiency virus

IC_{50-} Plant extracts concentration at which 50 % of ABTS or DPPH is inhibited

IK- Indigenous Knowledge

IMK-Indigenous Medicinal Knowledge

IPR- Intellectual Property Right

MIC- Minimal Inhibitory Concentration

PID-Pulmonary Inflammatory Disease

RCS- Reactive Chlorine Species

RNS- Reactive Nitrogen Species

ROS-Reactive Oxygen Species

STI – Sexually Transmitted Infections

STD-Sexually Transmitted Diseases

TK-Traditional Knowledge

TSS-Toxic Shock Syndrome

VD-Venereal Disease

WHO- World Health Organisation

WIPO-World Intellectual Property Organisation

CONTRIBUTION TO KNOWLEDGE

JOURNAL ARTICLES

Mongalo NI, Opoku AR and Zobolo AM (2012). Antibacterial and antioxidant activity of the extracts of *Waltheria indica* Linn. collected from Capricorn District, Limpopo Province, South Africa. Journal of Medicinal Plants Research 6(43):5593-5598.

Mongalo NI, Opoku AR and Zobolo AM (2013). Antibacterial and antioxidant activity of extracts from *Waltheria indica* L. Presented at 39th Annual South African Association of Botany Conference, 21-24 January 2013, Drakensberg, South Africa. (Article will be published in South African Journal of Botany).

Mongalo NI, Opoku AR and Zobolo AM (2013). Antibacterial activity of root and leaf extracts of *Jatropha zeyheri* Sond (Euphorbiaceae). African Journal of Biotechnology 12(5):476-480.

CONFERENCE PRESENTATIONS

Mongalo NI, Opoku AR, Zobolo AM (2009) Antibacterial and antioxidant properties of selected medicinal plants used to treat sexually transmitted infectionsin Blouberg area, Limpopo Province. Presented at the 4th Annual Faculty of Science and Agriculture, University of Zululand.

Mongalo NI, Opoku AR, Zobolo AM (2010) Antibacterial and antioxidant properties of medicinal plants from Fabaceae family. Seminar Presented to Faculty of Science and Agriculture, 17th September 2010.

Table of Contents

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

1.1 Overview of medicinal plants and traditional healing.

A medicinal plant is any plant with one or more of its organ(s) containing substance(s) that could be used for therapeutic purpose (treating and preventing) or from which a precursor for synthesis of useful drugs may be isolated. For centuries, medicinal plants have been widely used by man to treat a variety of illnesses - irrespective of pathogenic origin, hence serving as a fundamental component of indigenous health care systems. Even today, man depends solely on plants for survival and maintenance of health (Ramar *et al*., 2008). In South Africa, over 80 % of households use medicinal plants (Mulholland, 2005). This is because traditional medicines are more readily available, cheap, and are said to be “natural” thereby having less adverse or no side effects on patients, as Western medicines often do (Ibrahim *et al*., 2007).

It is important to note that medicinal plants form integral part of traditional medicine. Apart from pathogenic microbes, diseases can be caused by free radicals which may be accumulated due to the presence of by-products of biochemical processes in our bodies. Medicinal plants produce secondary metabolites which possibly scavenge such radicals and also exhibit antimicrobial activity, thereby serving as a source of primary health within a society.

Traditional healing mostly involves the use of knuckle bones and materials from both plants and animals to diagnose and treat illnesses respectively. There are ancient, traditional beliefs by lay people that specific plants can be used in the treatment of sexually transmitted infections (STIs).

1.2 Hypothesis and research questions

The first hypothesis for this study is that selected medicinal plants are active against selected bacterial strains, hence possessing both antibacterial and free radical scavenging activity.

Research questions include: Are there plant species used to treat STIs within Blouberg area? Are plant species largely active against Gram positive or Gram negative bacterial strains? Are the antioxidant properties of the selected plants equal or related in both ABTS and DPPH tests.

1.3 Aims and objectives

Aims of this study are:

- To identify and document medicinal plants utilised to treat STIs.
- To conduct a comprehensive investigation of ways and methods of treating STIs within the Blouberg area, Limpopo Province. This raises questions such as:
    - ❖ Is the genus or plant used alone or in combination with others?
    - ❖ What quantity of plant material is used for a single treatment?
    - ❖ Is it boiled or not?
    - ❖ What is the final volume?
    - ❖ Do you drink it or use it to wash the lesions on the genitals?

- ❖ What is the frequency of drinking or washing per day?
- ❖ How many spoons or cups do you drink at a time?

- To screen identified plant species for antibacterial activity, antioxidant properties and various classes of phytochemicals.
- To expand the quality and quantity of information available for research and development especially in the area of new drug discovery and development.
- To give feed back to the community about the efficiency of the plants they use on daily basis.

1.4 Motivation for the study

This study was motivated by the reportes high STI infections, teen pregnancy and HIV-AIDS infection rate in the Capricorn District. In Africa, 93 000 deaths were reportedly due to sexually transmitted diseases excluding HIV in 2002 and 65 million people are living with an incurable sexually transmitted disease (WHO, 2004). Sexually transmitted diseases are amongst the top five illnesses which need to be prioritised as a matter of urgency (WHO, 2001). To our knowledge, there are no medicinal plants documented among the Sepedi speaking communities that are used to treat STIs.

1.5 Scope of study

The scope of this work is as follows. Chapter 1 will introduce the topic, Chapter 2 will have the literature review, Chapter 3 will provide materials and methods, Chapter 4 will express the results, Chapter 5 is discussions and Chapter 6 will convey the conclusions of this research and the references will be in Chapter 7. The purpose of this dissertation is to document and screen the selected indigenous medicine(s) for antibacterial and free radicals scavenging activity.

CHAPTER 2

LITERATURE REVIEW

Each culture or community within a specific area, whether large or small, has its own, unique ethnobotanical perspective. To the best of the knowledge of this researcher, there are no medicinal plants used to treat STIs documented among the Sesotho speaking communities. Moreover, there are few researchers that report on both antibacterial activity and the documentation of medicinal plants used in the treatment of STIs. Although it is a known fact that communities rely heavily upon medicinal plants for their wellbeing, the effectivity and chemical spectrum of such plants is still not understood and remains unknown to traditional healers and plant traders as well as communities at large.

2.1 Traditional medicine

The world health organisation (WHO) observes that it is difficult to assign one definition to the broad range of characteristics and elements of traditional medicine, but that a working definition is essential. It thus concluded that traditional medicine includes diverse health practices, approaches, knowledge and beliefs incorporating plant, animal and or mineral based medicines, spiritual therapies, manual techniques and exercises applied singularly or in combination to maintain well-being, as well as to treat, diagnose or prevent illness (Richter, 2003). About 80 % of the population in the developing world, particularly in African countries, depend on traditional medicines for most of their healthcare issues (WHO, 2003). Traditional healing should thus become the subject of

intense research, with a view to establish new ways of strengthening collaboration between users of traditional medicines and modern healthcare systems (Ndubani and Bengt, 1999). In South Africa, traditional healers are potentially valuable partners in the delivery of healthcare. However, friction exists between Western medicines or biomedicines that look at material causation to understand and treat an illness, and traditional medicine that generally looks at the spiritual origin, such as witchcraft and displeasure of the ancestors in order to cure the ailment (Richter, 2003). Traditional health care has received attention largely in connection with the WHO's primary health care strategy (Wolfgang, 1979). Furthermore, the WHO supports the incorporation of indigenous health practitioners in government health programs (Edwards, 1988).

2.2 Secondary metabolites

Secondary metabolites, which may also be referred to as phytochemicals, are chemicals produced by means of secondary reactions resulting from primary carbohydrates, amino acids and lipids (Kayani *et al*., 2007). They may also be referred to as chemical substances which are not directly involved in the growth and development of plants (Achakzai *et al*., 2009). Such metabolites are known to participate in plant defense mechanisms (against herbivores, pathogens, and allelopathy) by their repellent or attractive properties, protection against biotic and abiotic stresses - which includes adaptation to changing environments and the maintenance of structural integrity (Edvera *et al*., 2008; Sa` *et al*., 2009; Achakzai *et al*., 2009). The most common classes of these chemicals are saponins, tannins, anthraqinones, flavonoids, and alkaloids which are widely

distributed amongst various plant families in abundant quantities. It is these secondary metabolites which attract so much attention from biological scientists due to their ability to inhibit the growth of microbes pathogenic to man (Pereira *et al*.,2009).

2.3 Drug discovery

Although traditional healers are rarely acknowledged for their knowledge on medicinal plants, which is their intellectual property right (IPR), their medicines have and will continue to contribute enormously towards the discovery of new drugs. Infact, the majority of modern medicines have their origins in plants that were often used in the treatment of illnesses and diseases. A notable example of such medicinal plants is *Salix mucronata* (Salicaceae), which is commonly known as wild willow. Its branch tips and leaves have been traditionally used to treat rheumatism and fever. It also has some anti-inflammatory and antipyretic properties. The anti-inflammatory property of the willow is ascribed to salicilin, which is converted to salicylic acid in the intestine (Van Wyk and Gericke, 2007).

Aspirin, an analgesic, is a modern product from *Salix mucronata* (Van Wyk and Gericke, 2005). Challenges in drug discovery include: high costs, lengthier and more complicated processes and scarce statistical chances of finding lead compounds (Balunas and Kinghorn, 2005). Moreover, it needs a multidisciplinary team with a holistic approach.

2.4 Conservation of medicinal plants

The world at large is experiencing extensive pressures on natural resources. Medicinal plant pressures are generally as a result of increased human populations, trading of plants, agricultural expansion increasing variety of diseases and antibiotic resistance to commonly used drugs, (Krog *et al*., 2006). It remains a challenge to sustain the ecosystems. Sustainability can be achieved through the complementary use of scientists with local and traditional ecological knowledge for joint management or co-management (Moller *et al*., 2004).

2.5 Intellectual property rights on traditional knowledge system.

Intellectual Property Rights (IPRs) are a bundle or set of exclusive rights over creations of the mind (intellect), both artistic (e.g. music, paintings and movies) and commercial. The terms traditional knowledge (TK) and indigenous knowledge (IK) may be used interchangeably. However, the world intellectual property organization (WIPO) defines traditional knowledge as tradition-based literary, artistic or scientific works, performances, inventions, scientific discoveries, designs, marks, names and symbols, undisclosed information, and all other tradition based innovations and creations resulting from intellectual activity in the industrial, scientific and artistic fields (WIPO, 1998-1999). Indigenous knowledge is localized to a specific community within the same geographical area while TK has international status. Possession is the key word for defining IPR. Possession of such knowledge may be individual, distributed or communal.

Across the cultures, knowledge about the use of medicinal plants exists in the form of local folklore available within families and tribes, handed down from one generation to the next. Some of this knowledge exists in written form, though usually in an indigenous vernacular which might be difficult to interpret. This improper and unstandardised documentation, coupled with communal knowledge (free to all members of the group) may result in patents granted to parties who are not sole owners or possessors of such medicinal knowledge, thereby undermining the Traditional Indigenous Knowledge. Moreover, part of the profits made by patent holders doesn't flow back to the local indigenous community. Traditional healers may therefore hold onto their knowledge, delaying the progress on drug discovery. It almost seems impossible to effectively and fairly patent knowledge on medicinal plants, due to a conflict of interests between indigenous knowledge systems and current legislature in South Africa. If herbal medicines are patented, either domestically or internationally, the medicines used as the first and last resort for health care by the poor may become unaffordable (Ng′etich, 2005).

2.6 Sexually transmitted diseases

2.6.1 What is sexually transmitted disease

A sexually transmitted disease (STD), sexually transmitted infection (STI) or a venereal disease (VD) is an illness that has a significant probability of transmission between humans or animals by means of sexual contact, including vaginal intercourse, oral sex and anal sex. However, some may be transmitted

non-sexually when pregnant mothers infect their babies during birth - commonly known as vertical transmission, or through blood and its products, and or the sharing of needles. When an infectious disease is transmitted from mother to child during birth or gestation, it is classified as a congenital infection (Carol, 2005). The most frequently observed congenital infections include syphilis, rubella, cytomegalovirus, chicken pox, herpes simplex viruses and Human Immunodeficiency Virus (HIV).

2.6.2 Causative agents of sexually transmitted infections

Microbes that cause sexually transmitted diseases may be bacteria, viruses, fungi, parasites or protozoa. However, the most common and frequent ones are caused by bacterial. Gonorrhoea, Chlamydia and Syphilis are among the most common highly infectious sexually transmitted diseases caused by *Neisseria gonorrhoea, Chlamydia trachomatis*, and *Treponema pallidum* respectively (Shim *et al*.,2010). Syphilis is commonly known as “pox” while gonorrhoea is called “the clap”. Chlamydia and gonorrhoea can spread to the uterus and fallopian tubes, causing pulmonary inflammatory diseases (PID) which may result in infertility and greatly increase the woman’s future risk of ectopic pregnancy. Syphilis may result in neurological and cardiovascular damage. The portal of entry for these microorganisms includes the mouth, genitalia, urinary meatus, rectum and skin (Porter *et al*., 2005).

Bacterial vaginitis, which may be caused by higher concentrations of *Prevotella* spp, *Mobiluncus spp* and *Mycoplasma hominis*, is the most prevalent cause of

vaginal discharge or malodour and may be asymptomatic (Austin et al.,2005). Such anaerobic bacterial strains replace the normal vaginal flora of *Lactobacillus spp*, resulting in vaginitis (MMWR, 2002). Vaginitis results in adverse pregnancy outcomes, including premature rupture of membranes, preterm labour, preterm birth, intraamniotic infection and post-partum endometritis.

2.6.3 Symptoms and spread of sexually transmitted infections.

Some factors contributing towards the spread of STDs include mobility and labour migration, prostitution, practice of polygamy, availability of birth control pills and other contraceptive methods cultural attitudes and practices, violence and poverty (Mulaudzi, 2005). This may be due to forgotten heritage, cultural beliefs and taboos. Westernization of indigenous people results in knowledge not being transferred from one generation to the next.

Today, sexually transmitted diseases continue in epidemic proportions in the entire world, particularly South Africa, judging by the South African Demographic Health Survey of 1997- in which 12 % of male adults reported symptoms that were suggestive of an STI (Reddy *et al*., 2003). This then serves as a problem statement for this study. Although signs and symptoms vary according to the type of infection, common symptoms includes itching or white milky discharge around genitals, pain during sex or when urinating or in the anus or in the pelvic area, painless sores around the tongue and body, swollen glands, fever and body aches.

South Africa therefore suffers a huge and largely hidden burden of sexually transmitted diseases (Wilkinson *et al*., 1997). Some of these STIs are asymptomatic. In general, STI and HIV infections are highly prevalent in South Africa (Colvin *et al*., 2004; Sturm *et al*., 1997) and are therefore a major public health concern and should therefore be highly prioritised as a matter of urgency. Sexually transmitted diseases are a major global cause of acute illness, infertility, long term disability and death, with severe medical and psychological consequences for millions of men, women and children (WHO, 2001). The WHO further states that "in developing countries, sexually transmitted diseases are among the top five disease categories for which adults seek health care." In women of childbearing age, sexually transmitted diseases, excluding HIV, are second only to maternal factors as causes of disease, death and a "healthy life lost". Because some strains develop resistance to antibiotics, there is a great need to screen and document the use of medicinal plants in South Africa (Van Wyk, 2004).

2.6.4 Challenges for effective STI control.

Although the quality of treatment has improved in South Africa, prevention control which includes the free provision of condoms still needs to be addressed as a matter of urgency. Some challenges that have a negative impact on control include partner treatment, inappropriate STI services - which involves diagnostic tests, drugs and staff numbers serve as barriers to effective control. Lack of male responsibility, poverty, migration, societal norms and values, educating teenagers, instability and politics (Mayaud and McCormick, 2001) may

collectively or singularly challenge the control of STIs. Stigmas around STI's persist, making contact tracing difficult (Reddy *et al*., 2003). There is an inaccurate recording of statistics about infection rates. That is, there is no single organisation that regularly collates STI statistics worldwide, and different countries have different types and levels of reporting systems.

Good social behaviour, such as listening to the elders and and fulfilment of moral expectations of a society based on cultural values such as sexual education, initiation schools, premarital counselling, polygamy and widow inheritance are believed to be the main strategies for combating sexually transmitted diseases (Mulaudzi, 2005).

Prevention of sexually transmitted diseases might be a key to yield lower HIV infection rates in the world. Abstinence is the practicable way of being safe from STIs. Faithfulness in relationships and condom use can also assist. Condoms only provide protection when used properly as a barrier to and from the area that it covers. Healthcare professionals suggest safer sex, such as the use of condoms, as the most reliable way of decreasing the risk of contracting sexually transmitted diseases during sexual activity, but safer sex should by no means be considered an absolute safeguard against STIs.

The most commonly used drugs in the treatment of STIs include ciprofloxacin, metronidazole and doxycycline. Some strains of gonorrhoea and chancroid are now resistant to all antibiotics generally available in poorer countries (Birley *et al*., 2002). Nowadays, the development of resistance by a pathogen to many of the

commonly used antibiotics provides an impetus for further attempt to search for new antimicrobial agents to combat infections and overcome problems of resistance and side effects of currently available microbial agents (Ali-Shtayeh *et al*., 1998).

2.6.5 Treatment of sexually transmitted infections.

Sexually transmitted infections are infections that are spread primarily through person to person sexual contact (WHO, 2007). Although people visit Western methods healthcare system, there is still a perception that the illnesses are not fully cured and should visit the traditional healer to cleanse the body and “purge out” diseases. (Tshikalange *et al*., 2005).

2.7 Free radicals

2.7.1 What is a free radical?

A free radical is a molecule that contains an unpaired electron in its outer orbit and that can exist independently (Clarkson and Thompson, 2000) and is highly reactive. Under normal conditions, the body’s natural antioxidant defense system, which is heavily reliant upon the intake of primary vitamins and minerals and the production of glutathione, easily counteracts the free radicals generated from a variety of both intrinsic and extrinsic processes and sources.

2.7.2 Common sources of free radicals

Free radicals like reactive oxygen species (ROS), reactive nitrogen species (RNS) and reactive chlorine species (RCS) are produced *in vivo* from various biochemical reactions (including metabolism) and respiratory chain as a result of occasional leakage (Adedapo *et al*., 2008) and are introduced into the body from outside sources of harmful chemicals in the environment- such as UV light, radiation, smoking, air pollution, unhealthy foods, stress, certain drugs and others. Such free radicals may lead to a variety of diseases.

2.7.3 Chemistry of free radicals formation within the body.

A reactive oxygen species (ROS) is a collective term, which includes not only the oxygen radicals (O_2 and OH) but also some non-radical derivatives of oxygen (Kumar *et al*., 2005). Because of high reactivity in nature, oxygen molecules which are diradicals may react with electrons, water and hydrogen in the body to form complexes that can be classified as superoxide, hyrdroperoxyl radicals, hydrogen peroxides, hyroxyl radicals (Clarkson and Thompson, 2000) as shown below.

$$O_2 + e^- \rightarrow O_2^-$$ Superoxide radicals

$$O^-_2 + H_2O \rightarrow HO^-_2 + OH^-$$ Hydroperoxyl radicals

$$HO^-_2 + e^- + H \rightarrow H_2O_2$$ Hydrogen peroxides

$$H_2O_2 + e^- \rightarrow {}^-OH + OH^-$$ Hydroxyl radicals

These free radicals may be envisaged as molecular sharks, which if not scavenged effectively and on time, are capable of damaging crucial biomolecules including those present in cell membranes, mitochondria and DNA and thus predisposing various pathophysiological states (Uddin *et al*., 2008) resulting in conditions such as ischemia, anaemia, asthma, arthritis, inflammation, haemorrhagic shock, Alzheimer's disease, arteriosclerosis, acute liver toxicity, cardiovascular disorder, heart damage, cancer, cystic fibrosis, gastro intestinal ulcerogenesis, neuro-degeneration, mongolism, Parkinson's disease, nephritis, diabetes, rheumatism, renal failure, brain dysfunction, lung damage, neoplastic disease, ageing process and perhaps dementia (Oke and Hamburger, 2002; Desai *et al*. 2008; Abdel-Hameed, 2009; Joshi *et al*., 2009).

2.8 Antioxidant properties of medicinal plants.

Phytochemicals, which contribute towards the antioxidative effect of medicinal plants, may be grouped into various classes, i.e. tannins, cardiac glycosides, flavonoids, alkaloids, saponins and others. Such phytochemicals, vitamins and other nutrients may be collectively called antioxidants. Antioxidants, which are often referred to as free radical scavengers, are molecules that can delay or prevent an antioxidative reaction catalysed by free radicals (Biapa *et al*, 2007). Increasing the intake of antioxidants can neutralize the free radicals and protect the body from cell damage. The antioxidative effect may be mainly due to the presence of phenolic components such as flavonoids, phenolic acids, tannins and phenolic diterpenes (Agbor *et al*., 2007).

Besides the prevention of a variety of diseases (Lugasi *et al*., 2003), antioxidants may play a role in protection of the nitrergic neurotransmitter (Colpaert and Lefebbvre, 2002), organ preservation and transplantation (Salehi *et al*., 2006), treatment of male infertility (Kefer *et al*, 2009), stimulation of mutagenic response (Corwin and Shloss, 1980), andrology and assisted reproductive technology (Sikka, 2004) and the control of lead pollution and enhancement of growth of specific biota in rivers (El-Shebly, 2009).

2.9 Review of related researches

Each culture or community within an area, whether large or small, has its own unique ethnobotanical perspective. To the best of the knowledge of this researcher, there are no medicinal plants documented among the Sesotho or Sotho speaking communities that are used to treat STIs. Although it is a known fact that communities heavily rely upon medicinal plants for their wellbeing, the effectivity and chemical spectrum of such plants is still not understood and remains unknown to traditional healers and plant traders as well as communities at large.

Buwa and van Staden (2006) studied the antibacterial and antifungal activity of traditional medicinal plants used against venereal diseases in South Africa. Plants were collected through consultations with a traditional healer and herbalist in the Eastern Cape Province while others were collected according to the literature from the KwaZulu-Natal Province. Thirteen medicinal plants belonging to twelve families were documented (Table 2.1).

Table 2.1 South African Medicinal plants used in the treatment of venereal diseases (Buwa and van Staden, 2006)

Family name	Botanical name	Plant part used	Uses and administration
Amaryllidaceae	*Cyrtanthus obliquus* Ait.	Bulbs	Venereal diseases, Decoction
Anacardiaceae	*Harpephylum caffrum* Bernh. Ex Kraus	Stem bark	Gonorrhoea, Decoction taken orally
Asclepiadaceae	*Xysmalobium undulatum* (L.) Ait.f.	Roots	Syphilis, decoction
Caesalpinaceae	*Albizia gummifera* (J.F. Gmel.)	Stem bark	Venereal diseases, Decoction
Capparidaceae	*Capparis tomentosa* Lam.	Roots	Infusion used as steam bath against lice
Gunneraceae	*Gunnera perpensa* Linn	Roots	Gonorrhoea, syphilis and urinary infections, Decoction
Hyacinthaceae	*Bowiea volubilis* Harv. Ex Hook	Bulbs	Poultice for the treatment of syphilis
	Ledebourea ovatifolia (Bak.) Jessop	Bulbs	Venereal diseases, Decoctions
Hypoxidaceae	*Hypoxis latifolia* Hook.	Roots and corm	Decoctions used as steam bath against lice
Liliaceae	*Albuca nelsonii* N.E. Br.	Bulbs	Gonorrhoea, Decoctions
Melianthaceae	*Bersama lucens* (Hochst.) Szyszyl	Stem bark	Decoctions used against lice
Ranunculaceae	*Knowltonia bracteata* Harv. Ex Zahlbr	Roots and leaves	Decoctions used as steam bath or orally (lice)
Rutaceaea	*Zanthozylum capense* (Thunb.)Harv.	Leaves	Infusion used for syphilis

Water, ethanol and ethyl acetate of plants were tested for antimicrobial activity against strains such as *Candida albicans, Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus*. *Gunnera perpensa*, *Harpephyllum caffrum*, *Hypoxis latifolia* and *Ledebouria ovatifolia* showed good antibacterial activity.

In studies carried out by Tshikalange *et al.* (2005) in the Venda region of the Limpopo Province (South Africa), roots and pods of six ethnobotanically selected

medicinal plants, *Terminalia sericea, Andredera cordifolia, Elaeodendron transvalense, Elephantorhiza burkei, Senna petersiana and Rauvolfia caffra* were extracted with chloroform, ethanol and distilled water respectively and tested for antibacterial activity against *Bacillus cereus, Bacillus pumilus, Bacillus subtilis Staphylococcus aureus*, *Enterobacter cloacae*, *Escherichia coli*, *Klebsiella pneumoniae, Pseudomonas aeruginosa, Serratia marcescens* and *Enterobacter aerogens.*

In general, water and chloroform extracts in the majority of plants were active against both Gram positive and Gram negative bacterial strains. Luteolin, a chemical compound which is a flavonoid was isolated from *Senna petersiana* and was found to be active against a variety of the selected bacterial strains. The other plant species which reportedly have the potential to treat sexually transmitted disease in Venda region of South Africa include *Annona senegalensis, Elaeodendron transvalenis, Ximenia caffra, Trichilia emetica, Trichilia dregeana* and *Bridelia micrantha*
(Mabogo, 1990).

Tshikalange *et al.* (2008), studied the *in vitro* anti HIV-1 properties of ethnobotanically selected south African plants used un the treatment of sexually transmitted diseases, in the Venda region. The plants were selected based on their traditional uses against syphilis, gonorrhoea, herpes and HIV-1. Ten medicinal plants belonging to eight families have been documented (Table 2.2).

Terminalia sericea extracts exhibited a considerable α-glucosidase inhibitory activity than acarbose. The plant also showed good activity in the reverse transcriptase assay.

Table 2.2 Medicinal plants investigated for anti-HIV activity (Tshikalange *et al*, 2008)

Family name	Scientific name	Part used for STD's
Basellaceae	*Andredera cordifolia* (Ten.) Steenis	stem tubers
Celastraceae	*Elaeodendron transvaalense* (Burt Davy)R.H. Archer	stem bark
Agavaceae	*Polianthes tuberose* L.	Roots
Apocynaceae	*Rauvolfia caffra* Sond.	stem bark
Rutaceae	Zanthozylum davyi (I. Verd.) P.G. Waterman	Roots
Combretaceae	*Terminalia sericea* Burch. Ex DC.	Roots
Fabaceae	*Senna petersiana* (Bolle) Lock	Roots
	Senna occidentalis (L)	Roots
Lamiaceae	Clerodendrum glabrum E. Mey var. Glabrum	Roots
	Rotheca myricoides (Hochst.) Vatke	Roots

Ethyl acetate extract of *Elaeodendron transvaalense* showed potent inhibitory qualities in the reverse transcriptase assay and Tat assay at a lower concentration. Acetone and chloroform extracts also showed good activity in NF-$_K$B and Tat assays.

In other studies carried out in the Guruwe District in Zimbabwe (Kambizi and Afolayan, 2001) plant species belonging to various families were documented (Table 2.3).

Table 2.3 Medicinal plants used for the treatment of sexually transmitted diseases in Zimbabwe (Kambizi and Afolayan, 2001)

Family name	Botanical name	Plant part used	Preparation
Fabaceae	*Acacia nilotica*	Root	Infusion/powder
	Erythrina abyssinica	Root	Infusion taken orally
	Cassia sangueana	Root	Infusion taken orally
	Cassia abbreviata	Bark	Infusion taken orally
	Dichrostachys cinerea	Friut	Infusion/powder
Saphindaceae	*Zanha africana*	Bark	Infusion taken orally
Asteraceae	*Vernonia amygdalina*	Root	Infusion taken orally
Liliaceae	*Aloe globuligena*	Leaves	Chopped fresh leaf applied on sores
Musaceae	*Musa spp* L	Root	Infusion taken orally
Olacaeceae	*Ximenia caffra*	Root	Infusion taken orally
Anacardiaceae	*Lamela edulis*	Root	Infusion taken orally
	Lamella discolor	Root	Infusion taken orally
Anonaceae	*Anona stenophylla*	Root	Infusion taken orally
Solanaceae	*Solanum incanum*	Fruit	Cut fruit is applied directly on affected part
Poaceae	*Phragmites mauritianus*	Leaves	Rub on affected area

Various plant organs that may be used and the method of administering the medication has been documented and the most commonly used plant material are roots, which may accelerate the death and general loss of such plant

species. In general, the minimum inhibitory concentrations of the selected plants species against the selected test organisms ranged from 0.5 to 5 mg/ml.

Indigenous Medicinal Knowledge (IMK) in the treatment of variety of ailments, including STIs in the rural Zimbabwe has also been documented (Chigora *et al*., 2007). Plant species used to treat sexually transmitted diseases - as shown in (Table 2.4),

Table 2.4 Plants used to treat sexually transmitted disease in Mutirikwi, Zimbabwe (Chigora *et al*, 2007).

Botanical name	Plant part used	Condition treated	Preparation
Acacia karoo	Root	Gonorrhoea, syphilis and aphrodisiac for men	Decoction
Androstachys johnsonii	Bulb	aphrodisiac for men	Decoction
Garciana huillensis	Fruit	aphrodisiac for men	Decoction
Macaranga capensis	Root	aphrodisiac for men	Decoction/with porridge
Commiphora marlothii	Root	Dropsy	Decoction
Entandorogma condatum	Fruit peels	Genital warts	Mix with Vaseline
Strychnos spinosa	Unripe fruit	Gonorrhoea and genital warts	Fruit boiled with water and applied
Spirostachys Africana	Roots	Venereal infection	Mixed with porridge

In related studies carried out in Zambia, a total of 19 different species of indigenous plants used to treat sexually transmitted diseases were documented (Table 2.5). The most commonly used plant parts in the documented plant species are roots and barks.

Table 2.5 Medicinal plants reported to have the potential to treat STDs in Zambia (Ndubani and Bengt, 1999).

Family name	Species name	Plant part used
Loganiaceae	*Strychnos spinosa*	Fruit and roots
	Strychnos cocculoides	Fruit and roots
Papilionaceae	*Ormocarpum trichocarpum*	Roots and leaves
Araliaceae	*Cussonia arboea*	Root and bark
Solanaceae	*Solanum incanum*	Root
	Solanum delagoese	Root
Leguminoceae	*Albizia antunesiana*	Root and leaves
	Cassia abbreviata	Root and bark
Apocynaceae	*Diplorynchus condylocarpon*	Root
	Rauvolfia caffra	Root
Bignoniacea	*Kigelia africana*	Root and bark
Olacaceae	*Ximenia caffra*	Root
Burseraceae	*Commiphora mossambicensis*	Root
Malvaceae	*Azanza garckeana*	Root and bark
Musaceae	*Musa spp*(cultivated)	Root and stem
Rutaceae	*Citrus limon*	Root and leaves
Euphorbiaceae	*Croton megelobotrys*	Root and bark
Caricaceae	*Carica papaya*(Cultivated)	Root and stem
Moraceae	*Fic sur*	Root

Cocks *et al*, (2006), studied the use and trade of medicinal plants in the Eastern Cape Province in South Africa amongst the Xhosa cultural group. Plants used to treat venereal diseases and impotence include *Cissampelos capensis, Bulbine abyssinica, Asparagus africanus, Bowiea volubilis*, *Pachycarpus concolor, Trichilia dregeana* and *Asparagus suaveolens.*

In other parts of Africa, the evaluation of extracts from *Anthlosteita djalonensis* of the family Loganiaceae, *Nauclea natifolia* of Rubiaceae and *Uvalia afzalii* of Arnonacea, for activity against bacterial isolates from cases of non-gonococcal urethritis were carried out (Okoli and Iroegbu, 2004).

In America, *Bixa orellana* (bark), *Casimiroa edulis* (root), *Clematis dioica* (whole plant), *Diphysa robinioides* (leaf), *Eupatorium odoratum* (leaf), *Gliricidia sepium* (leaf), *Parmentiera edulis* (fruit*), Physalis angualata* (leaf), *Piper aduncum* (leaf), and *Prosopis juluflora* (leaf) were found to be active and possible sources of drugs against *Neisseria gonorrhoea* in Gautemala (Caceres *et al.*, 1985).

Recently, interest in the search for natural antioxidants has emerged in Africa (Atawodi, 2005) and the world at large. There is considerable evidence to show foodstuffs (Aqil *et al.,* 2006; Siddhuraju and Becker, 2007; Thaipong *et al*., 2006; Yuan *et al.,* 2005) and medicinal plants (Miliauskas *et al*., 2004, , Mothana *et al*., 2008, Al-Mustafa *et al*., 2008) as sources of antioxidants. However, more interest seems to be on antioxidants from medicinal plants. This may be due to the fact that medicinal plants possess a variety of phytochemicals (flavonoids and other phenolic compounds), sugars, vitamins, saponins, ethereal oils, polyunsaturated fatty acids, phospholipids, enzymes and amino acids that contribute towards the antioxidative effect against free radicals.

Aqil *et al.* (2007) studied the antioxidant and free radical scavenging properties of twelve traditionally used Indian medicinal plants. Some plants showed more than 70 % of DPPH scavenging activity, but low lipid peroxidation. A variety of

phytochemicals responsible for such antioxidant properties were detected (alkaloids, flavonoids, sponins, glycosides, tannins, and phenols). *Delonix regia* (Leguminosae) possessed the highest total phenolic content.

Al Mustafa *et al.* (2008) investigated the antioxidant activities of some Jordanian medicinal plants used traditionally for the treatment of diabetes. Twenty-one medicinal plants were selected according to their reported frequency of use by traditional healers. Methanol and water extracts of such plant species were screened for antioxidant properties using both DPPH and ABTS free radical scavenging assays.

Miliauskas *et al.* (2004) investigated the radical scavenging activity of some medicinal and aromatic plant extracts from some plants growing in Central and Eastern Europe. Methanol extracts were the most effective radical scavengers, compared to those of acetone and ethyl acetate. *Salvea clarea*, *Salvia glatunosa*, *Salvia pratensis*, *Raphonticum carthamoides*, *Geranium macrorrhizum and Potentilla fruticosa* almost inhibited DPPH absorption and their percentage ranged from as high as 87.6 to 93.9 %. In the ABTS radical cation decolouration assay, similar results as those in the DPPH assay were obtained as extracts of *Geranium macrorrhizum* and *Potentilla fruticosa* showed higher scavenging activity.

Shyur *et al.* (2005), studied the antioxidant properties of extracts from twenty-six medicinal plants popularly used in Taiwan following the free radical scavenging activity (DPPH), superoxide scavenging activity (NBT) and analysis of hydroxyl

radical induced DNA strand scission. Extracts from *Ludwigia octovalvis*, *Vitis thunbergi*, *Rubus parvifolius*, *Lindendernia anagallii* and *Xanthozylum nitidum* exhibited strong activity on scavenging for DPPH free radicals, thus possessing lower IC_{50} (4.6-50.2 µg/ml) and were dose dependent.

Ayoola *et al.* (2008a)studied the phytochemical and antioxidant screening of some plants of the Apocynaceae family from South West Nigeria. There was a good correlation between the flavonoid content of *V. africana* leaf and DPPH (R^2 = 0.96) which suggested that such flavonoids may be contributing towards the free radical scavenging activity of the plant against DPPH.

The literature review above has highlighted that there are some plants used to treat sexually transmitted infections. In the selected medicinal plants below, some plants like Cassia abbreviata and Peltophorum africanum has been reported before this study. However, bacterial strains used were different to the ones in our study. Moreover, this literature review again showed that there is an increased interest in medicinal plants posessing antioxidant properties. Such plants may well scavenge free radicals which are mainly linked to various diseases in man.

2.10 Selected Medicinal Plants

The following medicinal plants as listed below were selected using structured interview and frequency of use.

2.10.1 *Elephantorrhiza burkei* Benth

Family: Fabaceae

Botanical description and other uses

It is a herb with compound leaves, bean shaped seed coats, greyish stem and a reddish underground rhizome which may be used in the treatment of a variety of illnesses. It has been reported elsewhere in the treatment of diarrhoea (Mathabe *et al.*, 2006). It occurs in grassland areas over large parts of South Africa. Its only known to possess tannins (van Wyk and Gericke, 2007).

2.10.2 *Peltophorum africanum* Sond

Family : Fabaceae

Botanical description and other uses

Small to medium sized deciduous tree with a pale brown wood and dark reddish heart. Leaves are bipinnate and alternate with oblong leaflets of 4-9 pairs (Coates Palgrave, 2005). Roots and bark may be used medicinally against sterility and backache (Pooley, 1993). Its distributed from Democratic Republic of Congo in the north to Kwazulu Natal in South Africa, mostly in wooded grasslands, wood lands and along margins of vleis (Venter and Venter, 2009). Bark is reported to posess tannins, norbegenin, coumaroylbergenin and bergenin (Mebe and Makuhunga, 1992).

2.10.3 *Elephantorrhiza elephantina* Burch Skeels

Family: Fabaceae

Botanical description and other uses

It is a herb with light yellow cluster of flowers and a brown rhizome which may be used for medicinal purposes. It can be used in the treatment of diarrhoea and dysentery, stomach disorder, haemmoroides and perforated peptic ulcer (van Wyk, 2009). It occurs in grassland areas over large parts of South Africa, Lesotho, Swaziland, Botswana, Namibia, Mozambique and Zimbabwe (van Wyk and Gericke, 2007, van Wyk, 2005a). Rhizome is reportedly possess compounds such as dihydrokaempferol, kaempferol, catechin, ethyl gallate, gallic acid, ethyl β -D-glucopyranoside and quercetin 3-O- β-D-glucopyranoside. Other reported compounds include 3-β -stigmast-5-en-3-ol (β-sitosterol), 3,4,5-trihydroxybenzoic acid (gallic), methyl gallate, quercetin 3'-O-glucoside and trans-3-o-galloyl-3,3',5,5',7-pentahydroxyflavan (Mthembu, 2007).

2.10.4 *Peucedanum sulcatum* Sond

Family : Apiaceae

Botanical description and other uses

It is an annual or perrenial herb with opposite leaves, creamy whitish leaves and light brownish underground stem which is whitish inside. It is distributed from West Coast of cape Town, extending into Northern Cape, further into Kruger National park and Limpopo province.

2.10.5 *Waltheria indica* Linn.

Family: Convolvulaceae

Botanical description and other uses

It is an erect perennial herb/shrublet up to ± 500 mm high, has stalked leaves with margins shallowly and irregularly toothed (van Wyk and Malan, 1998). Its flowers are yellow and occur in clusters. Roots extracts are reported to treat ailments such as diarrhoea, wounds and stomach ache (Ayantunde *et a.l*, 2009), while leaves are used as purgatives (Ganesan *et al.*, 2009). Whole plant may be used to treat coughs, haemorrhages, fever and malaria amongst others (Diallo *et al.*, 1999; Olowokudejo *et a*l., 2008). Globally, its distribution and habitat is mostly in subtropical and tropical zones and in scrub forests, inundated savannas, riverbanks, and sandy or clay soils and in disturbed or impoverished soils (Saunders, 2007). Flavonoids such as epicatechin, quercetin and tiliroside were isolated from whole plant extract and dose independently inhibits production of inflammatory mediator nitric oxide (NO), cytokines (TNF)-α and interleukin (IL)-12, in lipopolysaccharide and interferon activated murine peritoneal macrophages, without any cytotoxicity (Rao *et al.*, 2005).

2.10.6 *Harpagophythum procumbens* Burch. DC

Family: Pedaliaceae

Botanical description and other uses

It is a weedy, perrenial herb with stems spreading from tuberous fleshy roots. May be used to treat theumatism, stimulate appetidte, menstrual cramps,

diabetes, tuberculosis, arthritis and taken as an analgesic, especially during pregnancy (van Wyk, 2009; van Wyk and Gericke, 2007). It is mostly distributed in grasslands and savannah vegetations of the Limpopo Province and in Botswana. Isolated compounds include harpagogenin, harpagoside, harpagide, 8-(4-coumaroyl)-harpagide, procumbide, its 6-4-coumaroyl ester and procumboside (European Engine embassy, 2009).

2.10.7 *Urginea sanguinea* Schinz

Family: Hyacinthaceae

Botanical description and other uses

A herb with a single flowering stem and reddish scaly bulb which may be used in the treatment of asthma, as a blood purifier, for backache, hypertension, abdominal pains, venereal diseases, impotence, dysmenorrhoea, emetics, as a heart tonic, for bronchitis, asthma and during pregnancy (Marx *et al*., 2005). Water extract showed cytotoxicity in cell cultures L929 cell and primary embryonic neural cell cultures (Marx et al., 2006). Bulb is reported to be poisonous to both man and livestock (Foukaridis *et al*., 1995). It is distributed from the Eastern Cape, along the coast , extending to the Limpopo Province.Bulbs have been reported to contain stigmasterol, phloroglucinol, phloroglucinol 1-beta-D-glucopyranoside (phlorin), scillaren A, a novel compound 5-α-4,5-dihydroscillaren A, salicylic acid, and 3-hydroxy-4-methylbenzoic acid (Majinda *et al*., 1997).

2.10.8 *Senna italica* Mill. Subsp. Arachoides (Burch.) Lock

Family: Caesalpiniceae

Botanical description and other uses

It is a herb with yellow leaves and a black roots which are medicinally used. Anti-tick properties of the root extracts against *Hyalomma marginatum rufipes* has been reported (Magano *et al*., 2008). It occurs throughout Africa and eastwards towards India. It grows in the northern and eastern parts of South Africa (van Wyk,2009). Sennoside A and 1,5-dihydroxy-3-methyl-anthraquinone are known compounds (van Wyk,2009).

2.10.9 *Cissus quadrangularis* L.

Family: Vitaceae

Botanical description and other uses

It is an evergreen climber used by Zulu people as a drench for sick horses, wounds, skin diseases and as a tick repellent (Mcgaw and Eloff, 2008). It may also be in the management of weight loss and metabolic syndrome and epilepsy (Oben *et al*., 2006; Ngo Bum *et al*., 2008). It grows in KwaZulu Natal, Mpumalanga and Limpopo Provinces of South Africa. It is rich in carotenoids, flavonoids, triterpepenoids, stilbene derivatives, phytosterols, ascorbic acid, triterpene, piceatannol, pallidol perthenocissin, β-sisterol, asymmetrical tetracyclic triterpenoids, carotene A and others (Mishra *et al*., 2010).

2.10.10 *Jatropha zeyheri* Sond.

Family : Euphorbiaceae

Botanical description and other uses

It is a perenial herb with simple or sparingly branched stems and is mainly found in grasslands and in sandy soil. Infusion of the tuber may be used in the treatment of irregular periods, menstrual pains and during pregnancy to ensure a strong foetus (van Wyk, 2007). It may also be used to treat wounds and retain plancenta in animals (Luseba *et al*., 2007).

J. zeyheri is distributed mainly in the tropical and subtropical regions of America and Africa. A compound, jaherin, has been isolated from *J. zeyheri* root and has been reported to possess MIC of 8 mg/ml against *Streptococcus pyogenes* and 16 mg/ml against *Microsporum canis*, *Trichophyton rubrum*, *Trichophyton mentagrophytes* and *Sporotrichum schenkii* (Dekker *et al*., 1987).

2.10.11 *Cassia abbreviata* Oliv.

Family : Caesalpiniaceae

Botanical description and other uses

It is a shrub with elliptic opposite leaflets, yellow flowers (mostly in September), greyish stem, with cylindrical pods and long fruit. The bark and roots are used for medicinal purposes, in the treatment of blackwater fever, headache, toothache and stomach ache as well as a natural abortion agent (Schmidt, 2002). Mostly distributed in Botswana, Namibia, Zambia and Limpopo Province of South Africa.

2,4-trans-7, 4'-dihydroxy-4-methoxyflavan (Dehmlow *et al*., 1998)., guibourtinidol-(4β→8)-epiafzelechin, guibourtinidol-(4α→8)-epiafzelechin, guibourtinidol-(4α→8)-catechin, guibourtinidol-(4β→8)-epicachetin and ent- guibourtinidol-(4β→8)-epicachetin are some of the compounds isolated from extracts of *C. abbreviata* (Malan *et al*.,1996). Pure isolated alkaloids and the synthetic derivatives are used as a basic medicinal agent because of their analgesic, antispasmodic and bacterial properties (Njoku and Akumefula, 2007).

2.11 Selected bacterial strains

A total of twenty-three pathogenic bacterial strains have been selected. Eleven Gram negative strains, *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 7700), *Pseudomonas aeruginosa* (T3374), *Enterobacter cloacae* (ATCC 13047), *Klebsiella pneumoniae* ATCC 10031, *Klebsiella pneumoniae* (517298), *Klebsiella spp* (317302), *Serratia marscens* ATCC 9986, *Acinetobacter calcaoceuticus anitratus* CSIR and clinical isolates of *Shigella flexineri* KZN and *Samonella spp KZN* and twelve Gram positive, *Staphylococcus aureus* (ATCC 6538), *Staphylococcus aureus* (P12702), *Staphylococcus aureus* (P12763), *Staphylococcus aureus* (P12724), *Staphylococcus aureus* (B10808), Streptococccus viridans (517141), *Bacillus cereus* ATCC 10702, *Bacillus pumilus* ATCC 14884), and four clinical isolates of *Enterococcus faecalis* (KZN), *Staphylococcus aureus* (KZN), *Staphylococcus epididirmis* (KZN) and *Bacillus subtilis* (KZN). Clinical isolates were isolated from patients with various illnesses, including sexually transmitted infections and urinary tract infections within the KwaZulu-Natal Province while non-ATCC strains were multi-resistant from

Lancet Laboratories. Although *Staphylococcus aureus* is known to cause Urinary Tract Infections (UIT) in infants under the age of two years (Adjei *et al*., 2004),some of its strains such as Methicilin resistant *Staphylococcus aureus* may be transmitted sexually (Cook *et al*., 2007).

Table 2.11. Profile of selected bacterial strains

Bacteria	**Gram negative/positive**	**Type of strain**
Esherichia coli	Gram negative	ATTC 25922
Pseudomonas aeruginosa	Gram negative	ATCC 7700
Pseudomonas aeruginosa	Gram negative	T33774
Enterobacter cloacae	Gram negative	ATCC 13047
Klebsiella pneumoniae	Gram negative	ATCC 10031
Klebsiella pneumoniae	Gram negative	517298
Klebsiella spp	Gram negative	317302
Serratia marscens	Gram negative	ATCC 9986
Acinetobacter calcaoceuticals anitratus	Gram negative	CSIR strain
Shigella flexineri	Gram negative	Clinical isolate
Salmonella spp	Gram negative	Clinical isolate
Staphylococcus aureus	Gram positive	ATCC 6538
Staphylococcus aureus	Gram positive	P12702
Staphylococcus aureus	Gram positive	P12763
Staphylococcus aureus	Gram positive	P12724
Staphylococcus aureus	Gram positive	B10108
Staphylococcus aureus	Gram positive	Clinical isolate
Streptococcus viridans	Gram positive	517141
Bacillus cereus	Gram positive	ATCC 10702
Bacillus pumilus	Gram positive	ATCC 14884
Bacillus subtilis	Gram positive	Clinical isolate
Enterococcus faecalis	Gram positive	Clinical isolate
Staphylococcus epiderrmidis	Gram positive	Clinical isolate

Staphylococcus aureus is a Gram positive, primarily coagulase positive, facultative anaerobe, which appears as grape-like clusters when viewed through a microscope and has large, round, golden yellow colonies when grown on blood agar plates (Agston, 1984,Ryan *et al*., 2004). Although occurring as human indigenous microflora (Chomnaang *et al*., 2009), it may cause a variety of

illnesses, from skin infections such as pimples, impetigo, boils, cellulites, carbuncles, scalded skin syndrome, and abscesses, to life threatening diseases, such as pneumonia, meningitis, toxic shock syndrome (TSS), and septicaemia and is highly resistant to penicillin, vancomycin (Hiramatsu *et al*, 1997) and glycopeptides (Chang *et al*., 2003).

Escherichia coli is a Gram negative, facultative anaerobic and non-sporulating bacterium (Feng *et al*., 2002) and can cause serious food poisoning in humans (Vogt and Dippold, 2005) and urinary tract infections which may lead to morbidity, pyrexia and mortality (Adjei and Opoku, 2004).The harmless strains are part of the normal flora of the gut and can benefit the host by producing Vitamin K_2 (Bently and Meganathan, 1982, Todar, 2007) or by preventing the establishment of pathogenic bacteria within the intestine (Hadault *et al*., 2001).

Pseudomonas aeruginosa is a Gram-negative, aerobic, rod shaped bacterium with unipolar motility (Ryan and Ray, 2004). The word *pseudomonas* means “false unit” while *aeruginosa* means “copper rust” from Greek. It is an opportunistic human pathogen. It is also opportunistic to plants (Iglewski, 1996). responsible for 20% of Infections caused by *Pseudomonas aeruginosa may be* nosocomial infections (Ruftig, 1998) which are amongst the most difficult to treat with conventional antibiotics (Mathekga *et al*., 1998).

Enterobacter cloacae is a Gram-negative, facultative anaerobe which is rod shaped. It is oxidase negative, catalase positive, and it is part of normal flora

which may cause hospital acquired infections (Keller *et al*.,1998). It is sometimes associated with urinary tract and respiratory tract infections in humans.

Enterococcus faecalis is a Gram positive bacterial strain which can cause systemic infections like endocarditis, intra-abdominal sepsis, including bacteremia. Moreover, it's strains may produce β-lactamase and there is a need to understand the routes of infection, in the hope of preventing strains with these properties from becoming epidemic in our hospitals (Hall *et al*., 1992).

Acinetobacter calcoaceuticus anitratus is a non-fermenting, Gram negative bacterium commonly found in soil, water, latex gloves and on human skin and may cause pneumonia (Ahmed *et al*., 1994).

Shigella flexineri is a Gram negative causative agent of shigellosis which is a global human health problem (Yismaw *et al*., 2006). Diarrhoea and dysentery are among the major symptoms of the disease.

Salmonella spp is a Gram negative bacteria which may cause an infection called salmonellosis which may show symptoms which includes diarrhea, fever, vomiting and abdominal cramps. Infection may spread from the intestines to the blood stream, and then to other body sites and can cause severe dehydration, reactive arthritis, typhoid fever, gastroenteritis, bacteremia, and subsequent focal infection (Hohmann, 2001)**.** The most common species that may cause infections include *Salmonella typhimurium, Salmonella typhi* and *Salmonella enterica* and are common sources of food poisoning, detected in cheese and dairy products.

Klebsiella pneumoniae is one of the most important Gram negative bacterial pathogens that has caused world wide concern due to its ability to produce extended spectrum β–lactamases (ESBLs) which render it resistant to carbapenems (Falagas *et al*., 2007). Some of its isolates that produce KPC-2 are resistant to combinations of penicillin betalactamases inhibitors, as well as to ceftazidime and aztreonam and non-susceptible to cefoxitin, cefotaxime and cefepime (Giakoupi *et al*., 2009).

Bacillus genus contains nearly fifty species, most of which are soil organisms. Species of major medical importance include *Bacillus cereus*, *Bacillus subtilis* and *Bacillus anthracis* which cause anthrax. *Bacillus cereus* is a large, about four to ten µm, gram positive spore forming an encapsulated rod that respires aerobically (Mims, 1998).

Bacillus subtilis is a gram positive, obligate anaerobe, catalase-positive bacterium commonly found in soil. It is rod or bacilli shaped (Bauman, 2007) and able to form a tough, protective endospore, allowing the organism to tolerate extreme environmental conditions such as heat, salt, and acid.

Serratia marcescens is a Gram-negative, bacilli shaped, facultative anaerobe, which is motile and produces red pigment at room temperature. It occurs naturally in soil, water and intestines. It can cause nosocomial infection and is associated with urinary and respiratory tract infections, endocarditis, osteomyelitis, septicaemia, wound infections, eye infections and meningitis (Hejazi and Falkiner, 1997). Its mode of transmission is through direct contact,

droplets. It can grow on medical devices such as catheter and can infect other sterile materials.

Bacilus pumilus is a Gram positive, rod shaped, facultative anaerobe bacteria which may cause serious human infections, including endocarditis, sepsis, meningitis, pneumonia, endophthalmitis, primary cutaneous infections and surgical wound infections (Tena *et al*., 2007). Cutaneous infections arise from contact with infected animals or animal products such as hides and wool. It may also produce toxins which may be ingested from foodstuffs.

Staphylococcus epidermidis is a coagulase-negative, Gram positive, ubiquitous commensal in humans that normally inhabits the skin of the head, arms and legs. It is a common cause of bacteremia in immunocompromised patients and may result in localized infections in immunocompetent patients with indwelling medical devices (Ryan-Poirier *et al*., 1993). It may cause cervical adenitis.

Streptococcus viridans is a Gram positive cocci which is part of normal flora, also found in female genitals and most common cause of endocarditis and bacteremia (Shanson *et al*., 1984). It may also be isolated from patients suffering from osteomyelitis and endocarditis (Choudhurry *et al*. 2009).

CHAPTER 3

MATERIALS AND METHODS

3.1 Study area

Four villages in each of the two ethnoecological regions, within Limpopo Province, Blouberg and Molemole Municipalities, were selected as study sites under the Capricorn District (see Appendix 2) which is named after the Tropic of Capricorn ($23_{1/2}$° Southern altitude) which runs through the district (Magoro, 2008) and dominated by 88 % Sepedi speaking people (Statistics South Africa, 2001). The district has coordinates 23°53′S and 29°26′E. Rainfall is mostly experienced during hot summer days and is estimated to be between 380 and 550 mm annually. Blouberg area is commonly known as Hananwa, Ga-Malebogo. It is situated 30 kilometres north of Dendron and 95 kilometres from Polokwane. Geographically, it is a mountainous area located between the Waterberg Wetlands and the Dongola Trans-frontier and extends right up to the Botswana border. It occupies geographical land of approximately 5054 square kilometres and a total population of about 166 243 (Statistics SA, 2004-2006). Molemole Municipality covers an area of 3.347 kilometres and is located about 60 kilometres north of Polokwane.

3.2 Ethnobotanical survey

A total of thirteen traditional healers were randomly sampled in the Capricorn District (Molemole and Blouberg Municipalities) of Limpopo Province and

interviewed using a person-to-person structured interview (see Appendix 1(a)) to obtain information on indigenous plant species used to treat sexually transmitted infections amongst the Sepedi speaking ethnic group from April 2008 to August 2009. Ethics approval has been obtained from University of Zululand, ethics commitee. Consented (apendix 1b) healers were requested to give the names of medicinal plants, plant part used, ratio of plants, different methods of administering medicines, duration of treatment, estimated dosage and frequency, and other uses of identified medicinal plants (see Appendix 1). Healers identified the plants, using their respective vernacular names after building up a relationship of trust and mutual respect.

3.3 Collection of plant specimen.

Voucher specimens were then collected from the wild, dried, labelled, identified and deposited into the University of Zululand Herbarium, Department of Botany. Plant specimen which were unknown to staff were identified by SANBI, Pretoria. Conversations were aimed at the transfer of ethnomedicinal knowledge from one generation to the next, contribute to the improvement of the health within the immediate community and documenting the indigenous knowledge that would otherwise be lost.

3.4 Preparation of extracts.

Selected and identified plant materials were dried in the shade, ground into thin powder (2 mm mesh) using a hammer mill (Perten Instruments 3100, Sweden).

About 20 grams of dry powdered plant material was extracted (1:5w/v) with tap water, methanol, ethanol, ethyl acetate and acetone respectively using a mechanical shaker (Merck, South Africa) at 100 rpm for 24 hours. Extracts were filtered through Whatman No1 paper and the organic solvent extracts were concentrated using rotary evaporator. Dry extracts were weighed and kept in a refrigerator at 4ºC until needed.

3.5. Antibacterial tests

3.5.1 Disc Diffusion method

Plant extracts were tested for antibacterial activity by the disc diffusion method according to the National Committee for Clinical Laboratory Standard guidelines (NCCLS, 2001). Selected organisms were obtained form the Department of Chemistry, University of Zululand, and maintained on Muller-Hinton agar (MHA) (Oxoid) to obtain isolated colonies. A single colony was aseptically transferred with an inoculating loop to a 20 ml of fresh sterile saline broth in a test tube which was vortexed thoroughly and incubated overnight at 37°C. Turbidity was then spectrophotometrically adjusted (A_{625}=0.08-0.1) to that of 0.5 McFarland's standard.

About 100 µl of the inoculum was aseptically transferred to a labelled disposable Petri-dish containing 15 ml Muller-Hinton agar and spread thoroughly using a sterile glass spreader. Sterile paper discs of 5 mm (Mast Disks, UK) were impregnated with 10 µl of 5 mg/ml plant extract dissolved in 5 %

dimethylsulfoxide (DMSO) and gently placed individually on the seeded agar. Plates were allowed to dry for one hour and later incubated in an inverted position at 37 °C in over night.

Zones of inhibition were measured using a ruler in millimeters, including sterile paper disc. Streptomycin (10µg/disc), penicillin (10 µg/disc), and neomycin (10 µg/disc) were used as positive controls. Negative controls were performed using paper discs loaded with 10 µl of 5 % DMSO. Each experiment was repeated three times.

3.5.2 Determination of minimal inhibitory concentrations (MIC).

Extracts showing activity in Disc Diffusion were chosen to assay the minimal inhibitory concentration (Eloff, 1996) using micro plate broth dilution assay. The 24 hour old culture, as prepared in 3.1 was diluted 1:100 with saline broth. About 100 µl of extracts (50 mg/ml in 5 % DMSO) were added to a multi well plate containing 100 µl of freshly prepared broth and serially diluted, yielding 12.5 mg/ml in the first well. Plates were then incubated over night at 37 °C. About 40 µl of 2 mg/ml freshly prepared iodo-nitro-tetrazolium chloride were added to each well and incubated for 30 minutes at the same temperature. The MIC was defined as the lowest concentration of the extract to inhibit bacterial growth. Metronidazole and streptomycin sulphate were used as positive controls.

3.6 Free radical scavenging activity

3.6.1 DPPH assay

Plant extracts of different concentrations (1, 2, 3, 4, 5 mg/100ml) were prepared in methanol. DPPH (Sigma) solution was prepared by reacting 2 mg of 2, 2-diphenyl-1-picryl-hydrazyl. Test tubes were set in duplicates for each extract concentration and blank solution. About 2 ml of DPPH solution was mixed with 2 ml of crude extracts of different concentrations (Opoku *et al*., 2002). Absorbance was measured at 517 nm after 1 hour of incubation at room temperature against methanol. In cases where 1mg/ml inhibited DPPH within incubation time, dilutions were made from original solutions, yielding 0.25, 0.125, 0.083, 0.063, 0.005 mg/100ml. Ascorbic acid, at similar concentrations as plant extract was used as a positive control.

Percentage of inhibition was calculated as

$$\% \text{ Scavenging Inhibition} = [1-A_t/A_0] \text{ X } 100,$$

Where A_t represents the absorbance of the test sample, while A_0 represent absorbance of blank solution, which is the reaction of methanol and DPPH solution at 2 ml each.

3.6.2 ABTS assay

ABTS (2,2'-azinobis-3-ethylbenzithiazoline-6-sulfonic acid) assay was determined by modified method of Re *et al*, 1999 and Adedapo *et al*., 2009. Different concentrations of extracts were prepared as in the DPPH assay. About 7 mM was prepared in distilled water. An equivalent of 2.45 mM potassium persulfate was added to the mixture which was then incubated at room temperature in the dark for 16 hours. ABTS (Sigma) solution was diluted with methanol (1 ABTS: 60 MeoH). Test tubes were set as in DPPH assay, in duplicates. About 1 ml of ABTS was reacted with equal amounts of various concentrations of plant extract in a series of test tubes which were mixed and allowed to stand for 6 minutes. Absorbance was read at 734 nm, using methanol as blank. Ascorbic acid, at similar concentrations as plant extract was used as a positive control.

Percentage of inhibition was calculated as

$$\% \text{ Scavenging Inhibition} = [1-A_t/A_0] \text{ X } 100,$$

Where A_t represents the absorbance of the test sample, while

A_0 represents absorbance of blank solution, which is the reaction of methanol and ABTS solution at 2 ml each.

IC_{50} was recorded as a concentration that inhibit 50 % of the free radical (both in ABTS and DPPH assays) from the line graphs (extract concentration versus % ABTS or DPPH inhibition) constructed.

3.7 Preliminary phytochemical tests

Preliminary phytochemical tests were carried out (Harbourne, 1973; Trease and Evans, 1989; Ayoola *et al*., 2008; Singh *et al*., 2009.)

3.7.1 Test for alkaloids

About 0.5 g of extract was reacted with 5 ml of 1% (aq) Hydrochloric acid in a test tube which was carefully stirred in steam bath. Ingredients were filtered through Whatman's no. 1 filter paper. About 1.0 ml of filtrates were reacted with Meyers Reagent. Turbidity, sedimentation or precipitation indicates a positive result.

3.7.2 Test for saponins

About 5 g of extract was reacted with 10ml water and shaken properly in a test tube. Samples showing froth were warmed. Persistent frothing indicated the presence of saponin.

3.7.3 Test for tannins

About 5 g of plant extract was reacted with 10 ml water, stirred and filtered through Whatman's no. 1 filter paper. About 2 ml of the filtrates were reacted with two to three drops of 0.1 % $FeCl_3$ solution. Blue black, green or blue-green precipitate indicated a positive result.

3.7.4 Test for Anthraquinones (Borntrager's Test)

About 5 g of plat extract was reacted with 10 ml benzene, shaken properly and filtered through Whatman's no. 1 filter paper. Filtrates were reacted with 5 ml of 10 % ammonia solution and shaken properly. The presence of pink, red or violet colour in ammonia solution in the lower phase indicated a positive result.

3.7.5 Test for cardiac glycosides (Salkowski's Test)

About 0.5 g of extract was reacted with 2 ml chloroform and mixed carefully. About 2 ml of concentrated Sulphuric acid was carefully added to form a lower layer. Reddish brown colour at interface indicated the presence of a steroidal ring, glycone portion of a cardiac glycoside.

3.7.6 Test for Flavonoids (Lead acetate Test)

About 1 ml of extract was reacted with 1 ml of 10 % lead acetate. Reddish-brown colouration or precipitation indicated a positive result.

CHAPTER 4

RESULTS

An ethnobotanical survey was undertaken to identify and document medicinal plants used to treat sexually transmitted infections in Blouberg area, Limpopo Province, South Africa.

Villages such as Lethaleng, Ga-Nailana, Dilaeneng and Ga-Radimang falls within Blouberg municipality wheras Koekoek, Ga-Mokgehle, Maupye and Ga-Kgare are belonging to Molemole Municipality. Demographic information of informants revealed 65% of males compared to 38 % females (Table 4.1).

According to the demographic data information, the majority of sampled informants fell into the age interval of 61 years and above at 69.2 %, followed by 23% in the 40 to 60 year interval, with 7.7% in the 18 to 39 year age interval (Figure 4.1).

Table 4.1 Demographic information of informants

Municipal border	Villages	Number of Healers (n=13)	Sex		Age range
			Males	Females	
Blouberg	Lethaleng	1	0	1	61+
	Dilaeneng	1	0	1	61+
	Ga-Nailana	3	3	0	61+
	Ga-Radimang	1	1	0	61+
Molemole	Koek-koek	1	0	1	18-39
	Moletsi, Maupye	1	0	1	40-60
	Moletsi, Ga-Kgare	1	1	0	61+
	Moletsi, Ga-Mokgehle	4	1	1	40-60
			2	0	61+
Totals	8 villages	13	61.5 %	38.5%	

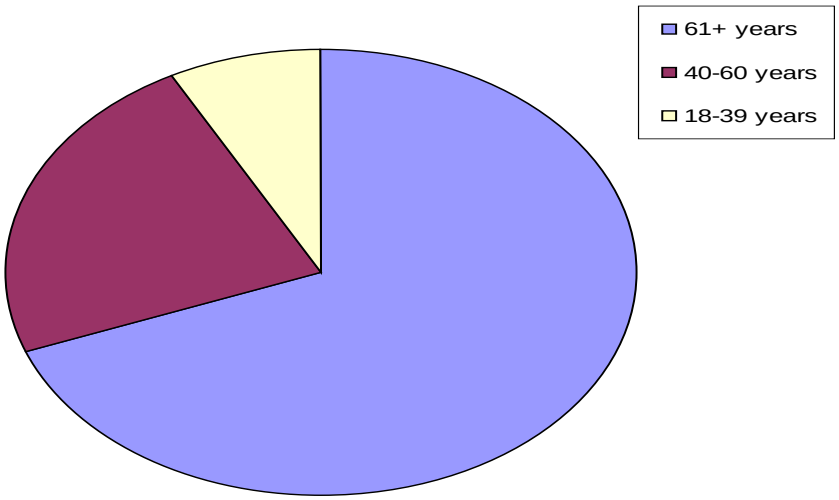


Figure 4.1 Range in age of selected interviewees.

Although dosage and ratio of plants is dependent upon severity of the illness, *Elephantorrhiza burkei*, *Elephantorrhiza* e*lephantine* and *Urginea sanguinea* are either double or triple the quantity of other plant species because of their blood cleansing power.

Eleven medicinal plants from 8 different families reportedly used to treat sexually transmitted infections have been documented (Table 2) with growth habits dominated by herbs (63.64 %), shrubs (18.18%) and trees and climbers at 9.09% each.Four out of the eleven plants commonly used by the traditional healers to treat STIs in the Limpopo province belong to the family Fabaceae; and the mode of preparation of all plants is through decoction, while others may also be administered via inhalation (Table 4.2). Some plant species are named according to their morphological features. *Urginea sanguinea* is indigenously known as *Sekanama* because of the blades or layers on its underground tuber that are easily removed like meat portions.

Stem bark of some trees like *Peltophorum africanum* may be collected from both the east and west sides of the plant, in the direction of wind, because wind is believed to carry power to heal illnesses. However, this might promote ring barking which may eventually result in loss of plant species. In our survey, the used plant parts are underground roots, bulbs or stem barks.

Although one plant can be administered differently, documented plants may both be taken orally and inhaled after boiling (Table 4.3). However, in cases of severe infections without any wounds, inhalation or steaming may be used. A

combination of various plants is boiled in “*pitsana*” which will then be inhaled by a patient. Powders may be licked with a tongue, added to half a cup of warm water or added to a decoction and then taken orally.

Pitsana- pot made up of clay. It may also be used to keep water cold

Elephantorrhiza elephantina , *Elephantorrhiza burkei* and *Urginea sanguinea* are the three most frequently used medicinal plants (Figure 2). *Cissus quadrangularis* showed a large error bar, because it is most used within the Blouberg area where it is abundant. The most common combinations of plants are reported in Table 4.3 and their frequency of use is dependent on type and severity of the illness.

Traditional healers use various combinations of plant species to treat different kinds of sexually transmitted infections. However, choice of plant species is dependent upon the type and nature of an illness *Peltophorum africanum* is regarded as the kingpin “purgative” and mostly used in small quantities. In cases of severe stomach disorders which may result in loss of appetite, *Senna italica* may be used in the same quantities as blood cleansing medicines.On average, the dosage for these medicines is half a cup three times a day.

Table 4.2 Plant species that are used to treat sexually transmitted infections in Limpopo Province, South Africa

Family name	Scientific name	Sotho name	Plant Part used	Mode of Preparation	Other Medicinal uses
Fabaceae	*Elephantorrhiza elephantina*	Mohauwane	Root	Decoction	Blood disorders
	Elephantorrhiza burkei	Mohauwane	Root	Decoction/Inhalation	Blood disorders
	Peltophorum africanum	Mosehla	Stem bark	Decoction	Stomach disorders, fire wood
	Senna italica	Morotelatshotshi	Root	Decoction/Inhalation	Stomach coolant.
Hyacinthaceae	*Urginea sanguinea*	Sekanama	Bulb	Decoction/Inhalation	Blood disorders
Euphorbiaceae	*Jatropha zeyheri*	Sefapabadia	Root	Decoction/Inhalation	Foot ache
Pedaliaceae	*Harpagophythum procumbens*	Moamare	Fleshy roots	Decoction	Induce pregnancy in women
Apiaceae	*Peucedatum sulcatum*	Mongamo	Root	Decoction	Womb disorders
Caesalpinioideae	*Cassia abbreviata*	Monepenepe	Stem bark	Decoction/powder	Combined as “thebele ya madi”. Doctoring homesteads annually.
Sterculiaceae	*Waltheria indica*	Mokhutesela	Root	Decoction	Treat gonorrhoea
Vitaceae	*Cissus quadrangularis*	Mohlabadipoo	Stem	Decoction/Inhalation	General medicine

“Thebele ya madi”- combination o f various dried plant materials which are powdered and used to cleanse blood. Such powders may be used in conjunction with/ as supplement to decoction made up of various combinations of plant materials.

Some of the plants used were in combination with other plants (Table 4.3) According to the number of reports, combination 1 (which comprised of *P. africanum*, *C. quadragularis*, *C abbreviata* and *E. burkei*) and 2 (*C. abbreviata*, *W. Indica, S.italica*) were most reported with a frequency of 6.

Table 4.3 Combinations of plants and mode of administration as reported by informants

Combination number	Plants combined	No. of plants	No. of reports	Mode of administration
1	*P. africanum, C. quadragularis, C abbreviata, E. burkei*	4	6	Mixture is boiled and inhaled for four days, and taken concurrently with combination 2 for treatment of severe gonorrhoea and syphilis without wounds.
2	*C. abbreviata, W. Indica, S.italica*	3	6	Boiled and taken orally, three quarters of a cup per day.
3	*P. africanum, E. burkei, W. Indica*	3	3	Boiled and taken orally for mild gonorrhoea infections.
4	*J. zeyheri, C. Abbreviata*	2	4	Powdered and licked

The most frequently used plants were *Elephantorrhiza burkei* and *Elephantorrhiza elephantine*, with a frequency of use at 78 %, while *Cissus quadrangularis* was the least (Figure 4.2). *Cissus quadrangularis* was only

reported by traditional healers in Blouberg Municipality, suggestng that it might be scarce in Molemole.

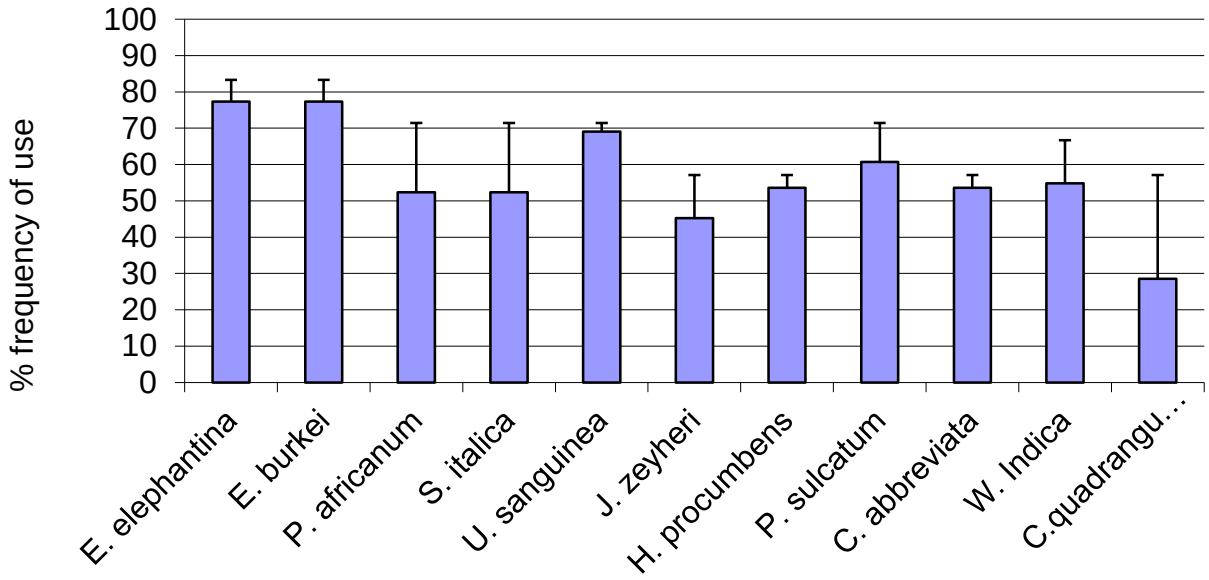


Figure 4.2. Frequency of use of medicinal plants reported by informants. Values

Results were recorded as mean±SE (Blouberg vs Molemole).

The zone of inhibition of the extracts of the stem bark and leaves of *C abbreviata* is presented in table 4.4 and 4.5 respectively. It is apparent that even though some microorganisms were resistant, the selected extracts exhibited antibacterial properties. Methanol extract of stem bark of *C. abbreviata* exhibited activity of 12.3±0.33 mm and 12.3±0.67 mm against *E. cloacae* and *Klebsiella spp* respectively while water extract of similar plant part exhibited zone of inhibition of 12.7±0.88 mm against *S.aureus* (P12724). Acetone leaf extract of the *C. abbreviata* leaf showed inhibition of 12.7±0.88 mm against both *S. aureus* and *B. pumilus*, while all gram negative strains were resistant to ethyl acetate extract (Table 4.5). In general, leaf extracts showed less activity compared to stem bark.

Table 4.4 Antibacterial activity of stem bark extracts from *Cassia abbreviata* using disc diffusion method (mm)

Bacterial strains	**Plant extracts**				
	Water	Acetone	Ethanol	Methanol	Ethyl acetate
E. coli	8.3±0.33	8.0±0.0	R	R	10.3±0.33
P. aeruginosa	9.3±0.33	R	R	R	R
P. aeruginosa (T3374)	R	R	R	R	R
E. cloacae	8.0±0.0	11.7±1.45	9.7±0.88	12.3±0.33	9.3±0.33
K. pneumoniae	R	R	R	R	R
K. pneumoniae (517298)	R	R	R	R	R
Klebsiella spp (317302)	9.3±0.67	R	R	12.3±0.67	R
S. marscens	8.0±0.0	R	R	7.3±0.67	7.3±0.67
S. flexineri *	R	R	7.7±1.15	R	R
Salmonella spp *	R	7.0±0.0	R	R	R
A. C. anitratus (CSIR)	7.3±0.33	R	R	7.7±0.67	R
S. aureus	8.0±0.0	R	9.7±1.33	11.3±1.20	9.0±0.0
S. aureus (P12702)	9.3±0.33	9.0±0.0	11.7±0.88	R	R
S. aureus (P12763)	8.0±0.0	7.0±0.0	R	7.7±1.15	9.0±0.0
S.aureus (P12724)	12.7±0.88	8.0±0.0	10.7±1.76	R	10.7±1.76
S. aureus (B10808)	8.0±0.0	R	11.7±0.67	10.0±0.0	R
S. aureus *	R	11.7±0.88	R	R	R
B. pumilus	10.3±1.67	9.7±1.33	7.7±0.33	R	R
B. cereus	R	7.3±0.33	10.3±1.53	8.0±0.0	8.0±0.0
B. subtilis *	R	R	R	R	7.7±0.33
S. epidirmidis *	R	R	R	R	R
E. faecalis *	R	R	7.3	R	R
S. viridans (517141)	R	R	R	R	R

Results were recorded as a mean of three replicates ± SE. Key: *E. coli- Escherichia coli, P. aeruginosa-Pseudomonas aeruginosa,E. cloacae- Enterobacter cloacae,K.pneumoniae- Klebsiella pneumoniae, Klebsiella spp- Klebsiella* specie,*S. marscens- Serratia marscens,S. flexineri-Shigella flexineri, Salmonella spp- Salmonella* specie, *A. C anitratus- Acinetobacter calcaoceticus anitrans, S. aureus-Staphylococcus aureus.B. pumilus- Baccilus pumilus, B. cereus- Baccilus cereus, B. subtilis- Baccilus subtilis, S. epidirmidis- Staphylococcus epidimidis,E. faecalis- Enterococcus faecalis,* , and *S. viridans-Streptococcus viridans.* *- clinical isolates, coded strains- reportedly resistant, strains with no codes-ATCC. R-Resistant

Table 4.5 Antibacterial activity of leaf extracts from *Cassia abbreviata* using disc diffusion method (mm)

Bacterial strains	**Plant extracts**			
	Acetone	Ethanol	Methanol	Ethyl acetate
E. coli	R	10.0±0.0	R	R
P. aeruginosa	R	R	R	R
P. aeruginosa (T3374)	R	R	R	R
E. cloacae	10.7±1.76	R	R	R
K. pneumoniae	R	R	R	R
K. pneumoniae (517298)	10.0±1.15	R	8.7±0.67	R
Klebsiella spp (317302)	R	R	10.0±0.0	R
S. marscens	R	R	R	R
S. flexineri *	9.0±0.0	R	R	R
Salmonella spp *	R	R	R	R
A. C. anitratus (CSIR)	R	R	R	R
S. aureus	12.7±0.88	R	10.7±1.76	10.0±0.0
S. aureus (P12702)	R	8.3±0.33	11.3±0.88	R
S. aureus (P12763)	R	R	R	R
S.aureus (P12724)	R	R	11.3±0.88	R
S. aureus (B10808)	R	8.7±0.67	10.0±0.0	10.0±0.0
S. aureus *	R	R	R	R
B. pumilus	12.7±0.88	8.7±0.67	R	R
B. cereus	R	R	R	9.0±0.0
B. subtilis *	R	R	8.0±0.0	R
S. epidirmidis *	R	R	R	R
E. faecalis *	R	10.7±1.20	12.7±0.67	8.7±0.67
S. viridans (517141)	11.7±1.45	9.0±0.0	12.7±1.20	R

Results were recorded as a mean of three replicates ± SE. Key: *E. coli- Escherichia coli, P.aeruginosa-Pseudomonasaeruginosa,E.cloacae-Enterobactercloacae,K.pneumoniae-Klebsiella pneumoniae, Klebsiella spp- Klebsiella* specie*,S. marscens- Serratia marscens,S. flexineri- Shigella flexineri, Salmonella spp- Salmonella* specie*, A. C anitratus- Acinetobacter calcaoceticus anitrans, S. aureus-Staphylococcus aureus.B. pumilus- Baccilus pumilus, B. cereus- Baccilus cereus, B. subtilis- Baccilus subtilis, S. epidirmidis- Staphylococcus epidimidis,E. faecalis- Enterococcus faecalis,* , and *S. viridans-Streptococcus viridans.* *- clinical isolates, coded strains- reportedly resistant, strains with no codes-ATCC. R-Resistant

E. coli, *S. aureus*, *B pumilus* and *E. faecalis* were suceptible to all extracts of *W. indica* root, while methanol extract exhibited the largest zone of inhibition of

15.2±2.18 mm against *B. pumilus* (Table 4.6). All Gram negative strains were susceptible to methanol root extract of *W. indica* except *Acinetobacter calcaoceuticals anitratus* and *Klebsiella pneumoniae.*

Antibacterial activity of *Jatropha zeyheri* root and leaf extracts are reported in Table 4.7 and 4.8 respectively. Ethyl acetate extract of *J. zeyheri* root exhibited antibacterial activity against a variety of selected strains, hence broad spectrum, compared to all selected plant extracts (Table 4.7). Although ethanol extract exhibited largest zone of inhibiton of 12.7±0.88 mm against *S. aureus*, it showed no activity against ten of the selected Gram positive bacterial strains. Acetone extract showed activity against some Gram negative strains and only two Gram positive strains (*S. aureus*).

Conversely, ethyl acetate extract of *J. zeyheri* leaf showed activity against only four of the selected bacterial strains (Table 4.8). However, acetone extract showed activity of 12.0±00 mm and 12.0±1.0 mm against *Enterobacter cloacae* and *Acinetobacter calcaoceutical anitratus* respectively. All *S aureus* strains were resistant to all leaf extracts of *Jatropha zeyheri*. In general, root extracts of this plant showed potent antibacterial activity against selected bacterial strains, compared to its leaf extracts.

Table 4.6 Antibacterial activity of root extracts from *Waltheria indica* using disc diffusion method (mm)

Bacterial strains	**Plant extracts**			
	Water	Acetone	Ethanol	Methanol
E. coli	13.6±0.38	8.0±0.0	11.1±0.18	10.1±0.85
P. aeruginosa	R	R	10.0±0.0	R
P. aeruginosa (T3374)	R	10.4±0.15	11.4±0.72	10.2±1.02
E. cloacae	R	R	10.0±0.0	10.0±0.0
K. pneumoniae	10.0±0.0	12.0±0.66	R	R
K. pneumoniae (517298)	R	10.4±0.90	9.4±1.0	9.9±0.73
Klebsiella spp (317302)	9.9±0.57	R	12.7±0.41	12.0±0.0
S. marscens	R	R	9.9±0.57	9.9±0.57
S. flexineri *	R	R	10.8±0.80	11.1±0.18
Salmonella spp *	R	R	12.2±0.67	R
A. C. anitratus (CSIR)	11.7±1.32	R	R	R
S. aureus	R	R	12.7±0.41	10.9±0.93
S. aureus (P12702)	9.9±0.57	R	11.7±0.88	R
S. aureus (P12763)	R	10.0±0.0	R	R
S.aureus (P12724)	10.0±0.0	R	R	11.0±1.26
S. aureus (B10808)	8.9±0.79	10.1±0.85	12.9±0.26	10.9±0.89
S. aureus *	12.0±0.0	R	10.0±0.0	R
B. pumilus	12.6±1.06	11.7±0.88	11.7±0.88	15.2±2.18
B. cereus	R	R	10.8±0.20	9.7±0.72
B. subtilis *	R	R	11.5±0.79	9.7±0.72
S. epidirmidis *	R	R	11.0±1.26	R
E. faecalis *	9.9±0.57	10.4±0.03	10.5±0.82	13.5±0.71
S. viridans (517141)	R	R	R	R

Results were recorded as a mean of three replicates ± SE. Key: *E. coli- Escherichia coli, P. aeruginosa-Pseudomonas aeruginosa,E. cloacae- Enterobacter cloacae,K.pneumoniae- Klebsiella pneumoniae, Klebsiella spp- Klebsiella* specie,*S. marscens- Serratia marscens,S. flexineri-Shigella flexineri, Salmonella spp- Salmonella* specie*, A. C anitratus- Acinetobacter calcaoceticus anitrans, S. aureus-Staphylococcus aureus.B. pumilus- Baccilus pumilus, B. cereus- Baccilus cereus, B. subtilis- Baccilus subtilis, S. epidirmidis- Staphylococcus epidimidis,E. faecalis- Enterococcus faecalis, ,* and *S. viridans-Streptococcus viridans.* *- clinical isolates, coded strains- reportedly resistant, strains with no codes-ATCC. R-Resistant

Table 4.7 Antibacterial activity of root extracts from *Jatropha zeyheri* using disc diffusion method (mm)

Bacterial strains	**Plant extracts**				
	Water	Acetone	Ethanol	Methanol	Ethyl acetate
E. coli	R	R	R	8.0±0.0	7.0±0.0
P. aeruginosa	8.0±0.0	8.7±2.08	10.7±1.76	R	8.0±0.0
P. aeruginosa (T3374)	R	10.0±0.0	R	R	9.7±1.33
E. cloacae	7.3±0.33	R	R	R	R
K. pneumoniae	R	R	R	R	10.7±0.58
K. pneumoniae (517298)	R	10.7±1.76	R	R	12.7±0.88
Klebsiella spp (317302)	R	R	R	R	10.0±0.0
S. marscens	7.0±0.0	7.7±1.15	R	8.3±1.15	R
S. flexineri *	R	R	R	7.0±0.0	9.7±0.58
Salmonella spp *	R	7.3±0.58	R	8.7±1.53	R
A. C. anitratus (CSIR)	R	R	7.3±0.33	8.7±1.53	8.0±0.0
S. aureus	9.3±0.33	9.0±0.0	9.0±0.0	8.3±1.15	9.0±0.0
S. aureus (P12702)	R	R	R	R	12.7±0.88
S. aureus (P12763)	8.0±0.0	11.7±0.88	R	R	8.7±0.67
S.aureus (P12724)	R	R	12.7±0.88	R	R
S. aureus (B10808)	R	R	R	R	8.7±0.67
S. aureus *	R	R	R	R	7.0±0.0
B. pumilus	8.0±0.58	R	R	8.7±1.53	8.0±0.0
B. cereus	R	R	R	R	9.7±0.58
B. subtilis *	R	R	R	R	9.7±0.58
S. epidirmidis *	R	R	R	R	7.3±0.58
E. faecalis *	R	R	R	R	9.7±0.58
S. viridans (517141)	R	R	R	10.7±1.76	10.0±0.0

Results were recorded as a mean of three replicates ± SE. Key: *E. coli- Escherichia coli, P. aeruginosa-Pseudomonas aeruginosa,E. cloacae- Enterobacter cloacae,K.pneumoniae- Klebsiella pneumoniae, Klebsiella spp- Klebsiella* specie*,S. marscens- Serratia marscens,S. flexineri-Shigella flexineri, Salmonella spp- Salmonella* specie*, A. C anitratus- Acinetobacter calcaoceticus anitrans, S. aureus-Staphylococcus aureus.B. pumilus- Baccilus pumilus, B. cereus- Baccilus cereus, B. subtilis- Baccilus subtilis, S. epidirmidis- Staphylococcus epidimidis,E. faecalis- Enterococcus faecalis, ,* and *S. viridans-Streptococcus viridans.* *- clinical isolates, coded strains- reportedly resistant, strains with no codes-ATCC. R-Resistant

Table 4.8 Antibacterial activity of leaf extracts from *Jatropha zeyheri* using disc diffusion method (mm)

Bacterial strains	**Plant extracts**			
	Acetone	Ethanol	Methanol	Ethyl acetate
E. coli	R	R	11.7±1.53	R
P. aeruginosa	R	10.0±0.0	10.7±0.58	R
P. aeruginosa (T3374)	R	10.0±0.0	R	R
E. cloacae	12.0±0.0	R	R	R
K. pneumoniae	10.7±0.58	R	R	R
K. pneumoniae (517298)	R	R	R	R
Klebsiella spp (317302)	R	8.0±0.0	R	R
S. marscens	10.3±1.53	R	R	9.7±0.58
S. flexineri *	R	R	R	9.0±0.0
Salmonella spp *	R	R	R	R
A. C. anitratus (CSIR)	12.0±1.0	R	R	10.3±1.53
S. aureus	R	10.0±0.0	10.7±0.58	R
S. aureus (P12702)	R	R	R	R
S. aureus (P12763)	R	R	R	R
S.aureus (P12724)	R	R	R	R
S. aureus (B10808)	R	R	R	R
S. aureus *	R	R	R	R
B. pumilus	10.7±0.58	R	R	R
B. cereus	R	R	8.7±1.53	9.7±0.58
B. subtilis *	R	R	R	R
S. epidirmidis *	R	R	R	R
E. faecalis *	R	R	12.0±0.0	R
S. viridans (517141)	R	R	R	R

Results were recorded as a mean of three replicates ± SE. Key: *E. coli- Escherichia coli, P. aeruginosa-Pseudomonas aeruginosa,E. cloacae- Enterobacter cloacae,K.pneumoniae- Klebsiella pneumoniae, Klebsiella spp- Klebsiella* specie*,S. marscens- Serratia marscens,S. flexineri-Shigella flexineri, Salmonella spp- Salmonella* specie*, A. C anitratus- Acinetobacter calcaoceticus anitrans, S. aureus-Staphylococcus aureus.B. pumilus- Baccilus pumilus, B. cereus- Baccilus cereus, B. subtilis- Baccilus subtilis, S. epidirmidis- Staphylococcus epidimidis,E. faecalis- Enterococcus faecalis, ,* and *S. viridans-Streptococcus viridans. **-* clinical isolates*,* coded strains- reportedly resistant*,* strains with no codes-ATCC. R-Resistant

Results for antibacterial activity of *Peltophorum africanum, Senna italica* and *Elephantorrhiza burkei* are reported in table 4.9, Table 4.10 and Table 4.11 respectively. *Klebsiella spp* and three *Staphylococccus aureus* species viz. P12702, P12763 and P12724 were susceptible to all the five extracts of *P. africanum*, with zones of inhibitions ranging from 8.7±0.67 to 14.3±0.33 mm, while *Staphylococcus epidirmidis*, *Staphylococcus aureus* (B10808), two strains of *Klebsiella pneumoniae* and *Streptococccus viridans* were resistant to all extracts of *P. africanum* (Table 4.9).

Ethyl acetate extract from *Senna italica* exhibited antibacterial activity against against all selected Gram negative strains except *Klebsiella pneumoniae* (517298) and *Acinetebacter calcaoceuticals anitratus* (Table 4.10). *Streptococcus viridans*, *Staphylococcus epidirmis*, *Bacillus subtilis*, *Staphylococcus aureus* (P127020, (P12763), (P12724), *Acinetobacter calcaoceuticals anitratus*, and *Klebsiella pneumoniae* (517298) were resistant to all the selected extracts of *S. italica*. Methanol extract only showed activity against *Pseudomonas aeruginosa*, *Enterobacter cloacae*, *Staphylococcus aureus*.

Ethyl acetate extract from *Elephantorrhiza burkei* only showed activity of 9.3±0.33 mm against *Enterobacter cloacae* and *Pseudomonas aeruginosa* and no activity against all other Gram negative strains (Table 4.11). Moreover, microorganisms such as *Streptococcus viridans*, *Enterococcus faecalis*, Staphylococcus epidirmidis, clinical isolate of *Staphylococcus aureus* and *Klebsiella pneumoniae* were resistant to all selected strains of *E. burkei*.

Table 4.9 Antibacterial activity of stem bark extracts from *Peltophorum africanum* using disc diffusion method (mm)

Bacterial strains	**Plant extracts**				
	Water	Acetone	Ethanol	Methanol	Ethyl acetate
E. coli	9.3±0.33	9.3±0.33	11.3±0.0	8.0±0.0	R
P. aeruginosa	9.3±0.33	8.7±0.67	R	8.0±0.0	7.7±0.0
P. aeruginosa (T3374)	R	R	10.0±1.15	R	R
E. cloacae	8.0±0.0	R	8.7±1.53	R	9.3±0.33
K. pneumoniae	R	R	R	R	R
K. pneumoniae (517298)	R	R	R	R	R
Klebsiella spp (317302)	11.7±1.20	14.3±0.33	13.7±0.67	12.7±1.20	13.3±0.88
S. marscens	R	8.7±0.33	R	10.7±0.58	8.0±0.0
S. flexineri *	R	7.7±0.33	12.3±0.67	R	R
Salmonella spp *	R	R	R	R	R
A. C. anitratus (CSIR)	9.0±0.0	R	R	8.3±0.33	R
S. aureus	R	8.0±0.0	R	7.3±0.33	8.3±0.33
S. aureus (P12702)	10±1.15	9.7±0.88	11.3±0.88	14.3±0.33	9.7±0.88
S. aureus (P12763)	9.3±0.88	13±0.58	8.7±0.67	13±0.58	10.7±0.67
S.aureus (P12724)	10.7±0.67	14.3±0.33	12±1.53	11.7±1.20	12.3±0.67
S. aureus (B10808)	R	R	R	R	R
S. aureus *	R	R	R	7.7±0.33	R
B. pumilus	R	8.3±0.33	9.0±0.0	R	R
B. cereus	R	R	R	R	8.3±0.33
B. subtilis *	11.0±0.0	R	R	R	R
S. epidirmidis *	R	R	R	R	R
E. faecalis *	7.0±0.0	R	R	R	R
S. viridans (517141)	R	R	R	R	R

Results were recorded as a mean of three replicates ± SE. Key: *E. coli- Escherichia coli, P. aeruginosa-Pseudomonas aeruginosa,E. cloacae- Enterobacter cloacae,K.pneumoniae-Klebsiella pneumoniae, Klebsiella spp- Klebsiella* specie,*S. marscens- Serratia marscens,S. flexineri-Shigella flexineri, Salmonella spp- Salmonella* specie*, A. C anitratus- Acinetobacter calcaoceticus anitrans, S. aureus-Staphylococcus aureus.B. pumilus- Baccilus pumilus, B. cereus- Baccilus cereus, B. subtilis- Baccilus subtilis, S. epidirmidis- Staphylococcus epidimidis,E. faecalis- Enterococcus faecalis, ,* and *S. viridans-Streptococcus viridans.* *- clinical isolates, coded strains- reportedly resistant, strains with no codes-ATCC. R-Resistant

Table 4.10 Antibacterial activity of root extracts from *Senna italica* using disc diffusion method (mm)

Bacterial strains	**Plant extracts**				
	Water	Acetone	Ethanol	Methanol	Ethyl acetate
E. coli	R	R	10.3±1.53	R	8.0±0.0
P. aeruginosa	8.0±0.0	R	R	9.0±0.0	10.3±1.53
P. aeruginosa (T3374)	R	R	R	R	8.7±0.67
E. cloacae	7.0±0.0	9.0±0.0	8.3±0.33	9.3±0.33	8.0±0.0
K. pneumoniae	R	R	R	R	7.3±0.33
K. pneumoniae (517298)	R	R	R	R	R
Klebsiella spp (317302)	R	R	R	R	8.7±0.67
S. marscens	10.3±0.33	R	R	R	8.7±0.67
S. flexineri *	R	R	7.7±0.33	R	7.7±0.33
Salmonella spp *	R	R	R	R	7.0±0.0
A. C. anitratus (CSIR)	R	R	R	R	R
S. aureus	7.0±0.0	7.0±0.0	8.0±0.0	8.0±0.0	8.0±0.0
S. aureus (P12702)	R	R	R	R	R
S. aureus (P12763)	R	R	R	R	R
S.aureus (P12724)	R	R	R	R	R
S. aureus (B10808)	R	R	9.7±0.88	R	R
S. aureus *	7.7±0.33	R	R	R	7.0±0.0
B. pumilus	R	8.7±0.67	R	R	R
B. cereus	10±0.0	R	7.0±0.0	R	8.0±0.0
B. subtilis *	R	R	R	R	R
S. epidirmidis *	R	R	R	R	R
E. faecalis *	R	R	7.3±0.33	R	R
S. viridans (517141)	R	R	R	R	R

Results were recorded as a mean of three replicates ± SE. Key: *E. coli- Escherichia coli, P. aeruginosa-Pseudomonas aeruginosa,E. cloacae- Enterobacter cloacae,K.pneumoniae- Klebsiella pneumoniae, Klebsiella spp- Klebsiella* specie,*S. marscens- Serratia marscens,S. flexineri-Shigella flexineri, Salmonella spp- Salmonella* specie*, A. C anitratus- Acinetobacter calcaoceticus anitrans, S. aureus-Staphylococcus aureus.B. pumilus- Baccilus pumilus, B. cereus- Baccilus cereus, B. subtilis- Baccilus subtilis, S. epidirmidis- Staphylococcus epidimidis,E. faecalis- Enterococcus faecalis, ,* and *S. viridans-Streptococcus viridans. **-* clinical isolates, coded strains- reportedly resistant, strains with no codes-ATCC. R-Resistant

Table 4.11 Antibacterial activity of root extracts from *Elephantorrhiza burkei* using disc diffusion method (mm)

Bacterial strains	**Plant extracts**				
	Water	Acetone	Ethanol	Methanol	Ethyl acetate
E. coli	R	8.0±0.0	R	8.0±0.0	R
P. aeruginosa	R	8.0±0.0	9.3±0.33	10.3±0.33	9.3±0.33
P. aeruginosa (T3374)	R	R	10.0±1.15	R	R
E. cloacae	7.7±0.33	8.0±0.0	10.3±1.53	9.3±0.33	9.3±0.33
K. pneumoniae	R	R	R	R	R
K. pneumoniae (517298)	R	13.0±1.0	R	R	R
Klebsiella spp (317302)	R	11.70.67	13.0±1.15	15.7±0.67	R
S. marscens	8.3±0.33	8.7±0.67	8.7±1.53	R	R
S. flexineri *	8.3±0.33	R	8.3±0.33	R	R
Salmonella spp *	R	R	R	7.0±0.0	R
A. C. anitratus (CSIR)	R	8.3±0.33	R	9.7±0.58	R
S. aureus	8.3±0.33	7.0±0.0	R	8.0±0.0	R
S. aureus (P12702)	R	12.0±1.15	R	9.7±0.88	12.0±1.0
S. aureus (P12763)	R	13.7±0.67	11.3±1.86	12.3±1.45	10.7±0.67
S.aureus (P12724)	R	10.3±0.88	14.0±0.58	11.7±0.88	11±0.58
S. aureus (B10808)	R	R	10.0±1.0	R	R
S. aureus *	R	R	R	R	R
B. pumilus	R	8.3±0.33	7.7±0.33	R	R
B. cereus	R	12.7±0.88	10.3±0.67	9.0±0.0	11.7±0.88
B. subtilis *	R	R	R	8.7±0.33	R
S. epidirmidis *	R	R	R	R	R
E. faecalis *	R	R	R	R	R
S. viridans (517141)	R	R	R	R	R

Results were recorded as a mean of three replicates ± SE. Key: *E. coli- Escherichia coli, P. aeruginosa-Pseudomonas aeruginosa,E. cloacae- Enterobacter cloacae,K.pneumoniae- Klebsiella pneumoniae, Klebsiella spp- Klebsiella* specie,*S. marscens- Serratia marscens,S. flexineri-Shigella flexineri, Salmonella spp- Salmonella* specie, *A. C anitratus- Acinetobacter calcaoceticus anitrans, S. aureus-Staphylococcus aureus.B. pumilus- Baccilus pumilus, B. cereus- Baccilus cereus, B. subtilis- Baccilus subtilis, S. epidirmidis- Staphylococcus epidimidis,E. faecalis- Enterococcus faecalis, ,* and *S. viridans-Streptococcus viridans.* *- clinical isolates, coded strains- reportedly resistant, strains with no codes-ATCC. R-Resistant

Antibacterial activity of *Elephantorrhiza elephantine*, *Hapargophythum procumbens* and *Cissus quadrangularis* are reported in Table 4.12, Table 4.13 and Table 4.14 respectively. *Enterobacter cloacae*, two *Klebsiella pneumonia* species, *Staphylococcus aureus* (B10808) and *Escherichia coli* were resistant to water, acetone and ethanol extracts of *Elephantorrhiza elephantine* while *Bacillus subtilis*, *Staphylococcus epidirmidis*, *Enterococcus faecalis*, *Salmonella spp* and *Klebsiella pneuminae* (517298) were found to be resistant to selected extracts of *Elephantorrhiza elephantine* (Table 4.12).

All seleceted Gram positive strains were resistant to water extract from Hapargophythum procumbens except *Bacillus cereus,* while *Staphylococcus epidirmidis*, *Enterococcus faecalis*, *Streptococcus viridans* and *Shigella flexineri* were resistant to all the selected extracts from *H. procumbens* (Table 4.13). Moreover, *Serratia marscens* and *Bacillus cereus* were susceptible to all selected extracts of *H. procumbens*.

Although ethyl acetate exract from *Cissus quadrangularis* exhibited maximum zone of inhibition of 12.7±0.88 against *Escherichia coli*, it showed no activity against all selected Gram positive bacterial strains except three strains of *Staphylococcus aureus* (Table 4.14). Moreover, *Enterococcus faecalis*, *Bacillus cereus* and *Acinetobacter calcaoceuticals anitratus* were resistant to all the selected extracts from *Cissus quadrangularis*.

Table 4.12 Antibacterial activity of root extracts *from Elephantorrhiza elephantina* using disc diffusion method (mm)

Bacterial strains	**Plant extracts**				
	Water	Acetone	Ethanol	Methanol	Ethyl acetate
E. coli	R	R	R	R	7.7±0.33
P. aeruginosa	8.0±0.0	R	R	9.7±1.33	7.0±0.0
P. aeruginosa (T3374)	R	R	R	R	R
E. cloacae	R	R	R	7.7±0.33	R
K. pneumoniae	R	R	R	R	7.7±0.33
K. pneumoniae (517298)	R	R	R	R	R
Klebsiella spp (317302)	13.0±1.15	13.0±1.0	12.0±0.58	14.7±0.33	R
S. marscens	R	9.7±0.33	R	R	8.7±0.67
S. flexineri *	R	7.3±0.33	R	R	8.0±0.0
Salmonella spp *	R	R	R	R	R
A. C. anitratus (CSIR)	R	8.7±0.33	R	R	R
S. aureus	8.3±0.33	7.7±0.33	9.7±0.33	8.0±0.0	8.0±0.0
S. aureus (P12702)	8.7±0.67	10.3±1.45	13.3±0.33	9.7±0.88	R
S. aureus (P12763)	11.7±0.88	10.7±1.76	12.3±0.67	12.0±1.15	11.7±0.88
S.aureus (P12724)	10.3±0.33	13.6±0.88	12.0±1.0	9.3±0.33	R
S. aureus (B10808)	R	R	R	10.0±1.15	R
S. aureus *	R	R	R	R	7.3±0.33
B. pumilus	R	8.3±0.67	R	R	R
B. cereus	10.3±1.53	10.7±1.76	11.7±0.88	11.3±1.20	10.3±1.53
B. subtilis *	R	R	R	R	R
S. epidirmidis *	R	R	R	R	R
E. faecalis *	R	R	R	R	R
S. viridans (517141)	8.6±0.67	9.3±1.33	R	10.7±0.67	R

Results were recorded as a mean of three replicates ± SE. Key: *E. coli- Escherichia coli, P. aeruginosa-Pseudomonas aeruginosa,E. cloacae- Enterobacter cloacae,K.pneumoniae- Klebsiella pneumoniae, Klebsiella spp- Klebsiella* specie,*S. marscens- Serratia marscens,S. flexineri-Shigella flexineri, Salmonella spp- Salmonella* specie, *A. C anitratus- Acinetobacter calcaoceticus anitrans, S. aureus-Staphylococcus aureus.B. pumilus- Baccilus pumilus, B. cereus- Baccilus cereus, B. subtilis- Baccilus subtilis, S. epidirmidis- Staphylococcus epidimidis,E. faecalis- Enterococcus faecalis,* , and *S. viridans-Streptococcus viridans.* *- clinical isolates, coded strains- reportedly resistant, strains with no codes-ATCC. R-Resistant

Table 4.13 Antibacterial activity of root extracts from *Harpagophythum procumbens* using disc diffusion method (mm)

Bacterial strains	**Plant extracts**				
	Water	Acetone	Ethanol	Methanol	Ethyl acetate
E. coli	8.0±0.0	7.0±0.0	7.3±0.33	R	R
P. aeruginosa	R	R	R	9.3±0.33	8.0±0.0
P. aeruginosa (T3374)	R	8.0±0.0	11.7±0.88	10.0±0.0	R
E. cloacae	R	R	9.3±0.33	R	R
K. pneumoniae	R	R	7.3±0.33	R	R
K. pneumoniae (517298)	10.0±0.0	R	R	R	R
Klebsiella spp (317302)	R	13.7±0.33	R	R	8.0±0.0
S. marscens	8.7±0.33	10.0±0.0	10.0±0.0	11.3±1.20	8.3±0.33
S. flexineri *	R	R	R	R	R
Salmonella spp *	R	R	R	R	10.3±0.33
A. C. anitratus (CSIR)	R	R	R	7.7±0.33	R
S. aureus	R	12.7±0.88	R	R	R
S. aureus (P12702)	R	R	R	10.0±0.0	R
S. aureus (P12763)	R	10.0±0.0	R	R	R
S.aureus (P12724)	R	R	R	12.0±0.0	11.7±0.88
S. aureus (B10808)	R	R	R	R	11.7±0.88
S. aureus *	R	R	R	8.3±0.33	R
B. pumilus	R	R	7.3±0.33	R	7.3±0.33
B. cereus	8.3±0.33	8.7±0.67	8.3±0.33	12.7±0.88	9.0±0.0
B. subtilis *	R	R	8.3±0.33	R	R
S. epidirmidis *	R	R	R	R	R
E. faecalis *	R	R	R	R	R
S. viridans (517141)	R	R	R	R	R

Results were recorded as a mean of three replicates ± SE. Key: *E. coli- Escherichia coli, P. aeruginosa-Pseudomonas aeruginosa,E. cloacae- Enterobacter cloacae,K.pneumoniae- Klebsiella pneumoniae, Klebsiella spp- Klebsiella* specie,*S. marscens- Serratia marscens,S. flexineri-Shigella flexineri, Salmonella spp- Salmonella* specie, *A. C anitratus- Acinetobacter calcaoceticus anitrans, S. aureus-Staphylococcus aureus.B. pumilus- Baccilus pumilus, B. cereus- Baccilus cereus, B. subtilis- Baccilus subtilis, S. epidirmidis- Staphylococcus epidimidis,E. faecalis- Enterococcus faecalis,* , and *S. viridans-Streptococcus viridans.* *- clinical isolates, coded strains- reportedly resistant, strains with no codes-ATCC. R-Resistant

Table 4.14 Antibacterial activity of stem extracts from *Cissus quadrangularis* using disc diffusion method (mm)

Bacterial strains	**Plant extracts**				
	Water	Acetone	Ethanol	Methanol	Ethyl acetate
E. coli	R	R	R	8.7±0.67	12.7±0.88
P. aeruginosa	R	10.3±1.53	8.0±0.0	9.3±0.33	8.7±0.33
P. aeruginosa (T3374)	R	R	R	10.0±0.0	12.3±0.67
E. cloacae	10.0±0.0	R	R	R	R
K. pneumoniae	R	12.3±1.20	R	R	R
K. pneumoniae (517298)	R	R	10.0±0.0	12.7±0.88	R
Klebsiella spp (317302)	R	R	R	10.0±0.0	8.7±0.33
S. marscens	8.7±0.67	R	R	7.7±0.33	9.0±0.00
S. flexineri *	R	R	R	9.3±0.33	R
Salmonella spp *	R	R	R	R	8.0±0.0
A. C. anitratus (CSIR)	R	R	R	R	R
S. aureus	R	R	8.0	12.3±0.67	10.7±0.67
S. aureus (P12702)	R	R	R	10.0±0.0	11.0±0.0
S. aureus (P12763)	R	R	12.0±0.0	R	R
S.aureus (P12724)	R	R	10.0±0.0	R	R
S. aureus (B10808)	R	10.0±0.0	R	10.0±0.0	R
S. aureus *	R	R	R	8.7±0.67	8.7±1.53
B. pumilus	10.7±0.33	R	R	R	R
B. cereus	R	R	R	R	R
B. subtilis *	10.0±0.0	R	R	R	R
S. epidirmidis *	R	10.7±1.76	R	R	R
E. faecalis *	R	R	R	R	R
S. viridans (517141)	R	10.0±0.0	R	10.0±1.0	R

Results were recorded as a mean of three replicates ± SE. Key: *E. coli- Escherichia coli, P. aeruginosa-Pseudomonas aeruginosa,E. cloacae- Enterobacter cloacae,K.pneumoniae- Klebsiella pneumoniae, Klebsiella spp- Klebsiella* specie*,S. marscens- Serratia marscens,S. flexineri-Shigella flexineri, Salmonella spp- Salmonella* specie*, A. C anitratus- Acinetobacter calcaoceticus anitrans, S. aureus-Staphylococcus aureus.B. pumilus- Baccilus pumilus, B. cereus- Baccilus cereus, B. subtilis- Baccilus subtilis, S. epidirmidis- Staphylococcus epidimidis,E. faecalis- Enterococcus faecalis, ,* and *S. viridans-Streptococcus viridans.* *- clinical isolates, coded strains- reportedly resistant, strains with no codes-ATCC. R-Resistant

Acetone extract from *Urginea sanguinea* did not show activity against all the selected Gram negative bacterial strains, while ethyl acetate extract showed activity against only two Gram positive bacterial strains such as *Staphylococcus aureus* and *Enteorcoccus faecalis* (Table 4.15). Moreover *Staphylococcus aureus* was the only strain susceptible to all the selected extracts from *U. sanguinea*.

Escherichia coli, *Salmonella spp*, *Staphylococcus epidirmidis* and *Enterococcus faecalis* were resistant to all the selected extracts from Peucedanum sulcatum (Table 4.16). Interestingly, water and ethyl acetate extracts exhibited zone of inhibition of 12.7±0.88 mm against *Pseudomonas aeruginosa* (T3374) and *Klebsiella spp* respectively. However, *Staphylococcus epidirmidis*, *Enterococcus faecalis*, *Salmonella spp*, *Serratia marscens* and *Escherichia coli* were resistant to all the selected P. sulcatum extracts.

Selected strains were more susceptible to streptomycin and neomycin than penicillin (Table 4.17). Moreover, the largest zone of inhibition of 29.3±0.57 was obtained from neomycin against *Escherichia coli*.

Table 4.15 Antibacterial activity of extracts from *Urginea sanguinea* using disc diffusion method (mm)

Bacterial strains	**Plant extracts**				
	Water	Acetone	Ethanol	Methanol	Ethyl acetate
E. coli	8.0±0.0	R	10.0±0.0	R	10.3±0.33
P. aeruginosa	R	R	R	8.0±0.0	13.0±0.0
P. aeruginosa (T3374)	12.7±0.88	R	R	7.7±0.33	R
E. cloacae	8.0±0.0	R	R	R	R
K. pneumoniae	R	R	R	R	R
K. pneumoniae (517298)	10.0±0.0	R	R	8.7±0.33	10.0±0.0
Klebsiella spp (317302)	R	R	8.7±0.67	R	R
S. marscens	7.0±0.0	R	R	7.7±0.33	8.7±0.67
S. flexineri *	R	R	7.7±0.33	R	R
Salmonella spp *	R	R	R	R	R
A. C. anitratus (CSIR)	8.3±0.33	R	R	9.3±0.33	R
S. aureus	7.0±0.0	8.3±0.33	7.7±0.33	7.7±0.33	10.3±0.33
S. aureus (P12702)	8.3±0.33	12.7±0.88	R	8.7±0.33	R
S. aureus (P12763)	R	8.0±0.0	10.7±1.76	R	R
S.aureus (P12724)	R	R	R	R	R
S. aureus (B10808)	9.7±1.33	8.0±0.0	R	9.7±0.58	R
S. aureus *	R	12.7±0.88	7.7±0.33	12.7±0.88	R
B. pumilus	7.7±0.33	7.7±0.33	R	R	R
B. cereus	R	11.7±0.88	8.0±0.0	R	R
B. subtilis *	8.0±0.0	7.0±0.0	R	R	R
S. epidirmidis *	7.7±0.33	R	R	R	R
E. faecalis *	R	R	R	R	7.3±0.33
S. viridans (517141)	12.7±0.88	R	R	R	R

Results were recorded as a mean of three replicates ± SE. Key: *E. coli- Escherichia coli, P. aeruginosa-Pseudomonas aeruginosa,E. cloacae- Enterobacter cloacae,K.pneumoniae- Klebsiella pneumoniae, Klebsiella spp- Klebsiella* specie,*S. marscens- Serratia marscens,S. flexineri-Shigella flexineri, Salmonella spp- Salmonella* specie, *A. C anitratus- Acinetobacter calcaoceticus anitrans, S. aureus-Staphylococcus aureus.B. pumilus- Baccilus pumilus, B. cereus- Baccilus cereus, B. subtilis- Baccilus subtilis, S. epidirmidis- Staphylococcus epidimidis,E. faecalis- Enterococcus faecalis,* , and *S. viridans-Streptococcus viridans.* *- clinical isolates, coded strains- reportedly resistant, strains with no codes-ATCC. R-Resistant

Table 4.16 Antibacterial activity of root extracts from *Peucedanum sulcatum* using disc diffusion method (mm)

Bacterial strains	**Plant extracts**				
	Water	Acetone	Ethanol	Methanol	Ethyl acetate
E. coli	R	R	R	R	R
P. aeruginosa	8.0±0.0	R	11.0±0.0	R	R
P. aeruginosa (T3374)	12.7±0.88	R	8.7±0.67	8.0±0.0	R
E. cloacae	9.7±0.58	R	10±0.0	11±0.0	9.7±0.33
K. pneumoniae	R	R	R	7.3±0.33	R
K. pneumoniae (517298)	R	R	9.7±0.58	R	9.7±0.33
Klebsiella spp (317302)	8.0±0.0	8.0±0.0	R	13.0±0.0	12.7±0.88
S. marscens	R	R	R	R	R
S. flexineri *	7.7±0.33	R	7.0±0.0	R	R
Salmonella spp	R	R	R	R	R
A. c. anitratus (CSIR)	R	R	7.7±0.33	R	8.3±0.33
S. aureus	7.0±0.0	8.0±0.0	R	7.0±0.0	R
S. aureus (P12702)	12.7±0.88	R	12.7±0.88	R	R
S. aureus (P12763)	R	9.0±0.0	9.0±0.0	R	R
S. aureus (P12724)	8.7±0.33	R	R	12.7±0.88	12.0±0.0
S. aureus (B10808)	8.7±0.33	7.3±0.33	R	8.0±0.0	R
S. aureus *	R	7.0±0.0	7.0±0.0	R	R
B. pumilus	R	R	R	R	8.7±0.67
B. cereus	R	R	R	7.0±0.0	R
B. subtilis *	8.3±0.33	R	R	R	R
S. epidirmidis *	R	R	R	R	R
E. faecalis *	R	R	R	R	R
S. viridans (517141)	12.7±0.88	R	R	11.7±0.88	R

Results were recorded as a mean of three replicates ± SE. Key: *E. coli- Escherichia coli, P. aeruginosa-Pseudomonas aeruginosa,E. cloacae- Enterobacter cloacae,K.pneumoniae- Klebsiella pneumoniae, Klebsiella spp- Klebsiella* specie,*S. marscens- Serratia marscens,S. flexineri-Shigella flexineri, Salmonella spp- Salmonella* specie, *A. C anitratus- Acinetobacter calcaoceticus anitrans, S. aureus-Staphylococcus aureus.B. pumilus- Baccilus pumilus, B. cereus- Baccilus cereus, B. subtilis- Baccilus subtilis, S. epidirmidis- Staphylococcus epidimidis,E. faecalis- Enterococcus faecalis, ,* and *S. viridans-Streptococcus viridans.* *- clinical isolates, coded strains- reportedly resistant, strains with no codes-ATCC. R-Resistant

Table 4.17 Susceptibility pattern of standard antibiotics (10 µg/disc) using disc diffusion method (mm)

Bacterial strains	**Standard antibiotic discs**		
	Streptomycin	Neomycin	Penicillin
E. coli	27.0±0.0	29.3±0.57	12.7±0.88
P. aeruginosa	19.3±0.67	24.0±0.0	14.3±0.88
P. aeruginosa (T3374)	24.3±1.16	26.7±1.53	16.3±0.67
E. cloacae	16.7±0.88	23.3±0.57	8.7±0.33
K. pneumoniae	16.7±1.52	21.0±0.0	13.3±0.33
K. pneumoniae (517298)	24.3±1.16	26.7±1.53	16.3±0.67
Klebsiella spp (317302)	16.0±0.67	23.3±0.57	16.3±0.67
S. marscens	17.3±0.33	27.7±1.52	11.3±0.33
S. flexineri *	12.3±0.33	20.3±0.57	12.7±0.88
Salmonella spp *	21.7±2.08	23.0±0.0	12.7±0.88
A. C. anitratus (CSIR)	22.0±0.0	26.7±1.53	12.0±0.0
S. aureus	17.7±1.53	20.3±2.47	11.3±0.33
S. aureus (P12702)	24.3±1.16	24.3±1.16	16.3±0.67
S. aureus (P12763)	17.3±0.33	24.3±1.16	11.7±0.67
S.aureus (P12724)	16.0±0.67	25.3±1.53	11.7±0.67
S. aureus (B10808)	27.7±1.52	27.7±1.52	14.0±0.0
S. aureus *	17.0±0.0	20.3±0.33	14.0±0.0
B. pumilus	18.7±0.67	22.7±1.16	11.3±0.88
B. cereus	22.7±1.16	21.7±1.52	12.3±0.67
B. subtilis *	16.3±0.67	24.3±1.16	11.7±0.67
S. epidirmidis *	18.3±0.33	20.7±1.16	14.3±0.67
E. faecalis *	12.7±1.16	21.7±2.08	11.7±1.52
S. viridans (517141)	16.5±1.41	20.3±2.47	9.7±0.88

Results were recorded as a mean of three replicates ± SE. Key: *E. coli- Escherichia coli, P. aeruginosa-Pseudomonas aeruginosa,E. cloacae- Enterobacter cloacae,K.pneumoniae- Klebsiella pneumoniae, Klebsiella spp- Klebsiella* specie*,S. marscens- Serratia marscens,S. flexineri-Shigella flexineri, Salmonella spp- Salmonella* specie*, A. C anitratus- Acinetobacter calcaoceticus anitrans, S. aureus-Staphylococcus aureus.B. pumilus- Baccilus pumilus, B. cereus- Baccilus cereus, B. subtilis- Baccilus subtilis, S. epidirmidis- Staphylococcus epidimidis,E. faecalis- Enterococcus faecalis, ,* and *S. viridans-Streptococcus viridans.* *- clinical isolates, coded strains- reportedly resistant, strains with no codes-ATCC. R-Resistant

Ethyl acetate extract of *Jatropha zeyheri* root and ethanol extract of *Waltheria indica* exhibited activity of 83 % and 73 % activity against selected strains (both Gram negative and Gram positive strains) respectively (Figure 4.3).

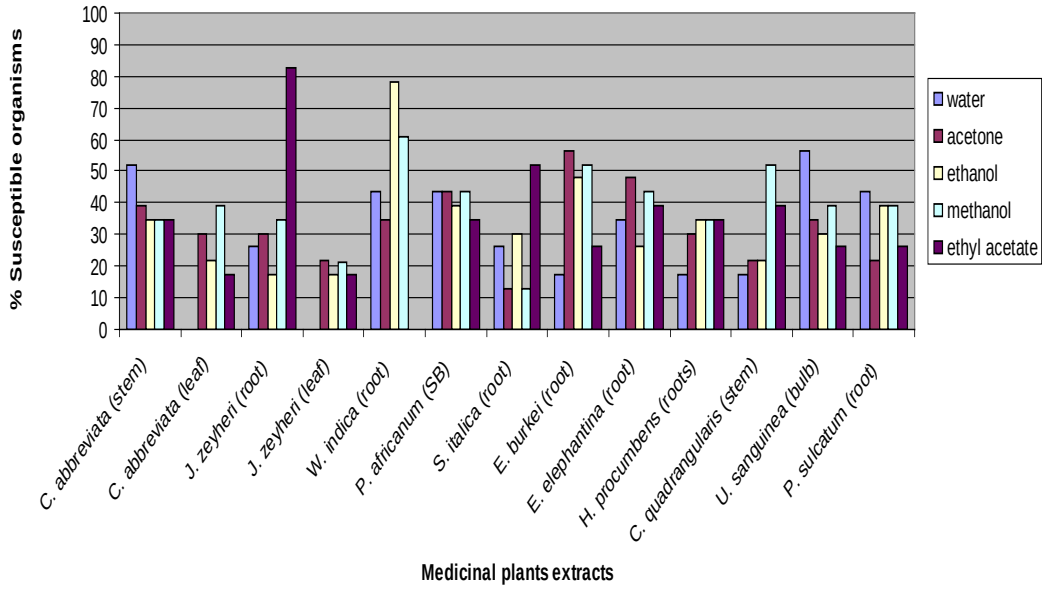


Figure 4.3 Susceptibility pattern of selected organisms to selected plant extracts

Most resistant bacterial strains were *Staphylococcus epidirmidis*, *Bacillus subtilis*, *Acinetobacter calcaoceuticals anitratus*, *Salmonella spp* and *Klebsiella pneumoniae* with resistance of grater than 80 % against the selected bacterial strains (Figur 4.4). Most susceptible organism was *S. aureus* with (29%) resistance against extracts of selected plants.

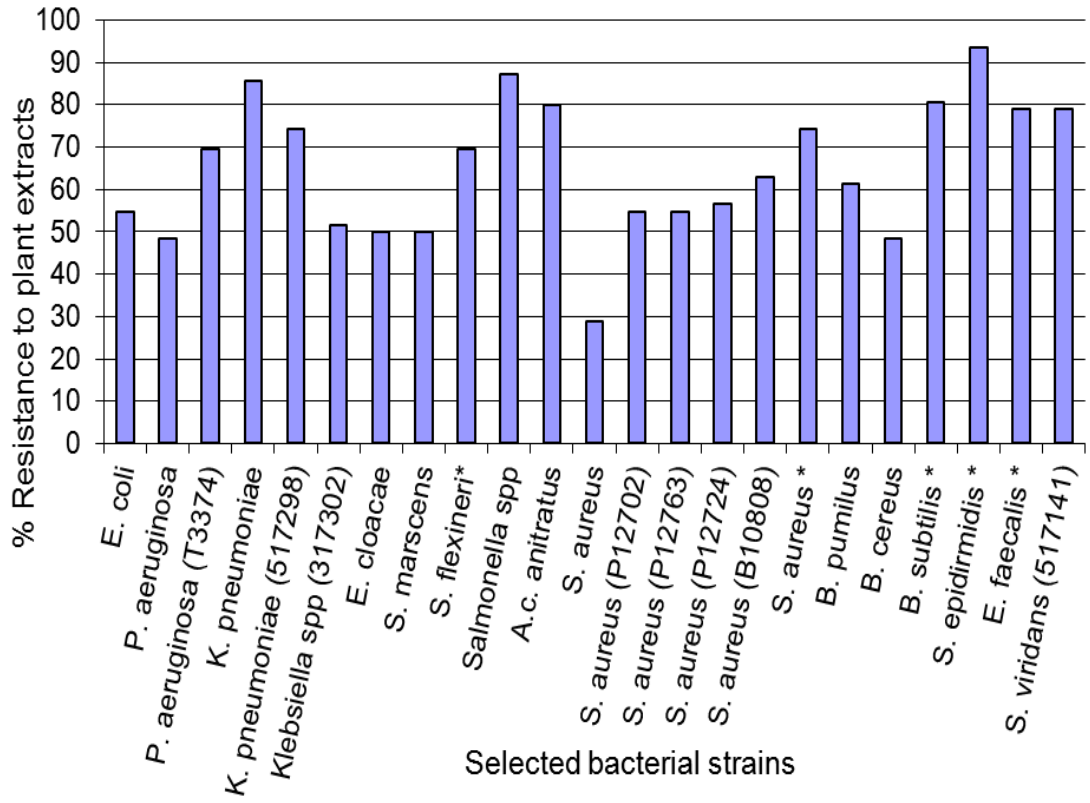


Figure 4.4 Resistance pattern of selected strains against plant extracts.

Ethanol extract from *Cassia abbreviata* stem bark exhibited minimal inhibitory concentration (MIC) of 0.78 mg ml against *Staphylococcus aureus*, 3.13 mg/ml against both *Bacillus pumilus* and *Bacillus cereus* and 6.25 mgml against both *Enterococcus faecalis* and *Staphylococcus aureus* P12702 (Table 4.18). Interestingly, water extract exhibited MIC of 0.39 mg/ml against *Staphylococcus aureus*. Moreover, selected extracts from *C. abbreviata* exhibited MIC values ranging 0.52 to 6.25 mg/ml against *Enterobacter cloacae*. Furhermore, acetone extract MIC of 0.52 against both *Bacillus cereus* and *Enterobacter cloacae,* while methanol extract exhibited 1.56 mg/ml against *Serratia marscens*.

Acetone extract from *Cassia abbreviata* leaf extract exhibited MIC of 2.08 mg/ml against Shigella flexineri (Table 4.19), while methanol extract exhibited MIC of

4.17 mg/ml against both *Enterococcus faecalis* and *Staphylococcus aureus* (12724). Moreover, in general, similar extract exhibited MIC 5.21 mg/ml against *Enterobacter cloacae*, *Klebsiella pneumoniae* (517298) and *Staphylococcus aureus*. However, antibacterial activity of the leaf extracts of this plant showed little activity compared to its stem bark extracts.

Ethanol extract from *Waltheria indica* exhibited MIC of 1.04 mg/ml against *Klebsiella pneumoniae* (517298), *Bacillus subtilis* and *Enterococcus faecalis* (Table 4.20), while methanol extract exhibited MIC OF 1.30 mg/ml against *Bacillus subtilis* and *Enterococcus faecalis*. Interestingly, water extract exhibied MIC of 2.08 mg/ml against *Escherichia coli*, while acetone extract exhibited 1.30 mg/ml against *Staphylococcus epidirmidis*.

Antibacterial activity of root and leaf extracts from *Jatropha zeyheri* are reported in Table 4.21 and 4.22 respectively. Ethyl acetate extract from root exhibited MIC of 0.52 mg/ml against *Streptococcus viridans*, *Staphylococcus aureus* (P12702), and Staphylococcus aureus (P12763). Moreover, methanol extract exhibited MIC of 0.78 mg/ml against *Serratia marscens*, while water extract exhibited activity of 3.13 mg/ml and 0.78 mg/ml against *Serratia marscens* and *Staphylococcus aureus* (P12763) respectively. Ethanol extract of the leaf exhibited MIC of 0.78 mg/ml against both *Pseudomonas aeruginosa* (T3374) and *Klebsiella spp* (Table 4.22). Moreover, acetone extract exhibited 6.25 mg/ml against *Klebsiella pneumoniae*, *Acinetobacter calcaoceuticals anitratus* and *Bacillus subtilis*.

Table 4.18 Antibacterial activity of stem bark extracts from *Cassia abbreviata* (MIC in (mg/ml)

Bacterial strains	**Plant extracts**				
	Water	Acetone	Ethanol	Methanol	Ethyl acetate
E. coli	>10	0.39	ND	ND	4.17
P. aeruginosa	6.25	ND	ND	ND	ND
E. cloacae	6.25	0.52	1.56	2.60	3.13
Klebsiella spp (317302)	4.17	ND	ND	>10	ND
S. marscens	6.25	ND	ND	1.56	>10
*S. flexineri**	ND	ND	3.65	ND	ND
*Salmonella spp**	ND	3.65	ND	ND	ND
A. c. anitratus (CSIR)	>10	ND	ND	>10	ND
S. aureus	>10	1.04	0.78	4.17	3.13
S. aureus (P12702)	0.39	ND	6.25	ND	ND
S. aureus (P12763)	>10	2.08	ND	5.21	4.17
S. aureus (P12724)	3.13	5.21	6.25	ND	>10
S. aureus (B10808)	>10	3.13	>10	>10	ND
B. pumilus	6.25	5.21	3.13	ND	ND
B. cereus	ND	0.52	3.13	6.25	4.17
*B. subtilis**	ND	1.56	ND	ND	1.56
*E. faecalis**	ND	ND	6.25	ND	ND

Results were recorded as a mean of three replicates. Key: *E. coli- Escherichia coli, P. aeruginosa-Pseudomonas aeruginosa,E. cloacae- Enterobacter cloacae,Klebsiella spp- Klebsiella* specie,*S. marscens- Serratia marscens,S. flexineri-Shigella flexineri, Salmonella spp- Salmonella* specie*, A. C anitratus- Acinetobacter calcaoceticus anitrans, S. aureus-Staphylococcus aureus.B. pumilus-Baccilus pumilus, B. cereus- Baccilus cereus, B. subtilis- Baccilus subtilis,E. faecalis- Enterococcus faecalis. *-* clinical isolates*,* coded strains- reportedly resistant*,* strains with no codes-ATCC. ND- not done

Table 4.19 Antibacterial activity of leaf extracts from *Cassia abbreviata* (MIC in mg/ml)

Bacterial strains	**Plant extracts**			
	Acetone	Ethanol	Methanol	Ethyl acetate
E. coli	ND	6.25	ND	ND
E. cloacae	5.21	ND	ND	ND
K. pneumoniae (517298)	5.21	ND	5.21	ND
Klebsiella spp (317302)	ND	ND	6.25	ND
S. flexineri *	2.08	ND	ND	ND
S. aureus	5.21	ND	>10	>10
S. aureus (P12702)	ND	4.17	4.17	ND
S. aureus (P12724)	ND	ND	>10	ND
S. aureus (B10808)	ND	>10	>10	6.25
B. pumilus	ND	>10	ND	ND
B. cereus	>10	ND	ND	6.25
B. subtilis *	ND	ND	>10	ND
E. faecalis *	ND	5.21	4.17	>10
S. viridans (517141)	>10	5.21	>10	ND

Results were recorded as a mean of three replicates ± SE. Key: *E. coli- Escherichia coli, E. cloacae- Enterobacter cloacae,K.pneumoniae-Klebsiella pneumoniae, Klebsiella spp- Klebsiella* specie*, S. flexineri-Shigella flexineri, S. aureus-Staphylococcus aureus.B. pumilus- Baccilus pumilus, B. cereus- Baccilus cereus, B. subtilis- Baccilus subtilis,E. faecalis- Enterococcus faecalis, ,* and *S. viridans- Streptococcus viridans.* *- clinical isolates*,* coded strains- reportedly resistant*,* strains with no codes- ATCC. Nd- not done.

Table 4.20 Antibacterial activity of root extracts from *Waltheria indica* (MIC in mg/ml)

Bacterial strains	**Plant extracts**			
	Water	Acetone	Ethanol	Methanol
E. coli	2.08	>10	3.65	6.25
P. aeruginosa	ND	ND	>10	ND
P. aeruginosa (T3374)	ND	>10	6.25	3.13
E. cloacae	ND	ND	4.12	4.17
K. pneumoniae	>10	6.25	ND	ND
K. pneumoniae (517298)	ND	1.82	1.04	0.65
Klebsiella spp (317302)	>10	ND	6.25	4.17
S. marscens	ND	ND	6.25	>10
*S. flexineri**	ND	ND	4.12	3.65
*Salmonella spp**	ND	ND	6.25	ND
A. c. anitratus(CSIR)	4.17	ND	ND	ND
S. aureus	ND	ND	5.21	4.17
S. aureus (P12702)	6.25	>10	5.21	ND
S. aureus (P12763)	6.25	ND	ND	ND
S. aureus (P12724)	>10	6.25	ND	6.25
S. aureus (B10808)	5.21	R	6.25	4.17
*S. aureus**	5.21	>10	>10	ND
B. pumilus	ND	ND	2.08	3.65
B. cereus	ND	ND	0.65	0.52
*B. subtilis**	ND	ND	1.04	1.30
*S. epidirmidis**	5.21	1.30	6.25	R
*E. faecalis**	ND	ND	1.04	1.30

Results were recorded as a mean of three replicates. Key: *E. coli- Escherichia coli, P. aeruginosa-Pseudomonas aeruginosa,E. cloacae- Enterobacter cloacae,K.pneumoniae-Klebsiella pneumoniae, Klebsiella spp- Klebsiella* specie,*S. marscens- Serratia marscens,S. flexineri-Shigella flexineri, Salmonella spp- Salmonella* specie*, A. C anitratus- Acinetobacter calcaoceticus anitrans, S. aureus-Staphylococcus aureus.B. pumilus- Baccilus pumilus, B. cereus- Baccilus cereus, B. subtilis-Baccilus subtilis, S. epidirmidis- Staphylococcus epidimidis,E. faecalis- Enterococcus faecalis. *-* clinical isolates*,* coded strains- reportedly resistant*,* strains with no codes-ATCC.ND- not done.

Table 4.21 Antibacterial activity of root extracts from *Jatropha zeyheri* (MIC in mg/ml)

Bacterial strains	**Plant extracts**				
	Water	Acetone	Ethanol	Methanol	Ethyl acetate
E. coli	ND	ND	ND	6.25	3.13
P. aeruginosa	>10	4.16	>10	ND	4.16
P. aeruginosa (T3374)	ND	4.16	ND	ND	4.16
E. cloacae	>10	ND	ND	ND	ND
K. pneumoniae	ND	ND	ND	ND	>10
K. pneumoniae (517298)	ND	6.25	ND	ND	4.16
Klebsiella spp (317302)	ND	ND	ND	ND	>10
S. marscens	3.13	3.13	ND	0.78	ND
S. flexineri *	ND	ND	ND	>10	4.16
Salmonella spp *	ND	0.39	ND	3.13	ND
A. c. anitratus (CSIR)	ND	ND	6.25	>10	>10
S. aureus	>10	>10	>10	>10	3.13
S. aureus (P12702)	ND	ND	ND	ND	0.52
S. aureus (P12763)	0.78	0.52	ND	ND	0.52
S. aureus (P12724)	ND	ND	>10	ND	ND
S.aureus (B10808)	ND	ND	ND	ND	0.52
S. aureus *	ND	ND	ND	ND	6.25
B. pumilus	>10	ND	ND	>10	>10
B. cereus	ND	ND	ND	ND	1.56
B. subtilis *	ND	ND	ND	ND	1.56
S. epidirmidis *	ND	ND	ND	ND	>10
E. faecalis *	ND	ND	ND	ND	1.56
S. viridans (517141)	ND	ND	ND	4.16	0.52

Results were recorded as a mean of three replicates *E. coli- Escherichia coli, P. aeruginosa-Pseudomonasa eruginosa,E.cloacae- Enterobacter cloacae,K.pneumoniae-Klebsiella pneumoniae, Klebsiella spp- Klebsiella* specie*,S. marscens- Serratia marscens,S. flexineri-Shigella flexineri, Salmonella spp- Salmonella* specie*, A. C anitratus- Acinetobacter calcaoceticus anitrans, S. aureus-Staphylococcus aureus.B. pumilus- Baccilus pumilus, B. cereus- Baccilus cereus, B. subtilis-Baccilus subtilis, S. epidirmidis- Staphylococcus epidimidis,E. faecalis- Enterococcus faecalis, ,* and *S. viridans-Streptococcus viridans.* *- clinical isolates*,* coded strains- reportedly resistant*,* strains with no codes-ATCC. ND-not done.

Table 4.22 Antibacterial activity of leaf extracts from *Jatropha zeyheri* (MIC in mg/ml)

Bacterial strains	**Plant extracts**			
	Acetone	Ethanol	Methanol	Ethyl acetate
E. coli	ND	ND	>10	ND
P. aeruginosa	ND	6.25	>10	ND
P. aeruginosa (T3374)	ND	0.78	ND	ND
E. cloacae	4.16	ND	ND	ND
K. pneumoniae	6.25	ND	ND	ND
Klebsiella spp (317302)	ND	0.78	ND	ND
S. marscens	>10	ND	ND	6.25
S. flexineri *	ND	ND	ND	4.16
A. c. anitratus (CSIR)	6.25	ND	ND	6.25
S. aureus	ND	6.25	3.13	ND
B. pumilus	6.25	ND	ND	ND
B. cereus	ND	ND	6.25	6.25
B. subtilis *	ND	ND	ND	ND
E. faecalis *	ND	ND	4.16	ND

Results were recorded as a mean of three replicates *E. coli- Escherichia coli, P. aeruginosa-Pseudomonasa eruginosa,E.cloacae- Enterobacter cloacae,K.pneumoniae-Klebsiella pneumoniae, Klebsiella spp- Klebsiella* specie*,S. marscens- Serratia marscens,S. flexineri-Shigella flexineri, S. aureus-Staphylococcus aureus.B. pumilus- Baccilus pumilus, B. cereus- Baccilus cereus, B. subtilis-Baccilus subtilis, E. faecalis- Enterococcus faecalis, **-* clinical isolates*,* coded strains- reportedly resistant*,* strains with no codes-ATCC. ND-not done.

Water extract from *Peltophorum africanum* stem bark exhibited MIC value of 0.78 mg/ml against *Enterococcus faecalis* and 2.08mg/ml against both *Escherichia coli* and *Pseudomonas aeruginosa* (Table 4.23), while acetone extract exhibited 0.20 mg/ml against *Escherichia coli.* Methanol extract exhibited MIC of 1.56 mg/ml against *Serratia marscens* and clinical isolate of *Staphylococcus aureus,* while ethyl

acetate extract exhibited MIC of 0.78 mg/ml against both *Serratia marscens* and *Bacillus cereus.*

Enterobacter cloacae and *Staphylococcus aureus* were susceptible to all selected extracts from *Senna italica* with MIC values ranging from 0.39 mg/ml to 6.25 mg/ml (Table 4.24). Interestingly, water extract exhibited activity of 1.30 mg/ml against both *Pseudomonas aeruginosa* and *Enterobacter cloacae*. Methanol extract exhibited activity of 1.56 mg/ml against both *Pseudomonas aeruginosa* and *Enterobacter cloacae*.

Acetone extract from *Elephantorrhiza burkei* exhibited antibacterial activity of 0.78 mg/ml against *Escherichia coli*, *Pseudomonas aeruginosa*, *Serratia marscens* and *Bacillus pumilus* (Table 4.25). Similar activity was exhibited by methanol extract against *Pseudomonas aeruginosa*, while ethanol extract exhibited 2.08 mg/ml against Enterobacter cloacae and ethanol extract against both *Enterobacter cloacae* and *Staphylococcus aureus* (P12724).

All selected extracts from *Elephantorrhiza elephantine* exhibited antibacterial activity against *Staphylococcus aureus*, *Bacillus cereus* and *Staphylococcus aureus* (P12763) with MIC values ranging from 0.20 mg/ml and >10 mg/ml (Table 4.26), while methanol extract further showed activity of 2.08 mg/ml against *Streptococcus viridans* and *Klebsiella spp*.

Table 4.23 Antibacterial activity of stem bark extracts from *Peltophorum africanum*

(MIC in mg/ml)

Bacterial strains	**Plant extracts**				
	Water	Acetone	Ethanol	Methanol	Ethyl acetate
E. coli	2.08	0.20	0.78	4.16	ND
P. aeruginosa	2.08	1.56	ND	2.08	73.13
P. aeruginosa (T3374)	ND	ND	0.39	ND	ND
E. cloacae	3.13	ND	4.16	ND	5.12
Klebsiella spp (317302)	>10	4.16	6.25	4.16	6.25
S. marscens	ND	1.56	ND	1.56	0.78
S. flexineri *	ND	2.08	2.08	ND	ND
A. c. anitratus (CSIR)	4.16	ND	ND	6.25	ND
S. aureus	ND	0.39	ND	4.16	4.16
S. aureus (P12702)	>10	5.21	1.30	1.04	1.04
S. aureus (P12763)	>10	0.52	5.21	6.25	>10
S. aureus (P12724)	4.16	5.12	5.12	>10	>10
S. aureus *	ND	ND	ND	1.56	ND
B. pumilus	ND	5.21	3.13	ND	ND
B. cereus	ND	ND	ND	ND	0.78
B. subtilis *	0.39	ND	ND	ND	ND
E. faecalis *	0.78	ND	ND	ND	ND

Results were recorded as a mean of three replicates *E. coli- Escherichia coli, P. aeruginosa- Pseudomonasa eruginosa,E.cloacae- Enterobacter cloacae, Klebsiella spp- Klebsiella* specie,*S. marscens- Serratia marscens,S. flexineri-Shigella flexineri, A. C anitratus- Acinetobacter calcaoceticus anitrans, S. aureus-Staphylococcus aureus.B. pumilus- Baccilus pumilus, B. cereus- Baccilus cereus, B. subtilis- Baccilus subtilis,E. faecalis- Enterococcus faecalis.* *- clinical isolates, coded strains- reportedly resistant, strains with no codes-ATCC. ND-not done.

Table 4.24 Antibacterial activity of root extracts from *Senna italica* (MIC in mg/ml)

Bacterial strains	**Plant extracts**				
	Water	Acetone	Ethanol	Methanol	Ethyl acetate
E. coli	ND	ND	0.39	ND	4.17
P. aeruginosa	1.30	ND	ND	1.56	>10
P. aeruginosa (T3374)	ND	ND	ND	ND	>10
E. cloacae	1.30	3.13	3.65	1.56	6.25
K. pneumoniae	ND	ND	ND	ND	>10
Klebsiella spp (317302)	ND	ND	ND	ND	>10
S. marscens	6.25	ND	ND	ND	5.21
S. flexineri *	ND	ND	5.21	ND	6.25
Salmonella spp *	ND	ND	ND	ND	4.17
S. aureus	0.78	1.30	0.39	0.52	6.25
S. aureus (B10808)	ND	ND	>10	ND	ND
S. aureus *	3.13	ND	ND	ND	3.13
B. pumilus	ND	6.25	ND	ND	ND
B. cereus	1.56	ND	0.78	ND	4.17
E. faecalis *	ND	ND	4.17	ND	ND

Results were recorded as a mean of three replicates *E. coli- Escherichia coli, P. aeruginosa-Pseudomonasa eruginosa,E.cloacae- Enterobacter cloacae,K.pneumoniae-Klebsiella pneumoniae, Klebsiella spp- Klebsiella* specie*,S. marscens- Serratia marscens,S. flexineri-Shigella flexineri, Salmonella spp- Salmonella* specie*,S. aureus-Staphylococcus aureus.B. pumilus- Baccilus pumilus, B. cereus- Baccilus cereus, E. faecalis- Enterococcus faecalis* *- clinical isolates, coded strains- reportedly resistant, strains with no codes-ATCC. ND-not done.

Table 4.25 Antibacterial activity of root extracts from *Elephantorrhiza burkei* (MIC in mg/ml)

Bacterial strains	**Plant extracts**				
	Water	Acetone	Ethanol	Methanol	Ethyl acetate
E. coli	ND	0.78	ND	1.30	ND
P. aeruginosa	ND	0.78	4.17	0.78	1.04
P. aeruginosa (T3374)	ND	ND	>10	ND	ND
E. cloacae	>10	1.04	2.08	1.56	3.13
K..pneumoniae (517298)	ND	5.21	ND	ND	ND
Klebsiella spp (317302)	ND	>10	>10	2.08	ND
S. marscens	>10	0.78	1.56	ND	ND
S. flexineri *	4.17	ND	0.39	ND	ND
Salmonella spp *	ND	ND	ND	0.52	ND
A. c. anitratus (CSIR)	ND	1.56	ND	0.52	ND
S. aureus	1.04	1.30	ND	0.20	ND
S. aureus (P12702)	ND	>10	ND	>10	6.25
S. aureus (P12763)	ND	>10	>10	5.21	>10
S.aureus (P12724)	ND	>10	2.08	0.52	>10
S. aureus (B10808)	ND	ND	>10	ND	ND
B. pumilus	ND	0.78	0.52	ND	ND
B. cereus	ND	0.52	0.52	0.52	4.17
B. subtilis *	ND	ND	ND	1.30	ND

Results were recorded as a mean of three replicates *E. coli- Escherichia coli, P. aeruginosa-Pseudomonasa eruginosa,E.cloacae- Enterobacter cloacae,K.pneumoniae-Klebsiella pneumoniae, Klebsiella spp- Klebsiella* specie*,S. marscens- Serratia marscens,S. flexineri-Shigella flexineri, Salmonella spp- Salmonella* specie*, A. C anitratus- Acinetobacter calcaoceticus anitrans, S. aureus-Staphylococcus aureus.B. pumilus- Baccilus pumilus, B. cereus- Baccilus cereus, B. subtilis-Baccilus subtilis* *- clinical isolates*, coded strains- reportedly resistant*, strains with no codes-ATCC. ND-not done

Table 4.26 Antibacterial activity of root extracts from *Elephantorrhiza elephantina* (MIC in mg/ml)

Bacterial strains	**Plant extracts**				
	Water	Acetone	Ethanol	Methanol	Ethyl acetate
E. coli	ND	ND	ND	ND	1.56
P. aeruginosa	1.56	ND	ND	1.04	>10
E. cloacae	ND	ND	ND	1.30	R
K. pneumoniae	ND	ND	ND	R	1.56
Klebsiella spp (317302)	6.25	>10	2.08	2.08	R
S. marscens	ND	0.39	ND	ND	0.39
S. flexineri *	ND	1.04	ND	ND	4.17
A. c. anitratus (CSIR)	ND	2.08	ND	ND	ND
S. aureus	1.56	1.04	0.20	0.20	0.20
S. aureus (P12702)	>10	>10	4.17	6.25	ND
S. aureus (P12763)	6.25	1.04	4.17	>10	6.25
S. aureus (P12724)	5.21	3.13	3.13	0.39	ND
S. aureus (B10808)	ND	ND	ND	>10	ND
S. aureus *	ND	ND	ND	ND	1.56
B. pumilus	ND	0.78	ND	ND	ND
B. cereus	1.56	1.56	1.04	0.78	1.04
E. faecalis *	ND	ND	ND	ND	ND
S. viridans (517141)	4.17	>10	ND	2.08	ND

Results were recorded as a mean of three replicates *E. coli- Escherichia coli, P. aeruginosa-Pseudomonasa eruginosa,E.cloacae- Enterobacter cloacae,K.pneumoniae-Klebsiella pneumoniae, Klebsiella spp- Klebsiella* specie*,S. marscens- Serratia marscens,S. flexineri-Shigella flexineri,A. C anitratus- Acinetobacter calcaoceticus anitrans, S. aureus-Staphylococcus aureus.B. pumilus- Baccilus pumilus, B. cereus- Baccilus cereus, E. faecalis- Enterococcus faecalis, ,* and *S. viridans-Streptococcus viridans.* *- clinical isolates, coded strains- reportedly resistant, strains with no codes-ATCC. ND-not done.

Bacillus cereus and *Serratia marscens* were susceptible to all extracts from *Harpagophythum procumbens* with MIC values ranging from 0.20 mg/ml to 6.25 mg/ml (Table 4.27), with both ethanol and methanol extracts recording MIC of 0.20 mg/ml against *Serratia marscens*. Moreover, ethyl acetate extract exhibited 0.52 mg/ml against both *Serratia marscens* and *Salmonella spp*.

Ethyl acetate extract from *Cissus quadrangularis* exhibited MIC of >10mg/ml against *Escherichia coli*, *Pseudomonas aeruginosa*, *Pseudomonas aeruginosa* (T3374) and *Staphylococcus aureus* (Table 4.28). Methanol extract exhibited 1.56 mg/ml against both *Escherichia coli* and *Pseudomonas aeruginosa* (T3374), while the water extract exhibited 3.13 mg/ml against *Enterobacter cloacae*.

Water extract from *Urginea sanguinea* exhibited MIC of 0.78 mg/ml and 0.39 mg/ml against *Escherichia coli* and *Enterobacter cloacae* respectively (Table 4.29), while acetone extract exhibited 0.20 mg/ml against *Bacillus cereus*. The ethanol extract exhibited MIC of 3.13 mg/ml against both *Escherichia coli* and *Shigella flexineri* while methanol and ethyl acetate extracts *Klebsiella pneumoniae* respectively.

Acetone and ethanol extracts from *Peucedanum sulcatum* showed MIC of 0.78 and 0.52 mg/ml against clinical isolate of *Staphylococcus aureus* respectively (Table 4.30). Moreover, water extract exhibited 3.13 mg/ml against *Shigella flexineri* and 4.17 mg/ml against both *Klebsiella spp* (317302) and *Staphylococcus aureus,* while ethyl acetate extract exhibited 0.52 mg/ml against *Klebsiella spp* (317302).

Table 4.27 Antibacterial activity of extracts from *Harpagophythum procumbens* (MIC in mg/ml)

Bacterial strains	**Plant extracts**				
	Water	Acetone	Ethanol	Methanol	Ethyl acetate
E. coli	6.25	1.30	1.30	ND	ND
P. aeruginosa	ND	ND	ND	>10	6.25
P. aeruginosa (T3374)	ND	6.25	>10	5.21	ND
E. cloacae	ND	ND	>10	ND	ND
K. pneumoniae	ND	ND	>10	ND	ND
K. pneumoniae (517298)	>10	ND	ND	ND	ND
Klebsiella spp (317302)	ND	4.17	ND	ND	5.21
S. marscens	4.17	4.17	0.20	0.20	0.52
Salmonella spp *	ND	ND	ND	ND	0.52
A. c. anitratus (CSIR)	ND	ND	ND	>10	ND
S. aureus	ND	4.17	ND	ND	ND
S. aureus (P12702)	ND	ND	ND	>10	ND
S. aureus (P12763)	ND	4.17	ND	ND	ND
S. aureus (P12724)	ND	ND	ND	5.21	0.52
S. aureus (B10808)	ND	ND	ND	ND	0.52
S. aureus *	ND	ND	ND	4.17	ND
B. pumilus	ND	ND	6.25	ND	>10
B. cereus	6.25	3.13	6.25	6.25	6.25
B. subtilis *	ND	ND	0.39	ND	ND

Results were recorded as a mean of three replicates. Key: *E. coli- Escherichia coli, P. aeruginosa- Pseudomonas aeruginosa,E. cloacae- Enterobacter cloacae,K.pneumoniae-Klebsiella pneumoniae, Klebsiella spp- Klebsiella* specie*,S. marscens- Serratia marscens,S. flexineri-Shigella flexineri, Salmonella spp- Salmonella* specie*, A. C anitratus- Acinetobacter calcaoceticus anitrans, S. aureus- Staphylococcus aureus.B. pumilus- Baccilus pumilus, B. cereus- Baccilus cereus, B. subtilis- Baccilus subtilis* *- clinical isolates, coded strains- reportedly resistant, strains with no codes- ATCC.ND- not done.

Table 4.28 Antibacterial activity of extracts from *Cissus quadrangularis* (MIC in mg/ml)

Bacterial strains	**Plant extracts**				
	Water	Acetone	Ethanol	Methanol	Ethyl acetate
E. coli	ND	ND	ND	1.56	>10
P. aeruginosa	ND	6.25	4.17	>10	>10
P. aeruginosa (T3374)	ND	ND	ND	1.56	>10
E. cloacae	3.13	ND	ND	ND	ND
K. pneumoniae	ND	>10	ND	ND	ND
K. pneumoniae(517298)	ND	ND	>10	5.21	ND
Klebsiella spp (317302)	ND	ND	ND	5.21	3.65
S. marscens	>10	ND	ND	>10	>10
S. flexineri *	ND	ND	ND	3.13	ND
Salmonella spp *	ND	ND	ND	ND	3.13
S. aureus	ND	ND	>10	>10	>10
S. aureus (P12702)	ND	ND	ND	>10	5.21
S. aureus (P12763)	ND	ND	5.21	ND	ND
S. aureus (P12724)	ND	ND	5.21	ND	ND
S. aureus (B10808)	ND	>10	ND	6.25	ND
S. aureus *	ND	ND	ND	0.78	0.52
B. pumilus	>10	ND	ND	ND	ND
B. subtilis *	>10	ND	ND	ND	ND
S. epidirmidis *	ND	6.25	ND	ND	ND
S. viridans (517141)	ND	6.25	ND	6.25	ND

Results were recorded as a mean of three replicates. Key: *E. coli- Escherichia coli, P. aeruginosa-Pseudomonas aeruginosa,E. cloacae- Enterobacter cloacae,K.pneumoniae-Klebsiella pneumoniae, Klebsiella spp- Klebsiella* specie,*S. marscens- Serratia marscens,S. flexineri-Shigella flexineri, Salmonella spp- Salmonella* specie*, S. aureus-Staphylococcus aureus.B. pumilus- Baccilus pumilus, B. cereus- Baccilus cereus, B. subtilis- Baccilus subtilis, S. epidirmidis- Staphylococcus epidimidis, S. viridans- streptococcus viridans* *- clinical isolates, coded strains- reportedly resistant, strains with no codes-ATCC.ND- not done.

Table 4.29 Antibacterial activity of extracts from *Urginea sanguinea* (MIC in mg/ml)

Bacterial strains	**Plant extracts**				
	Water	Acetone	Ethanol	Methanol	Ethyl acetate
E. coli	0.78	ND	3.13	ND	>10
P. aeruginosa	ND	ND	ND	0.78	>10
P. aeruginosa (T3374)	>10	ND	ND	5.21	ND
E. cloacae	0.39	ND	ND	ND	ND
K. pneumoniae (517298)	>10	ND	ND	6.25	0.52
Klebsiella spp (317302)	ND	ND	>10	ND	ND
S. marscens	>10	ND	ND	6.25	>10
S. flexineri *	ND	ND	3.13	ND	ND
A. c. anittratus (CSIR)	>10	ND	ND	>10	ND
S. aureus	>10	>10	6.25	>10	>10
S. aureus (P12702)	6.25	>10	ND	>10	ND
S. aureus (P12763)	ND	5.21	6.25	ND	ND
S. aureus (B10808)	5.21	6.25	ND	>10	ND
S. aureus *	ND	>10	3.13	6.25	ND
B. pumilus	>10	>10	ND	ND	ND
B. cereus	ND	0.20	>10	ND	ND
B. subtilis *	6.25	>10	ND	ND	ND
S. epidirmidis *	>10	ND	ND	ND	ND
E. faecalis *	ND	ND	ND	ND	>10
S. viridans (517141)	0.52	ND	ND	ND	ND

Results were recorded as a mean of three replicates *E. coli- Escherichia coli, P. aeruginosa-Pseudomonasa eruginosa,E.cloacae- Enterobacter cloacae,K.pneumoniae-Klebsiella pneumoniae, Klebsiella spp- Klebsiella* specie*,S. marscens- Serratia marscens,S. flexineri-Shigella flexineri, A. C anitratus- Acinetobacter calcaoceticus anitrans, S. aureus-Staphylococcus aureus.B. pumilus-Baccilus pumilus, B. cereus- Baccilus cereus, B. subtilis- Baccilus subtilis, S. epidirmidis-Staphylococcus epidimidis,E. faecalis- Enterococcus faecalis, ,* and *S. viridans-Streptococcus viridans. **- clinical isolates, coded strains- reportedly resistant, strains with no codes-ATCC. ND-not done.

Table 4.30 Antibacterial activity of root extracts from *Peucedanum sulcatum* (MIC in mg/ml)

Bacterial strains	**Plant extracts**				
	Water	Acetone	Ethanol	Methanol	Ethyl acetate
P. aeruginosa	6.25	ND	1.56	ND	ND
P. aeruginosa (T3374)	0.52	ND	0.52	6.25	ND
E. cloacae	6.25	ND	>10	>10	>10
K. pneumoniae	ND	ND	ND	>10	ND
K. pneumoniae (517298)	ND	ND	3.65	ND	4.17
Klebsiella spp (317302)	4.17	6.25	ND	>10	0.52
S. flexineri *	3.13	ND	3.13	ND	ND
A. c. anitratus (CSIR)	ND	ND	>10	ND	>10
S. aureus	>10	>10	ND	>10	ND
S. aureus (P12702)	6.25	ND	3.65	ND	ND
S. aureus (P12763)	ND	4.17	3.65	ND	ND
S. aureus (P12724)	6.25	ND	ND	>10	6.25
S. aureus (B10808)	4.17	>10	ND	>10	ND
S. aureus *	ND	0.78	0.52	ND	ND
B. pumilus	ND	ND	ND	ND	6.25
B. cereus	ND	ND	ND	>10	ND
B. subtilis *	>10	ND	ND	ND	ND
E. faecalis *	ND	ND	ND	ND	ND
S. viridans (517141)	6.25	ND	ND	6.25	ND

Results were recorded as a mean of three replicates. *P. aeruginosa-Pseudomonasa eruginosa,E.cloacae- Enterobacter cloacae,K.pneumoniae-Klebsiella pneumoniae, Klebsiella spp-Klebsiella* specie, *S. flexineri-Shigella flexineri, Salmonella spp- Salmonella* specie, *A. C anitratus-Acinetobacter calcaoceticus anitrans, S. aureus-Staphylococcus aureus.B. pumilus- Baccilus pumilus, B. cereus- Baccilus cereus, B. subtilis- Baccilus subtilis, E. faecalis- Enterococcus faecalis, ,* and *S. viridans-Streptococcus viridans.* *- clinical isolates, coded strains- reportedly resistant, strains with no codes-ATCC. ND-not done.

All the selected organisms were susceptible to both metronidazole and streptomycin sulphate, with MIC values ranging from 0.08 to 0.63 mg/ml and 0.08 to 0.32

respectively. Moreover, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Klebsiella spp* (317302), *Shigella flexineri*, *Acinetobacter calcaoceuticals anitratus*, *Staphylococcus aureus* and *Staphylococcus epidirmidis* were most susceptible to metronidazole (0.08 mg/ml) while *Klebsiella pneumoniae* (517298) was the most susceptible strain to streptomycin sulphate with MIC of 0.01 mg/ml (Table 4.31).

About 82 % of methanol extracts of the selected plants contained flavanoids, while 73 % contained both saponins and tannins (Table 4.32). Moreover, 55 % of methanol extracts of the selected plants contained both cardiac glycosides and alkaloids.

Although methanol extracts from *Peucedanum sulcatum* and *Cissus quadrangularis* exhibited IC_{50} of >5 mg/100 ml, root extract from *Elephantorrhiza burkei* exhibited $IC_{50\ of}$ 0.10±0.02 and 0.36±0.02 mg/100 ml against DPPH and ABTS respectively (Table 4.33). Furthermore, methanol extract from stem bark of Cassia abbreviata exhibited lowest IC_{50} of 0.16±0.02 mg/100 ml against ABTS.

Table 4.31 Susceptibility pattern control drugs

Bacterial strains	**Antibiotics**	
	Metronidazole	streptomycin sulphate
E. coli	0.32	0.04
P. aeruginosa	0.08	0.04
P. aeruginosa (T3374)	0.63	0.02
E. cloacae	0.63	0.32
K. pneumoniae	0.08	0.04
K. pneumoniae (517298)	0.08	0.01
Klebsiella spp (317302)	0.08	0.04
S. marscens	0.63	0.32
S. flexineri *	0.08	0.03
Salmonella spp *	0.04	0.02
A. c. anitratus (CSIR)	0.08	0.08
S. aureus	0.32	0.08
S. aureus (P12702)	0.32	0.32
S. aureus (P12763)	0.04	0.04
S. aureus (P12724)	0.04	0.04
S. aureus (B10808)	0.04	0.04
S. aureus *	0.08	0.04
B. pumilus	0.08	0.08
B. cereus	0.63	0.02
B. subtilis *	0.63	0.03
S. epidirmidis *	0.08	0.08
E. faecalis *	0.63	0.03
S. viridans (517141)	0.04	0.04

Results were recorded as a mean of three replicates *E. coli- Escherichia coli, P. aeruginosa-Pseudomonasa eruginosa,E.cloacae- Enterobacter cloacae,K.pneumoniae-Klebsiella pneumoniae, Klebsiella spp- Klebsiella* specie,*S. marscens- Serratia marscens,S. flexineri-Shigella flexineri, Salmonella spp- Salmonella* specie*, A. C anitratus- Acinetobacter calcaoceticus anitrans, S. aureus-Staphylococcus aureus.B. pumilus- Baccilus pumilus, B. cereus- Baccilus cereus, B. subtilis-Baccilus subtilis, S. epidirmidis- Staphylococcus epidimidis,E. faecalis- Enterococcus faecalis, ,* and *S. viridans-Streptococcus viridans. **- clinical isolates, coded strains- reportedly resistant, strains with no codes-ATCC. ND-not done.

Table 4.32 Preliminary phytochemical screening of medicinal plants indigenously used to treat STIs

Medicinal plants	Phytochemicals					
	Alkaloid (Meyer's reagent)	Saponin	Tannin	Anthraquinone	Cardiac glycoside (Salkowski's test)	Flavonoid (Lead acetate test)
E. elephantine	—	++++	++	—	—	+++
Elephantorrhiza burkei	—	++	++	—	++++	+++
Peltophorum africanum	+++	++++	++	—	++++	++++
Senna italica	_	++	_	+++	_	—
Urginea sanguinea	_	—	_	—	++	++
Jatropha zeyheri (root)	++	++	+++	—	++++	++++
Harpagophythum procumbens	+++	_	++++	—	++++	++
Peucedatum sulcatum	++	+++	_	—	—	++++
Cassia abbreviata (stem bark)	_	_	++++	—	—	+++
Waltheria indica	+++	++	+++	—	+++	+++
Cissus quadrangularis	++	++	++	—	—	—

Key: – = negative test, ++ = present in small quantity, +++ = present in moderate quantity, ++++ = present in large quantity.

Table 4.33 Antioxidant activity of methanol extracts of selected medicinal plants, showing IC_{50}

Medicinal plants	DPPH IC_{50}	ABTS IC_{50}
E. elephantina (root)	0.24±0.05	0.29±0.02
E. burkei (root)	0.10±0.02	0.36±0.03
P. africanum (SB)	0.50±0.11	0.26±0.04
S. italica (root)	2.33±0.59	1.33±0.23
U. sanguinea (bulb)	1.80±0.04	0.50±0.11
Jatropha zeyheri (root)	1.35±0.06	0.80±0.10
H. procumbens (root)	3.67±0.71	3.50±1.12
P. sulcatum (root)	>5	>5
C. abbreviata (SB)	1.87±0.25	0.16±0.02
C. quadrangularis (stem)	>5	>5
Waltheria indica (root)	0.33±0.06	0.42±0.06
Control: Ascorbic acid	0.53±0.02	0.80±0.10

Results were recorded as a mean of three replicates ±SD Key, SB-stem bark

CHAPTER 5

DISCUSSIONS

5.1 Ethnobotanical Survey

The common name for sexually transmitted infections is "*go wela*". In this study, a total of 13 traditional healers from two close municipal borders in 8 different villages were interviewed at their respective homes. Selected informants were dominated by males, 61.5% (Table 4.1). Men have more knowledge and dominate the practice of traditional medicine because they treat both children and adults while women are mostly restricted to treating child diseases (Togola *et al*., 2005).

According to informants, infections seem to be more prevalent in males than females. High proportions of sexually transmitted infections are asymptomatic in women and such patients are less likely to seek medical care and may develop complications (Bozicevic *et al*., 2006). According to reports, healers are frequently visited by patients and the most common infections include syphilis, gonorrhoea, *makgoma* and opportunistic infections associated with HIV-AIDS. Washing, purging and springling all feature strongly as modes of action in purification rituals (Hutchings, 2007).

"*Makgoma*", is an illness due to having intercourse with the deceased's wife or husband who has not undergone ritual cleansing.

Plants are mostly collected from April to July in their matured stage. This time frame serves as a safety measure against snake bites when collections are made in the wild and mountains. Moreover, it allows healing of ring barks and growth of seedlings. After collections, plant materials are chopped into small portions which are exposed to full sun for drying and are ready to serve.

Patients are diagnosed through the use of knuckle bones which reveal the nature and severity of the illness. Moreover, such bones will assist in identifying medicines to be used and whether or not to perform rituals.

Whenever a n'anga collected annuals for medicinal use, had to leave behind some individuals of the species at the collection site because it is believed that if a species is completely destroyed, then the patient to whom the medicine from the species was administered would also die (Mavi and Shava, 1997). However, some healers believe that such practices will have devastating effects on the patient either causing death or bringing about an only partial cure. A strategy which would satisfy the requirements of sustainable harvesting, yet simultaneously provide for primary health care needs, would be the substitution of bark or underground parts with the leaves of the same plant (Zschoke *et al*.,2000).

Major factors that may be considered as threats to biodiversity include large scale international trade of medicinal plants, agricultural expansion, leisure and

deforestation. These factors result in harvesting pressures on resources in South Africa where several medicinal plants are red listed (Krog *et al*., 2006). The removal of wood, roots or the whole plant generally leads to death of an individual plant , as does the cutting of bark when ring barking takes place (van Andel and Havinga 2008). Harvesting of such plant parts will result in either extinction or the plant speces becoming endangered. Although each community has its own particular approach to health and disease, even at the level of ethno-pathogenic perceptions of the diseases and therapeutic behaviour, multi-purpose plant species are likely to be vulnerable to extinction due to high demands and likely to be traded bulk in cities. The conservation status of plant species in this study suggests that some plant species such as, *Harpagophythum procumbens, Cassia abbreviata* and *Peltophorum africanum* are likely to become extinct due to unsustainable harvesting. Domestication and cultivation have been mooted as key strategies in meeting the demand for medicinal plants (Jiofack *et al*., 2008). However, slow growth and specific environmental and ecological conditions remain challenges to such practices. Moreover, stake holders such as plant traders, traditional healers, kingship and relevant authorities, including national, provincial and local government, should collectively contribute towards sustainable harvesting. Construction of small scale protected areas at various localities may assist in retaining indigenous ecosystems, by allowing seedlings to grow and mature. Such areas should therefore be monitored by concerned stakeholders.

5.2 Antibacterial activity

The investigation of plant extracts may be a first step towards finding new therapeutic agents against resistant human pathogens of bacterial origin. Pathogenic bacterial strains which may be etiologic agents of sexually transmitted infections include *E. coli*, *K. pneumoniae*, *Enterobacter* species and *Pseudomonas* species (Richens, 2004). *Shigella* and *Salmonella species* are also known to cause sexually transmitted enteric infections while *S. aureus* and *Escherichia coli* are orally transmissible. Together with *Streptococcus faecalis* and other common etiologic agents, these strains were prevalent in genital infections among pregnant women (Akerele *et al*, 2002). The selected strains have been used elsewhere for similar research; (Kambizi and Afolayan, 2001; Okoli and Iroegbu, 2004;Tshikalange *et al*., 2005; Buwa and van Staden, 2006), as shown in the literature review in chapter 2. All these microbes have shown resistance to a variety of antibiotics (Edoh and Mensah, 2007). Microbes that serve as etiologic agents of sexually transmitted infections may cohabit or infect the host concurrently. Although not the most common, these microbes are often isolated from patients of such infections and are usually not found outside the traditional sphere of venereal infections. Besides sexual contact, these strains may be transmitted through the mouth and anus or rectum.

Bacterial strains such as *S. aureus*, *Enterococcus*, spp *P. aeruginosa*, *Acinetobacter*, *E. coli*, *Klebsiella* are among the causative agents of microbes in

hospital acquired infections, with the stethoscope being a possible vector (Randrianirina *et al*., 2010; Killic *et al*., 2011) and further, it may cause urinary tract infections cohabiting with *Proteus* and *Serratia* species. Like sexually transmitted infections, causative agents of urinary tract infections may infect the urethra, prostate, bladder, or kidneys and symptoms may be absent or include urinary frequency and urgency, dysuria, lower abdominal pain, and flank pain. Systemic symptoms and even sepsis may occur with kidney infections. Within Western methods of healing, diagnosis is based on analysis and culture of urine sample while treatment may involve the use of metronidazole and ciprofloxacin. Moreover, urinary tract infections are prevalent in clinics for sexually transmitted infections (Mead and Gruneberg, 1978).

The maximum zone of inhibition was exhibited by methanol extract of *E. burkei* against *Klebsiella spp* at 15.7±0.67 mm (Table 4.11) and is comparable to both streptomycin and penicillin standard antibiotic discs which yield 16.3±0.67 (Table 4.17) respectively. Moreover, all extracts of *E. burkei* showed activity against two strains of *S. aureus* (P12763 and P12724) with zones of inhibition ranging from 10.3±0.88 to 14.0±0.58 mm (Table 4.11). Methanol extract of *W. indica* roots exhibited inhibitory activity of 15.2±2.18 mm and 13.5±0.71 mm (Table 4.6) against *B. pumilus* and clinical isolate of *E. faecalis* respectively. These results are potent, compared to penicillin standard discs against similar organisms. These organisms may also cause infective endocarditis - a serious complication of bacteremia (Kamalakannan *et al*., 2007). *E. faecalis* may cause

endophthalmitis and some of it´s isolates are reported to express toxins and cytolysin which are plasmid encoded (Booth *et al*., 1998).

All extracts of *P. africanum* bark were active against all four *S. aureus* and *Klebsiella spp* resistant strains with zones of inhibitions ranging from 8.7±0.67 to 14.3±0.33 mm (Table 4.9). Moreover, *P.africanum* has recently been reported to possess anthelmintic activity and is non-toxic, hence used in ethnoveterinary medicine (Bizimenyera *et al*., 2006; Bizimenyera *et al*., 2008). Moreover, its various extracts have exhibited activity against a variety of *Helicobacter pylori* strains (Okeleye *et al*., 2011).

All extracts of *C. abbreviata* stem and *S. italica* had activity against *E. cloacae* (Table 4.4 and 4.10 respectively). *E. cloacae* has recently been reported to cause infections among burn victims, immunocompromised patients and patients with malignancy and may cause bacteremia, urinary tract and pulmonary infections (Musil *et al*., 2010), Moreover, it is reported to resist expanded-spectrum cephalosporins and such resistance may be caused by stable derepression of the chromosomal Ampler class C β-lactamase (Kartali *et al*., 2002). *C abbreviata* bark inhibited IC_{50} of 0.6 mg/ml against yeast α-glucosidase (Shai *et al*., 2010) and had high inhibitory against mammalian α-glucosidase (Shai *et al*., 2011*).* Further investigations revealed that ethyl acetate extract *C. abbreviata* of the root exhibited good antimalarial activity with an IC_{50} of 39 µg/ml against *Plasmodium falciparum* (Gessler *et al*., 1994). Ether fraction of the root

was reported active against *Staphylococcus aureus* (Khan, 2001) and compounds such *as* 2,4-trans-7, 4'-dihydroxy-4-methoxyflavan (Dehmlow *et al*., 1998), guibourtinidol-(4β→8)-epiafzelechin, guibourtinidol-(4α→8)-epiafzelechin, guibourtinidol-(4α→8)-catechin, guibourtinidol-(4β→8)-epicachetin and ent-guibourtinidol-(4β→8)-epicachetin (Malan *et al*.,1996) have been isolated from extracts of *C. abbreviata. S. Italica* has been reported to possess anti-tick properties and compounds such as 1,2 benzenedicarboxylic acid, butyl ester, 1,8-dihydroxy-3-methylanthraquinone, hexadecanoic acid, 9-hexadecanoic acid and bis(2-ethyl hexyl) ester have been isolated (Magano *et al*., 2008). Moreover, aqueous extract exhibited activity ranging from 11.50 to 14.00 mm against *S aureus*, *Salmonella typhi*, *E.coli*, *P. aeruginosa* and *Stretococcus pneumoniae* at a concentration of 30 mg/ml (Dabai *et al*., 2012).

Water, acetone, ethanol and methanol extracts of *E. elephantina* showed high inhibitory against three *S. aureus* strains (Table 4.12) with zones of inhibition ranging from 8.7±0.67 to 13.6±0.88 mm, while all extracts of *H. procumbens* only showed activity against *S. marscens* and *B. cereus* (Table 4.13). *H. procumbens* roots reportedly contained two acetyl phenolic glycosides such as 6-acetylacteoside and 2,6-diacetyl acteoside (Munkombwe, 2003).

Although water extract of *U. sanguinea* is reported to be cytotoxic in cell cultures of L929 cell and primary embryonic neural cultures (Markx *et al*., 2006), it showed potent activity against *S. viridans* with a zone of inhibition of 12.7±0.88

mm (Table 4.15) , while methanol extract of *C. quadrangularis* exhibited a similar zone of inhibition against *K. pneumoniae* 517298 (Table 4.14). Interestingly, water extract of *P. sulcatum* exhibited a similar zone of inhibition against *P. aeruginosa* T3374 and *S. aureus* P12702 (Table 4.16). Contrarary to inactivity in the current study, ethanol extract of *C. quadragularis* reportedly exhibited activity at a zone of 10 mm against *E. coli* (Merinal and Viji, 2012), and contained high amounts of carotene A, triterpene, β-sitosterol and ketosteroid as the main constituents, and posessed bonefracture healing, analgesic, antiosteoporotic antiulcer, paraasympathomimetic, antihemorrhoidal, androgenic, anti-inflammatory and gastroprotective activity (Mishra *et al*., 2010). Moreover, *C. quadrangularis* yielded hexadecanoic acid, piceid, amyrin acetate and trans-resveratrol-3-O-glucoside (Thakur *et al*., 2009). Transvaalin, a member of cardiac glycosides, stigmasterol, phloroglucinol, salicylic acid, phlorin and 3-hydroxy-4-methylbenzoic acid have been identified from fresh bulbs of *U. sanguinea* (Marx *et al.*, 2005).

According to Mathekga and Meyer (1998), *P. aeruginosa* which mainly infects the pulmonary tract and urinary tract is among the most difficult to treat with conventional antibiotics, while *K. pneumoniae* has been associated with nosocomial infections and may produce extended spectrum β–lactamases (ESBLs) which renders it resistant to carbapenems (Falagas *et al*., 2007). Although *S. viridans* is mostly prevalent in oral cavities, it may reside in the upper

respiratory tract and can lead to life threatening diseases which include endocarditis and pneumonia (Tunkel and Sepkowitz, 2002; Refoua *et al*., 2005).

Susceptibility patterns of selected bacterial strains for plant extracts is shown in Figure 4.3. Ethyl acetate extract of *J. zeyheri* root extract exhibited activities (83 %) against bacterial strains which comprise of Gram positive and Gram negative strains, hence broad spectrum. Genus *Jatropha* is known to produce diterpenes which mostly belong to rhamnofolane, daphnane, lathyrane, tigliane, dinorditerpene, deoxy preussomerin and pimarane skeletal structures (Devappa *et al*., 2011). A daphnane compound known as jaherin has been isolated from *J. zeyheri* root and reportedly possessed MIC of 8 mg/ml against *Streptococcus pyogenes* and 16 mg/ml against *Microsporum canis*, *Trichophyton rubrum*, *Trichophyton mentagrophytes* and *Sporotrichum schenkii* (Dekker *et al*., 1987). Besides antibacterial activity, dichloromethane and methane extracts of the root have been reported to possess both anti-inflammatory and mutagenic effects (Luseba *et al*., 2007).

Ethanol extract of *W. indica* has also exhibited antibacterial activity of 73 % against a variety of strains. Elsewhere, ethanolic extracts of stems, roots and leaves of *W. indica* have recenlty been reported to possess potent activity against a variety of Gram negative strains, with the largest zone of inhibition of 15 mm against *Citrobacter freundii* (Olajuyigbe *et al*., 2011). The current work therefore validates the effectivity of this extract and it is worth doing further trials

for the isolation of individual compounds and to investigate the effectivity of such compounds against a variety of strains. Flavonoids such as epicatechin, quercetin and tiliroside were isolated from whole plant extract and dose independently inhibits production of inflammatory mediator nitric oxide (NO), cytokines (TNF)-α and interleukin (IL)-12, in lipopolysaccharide and interferon activated murine peritoneal macrophages, without any cytotoxicity (Rao *et al*., 2005). In the current study, *W. indica* exhibited high activity with zones ranging from 8.0±0.0 mm (acetone extract) to 13.6±0.36 mm (water extract) and these findings are in agreement with reports by Zailani *et al.* (2010) and Bala *et al.* (2011) which reported high activity against *E. coli, P aeruginosa* and trypanosome parasites.

Although *C. abbreviata* leaf extracts had activity against *Streptococcus viridans* and *Klebsiella pneumoniae* (Table 4.5), while *C. abbreviata* bark extracts were inactive against similar strains, stem bark extracts showed pronounced activity against a variety of strains compared to those of leaves. Generally, the *J. zeyheri* root had good activity compared to leaf extracts. These findings are contrary to recent studies by Adeshina *et al.* (2010) who reported relatively equal zones of inhibitions displayed by leaf, root and bark of the same plant . Moreover, there are reports which show that dried leaves of *J. zeyheri*, as in our study, exhibit no activity against both Gram negative and Gram positive strains while green leaves do (Rahman *et al*., 2009) suggesting that active ingredients might be present mostly in large quantities in fresh green leaf extracts.

It should also be noted that water extracts of only four plants, *C. abbreviata*, *P. sulcatum P. africanum*, and *U. sanguinea,* possessed higher or equal activity compared to organic extracts. Inactivity of other water extracts may be attributed to freeze drying which is not applicable to indigenous traditional medicine. However, it is possible that traditional healers do not extract all of the active compounds that are present in the plant and consequently the prepared drug would not contain all the pharmacologically active compounds (Kelmanson *et al*., 2000). Pronounced activity of aqueous extracts in comparison to organic extracts is documented elsewhere (Kumaraswamy *et al*., 2008). Activity of water extracts, in a way, validates the use of this plants in the treatment of a variey of infections which may be sexually transmissible. Moreover, some plants are used in combined form, as shown in Table 4.3. Recent reports in the literature relate combined medicinal plants with an improved antibacterial activity compared to single plant extract (Karmegam *et al.*, 2012; Mabrouk, 2012,).

The resistance pattern of selected bacterial strains is shown in figure 4. Clinical isolate of *S. epidirmidis* was most resistant (93.5 %) while ATCC strain of *S. aureus* was more susceptible (29 %) to plant extracts. Clinical isolates were most resistant than other strains. Gram negative strains exhibited slightly higher resistance compared to Gram positives. Simmilar trend have been reported elsewhere (Lall and Meyer, 2000, Morales *et al*, 2008; Bishnu *et al*, 2009) and gram negative strains are known to be resistant to plant extracts, due to the

presence of an outer membrane that possesses hydrophilic polysaccharide chains as a barrier or outer lipid membrane (Chan *et al*., 2008).

Minimal inhibitory concentrations of plant extracts which showed activity in the disc diffusion test are reported. The lowest MIC of 0.2 mg/ml was exhibited by a number of extracts including acetone extract of *P. africanum* against *E. coli* (Table 4.23), methanol extract of *E. burkei* against *S. aureus* (Table 4.25), ethanol, methanol and ethyl acetate of *E. elephantina* against S. aureus (Table 4.26) and both ethanol and methanol extracts of *H. Procumbens* against *S. marscens.* Bizimenyera *et al.* (2005), contrarily reported acetone bark extract of *P. africanum* to possess an MIC of 0.63 mg/ml against *E. coli* and 0.16 mg/ml against both *E. faecalis* and *P. aeruginosa* while Steenkamp *et al.* (2007) reported a methanolic root extract of *P. africanum* to posess activity of 0.50 and 2.00 mg/ml against *S. epidermidis and S. aureus,* respectively. Although compounds such as norbergenin, catechin, betulinic acid and its analogues, which may also inhibit HIV-1 (Mebe and Makuhunga,1992; Theo *et al*., 2009,), have earlier been reported as some of the soluble metabolites from *P. africanum,* it is still worth introspecting for new antimicrobial drugs because there is either no or little information on biological activity of isolated compounds from all the selected plants for this study. Contradiction of MIC values may be due to environmental factors, differences in collection times, locality, soil types, different types of strains and other factors. Bergenin from other plant sources reportedly exhibited MIC values ranging from 125 µg/ml to >500 µg/ml against a variety of

fungal strains including *Aspergillus niger* and showed no activity against *B. subtilis*, *S. aureus*, *E. coli*, *S. epidirmidis*, *E. faecalis*, *P. aeruginosa*, *K. pneumoniae* (Raj *et al*., 2012). Betulic acid from various plants reportedly posess anticancer and antimalarial activity (Moghaddam *et al*.,2012). Ethyl acetate extract from *P. africanum* exhibited potent activity against isolates of *Helicobacter pylori* (Okeleye *et al*., 2010), while acetone extract exhibited activity against *Mycobacterium tuberculosis* (Green *et al*., 2010).

Extracts of *Cassia abbreviata* stem bark exhibited MIC values ranging from 0.52 to 6.25 mg/ml against *E. cloacae* (Table 4.18), while acetone extract of the leaf exhibited a notably potent MIC of 2.08 mg/ml against *S. flexineri* (Table 4.19). Moreover, methanol and ethanol extracts of stem bark exhibited notable MIC values of 1.56 and 0.78 mg/ml against *S. marscens* and *S. aureus* respectively. Besides being broad spectrum, ethanol extract of *W. indica* exhibited a potent MIC value of 1.04 mg/ml against *B. subtilis*, *K. pneumoniae* and one of the most resistant organisms *E. faecalis*. According to Aliyu *et al.* (2008), an MIC of 3 mg/ml is of high potency. This study, therefore validates the reports on the use of selected medicinal plants against human pathogenic strains which may resist commonly used antibiotics in our health care services.

Ethyl acetate extract of *J. zeyheri* root exhibited an MIC value of 0.52 mg/ml against three *S. aureus* strains (Table 4.21), while methanol leaf extract had a yield of 0.78 mg/ml against *P. aeruginosa* and *Klebsiella spp* (Table 4.22). Water

and ethanol extracts of *P.* sulcatum exhibited an MIC of 0.52 against *P. aeruginosa* (Table 4.30). To our knowledge, antibacterial activity of *P. sulcatum* root, *C. abbreviata* leaves and *J. zeyheri* leaves were not reported elsewhere. However, Luseba *et al* (2007), reported the MIC of 90 % methanolic extract of *J. zeyheri* root as 0.63 mg/ml against *E. coli* and 2.5 mg/ml against both *S. aureus* and *P. aeruginosa*. It is difficult to compare these results to this study due to the differences in variables such as solvent type, extraction procedure and other environmental conditions. Moreover, Aqueous extract of *J. zeyheri* root combined with *Warburgia salutaris* bark and *Pentanisia prunelloides* has been reported to possess an MIC of >2mg/ml against *B. subtilis* and *S. aureus* (Jager, 2003).

Although aqueous extracts of most plants exhibit no activity against microbes, water extract of *Senna italica* exhibited a notably potent MIC of 0.78 against *S. aureus* and 1.30 mg/ml against both *P. aeruginosa* and *E. cloacae (*Table 4.24) while water extract of *U. sanguinea* exhibited 0.78 mg/ml against *E. coli* and 0.39 mg/ml against *E. cloacae* (Table 4.29). Acetone extract of *S. italica* reportedly exhibited an average MIC of 0.16 mg/ml against both *P.aeruginosa* and *E. coli* (Masoko *et al*., 2010).

Methanol extract of *C. quadrangularis* exhibited MIC values of 0.78 and 1.56 mg/ml against *S. aureus* and both *E.coli* and *P. aeruginosa* respectively (Table 4.28). Elsewhere, a similar extract reportedly exhibited LD_{50} of 2000 mg/ml in an acute cytotoxicity study (Swamy *et al*., 2012), while water extract of the same

plant reportedly possessed both sedative and anticolvusant properties in mice (Ngo Bum *et al*., 2008).

5.3 Antioxidant and phytochemical properties

E. burkei exhibited the most potent IC_{50} against DPPH 0.10±0.02 while *J. zeyheri* showed the lowest IC_{50} against ABTS at 0.80±0.10 mg/100 ml (Table 4.33). IC_{50} is the concentration of plant sample at which 50 % of a free radical is scavenged. *P africanum*. exhibited $IC_{50\ of}$ 0.50±0.11 mg/100 ml, while Bizimenyera *et al* (2007) reported IC_{50} acetone extract of leaf, bark and toot of the same plant inhibiting DPPH at 6.54, 4.34 and 3.82 respectively. Other plants species such as *E. elephantina, S. italica, U. sanguinea, C. abbreviata and Waltheria indica* showed potent *inhibition while H. procumbens* exhibited moderate activity against both DPPH and ABTS. Methanolic extract of *H. procumbens* exhibited ferric ion-chelating activity of 53.99 % at 100 µg/ml, while verbascoside, the most abundant phenylethanoid compound found in *H. procumbens* cell cultures was inactive (Georgiev, 2012). *Moreover, H. procumbens* extract, Pascoe® -Agil, inhibits the LPS- induced release of TNFα and IL-6PGE2 in primary monocytes, hence possessed anti-inflammatory activity (Fiebich *et al*., 2012).

P.sulcatum and *C. quadrangularis* exhibited inhibition >5 mg/100 ml against both DPPH and ABTS, All the other plant species showed inhibition (IC_{50}) of less than 5 mg/100ml in both assays, suggesting that selected plants inhibit ABTS

and DPPH equally as in cases reported by Al Mustafa *et al* (2008) and Miliauskas *et al* (2004) as reported in Chapter 2.

Results for the phytochemicals screened are presented in Table 4.32. Tested plant extracts exhibited 82 % flavonoids, 73 % of both saponins and tannins, 55% of both Cardiac glycosides and alkaloids and only 9 % anthraquinone. Plants such as *Peltophorum africanum* and *Elephantorrhiza burkei* posessed highest quantities of both saponins and flavonoids, while *H. Procumbens* and and *J. zeyheri* root posessed high cardiac glycosides and tannins. These compounds may account for both antibacterial and free radical scavenging activity of the plant as reported in this research. Some of the secondary metabolites detected in aqueous and powdered root extracts of *Waltheria indica* include tannins, saponins and cardiac glycosides (Zailani *et al*., 2010). These results are in agreement with the current study. Tannins may selectively inhibit HIV replication and are widely known to make trees and shrubs a difficult meal for caterpillars and other organisms due to an astringent taste (Ishikawa *et al*., 2008). Furthermore, tannins may prevent the development of microorganisms by precipitating microbial protein and making nutritional proteins unavailable (Prasad *et al*., 2008). Moreover, they may hasten the healing of wounds and inflamed mucous membranes (Njoku and Akumefula, 2007). Saponins have detergent properties and serve as lytic agents and exhibit anti-inflammatory properties (Abukakar *et al*., 2008). Cardiac glycosides are known to work by inhibiting the (Na^+/K^+) pump, thereby increasing the amount of Ca^{2+} ions available for the contraction of heart muscles which improves cardiac output and

reduces distensions of the heart, thus is used in the treatment of congestive heart failure and cardiac arrhythmia (Ngbede *et al*., 2008). Complete absence of cardiac glycosides in extracts from both *P. sulcatum* and *C. quadrangularis* may well explain the reason for these plants exhibiting poor antioxidant activity (5 mg/100 ml) against both ABTS and DPPH.

Besides playing a functional role in plant heat acclimatization and protecting plants from both biotic and abiotic stress, flavonoids are known to be highly potent antioxidant compounds that help reduce the incidence of strokes, heart failure, diabetes and cancer (Sharma, 2006; Ghasemzadeh and Ghasemzadeh, 2011). Moreover, they may also posess antimicrobial, anti-inflammatory, enzyme inhibition, and oestrogenic activity (Cushnie and Lamb, 2005).

CHAPTER 6

CONCLUSIONS

6.1 Findings of the current work

This current work documented medicinal plants used for the treatment of sexually transmitted infections. The hypothesis that people in the Blouberg area use medicinal plants to treat STIs is accepted. The antibacterial tests carried out suggest that these plants are vital in the treatment of cited STI infections as well as other infections which may arise d€ue to selected organisms. Moreover, methanol extracts posess highly potent antioxidant activity against DPPH and ABTS, and inhibit such free radicals equally. The first hypotheses that medicinal plants used to treat sexually transmitted infections are active against selected bacterial strains, hence possess free radical scsvenging activity is accepted. €Such biological activity of selected plants may be attributed to flavonoids, alkaloids, cardiac glycosides and saponins as identified by standard tests. More over, methanol extracts of selected extracts inhibits both DPPH and ABTS equally.

According to information obtained from traditionall healers, some common combinations of plants may be used to treat STIs, depending on the type and nature of an illness of a patient. The second hypothesis is therefore accepted as combinations are reported (Table 4.3).

6.2 Suggestions for further study

Although selected plants possess both antibacterial activity and antioxidant effects, there is a need to screen the selected medicinal plants against major etiologic agents of sexually transmitted infections like *Candida albicans* and *Neisseria gonorrhoea.* Moreover, biological effects of reported combinations of these plants needs to be investigated.

The current study was only limited to the selected bacterial strains. Antioxidant properties of selected methanol extracts was also limited to DPPH and ABTS assays.

CHAPTER 7

REFERENCES

Abdel-Hameed ES (2009).Total phenolic contents and free radical scavenging activity of certain Egyptian *Ficus* species leaf sample. Food Chemistry 114:1271-1277.

Abukakar MG, Ukwuani AN, Shehu RA (2008). Phytochemical screening and antibacterial activity of *Tamarindus indica* pulp extract. Asian Journal of Biochemistry 3(2):134-138.

Achakzai AK, Achakzai P, Masood A, Kayani SA, Tareen RB (2009). Response of plants parts and age on the distribution of secondary metabolites on plants found in Quetta. Pakistan Journal of Botany 41(5):2129-2135.

Adedapo AA, Jimoh FO, Koduru S, Afolayan AJ, Masika PJ (2008). Antibacterial and antioxidant properties of the methanol extracts of the leaves and the stems of *Calpurnia aurea*. BMC Complementary and Alternative Medicine 8:53**,** Retrieved on 12/06/2009, Available at http:www.biomedcentral.com/1472-6882-8-53.

Adedapo AA, Jimoh FO, Koduru S, Masika PJ, Afolayan AJ (2009). Assessment of the medicinal potentials of the methanol extracts of the leaves and the stems of Buddleja saligna. BMC Complementary and Alternative Medicine 9:21 DOI:10.1186/1472-6882-9-21

Adeshina G, Onujagbe O, Onaolapo J (2010). Comparative antibacterial studies on the root, stem bark and leaf extracts of *Parkia clappertoniana*. The Internet Journal of Alternative Medicine 8(2):DOI:10.5580/298a.

Adjei O, Opoku C (2004). Urinary Tract Infections in African Infants. International Journal of Antimicrobial Agents 24S (2004) S32-S34.

Agbor GA, Kuate D, Oben JE (2007). Medicinal Plants can be a good source of antioxidants: Case study in Cameroon. Parkistan Journal of Biological Sciences 10(4):534-544.

Agston A (1984). “On abscesses”, Classics in infectious diseases, Rev. Infectious Diseases 6 (1):122-128

Ahmed J, Brutus A, D´Amato RF, Glatt AE (1994). *Acinetobacter calcoaceticus anitratus* outbreak in the intensive care unit traced to a peak flow meter, American Journal of Infections control 22:319-321.

Akerele J Abhulimen P, Okonofua F (2002). Prevalence of asymptomatic genital infection among pregnant women in Benin City-Nigeria. African Journal of Reproductive Health 6(3):93-97.

Aliyu AB, Musa AM, Abdullahi MS, Oyewale AO, Gwarzo US (2008). Activity of plant extracts used in Northern Nigerian traditional medicine against methicillin-resistant *Staphylococccus aureus* (MRSA). Nigerian Journal of Pharmaceutical Science 7(1):1-8.

Ali-Shtayeh MS, Reem M, Raghmour R. Faidi YR, Khalid S, Alnuri MA (1998). Antimicrobial activity of twenty plants used in folkloric medicine in the Palestinian area. Journal of Ethnopharmacology 60(3):265-271.

Al-Mustafa AH, Al-Thinubat OY (2008). Antioxidant activity of some Jordanian medicinal plants used traditionally for treatment of diabetes, Pakistan Journal of Biological Sciences 11 (3):351-358.

Aqil F, Ahmad I, Mehmood Z (2007). Antioxidant and free radical scavenging properties of twelve traditionally used Indian medicinal plants. Turkish Journal of Biology 30 :177-183.

Atawodi SE (2005). Antioxidant potential of African medicinal plants. African Journal of Biotechnology 4(2):128-133.

Austin MN, Beigi RH, Meyn LA, Hillier SL (2005). Microbiologic response to treatment of bacterial vaginosis with topical clindamycin or mtronidazole. Journal of clinical Microbiology 43(9):4492-4497.

Ayantunde AA, Hiernaux P, Briejer M, Udo H, Tabo R (2009). Uses of local plant species by Agropastoralists in South-western Niger. Ethnobotany Research & Applications 7:053-066.

Ayoola GA, Folawewo AD, Adesegun SA, Abioro OO, Adepoju-Bello AA, Coker HAB (2008). Phytochemical and antioxidant screening of some plants of Apocynaceae from South West Nigeria. African Journal of Plant Science 2 (9):124-128.

Balunas MJ, Kinghorn AJ (2005). Drug discovery from medicinal plants. Life Sciences 78:431-441.

Bauman RW, Machunis-Masuoka E, Tizard I (2007). Microbiology with Diseases by Taxonomy, Second Edition, Pearson Benjamin Cummings Publishers, San Francisco, New York, pp 77.

Bently R, Meganathan R (1982). Biosynthesis of vitamin K (menaquenone) in bacteria. Bacteriological Reviews 46 (3):241-280.

Biapa PN, Agbor GA, Oben JE,Ngogang JY (2007). Phytochemical studies and antioxidant properties of four medicinal plants used in Cameroon. African Journal of Traditional, Complementary and Alternative Medicines 4(4):495-500.

Birley H, Duerden B, Hart CA (2002). Sexually transmitted diseases: Microbiology and Management proceedings of Seventh Liverpol Tropical School Bayer, Symposium on Microbial Disease. Journal of Medical Microbiology 51:792-807.

Bishnu J, Sunil L, Anuja S (2009). Antibacterial property of different medicinal plants: *Ocimum sanctum*, *Cinnamomum zeylanicum*, *Xanthoxylum armatum* and *Origanum majorana.* Kathmandu University Journal of Science, Engineering and Technology 5(1):143-150.

Bizimenyera ES, Swan GE, Chikoto H, Eloff JN (2005). Rationale for using Peltophorum africanum (Fabaceae) extracts in veterinary medicine. Tydskr. S. Afr. Vet Ver. 76(2):54-58.

Bizimenyera ES, Githiori JB, Eloff JN, Swan GE (2006). *In vitro* activity of *Peltophorum africanum* Sond. (Fabaceae) extracts on the egg hatching and larval development of the parasitic nematode *Trichostrongylus colubriformis*, Veterinary Parasitology 142:336-343.

Bizimenyera ES, Aderogba MA, Eloff JN, Swan GE (2007). Potential of neuropretective antioxidant-based therapeutics from *Peltophorum africanum* Sond (Fabaceae). African Journal of Traditional, Complementary and Alternative Medicines 4(1):91-96.

Bizimenyera ES, Meyer S, Naidoo V, Eloff JN, Swan GEM (2008). Efficacy of *Peltophorum africanum* Sond. (Fabaceae) extracts on *Haemonchus contortus* and *Trichostrongylus colubriformis* in sheep. Journal of Animal and Veterinary Advances 7(4):364-371.

Booth MC, Hatter KL, Miller D, Davis J, Kowalski R, Parke DW, Chodosh J, Jett BJ, Callegan MC, Penland R, Gilmore MS (1998). Molecular epidermiology of *Staphylococcus aureus* and *Enterococcus faecalis* in Endophthalmitis. Infection and Immunity 66(1):356-360.

Bozicevic I, Fenton KA, Martin IMC, Rudd EA, Ison CA, Nanchahal KA, Wellings K (2006). Epidemiological correlates of asymptomatic gonorrhoea. Sexually Transmitted Diseases 33(5):289-295.

Buwa LV, van Staden J (2006). Antibacterial and antifungal activity of traditional medicinal plants used against venereal diseases in South Africa. Journal of Ethnopharmacology 103 :139-142

Caceres A, Menendez H, Mendez E, Cohobon E, Samayoa BE, Jauregui E, Peralta E, Carrillo G (1985). Antigonorrhoeal Activity of plants used in Gautemala for the treatment of Sexually Transmitted Diseases. Journal of Ethnopharmacology 48:85-88.

Carol MP (2005). Pathophysiology-Concepts of altered health states, Seventh Edition, Lippincott Williams and Wilkins Publishers, Philadelphia, Page 351.

Chang S, Sievett DM, Hageman JC, Boulton ML, Tenover FC, Downes FP, Shah S, Rudrik JT, Pupp GR, Brown WJ, Cardo D, Fridkin SK (2003). Infection with vancomycin-resistant *Staphylococcus aureus* containing van A resistant gene. New England Journal of Medicine 348 (14) :1342-1347.

Chomnawang MT, Surassmo S, Wongsaria K, Bunyapraphatsara N (2009). Antibacterial activity of Thai medicinal plants against methicilin-resistant *Staphylococus aureus*. Fitoterapia 80:102-104.

Choudhury M, Patel BR, Patel M, Bashir T (2009). *Streptococcus viridans* osteomyelitis and endocarditis following dental treatment: a case report. Case Journal 2:6857(http://casesjournal.com/casesjournal/article/view/6857). Retrieved: 20/12/2010).

Clarkson P, Thompson H (2000). Antioxidants: what role do they play in physical activity and health? American Journal of Clinical Nutrition (72):637S-638S.

Coates Palgrave M (2005). Keith Coates Palgrave Trees of Southern Africa, Third Edition, Third Impression, Struik Publishers, Cape Town, Republic of South Africa,

Colpaert EE, Lefebvre R (2002). Role of antioxidants in the protection of nitrergic neurotransmitter. Acta neurol. Belg. 102:68-72.

Colvin M, Shisana O, Connolly C, Shimbali L (2004). The association between HIV and STIs in South Africa, Medical Research Council of South Africa, International Conference on AIDS (15th:2004, Bangkok, Thailand).

Cook H, Furura E, Larson E, Vasquez G, Lowly F (2007). Heterosexual Transmission of community-associated methicilin–resistant *Staphylococcus aureus*. Clinical Infectious Diseases 44 (3):410-413.

Corwin LM and Shloss J (1980). Role of antioxidants on the stimulation of the mitogenic response. Journal of Nutrition 110:2497-2505.

Chan LW, Cheah ELC, Saw CLL, Weng W, Heng PWS (2008). Antimicrobial and antioxidant properties of Cortex *Magnoliae officinalis* and some other medicicnal plants commonly used in South-East Asia, Chinese Medicine 3:15 (doi10.1186/1749-8546-3-15) Retrieved on 23/04/2010).

Cushie TPP and Lamb AJ (2005). Antimicrobial activity of flavonoids. International Journal of Antimicrobial agents 26:343-356.

Dabai YU, Kawo AH, Aliyu RM (2012). Phytochemical screening and antibacterial activity of leaf and root extracts of *Senna italica*. African Journal of Pharmacy and Pharmacology 6(12):914-918.

Dehmlow EV, Van Ree T, Guntenhoner M (1998). 2,4-Trans-7,4'-dihydroxy-4-methoxyflavan from *Cassia abreviata*. Phytochemistry 49(6):1805-1806.

Dekker TG, Fourie TG, Mathee E, Snyckers FO (1987). Studies of South African plants. Part 4. jaherin, a new daphthanane diterpene with antimicrobial properties from *Jatropha zeyheri.* South African Journal of Chemistry 40(1):74-76.

Desai PV, Wadekar RR, Kedar GH, Patil KS (2008). Free radical scavenging activity of aqueous extract of roots of *Baliospermum montanum* Muel-Arg, International Journal of Green Pharmacy (January-March 2008):31-33

Devappa RK, Makkar HPS, Becker K (2011). *Jatropha* Diterpenes: A Review. J. Am. Oil. Chem. Soc. 88:301-322.

Diallo D, Hveem B, Mahmoud MA, Berge G, Paulsen BS, Maiga A (1999). An ethnobotanical survey of herbal drugs of Gourma District, Mali. Pharmaceutical Biology 37(1):80-91.

Edoh D, Mensah A (2007). Incidence of antibiotic resistant microbes in Accra West, Ghana, African Journal of Science and Technology 8(2):103-109.

Edvera A, Velikova V, Tsonev T, Dagnon S, Gurel A, Aktas L and Gesheva V (2008). Stress protective role of secondary metabolites: Diversity of functions and mechanisms. Genetics and Applied plant Physiology 34(1-2):67-78.

Edward CG (1988). Can Collaborative Programs between biomedical and African Indigenous health practitioners succeed? Social science and Medicine 27(1):1125-1130.

Eloff JN (1998). A sensitive and quick method to determine minimal inhibitory concentrations of plant extracts for bacteria. Planta Medica 64:711-714.

EL-Shebly AA (2009). The role of antioxidant (Vitamin E) in the control of lead (Pb) pollution and enancement of growth within Nile tilapia (*Oreochromis niloticus*). International Journal Applied Research in Veterinary Medicine 7(3):97-101.

European Engine embassy (Viguet poupelloz J, Lavergne F.Gueho S, Deleau N (assesors), Saint-salvi B (Interactions) (2009). Assessment report on *Harpagophytum procumbens* dc. and/or *Harpagophytum zeyheri* decne, radix, London. DOC. Ref.: EMEA/HMPC/251324/2006 *Corr (*Retrieved on 16/12/2010).

Falagas ME, Rafailidis PI, Kofteridis D, Virtzili S. Chelvatzoglou FC, Papaioannou V, Maraki S, Samonis G, Michalopoulos A (2007). Risk actors of carbapenem-resistant *Klebsiella pneumoniae* infections: a matched case-control study. Journal of Antimicrobial Chemotherapy 60:1124-1130.

Falagas ME, Rafailidis PI, Kofteridis D, Virtzili S, Chelvatzoglou FC, Papaioannou V, Maraki S,Samonis G and Michalopoulos A (2007). Risk factors of carbapenem-resistant *Klebsiella pneumoniae* infections: a matched case-control study, Journal of Antimicrobial Chemotherapy 60:1124-1130.

Feng P, Weagant S, Grant M (2002). Enumeration of *Escherichia coli* and the coliform bacteria. Bacterilogical Analytical Manual (8th edition) FDA/Center for Food Safety and Nutrition (http://www.cfsan.dfa.gov/~ebam/bam-4html) retrieved on 29/05/2009.

Foukaridis GN, Osuch E, Mathibe L, Tsipa P (1995). The ethnopharmacology and toxicity of *Urginea sanguinea* in the Pretoria area. Journal of Ethnopharmacology 49:77-79.

Fiebich BI, Muṅoz E, Rose T, Weiss G, McGregor GP (2012). Molecular targets of the anti-inflammatory *Harpagophythum procumbens* (Devil's Claw): Inhibition of TNFα and COX-2 gene expression by preventing activation OF AP-1. Phytotherapy Research 26:806-811.

Ganesan S, Ponnuchamy M, Kesavan L, Salvaraj A (2009). Floristic compositin and practices on the selected sacred groves of Pallatty village (Reserved forest), Tamil Nadu. Indian Journal of Traditional Knowledge 8(2):154-162.

Giakoupi P, Maltezou H, Polemis M, Pappa O, Saroglou G, Vatopoulos A and The Greek System for the Surveillance of Antimicrobial Resistance (2009). KPC-2-Producing *Klebsiella pneumoniae* infections in Greek Hospitals are mainly due to hyperepideemic clone. Eurosurveillanc*e* 14(21):1-5.

Germishuizen G, Meyer NL (2003). Plants of Southern Africa: An annotated checklist, Strelitzia, National Botanical Institute, Pretoria.Gergiev MI, Alipieva K, Orhan IE (2012). Cholinesterase inhibitory and antioxidant activities of *Harpagophythum procumbens* from *in-vitro* systems. Phytotherapy Research 26:313-316.

Gessler MC, Nkunya MHH, Mwasumbi LB, Heinrich M, Tanner M (1994). Screening Tanzanian medicinal plants for antimalarial activity. Act. Trop. 56:65-77.

Ghasemzadeh A, Ghasemzadeh N (2011). Flavanoids and phenolic acids: Role and biochemical activity in plants and human. Journal of Medicnal Plant Research 5(31):6697-6703.

Green E, Samie A, Obi CL, Bessong PO, Ndip RN (2010). Inhibitory properties of selected South African medicinal plants against *Mycobacterium tuberculosis*. Journal of Ethnopharmacology 130:151-157.

Hadault S, Guignot J, Serwin AL (2001). *Escherichia coli* strains colonising the gastrointerstinal tract protect germ free mice against *Salmonella typhimurium* infection. *Gut* 49:47-55.

Hall LMC, Duke B, Urwin G, Guiney M (1992). Epidemiology of *Enterococcus faecalis* urinary tract infections in a teaching hospital in London, United Kingdom, Journal of Clinical Microbiology 30(8):1953-1997.

Harbourne JB (1973). Phytochemical Methods. A guide to Modern Techniques of Plant Analysis, Chapman and Hall, London, Pp. 221-232.

Hejazi A, Falkiner FR (1997). *Serratia marcescens*. Journal of Medical Microbiology 46:903-912

Hiramatsu K, Hanaki H, Ino T, Yabuta K, Oguri T, Tenover FC (1997). Methicilin-resistant *Staphylococcus aureus* clinical strain with reduced vancomycin susceptibility, Journal of Antimicrobial Chemotherapy 40 (1):143-144.

Hohmann EL (2001). Nontyphoidal Salmonellosis. Clinical Infectious Diseases 32:263-269.

Hutchings A (2007). Ritual Cleansing, Incense and the tree of life- Observations on some indigenous plant usage in Traditional Zulu and Xhosa Purification and ritual rites. Alteration 14(2):189-217.

Ibrahim JA, Muazzam I, Jegege IA, Kunle OF, Okogun JI (2007). Ethno-Medicinal plants and methods used by Gwandara Tribe of Sabo Wuse in Niger State, Nigeria. African Journal of Traditional, Complimentary and Alternative Medicines- African Ethno Medicines Network 4(2):211-218.

Iglewski BH (1996). *Pseudomonas*. In: Baron's Medical Microbiology, (Baron S *et al* eds) Fourth Edition, University of Texas Medical Branch. ISBN 0-9631172-1-1.

Ishikawa K, Kato ETM, Yoshida M, Kaneko TM (2008). Morphoanatomic aspects and phytochemical screening of *Plinia edulis* (Vell.) Sobral (Myrtaceae). Brazillian Journal of Pharmaceutical Sciences 44(3):515-520.

Jager AK (2003). Evaluation of antibacterial activity of traditionally prepared South African remedies for infections. South African Journal of Botany 69(4):595-598.

Joshi YM, Kadam VJ and Kaldhone PR (2009). *In-vitro* Antioxidant activity of methanolic extracts of aerial parts of *Canna indica* L. Journal of Pharmacy Research 2(11):1712-1715.

Jiofack T, Fokunang C, Kemeuze V, Fongnzossie E, Tsabang N, Nkuinkeu R, Mapongmetsem PM, Nkongmeneck BA (2008). Ethnobotany and phytopharmacopoea of the South West ethnoecological region of Cameroon. Journal of Medicinal Plants Research 2 (8):197-206.

Kamalakannan K, Pai RM, Johnnson LB, Gardin JM, Saravolatz LD (2007). Epidermiology and clinical outcomes of infective endocarditis in haemodialysis patients. Annals of Thoracic Surgery 83:2081-2086.

Kambizi L, Afolayan AJ (2001). An ethnobotanical study of plants used for the treatment of Sexually Transmitted Diseases (njovhera) in Guruwe District, Zimbabwe, Journal of Ethnopharmacology 77(1):5-9.

Karmegam N, Jayakumar M, Karuppusamy S (2012). Synergistic antibacterial activity of four medicinal plants collected from Dharapuram Taluk of Tirrupur District, South India. Journal of Plant Science 7(1):32-38

Kartali G, Tzelepi E, Pournaras S, Kontopoulou C, Kontos F, Sofianou D, Maniatis AN, Tsakris A (2002). Outbreaks of infections caused by *Enterobacter cloacae* producing integron-associated β-lactamase IBC-1 in a neonatal intensive care unit of greek hospital. Antimicrobial Agents and Chemotherapy 46(5):1577-1580.

Keller R, Pedrosso MZ, Ritchmann R, Silva RM (1998). Occurence of virulence-associated properties in Enterobacter cloacae. Infection and Immunity 66(2):645-649.

Kelmanson JE, Jager AK, van Staden J (2000). Zulu medicinal plants with antibacterial activity. Journal of Ethnopharmacoclogy 69:241-246.

Khan MR (2001). Antibacterial activity of some Tanzaniana medicinal plants. Pharmaceutical Biology 39(3):206-212.

Killic IH, Ozaslan M, Karagoz ID, Zer Y, Savas E, Davutoglu V (2011). The role of stethoscopes in the transmission of hospital infections. African Journal of Biotechnology 10(30):5769-5772.

Krog M, Falcao MP, Olsen CS (2006). Medicinal plant markets and trade in Maputo, Mozambique, Forest and Landscape, Working Papers NO. 16-2006:18.

Kumaraswamy MV, Kavitha HU, Satish S (2008). Antibacterial evaluation oand phytochemical analysis of *Betula utilis* D. Don against some human pathogenic bacteria. World Journal of Agricultural Sciences 4(5):661-664.

Lall N, Meyer JJM (2000). Antibacterial activity of water and acetone extracts of the roots of *Euclea natalensis*. Journal of Ethnopharmacology 72: 313-316.

Lugasi A, Hovari J, Sagi KV, Biro L (2003). The role of antioxidant phytonutrients in the prevention of diseases. Acta Biologica Szegediensis 47(1-4):119-125.

Luseba D, Elgorashi EE, Ntloedibe DT and van Staden J (2007). Antibacterial, antiinflammmatory and mutagenic effects of some medicinal plants used in South Africa for the treatment of wounds and retained placenta in livestock. South African Journal of Botany 73(3):378-383.

Mabogo DEN (1990). The ethnobotany of Vhavenda, Unpublished Master of Science Thesis, University of Pretoria, Pretoria.

Mabrouk MI (2012). Synergistic and antibacterial activity of six medicinal plants used in folklore medicine in Egypt against *E.coli* O157:H7. Journal of Applied Science Research 8(2):1321-1327.

Magano SR, Thembo KM, Ndlovu SM and Makhubela NFH (2008). The anti-tick properties of the root extracts of *Senna italica* subsp. *Arachoides,* African Journal of Biotechnology 7(4):476-481.

Magoro MD (2008). Health Practitioner's practices and the sustainability of extinction-prone Traditional Medicinal Plants, MSc. Thesis, University of South Africa, page 37.

Majinda RR, Waigh RD, Waterman PG (1997). Bufadienolides and other constituents of *Urginea sanguinea*. Planta Medica 63(2):188-190.

Malan E, Swinny E, Ferreira D, Steynberg P (1996). Structure and synthesis of proguibourtinidins from *Cassia abbreviata*. Phytochemistry 41(4):1209-1213.

Marx J, Pretorius E, Espang WJ, Bester MJ (2005). *Urginea sanguinea*: medicinal wonder or death in disguise? Environmental Toxicology and Pharmacology 20:26-34.

Marx J, Pretorius E, Bester MJ (2006). Effects of *Urginea sanguinea*, a traditional asthma remedy on embryo neuronal development. Journal of Ethnopharmacology 104(3):315-321.

McGaw LJ, Eloff JN (2008). Ethnoveterinary use of southern African plants and scientific evaluation of their medicinal properties. Journal of Ethnopharmacology 119:559-574.

Masoko P, Gololo SS, Mokgotho MP, Eloff JN, Howard RL, Mampuru LJ (2010). Evaluation of the antioxidant, antibacterial and antiproliferative activities of the acetone extract of the roots of *Senna italica* (Fabaceae). African Journal of Traditional, Complementary and Alternative Medicines 7(2):138-148.

Mathabe MC, Nikolova RV, Lall N, Nyazemba NZ (2006). Antibacterial activities of medicinal plants used for the treatment of diarrhoea in Limpopo Province South Africa. Journal of Ethnopharmacology 105 (1-2):286-293.

Mathekga ADM, Eloff JJM (1998). Antibacterial activity of South African *Helichrysum* species. South African Journal of Botany 64 (5) 293-295.

Mavi S, Shava S (1997). Traditional methods of conserving medicinal plants in Zimbabwe, BGCI, *BGC News* 2(8)- July 1997. Available at: http://www.bgci.org/index.php, Retrieved on 25/04/2009.

Mayaud P, McCormick D (2001). Interventions against sexually transmitted infections (STI) to prevent HIV infection, British Medical Bulletin 58:129-153.

Mead MG, Gruneberg RN (1978). Urinary tract infection in a clinic for sexually transmittrd diseases. British Journal of Venereal Diseases 54:274-277.

Mebe P P, Makuhunga P (1992). ll-(E)-p-Coumaric acid ester of bergenin from *Peltophorum africanum.* Phytochemistry 31(9):3286-3287.

Merina S, Viji SBG (2012). *In-vitro* screening of antimicrobial potentials of *Cissus quadrangularis* L. Asian Journal of Plant Science and Research 2(1):58-62.

Miliauskas G, Venskutonis PR and van Beek TA (2004). Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chemistry 85 (2004): 231-237.

Mims C, Playfair J, Roitt I, Wakelin J, Williams R (1998). Medical Microbiology, Second Edition, Mosby Publishers Limited, Barcelona, Page 518-519.

Mishra G, Srivastava S, Nagori BP (2010). Pharmacoclogical and therapeutic activity off *Cissus quadrangularis* : An overview. International Journal of PharmTech Research 2(2):1298-1310.

Moghaddam MG, Ahmad FBH, Samzadeh-Kermani A (2012). Biological activity of betulinic acid: A review. Pharmacology and Pharmacy 3:119-123.

Moller H, Berkes F, Lyver PO and Kislalioglu M (2004). Combining Science and Traditional ecological knowledge: Monitoring populations for co-management, *Ecology and Society* 9(3):2. retrieved on 13/08/2009. http://www.ecologyandsociety.org/vol9/iss3/art2. Retrieved on 15/09/2009.

MMWR (2002). VOL 51, NO RR-15, Division of STD Prevention, National centre for HIV-AIDS, Viral hepatitis, STD and TB Prevention.

Morales G, Peredes A, Sierra P, Loyola LA (2008). Antimicrobial activity of three Baccharis species used in the traditional medicine of Northern Chile. Molecules 13:790-794.

Mothana RAA, Abdo SAA, Hasson S, Althawab FMN, Alaghbari SAZ, Lindequist U (2008). Antimicrobial, antioxidant and cytotoxic activities and phytochemical screening of some Yemeni medicinal plants, Evidence-based Complementary and Alternative Medicine. Document: DOI:10.1093/ecam/nen007.

Mthembu XS (2007). A phytochemical study of *Schefflera umbellifera* and *Elephantorrhiza elephantina*, Master of Science Dissertation, University of Kwazulu Natal, Pietermaritzburg.

Mulaudzi FM (2005). Attitudes, believes and practices of the Vhavenda in sexually transmitted diseases, Indilinga. African Journal of Indigenous Knowledge Systems 4(1):323-337.

Mulholland DA (2005). The future of ethnopharmacology: A South African perspective. Journal of Ethnopharmacology 100 (1-2):124-126.

Munkombwe NM (2003). Acetylated phenolic glycosides from *Harpagophythum procumbens*. Phytochemistry 62:1231-1234.

Musil I, Jensen V, Schilling J, Ashdown B, Kent T (2010). *Enterobacter cloacae* infection of an expanded polytetrafluoroethylene femoral-popliteal bypass graft: A case report. J. Med. Case Rep. 4:131 http//www.jmedicalcasereports.com/content/4/1/131

NCCLS, National Committee for Clinical Laboratory Standard guidelines (2001). Performance standards for anti-microbial susceptibility testing: 11th informational supplement. Document M100.

Ndubani P, Bengt Hojer (1999), Traditional Healers and the treatment of sexually transmitted diseases in rural Lusaka, Journal of Ethnopharmacology 67(1):135 -144.

Ngo Bum E, Ngoupaye GT, Talla E, Dimo T, Nkantchoua GCN, Pelanken MM, Taiwe S (2008). The anticonvulsant and sedative properties of stems of *Cissus quadrangularis* in mice. African Journal of Pharmacy and Pharmacology 2(3):042-047.

Ng'etich KA (2005). Indigenous knowledge, alternative medicines and intellectual property rights concerns in Kenya, 11th General Asssembly, Maputo Mozambique, 6-10 December 2005.

Ngbede J, Yakubu RA, Nyam DA (2008). Phytochemical screening for active compounds in *Canarium schweinfurthii* (Atile) leaves from Jos North, Plateau State Nigeria. Research Journal of Biological Science 3(9):1076-1078.

Njoku PC, Akumefula MI (2007). Phytochemical and nutrient evaluation of *Spondias mombin* leaves. Pakistan Journal of Nutrition 6(6):613-615.

Ngo Bum E, Ngoupaye GT, Talla E, Dimo T, Nkantchoua GCN, Pelanken MM, Taiwe GS (2008). The anticonvulsant and sedative properties of stems of *Cissus quadrangulanris* in mice. African Journal of Pharmacy and Pharmacolgy 2(3):042-047.

Oben J, Kuate D, Agbor G, Momo C and Talla X (2006). The use of a *Cissus quadrangularis* formulation in the management of weight loss and metabolic syndrome, *Lipids in Health and Disease* 5:24. Available at http:www.lipidworld.com/content/5/1/24, Retrieved on 20/07/2010

Oke JM, Hamburger MO (2002). Screening of some Nigerian medicinal plants for antioxidant activity using 2, 2, diphenyl- picryl-hydrazyl radical. African Journal of Biomedical Research 5:77-79.

Okeleye BI, Samie A, Bessong PO, Mkwetshana NF, Green E, Clarke AM, Ndip RN (2010). Crude ethyl acetate extract of the stem bark of *Peltophorum africanum* (Sond, Fabaceae) possessing *in vitro* inhibitory and bactericidal activity against clinical isolates of *Helicobacter pylori*. Journal of Medicinal Plants Research 4(14):1432-1440.

Okoli AS, Iroegbu CU (2004). Evaluation of extracts of *Anthlocleista djalonensis*, *Nauclea latifolia* and Uvaria *afzalii* for activity against bacterial isolates from cases of nongonococcal urethritis. Journal of Ethnopharmacology 92 :135-144.

Olajuyigbe OO, Babalola AE, Afolayan AJ (2011). Antibacterial and phytochemical screening of crude ethanolic extract of *Waltheria indica* L. African Journal of Microbiology Research 5(22):3760-3764.

Olowokudejo JD, Kadiri AB, Travih VA (2008). An ethnobotanical survey of herbal markets and medicinal plants in Lagos. Ethnobotanical Leaflets 12:851-865

Opoku AR, Maseko NF, Terblanche SE (2002). The *In Vitro* antioxidative activity of some traditional Zulu medicinal plants, Phytotherapy Research 16: S51-S56.

Pereira DM, Valentão P, Pareira JA, Andrade PB (2009). Phenolics : From chemistry to biology. Molecules 14(6):2202-2211.

Pooley E (1993). The complete field guide to the Trees of Natal, Zululand and Transkei, First Edition, Natal Flora Publications Trust, Pietermaritzburg, Republic of South Africa. Page165.

Pooley E (2003), Mountain flowers:A field guide to the flora of the Drakensberg and Lesotho, First Edition, The flora Publication Trust, Durban, Republic of South Africa.

Porter E, Yang H, Yavagal S, Preza GC, Murillo O, Lima H, Greene S, Mahoozi L, Klein PM, Diamond G, Gulati S, Ganz T, Rice PA, Quayle AJ (2005). District difensin profiles in Neisseria gonorrhoeae and Chlamidia trachomatis urethritis reveal novell epithelial cell-neutrophil ineractions. Infection and Immunity 73(8):4823-4833.

Prasad RN, Viswanathan S, Devi JR, Nayak V, Swetha VC, Archana BR, Parathasarathy N, Rajkumar J (2008). Preliminary phytochemical screening and microbial activity of *Samaea saman*. Journal of Medicinal Plants Research 2(10):268-270.

Radrianirina F, Vaillant L, Ramarokoto CE, Rakotoarijaona A, Anriamanarivo ML, Razafimahandry HC, Radrianomenjanahary J, Raveloson JR, Hariniaina ER, Carod JF, Talarmin A, Richard V (2010). Antimicrobial resistance in pathogens causing nosocomial infections in surgery and intensive care wards in Antananarivo, Madagascar. J. Infect. Dev. Ctries 4(2):074-082.

Rahman MM, Sheikh MMI, Sharmin SA, Islam MS, Rahman MA, Rahman MM, Alam MF (2009). Antibacterial activity of leaf juice and extracts of *Moringa oleifera* Lam. against some human pathogenic bacteria. CMU Journal of Natural Sciences 8(2):219-227.

Raj MK, Duraipandiyan V, Agustin, Ignacimuthu S (2012). Antimicrobial activity of bergenin isolated from *Peltophorum pterocarpum* DC. Flowers. Asian Paxific Journal of Tropical Biomedicine S901-S904.

Ramar P.S, Maung Maung T, Ponnampalam G and Savarimithi I (2008). Ethnobotanical Survey of plants for the treatment of snakebites in Southern part of Tamilnadu, India, Journal of Ethnopharmacology 115(2):302-312

Rao YK, Fang S, Tzeng Y (2005). Inhibitory effects of the flavonoids isolated from *Waltheria indica* on the production of NO, TNF-α and IL-12 in activated macrophages, Biological and Pharmaceutical Bulletin 28(5):912-915.

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999). Antioxidant activity applying an Improved ABTS radical cation decolorising assay. Free Radical Biology and Medicine 26:1231-1237.

Reddy PS, Mbewu AD and Nogoduka CM (2003). Commentary: Sexually Transmitted Infections in South Africa: 50 years after Sydney Kark. International Journal of Epidemiology 32 (2):187-189.

Refoua Y (2005). A study of *Streptococccus viridans* in the maxillofacial region. Journal of Dentistry 2(4):174-177.

Richens J (2004). ABC of sexually transmitted infections: Main presentations of sexually transmitted infections in men. BMJ 328:1251-1253.

Richter M (2003). Traditional medicine and Traditional healers in South Africa, Discussion paper prepared for the Treatment Action Campaign and AIDS-Law Project, 27 November 2003, page 12.

Ruftig RB (1998). Microbiology and Immunology, Lippincott-Raven Publishers, Washington-Philadelphia, Pp.74.

Sa' RA, Argolo ACC, Napoleao TH, Gomes FS, Santos NDL, Melo CML, Albuquerque AC, Xavier HS, Coelho LCBB, Bierber LW, Paiva PMG (2009). Antioxidant, Fusarium Growth inhibition and Nasutitermes corniger repellent activities of Secondary metabolites from *Myractrodruom urundeuva* heartwood, International Biodeterioration and Biodegradation 63 :470-477.

Salehi P, Walker J, Madsen K, Churchill TA (2006). Control of oxidative stress in small bowel: relevance to organ preservation. Surgery 139:317-323.

Samie A, Obi CL, Bessong PO, Namrita L (2005). Activity profiles of fourteen selected medicinal plants from rural Venda communities in South Africa against fifteen clinical bacterial species. African Journal of Biotechnology 4(12):1443-1451.

Saunders JG (2007). Sterculiaceae of Paraguay II. *Waltheria*. Bonplandia 16(1-2):143-180.

Schmidt S, Lotter M, McCleland W (2002). Trees and shrubs of Mpumalanaga and the Kruger National Park. Jacana, Johannesburg, Page 184.

Shanson DC, Thomas F, Wilson D (1984). Effect of volume of blood cultured on detection of *Streptococcus viridans* bacteremia. Journal of Clinical Pathology 37:568-570.

Shim H, Noh S, Park A, Lee Y, Kim J, Chung H, Kang K, Cho NH (2010). Detection of sexually transmitted infection and human papillomavirus in negative cytology by multiplex-PCR. BMC Infectious Diseases 10:284. DOI:1186/1471-2334-10-284.

Shyur L, Tsung J, Chen J, Chiu C, Lo C (2005). Antioxidant properties of Extracts from medicinal plants popularly used in Taiwan. International Journal of Applied Science and Engineering 3 (3):195-202.

Siddhuraju P, Becker K (2007). The antioxidant and free radical scavenging activities of processed cowpea (*Vigna unguiculata* (L) Walp.) seed extracts. Food Chemistry 101 (2007):10-19.

Sikka SC (2004). Role of antioxidative stress and antioxidants in Andrology and assisted reproductive technology. Journal of Andrology 25(1):5-18

Singh V, Patel JR, Gaur K, Tyagi LK, Kori ML (2009). In vitro antioxidant activity and phytochemical analysis of stem bark of *Balanites roxburghii* Planch. Advances in Biological Research 3(5-6):242-246.

Statistics South Africa (2001). Census in Brief, Pretoria, Republic of South Africa.

Statistic South Africa (2004-2006). Semi-permanent data estimated by National Department of Health in Mid-2006 by Disaggregating Province and District estimates using data from Small Area Layer.

Sturm AW, Wilkinson D, Ndovela N, Bowen S, Conolly C (1997). Australasian Society for HIV Medicine, Conference, 1997 November 13-16, 9:151(Poster P67).

Shai LJ, Masoko P, Mokgotho MP, Magano SR, Mogale AM, Boaduo N, Eloff JN (2010). Yeast alpha glucosidase inhibitory and antioxidant activities of six medicinal plants collected in Phalaborwa, South African Journal of Botany 76:465-470.

Shai LJ, Magano SR, Lebelo SL, Mogale AM (2011). Inhibitory effects of five medicinal plants on rat alpha-glucosidase: Comparison with their effects on yeast alpha-glucosidase. Journal of Medicinal Plant Research 5(13):2863-2867.

Sharma DK (2006). Pharmacological properties of flavonoids including flavonolignans-Integration of petrocrops with drug development from plants. Journal of Scientific and International Research 65:477-484.

Steenkamp V, Fernandes AC, van Rensburg CEJ (2007). Antibacterial activity of Venda medicinal plants. Fitoterapia 78:561-564.

Swamy AHMV, Koti BC, Thippeswamy AHM, Sadiq AJ, Praveen DM, Patil M (2012). Possible mode of action of *Cissus quadrangularis* in experimental induce nociception in mice. African Journal of Pharmacy and Pharmacology 6(14):1088-1091.

Tena D, Martinez-Torres JA, Perez-Pomata MT, Saez-Nieto JA, Rubio V, Bisquert J (2007). Cutaneous infection due to *Bacillus pumilus*: Report of 3 case., Clinical Infectious Diseases 44:e40-2.

Thaipong K, Boonprakob U, Crosby K, Cisneros-Zevallos L, Byrne DH (2006). Comparison of ABTS, DPPH, FRAP and ORAC assays for estimating antioxidant activity from guava fruit extracts. Journal of Food Composition and Analysis 19 :669-675.

Thakur A, Jain V, Hingorani L, Laddha KS (2009). Phytochemical studies on *Cissus quadrangularis* Linn. Pharmacognosy Research 1(4):213-215.

Theo A, Masebe T, Suzuki Y, Kikuchi H, Wada S, Obi CL, Bessong PO, Usuzawa M, Oshima Y, Hattori T (2009). *Peltophorum africanum*, A traditional South African medicinal plant, contains an anti HIV-1 constituent, betulinic acid. Tohoku Journal of Experimental Medicine 217:93-99.

Todar K (2007). Pathogenic *Escherichia coli* Online Textbook of Bacteriology, University of Wisconsin-Madison Department of Bacteriology. (http://www.textbookofbacteriology.net/e.coli.html). Retrieved on 2009/11/30

Togola A, Diallo D, Dembele S, Barsett H, Paulsen BS (2005). Ethnopharmacological survey of different uses of seven medicinal plants from Mali, (West Africa) in the regions Doila, Kolokani and Siby, Journal of Ethnobiology and *Ethnomedicine* 1:7 Available at http://www.ethnobiomed.com/content/1/1/7, Retrieved on 17/05/2009.

Trease GE, Evans WC (1989). Pharmacognosy, 13th Edition, Bailliere Tindall, London, Pp. 176-180

Tshikalange TE, Meyer JJM, Hussein AA (2005). Antimicrobial Activity, toxicity and isolation of bioactive compound from plants used to treat sexually transmitted diseases. Journal of Ethnopharmacology 96(3):515-519.

Tunkel AR, Sepkowitz KA (2002). Infections caused by *Viridans Streptococci* in patients with neutropenia. Emerging Infections 34:1524-1529.

Udin SN, Akond MA, Mubassara S, Yesmin N (2008). Antioxidant and antibacterial activities of *Trema cannabina*. Middle-East Journal of Scientific Research 3(2):105-108.

van Andel T, Havinga R (2008). Sustainability aspects of commercial plant harvesting in Suriname. Forest Ecology and Management 245:1540-1545.

van Wyk B, van Oudtshoorn B, Gericke N ($2005_{(a)}$). Medicinal plants of South Africa, Briza Publications, Pretoria, RSA, pp 116.

van Wyk B, Gericke N (2007). People's plants, A guide to useful plants of Southern Africa, Briza Publications, Pretoria, RSA.

Van Wyk B, Malan S (1998). Field guide to the wild flowers of the Highveld. Struik publishers. Capetown. Pp 178.

Venter F, Venter J (2009). Making the most of indigenous trees, Revised Edition. Briza Publications, Pretoria, RSA Pp 228

Vogt RL, Dippold L (2002). *Escherichia coli 0157:H7* Outbreak as associated with consumption of ground beef, Public Health Reports 2:174-178 .

WHO, World Health Organisation (2001). STD Statistics Worldwide, Prevalence and incidence of selected curable Sexually Transmitted infections, Overview and Estimates, Last updated in 2008-http://www.avert.org/stdstatisticsworldwide.htm.

WHO, World Health Organisation (2003) Traditional medicine, Fact sheet NO. 134.

WHO, World Health Organisation (2004). Statistics about sexually transmitted diseases, the world Health Report.

WHO, World Health Organisation (2007). sexually transmitted infections, Factsheet NO 110, Revised October 2007, http:www.who.int/mediacentre/factsheets/fs110/en/index.html.

Wilkinson D, Ramjee G, Sturm AW, Abdool KSS (1997). Reducing South Africa's hidden epidemic of Sexually transmitted infections, Case study in Hlabisa District-KwaZulu Natal Province. Medical Research Council Newsletter.

WIPO (1998-1999). Interlectual property needs and expectations of traditional knowledge holders. WIPO report on fact-finding missions on intelllectual property and traditional knowlwdge, Pp 25.

Wolfgang B. (1979). Primary health acre and traditional medicine- considering the background of changing health care concepts in Africa. Social Science and Medicine, Part B: Medical Anthropology 13(3):175-182.

Yismaw G, Negeri C and Kassu A (2006). A five year antimicrobial resistance pattern observed in *Shigella* species isolated from stool samples in Gondar University Hospital, northwest Ethiopia. Ethiopian Journal of Health Development 20(3):194-198.

Yuan YV, Bone DA and Carrington MF (2005). Antioxidant activity of *dulse* (*Palmaria palmata*) extract evaluated *in vitro*, Food Chemistry 91 :485-494.

Zailani AH, Jada SM, Wurochekke UA (2011). Antimicrobial activity of *Waltheria indica*. Journal of American Sciences 6(12):1591-1594.

Zchocke S, Rabe T, Taylor JLS, Jager AK, van Staden J (2000). Plant part substitution- a way to conserve endangered medicinal plants? Journal of Ethnopharmacology 71 : 281-292.

APPENDICES

Appendix 1(a)

Name of interviewer								
Name of Interviewee							Gender:	
Age in years	18-39		40-60		60 +			
District :				Village:				
Name Indigenous plants used to treat Sexually Transmitted Diseases in your area?								
Name of plant	Plant part used	Is it or used alone or combined	Ratio of plants and dosage-frequency and quantity	Other uses of the plant				
Which of the above named plants are the best or core species?								
Name medicinal plants that may be used to treat Sexually Transmitted Diseases that are scarce or no longer available in the area?								
From which place are you obtaining them?								
In which sate of growth would you preferably collect the plant materials?								
Young		Mature						
After digging or collecting plant roots and tubers, would you leave the hole open?								
Close the hole		Leave the hole open						
Why so?								
How often would you have a patient suffering from Sexually Transmitted Disease?								
Every Week		Two weeks		Every Month				
Are your patients males or females?								
Males		Females						
Which sexually transmitted diseases are more common than others?								

Appendix 1(b)

Consent form

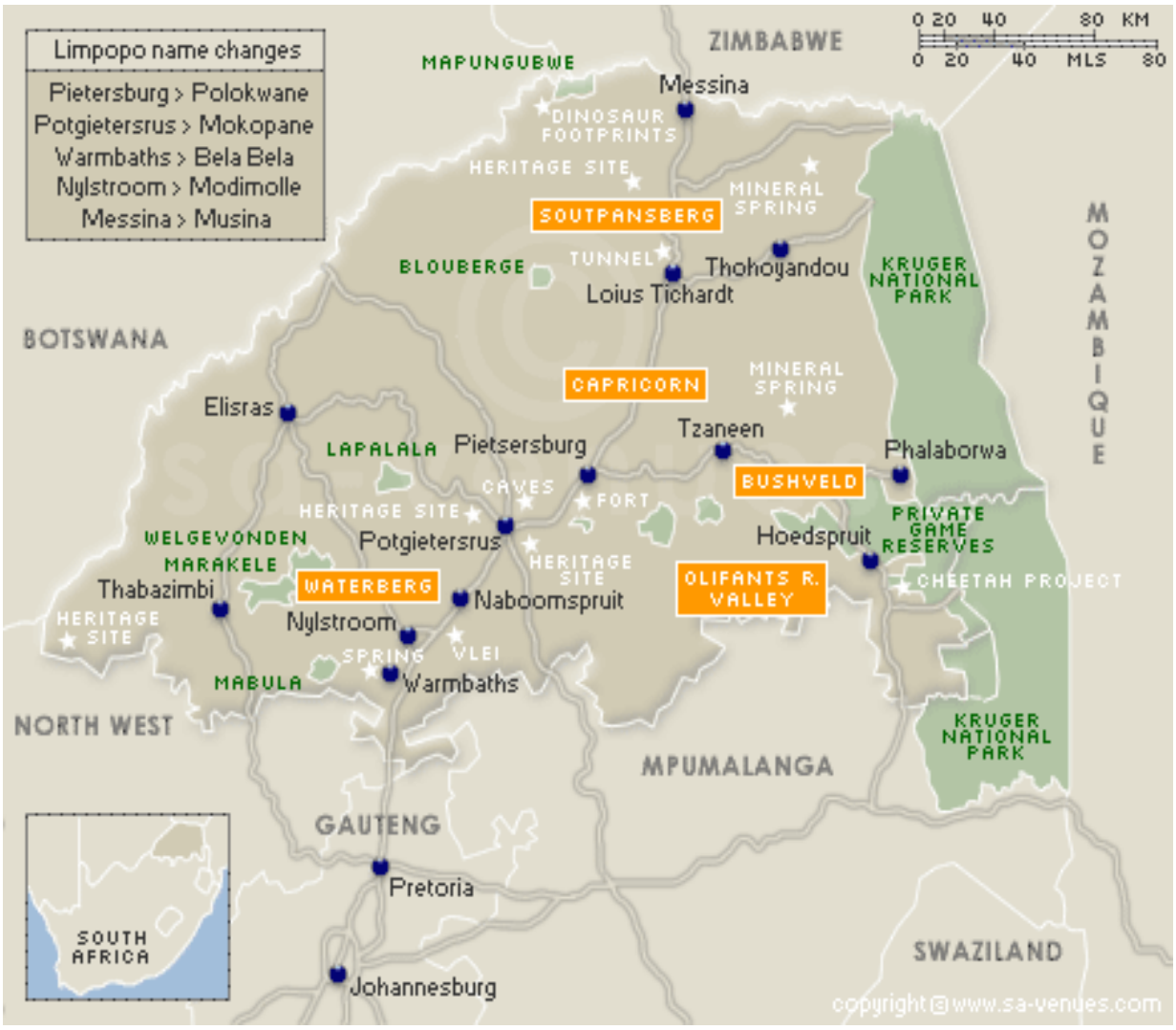
I .....(traditional healer's name) of the BLOUBERG/ MOLEMOLE MUNICIPALITY (delete one) residing at .....(physical adress) agree to be interviewed by Nkoana Mongalo, a student at University of Zululand (KWAZULU-NATAL PROVINCE). The information given to him is based on my knowledge in the ethnomedicine practice and that information contained in the interview will only be used for studies/research purpose as his project is based on SEXUALLY Transmitted Infections.

....

Signature of healer Signature of student

Date..... Date.....

APPENDIX 2



Map showing Blouberg area

APPENDIX 3 (a)

Abtract ACCEPTED AND PRESENTED AT 39TH South African Association of Botany (19-24) January 2013.

Nkoana Ishmael Mongalo, E-mail:nmongalo@pan.uzulu.ac.za
Oral
Session: Ethnobotany

Antibacterial and antioxidant activity of extracts from *Waltheria indica* L.

N.I. Mongalo[1], A.R. Opoku[2], A.M. Zobolo[1]

[1]Department of Botany, University of Zululand, Private Bag X1001, KwaDlangezwa, 3886, South Africa.
[2]Department of Biochemistry and Microbiology, University of Zululand, Private Bag X1001, KwaDlangezwa, 3886, South Africa.

Waltheria indica L., a member of Sterculiaceae family, is widely used traditionally to treat a variety of infections in humans. Roots of *W. indica* were collected from William Show farm, Blouberg area- Limpopo Province. Antibacterial activity of water, acetone and methanol extracts were tested against clinical isolates three Gram negative and two Gram positive bacterial strains. Largest zone of inhibition was exhibited by methanol extract 15.5±0.82 at 20mg/ml against *Salmonella spp* while *Staphylococcus aureus* was susceptible to all of selected extracts at 5, 10 and 20 mg/ml. Potent minimal inhibitory concentration (MIC) was exhibited by methanol extract at 2.08 mg/ml against *Proteus vulgaris*. Methanol extract was also tested for antioxidant activity using DPPH radical scavenging assay and exhibited 75.45±2.76 at a concentration of 0.75 mg/100ml. DPPH inhibition was also found to be dose dependent. These biological activities observed in the selected extracts validate ethnomedicinal use of *Waltheria indica.*