ANTIMICROBIAL ACTIVITY AND QUALITATIVE PHYTOCHEMICAL COMPOSITION OF CRUDE EXTRACTS FROM MEDICINAL PLANTS AGAINST SELECTED ENTERIC BACTERIAL PATHOGENS AND *Candida albicans*

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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DEDICATION

This thesis is dedicated to my parents; Noah Odek Opinde and Hilda Akinyi Opinde for their guidance and moral support since I started my education journey.

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Thanks to Almighty God for this far He has brought us, his blessings and the gift of a healthy life. Despite the many challenges we have been through He has been good to us and we have been able to overcome them. I sincerely thank my supervisors; Dr. Anthony Kebira and Dr. Grace W. Gatheri for their relentless support, encouragement, relentless support and guidance during my research work. Special appreciation Professor L.E. Newton for the help and guidance offered in the process of identification of the plants. I would also like to dearly appreciate the support and input from my fellow colleagues; Philip Ogola, Japheth Wambani, Nathan Kiboi, James Kimani, Wycliffe Arika, Majorie Oruru, Christine Nakhumincha and Gilbert Koskey. May almighty God give you good health and continue blessing you in your endeavor's throughout your lives. I acknowledge the support and assistance from Mr. Daniel Ng`ang`a Ms. Mary Njeri, Mr. Roy Mulanda, and the entire Microbiology Laboratory staff. May Almighty God shower you with his everlasting blessings, we made it at last. Finally, thanks to Almighty God again for in Him, we find the strength to explore new horizons; Amen.

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ABBREVIATIONS AND ACRONYMS

AB	*Aloe secundiflora* and *Bulbine frutescens* plant extract combination
ANOVA	Analysis of variance
AT	*Aloe secundiflora* and *Tagetes minuta* plant extract combination
AV	*Aloe secundiflora* and *Vernonia lasiopus* plant extract combination
BT	*Bulbine frutescens* and *Tagetes minuta* plant extract combination
BV	*Bulbine frutescens* and *Vernonia lasiopus* plant extract combination
$DMSO_4$	Dimethyl sulphoxide
$FeCl_3$	Iron (III) chloride
HCl	Hydrochloric acid
INT	2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyl-2H-tetrazolium chloride
MBC	Minimum bactericidal concentration
MIC	Minimum inhibitory concentration
SAS	Statistical analysis software
VT	*Vernonia lasiopus* and *Tagetes minuta* plant extract combination
W.H.O	World Health Organization

ABSTRACT

Plant extracts with medicinal value have been used to treat many diseases that can either be bacterial, fungal or parasitic among many others. Plants with medicinal value produce certain chemical elements known as phytochemicals that have antimicrobial activity. Enteric bacterial pathogens are disease-causing microorganisms that are usually located in the intestinal tracts of either animal or human beings. The pathogenic members are usually associated with infections that are characterized by; enteric fevers, abdominal pain, diarrhoea, and vomiting. *Candida albicans* is a yeast fungus that is mainly found in the mucosal cavity of the vagina and intestinal tract as a normal microbiota but it can cause systematic infections in immunocompromised individuals. This study was aimed at determining the antimicrobial activity, combined effects of the selected plant leaf extracts of *Tagetes minuta*, *Aloe secundiflora*, *Vernonia lasiopus* and *Bulbine frutescens* against selected clinical isolates of *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Shigella flexineri*, *Enterococcus faecalis* and *Candida albicans* obtained from Kenyatta University Health Centre; using the Kirby-Bauer method. In addition, qualitative analysis of the phytochemicals present in the extracts was also determined. The plants materials were obtained from Kenyatta University arboretum and identified by Taxonomist; Prof L.E. Newton and voucher specimen deposited in the University herbarium. The collected data was then analyzed in SAS version 9.1 using ANOVA and further subjected to a post hoc test with $P<0.05$ being considered significant. When used singly and in combinations against the test microorganisms the average zones of inhibition were found to be significant at $P<0.05$. When the plant extracts were used in low concentrations against the test microorganisms; *Vernonia lasiopus* was more active against *Shigella flexineri* (MIC 3.3µg/ml, MBC 7.1µg/ml), *Bulbine frutescens* against *Shigella flexineri* (MIC 3.2µg/ml, MBC 6.2µg/ml), *Aloe secundiflora* against *Shigella flexineri* (MIC 3.7µg/ml, MBC 8.0µg/ml) and *Tagetes minuta* against *Enterococcus faecalis* (MIC 5.1µg/ml, MBC 6.3µg/ml)*. The combining of the extracts also showed an increased and decreased antimicrobial activity with the interactions being significant; $P<0.05$. The average zone of inhibition formed by *Aloe secundiflora* and *Tagetes minuta* plant leaf extracts combination (8.67±1.86mm) showed a decrease in antimicrobial activity as compared to when *Tagetes minuta* (15.17±2.71mm) and *Aloe secundiflora* (17.00±2.10mm) respectively when used against *Candida albicans*. The qualitative phytochemical analysis showed the presence of four phytochemicals; saponins, tannins, alkaloids, and flavonoids. The study provides insight into the antimicrobial activities of the plant extracts and their use in the treatment of bacterial or fungal infections. This information might be used in herbal medicine in making concoctions to maximize their effectiveness. There is a need to elucidate the actual compounds in the plant leaf extracts responsible for the antimicrobial activity so that can be used in drug development.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Medicinal plants are used by almost 80% of the world's population for their basic health care because of their low cost and ease in availability (Shazadi *et al*., 2010). From the dawn of civilization, people have developed a great interest in plant-based drugs and pharmaceutical products (Shazadi *et al*., 2010). In the last few decades, many bacterial organisms have continued to show increasing resistance against current antimicrobial agents (Nascimento *et al*., 2000). Herbal drugs made from medicinal plants have been used from ancient times to treat various diseases and their antimicrobial properties make them a rich source of many potent drugs (Srivastava *et al*., 2005).

The use of herbal medicinal plants has always played a positive role in the control or prevention of diseases such as diabetes, heart disorders and various cancers (Mohanta *et al*., 2003). Some medicinal plants have been used in the production of various drugs singly or in combination and even as principal raw material for the production of other conventional medicines (Tahir and Khan, 2012). *Tagetes minuta L.* is also known as Southern Cone Marigold, Stinking Roger or black mint. It is a tall upright plant, with small flowers, native to the southern half of South America (Everett, 1982). The genus comprises of 56 species which grow

either annually or perennially and mostly herbaceous plants (Soule, 1996). Other common species of the family include *Tagetes erecta*, *Tagetes patula* and *Tagetes tenuifolia* (Tereschuk *et al*., 1997). The herbaceous plants were mostly found in North and South America but some species have become naturalized around the world (Soule, 1996). *Tagetes minuta* extracts have been used as medicinal tea in some areas (Soule, 1993). The total extracts from leaves, flowers, stem and other parts of the plant have shown antibacterial activity against Gram positive and Gram negative bacteria (Tereschuk *et al*., 1997). Extracts from the other common species have also been used as medicine in treating various illnesses such as stomach problems and intestinal disorders (Broussalis *et al*., 1999).

Some of the components contained in *Tagetes minuta* extracts such as flavonoids have been tested and proved to have antimicrobial activity against not only bacteria but also fungi and some nematodes (Cushnie and Lamb, 2005). The genus *Aloe* belongs to Aloeaceae (Liliaceae) family which has around 360 to 400 different species (Newall *et al*., 1996). Aloe species have antibacterial, antifungal, anticancer, antiviral and immunomodulatory properties (Holzmuller *et al*., 2002). *Aloe secundiflora* Engl. is also known as *Aloe floramaculata*, *Aloe engleri* and *Aloe marsabitensis* (Kaingu *et al*., 2013). Other common species include *Aloe arborescens, Aloe chabaudii*, *Aloe turkanensis, Aloe greatheadii*, *Aloe cameronii and Aloe excels*. *Aloe secundiflora* leaf components have been credited for antibacterial, antifungal and antiviral and antihelmintic medicinal properties

(Mwale *et al*., 2005). *Bulbine* is a genus of plants in the family Xanthorrhoeaceae and subfamily asphodeloideae and its members are well known for their medicinal value (Acock, 1988). They are succulent plants with most species having yellow flowers while others are white, orange or pink flowers. The most common species is *Bulbine frutescens* wild which is popularly grown in flower gardens (Van Wyk, 2008). Many species have bulb shaped tuber and they are chiefly found in South Africa with a few species extending to the tropics of Africa and Australia (Coopoosamy *et al*., 2000). *Vernonia lasiopus* O. Hoffman belongs to the tribe Vernonieae in the family Asteraceae which mostly contains herbaceous plants (Keeley, 2007). *Vernonia* is a shrub that grows in tropical Africa and has a height of about 2-5 metres, elliptical leaves of up to 20 centimetres and a rough bark (Ijeh and Ejike, 2011).

The plants in this family usually have a bitter taste and in English, they are called bitter leaf (Ijeh and Ejike, 2011). Studies carried out have shown some of the phytochemical components found in their extracts have the antimicrobial capability (Koul *et al*., 2003). *Vernonia lasiopus* decoctions from the stems and leaves have been traditionally used by herbalists in East Africa to treat, malaria, worms and gastrointestinal problems (Kareru *et al*., 2007). The study of contemporary medicine has yielded promising and commendable results on the antibacterial activity of medicinal plant extracts as potential drugs that can be added along with the contemporary drugs (Coopoosamy *et al*., 2007).

1.2 Problem statement

The increase in resistance to many commercially produced synthetic antimicrobial agents by microorganisms has been increasing with time hence the need of searching for new antimicrobial agents (Ramesh and Okigbo, 2008). There has been a need to increase alternative antimicrobial agents leading interests of evaluating extracts from plants known to have medicinal value for the manufacturing of herbal antimicrobial agents by Pharmaceuticals Company. Most of the bacterial and fungal pathogens have shown the capability of developing resistance to some of the available commercially available antimicrobial agents.

This has led to the search of new raw materials that can be used in developing new antimicrobial agents that can combat the increasing resistance by the pathogenic microbes. Extracts obtained from medicinal plants have been used over some time to in the manufacturing of herbal antimicrobial agents by pharmaceutical companies. However, over time some of the pathogenic microorganisms have been gaining resistance against some of the commercially produced herbal antimicrobial agents. This has led to the need for improving the antimicrobial capability of both commercially produced herbal and synthetic/conventional antimicrobial agents. Evaluation of the combined effect of herbal extracts with commercially produced antimicrobial agents and formulation of herbal extracts concoctions as a means of developing new and improved antimicrobial agent.

1.3 Justification

The use of herbal plant extracts with medicinal value has increasingly been advocated and incorporated in the production of antimicrobial agents. This is due to the need of increasing the base of already available antimicrobial agents that are commercially produced. Plants with medicinal value have been used to make homemade concoctions that have not been scientifically validated to have antimicrobial activity. Furthermore, different parts of the same medicinal plants have been used in making antimicrobial agents giving contrasting antimicrobial activity against pathogenic microorganisms. In this study, the antimicrobial activity of crude extracts from the leaves of *Tagetes minuta, Aloe secundiflora, Bulbine frutescens* and *Vernonia lasiopus* were tested for antimicrobial activity against pathogenic bacteria and fungus *Candida albicans*.

The plants leaves were selected because in some other similar studies carried out showed extracts from the other plants parts; *Tagetes minuta* seeds (Shazadi *et al*., 2010), *Aloe secundiflora* roots (Induli *et al*., 2012), *Bulbine frutescens* roots (Van Wyk *et al*., 1997) and *Vernonia lasiopus* roots (Toyang and Verpoorte, 2013). The combination of the plant extracts was done so as to determine if, the combining of the extracts can improve the antimicrobial activity. This was done because there are no other studies carried out on antimicrobial activity of combined plant extracts. The qualitative analysis of the phytochemical composition of the plant leaf extracts was also done to determine their presence.

This is because phytochemicals have been known to be secondary metabolites that are usually produced by plants and accumulated in different parts of the plant. Some of these phytochemicals have been thought to be responsible for the antimicrobial activity shown by extracts from various herbal plants. The finding in the study could provide an insight into the antimicrobial activity of the plant leaf extracts against the test pathogenic bacteria and fungus *Candida albicans.* Furthermore, the combination of the plant extracts will provide an insight if there is any combined effect of the plant extracts against the test microorganism. This may lead to the future formulation of herbal concoctions that are scientifically validated not only at home but also for commercial production hence increasing the base of antibacterial agents available. This may provide an effective and alternative available antimicrobial agent for people who cannot afford conventionally synthetically produced medicine.

1.4 Research questions

i) Do the extracts from *Tagetes minuta*, *Aloe secundiflora*, *Vernonia lasiopus* and *Bulbine frutescens* have any antimicrobial activity against the bacterial pathogens and fungus *Candida albicans* when used separately?

ii) Combined extracts of *Tagetes minuta*, *Aloe secundiflora*, *Vernonia lasiopus* and *Bulbine frutescens* have synergistic antimicrobial activity against bacterial pathogens and *Candida albicans* than when used separately?

iii) What are the phytochemicals present in the extracts of *Tagetes minuta*, *Aloe secundiflora*, *Vernonia lasiopus* and *Bulbine frutescens*?

1.5 Hypotheses

i) Extracts from *Tagetes minuta*, *Aloe secundiflora*, *Vernonia lasiopus* and *Bulbine frutescens* do not have a bactericidal effect on bacterial pathogens and fungicidal effect on *Candida albicans*.

ii) The combinations of extracts from *Tagetes minuta*, *Aloe secundiflora*, *Vernonia lasiopus* and *Bulbine frutescens* does not have different antimicrobial activity against bacterial pathogens and fungus *Candida albicans* than when used separately.

iii) Phytochemicals present in the crude extracts from *Tagetes minuta*, *Aloe secundiflora*, *Vernonia lasiopus* and *Bulbine frutescens* are not known.

1.6 Objectives of the study

1.6.1 General objective

To determine the antimicrobial effect of *Tagetes minuta*, *Aloe secundiflora*, *Vernonia lasiopus*, and *Bulbine frutescens* extracts on bacterial pathogen and fungus *Candida albicans* and to evaluate their qualitative phytochemical composition.

1.6.2 Specific objectives

i) To determine the antimicrobial activity of plant extracts from *Tagetes minuta*, *Aloe secundiflora*, *Vernonia lasiopus* and *Bulbine frutescens* against bacterial pathogens and *Candida albicans*.

ii) To determine the impact of combined plants extracts against the bacterial pathogens and *Candida albicans*.

iii) To determine the qualitative phytochemical present in the plant extracts obtained from *Tagetes minuta*, *Aloe secundiflora*, *Vernonia lasiopus* and *Bulbine frutescens*.

1.7 Significance of the study

The main aim of the study was to determine if the plant extracts from *Tagetes minuta*, *Aloe secundiflora*, *Vernonia lasiopus* and *Bulbine frutescens* have antimicrobial tendencies against the bacterial pathogens and *Candida albicans*. The study also focused on finding out if there was any combined effect when the plant extracts were mixed and used against the microorganisms. This aided in determining whether the plant extracts are effective when used as antimicrobial agents singly or in combinations. The determination of phytochemicals present in the extracts was to propose the use of the herbal plant extracts as the primary material in the development of antimicrobial agents. This also provides an alternative to the conveniently available conventional drugs.

CHAPTER TWO

LITERATURE REVIEW

2.1 Medicinal plants

Medicinal plants have been identified and used throughout human history (Lichterman, 2004). The use of medicinal plants to treat diseases is almost universal among non-industrialized societies and is often more affordable than purchasing expensive conventional drugs (Fabricant and Farnsworth, 2001). The World Health Organization (WHO) estimates that 80% of the world population especially Asian and African countries use herbal medicine for some aspect of primary health care (http://www.traffic.org/medicinal-plants, 30th march 2014). Over 120 active compounds currently isolated from the higher plants are widely used in modern medicine and 80% of these show a positive correlation between their modern therapeutic use and the traditional use of the plants from which they are derived (Fabricant and Farnsworth, 2001).

2.1.1 *Tagetes minuta* (Southern cone marigold)

The genus *Tagetes* belongs to the Asteraceae family which presently comprises of 56 species, 27 biennials, and 29 perennials. *Tagetes* species are grown all over the world as multipurpose plants. The most common species are *Tagetes minuta*, *Tagetes patula*, *Tagetes erecta* and *Tagetes tenuifolia* (Soule, 1993). *Tagetes* species and chemotypes from its genus have been largely examined for biologically active metabolites that can be used in industry and medicine (Green *et al*., 1991). Compounds that have antimicrobial activity in the *Tagetes minuta*

plant are said to be accumulated in the organs of the plant and their essential oils have not only antimicrobial effect but also insecticidal properties (Piccaglia *et al*., 1997). Plant parts such as flowers and leaves have been known to contain flavonoids that are scavengers for free radicals which enhances the antimicrobial activity of the *Tagetes minuta* extracts (Rivas, 1991). Phytochemicals from the plant such as carotenoids have also been used in pharmacological preparations and they have been found to contain anti-aging and anticancer effects (Bennish *et al*., 1992). The plant extracts have been used in treating intestinal and stomach problems (Tereschuk *et al*., 1997; Broussalis *et al*., 1999). *Tagetes minuta* extracts such as its volatile oil and other components have been used in the flavoring of food products and as perfumes.

The plant has also shown inhibitory activity against some pathogens and insects (Green *et al*., 1991). Studies carried out have shown that leaf extracts from most of the *Tagetes* species including *Tagetes minuta* contain flavonoids that have shown antimicrobial potential against both Gram positive and Gram negative bacteria (Tereschuk *et al*., 1997). Extracts from *Tagetes minuta* leaf flowers and stem extracted using methanol have shown to contain secondary metabolites including terpenes which are thought to be responsible for antibacterial activities (Lopez *et al*., 2008). Components of some of the essential oils extracted from *Tagetes minuta* around the world have been found to contain a compound called

camphene which has been found to be effective against *Erwinia amylovora* and *Staphylococcus aureus* (Facon, 1996).

2.1.2 *Aloe secundiflora*

Aloes are perennial succulent xerophytes which develop water storage tissues in leaves to survive in areas with low or erratic rainfall (Talmadge *et al*., 2004). The plant is mainly found in cultivation, having no naturally occurring population although closely related Aloes do grow in northern parts of Africa (Akinyele and Odiyi, 2007). The plant is an almost sessile perennial herb that has leaves 30-50 centimetres long and 10 centimetres broad at the base, bright yellow tubular flowers 25-35centimetres in length arranged in a slender loose spike (WHO, 1999). The genus Aloe is common in Kenya; with about 60 taxa recognized (Hendricks and Wright, 1979).

Aloe secundiflora has been used in treating ailments including; chest problems, polio, malaria and stomach ache by herbalists in the Lake Victoria region (Kigondu *et al*., 2009). *Aloe secundiflora* leaf components have been credited for antibacterial, antifungal and antiviral and antihelmintic medicinal properties (Kaingu *et al*., 2013). *Aloe* extracts have been used for many centuries for their curative and therapeutic properties (Habeeb *et al*., 2007). *Aloe* products have also been used in pharmaceuticals, cosmetic and food industries (Eshun and He, 2004). *Aloes* contain over 75 nutrients and 200 active compounds including enzymes, vitamins, minerals, lignin, sugars, saponins, anthraquinones amino acids and

salicylic acid (Park and Jo, 2006). Extracts of *Aloes* especially its leaf gel have shown antibacterial activity by inhibiting the growth of both Gram-negative bacteria and Gram-positive bacteria (Ferro *et al*., 2003). Specific compounds isolated from *Aloe vera* such as anthraquinones (Garcia *et al.*, 2006) and dihydroxyanthraquinones (Wu *et al*., 2006) as well as saponins (Reynolds and Dweck, 1999) have been proposed to have antibacterial activity. Studies carried out have also shown that Gram-negative bacteria are more susceptible to most Aloes extracts (Cock, 2008). Crude extracts of Aloes show a broad range of antimicrobial activity because it contains all the constituent of the sap (Atherton, 1997). Previous studies have shown some antibacterial activity of crude extract of *Aloe secundiflora* (Waihenya *et al*., 2002).

2.1.3 *Bulbine frutescens*

Bulbine is a genus of plants in the family Xanthorrhoeaceae and subfamily asphodeloideae and its members are well known for their medicinal value (Acock, 1988). *Bulbine frutescens* wild and *Bulbine natalensis* baker are the most common species known (Van Wyk and Gericke, 2000). *Bulbine* plant has been used for medicinal purposes in the early stages of the eighteen century by Dutch and British settlers of South Africa in treating various ailments (Coopoosamy *et al*., 2000). Many species have bulb shaped tuber. It's chiefly found in South Africa with a few species extending to the tropics of Africa and Australia (Coopoosamy *et al*., 2000). They are succulent plants with most of the species having yellow flowers whereas some of them have white, orange or pink flowers. *Bulbine*

frutescens is mostly grown as an ornamental plant in the flower garden at homes in South Africa (Van Wyk and Gericke, 2000). The leaves of the plant have been used in the treatment of wound thought to be infected with bacterial pathogens and it has shown antibacterial properties (Kelmanson *et al*., 2000). Some of the species of the plant found in South Africa have been used for blood cleansing, treatment of ringworms and gravel rush by some local communities such as the Xhosa (Coopoosamy *et al*., 2000). A decoction of bulbs and roots of some of the species has been used in the treatment of some of the venereal diseases in women and stomach upsets (Van Wyk, 2008).

2.1.4 *Vernonia lasiopus*

Vernonieae is a tribe which has about 1300 species and in the family Asteraceae (Compositae) which mostly contains herbaceous plants (Keeley, 2007). *Vernonia* shrubs grow in tropical Africa and have a height of about 2-5 metres, elliptical leaves of up to 20 centimetres and a rough bark (Ijeh and Ejike, 2011). The plants in this genus usually have a bitter taste and in English, they are called bitter leaf (Ijeh and Ejike, 2011). Some of the common African names of plants in this genus are Olusia (Luo), Mululuza (Luganda), Onugu (Igbo), Grawa (Amharic) and Chusar-doki (Hausa) (Kokwaro, 2009). *Vernonia lasiopus* decoctions from the stems and leaves have been traditionally been used by herbalists in East Africa to treat, malaria, worms and gastrointestinal problems (Kareru *et al*., 2007). In Kikuyu community, it's traditionally known as Mucatha and it has been used in treating diarrhoea problems (Kareru *et al*., 2007). Studies carried out have shown

some of the phytochemical components found in its extracts have the antimicrobial capability (Koul *et al*., 2003). Its extracts have also been used in treating some of the sexually transmitted diseases in southern parts of Africa (Kambizi and Afolayan, 2001). In North America some of the species of the genus *Vernonia* such as *Vernonia altissima*, *Vernonia fasciculata*, and *Vernonia flaccidifolia* have been found to contain effective properties for them to be used as blood purifiers, uterus toner and also contain sesquiterpene lactone which can also help in preventing atherosclerosis (Crellin *et al*., 1989). In Brazil *Vernonia condesata* commonly known to locals as necroton or figatil has been used in traditional medicine to treat analgesic, anti thermal, anti-anemic, anti-inflammatory and as an antibacterial agent (Da Silva *et al*., 2013).

2.2 Test microorganisms

2.2.1 *Escherichia coli*

Escherichia coli are normal flora in the body of human beings and they can be non-pathogenic, commensal or pathogenic (Kaper *et al*., 2004). When pathogenic they usually cause uinary tract infections, systematic infections and enteric infections (Mandell *et al*., 2005). The development of resistance by *Escherichia coli* due to increasing in the use of antimicrobial agents has led to the use of medicinal plants extracts against it (Akram *et al*., 2007). Medicinal plant extracts have shown to have antimicrobial activity against enteropathogenic *Escherichia coli* found in food material (Fullerton *et al*., 2011). Traditional products used in food preserving (spices) have antimicrobial activity against multiple antibiotic

resistant *Escherichia coli* isolated from water (Rahman *et al*., 2011). Other studies carried out on plants with a medicinal value such as *Allium sativum* has shown antimicrobial activity against *Escherichia coli* (Ziarlarimi *et al*., 2011).

2.2.2 *Salmonella typhi*

Salmonella typhi is a Gram-negative bacterial pathogen that causes gastroenteritis in humans (Ibarra and Steele, 2009). In developing countries, it is mainly associated with causing typhoid fever (Watson and Holden, 2010). Typhoid fever is a major cause of death around the world in a limited setting and globally remains as one of the most infectious diseases (Buckle *et al*., 2012). The disease is estimated to be responsible for about 26.9 million infections and 269,000 deaths in 2010 (Buckle *et al*., 2012). Studies carried out have shown that herbal extracts and dietary spices from medicinal plants have antimicrobial activity against *Salmonella typhi* (Shan *et al*., 2007). Other studies have shown that herbal extracts from medicinal plants not only have antimicrobial activity on *Salmonella typhi* found in vegetables but also against other disease-causing bacteria pathogens such as enteropathogenic *Escherichia coli* and *Listeria monocytogenes* (Cutter, 2000).

2.2.3 *Staphylococcus aureus*

Staphylococcus aureus is Gram-positive bacteria that cause diseases such as skin and soft tissues infections as well as food poisoning and toxic shocks (Perez *et al*., 2009). The rate of mortality associated with *Staphylococcus aureus* in developing

world exceeds one of the developed countries (Nickerson *et al*., 2009). The increasing use of antimicrobials against *Staphylococcus aureus* has led to the development of resistance hence need to develop new antimicrobial agents (Kwon *et al*., 2007). Medicinal plant extracts have shown a wide range of antimicrobial activity against both bacterial and fungal pathogens (Manvi *et al*., 2010). Studies carried out have shown that some edible plants extracts also have antimicrobial activity against *Staphylococcus aureus* (Alzoreky *et al*., 2003). Other studies carried out have shown a great synergistic activity of plant extracts and spices when used against not only pathogenic, probiotic and food spoilage pathogens such as *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli* and other bacteria organisms, both Gram positive and Gram negative (Das *et al*., 2012).

2.2.4 *Candida albicans*

Candida albicans is a normal microbiota mainly found in the mucosal cavity, vagina and gastrointestinal tract of an individual (Shao *et al*., 2007). They are a yeast-like fungus that are commensals in healthy humans but can cause systemic infections in immunocompromised individuals (Pfaller *et al*., 2007). The incidence of fungal infections has led to increased antimicrobial resistance hence making a few antifungal agents active (Arendrup *et al*., 2005; Espinel - Ingruff *et al*., 2009). There has been an increase in resistance by *Candida albicans* to conventionally produced antimicrobials recently, leading to the search of a new antifungal agent (White *et al*., 1998; Sardi *et al*., 2011). Herbal extracts from medicinal plants have been used to treat infectious diseases such as candidiasis in

developing nations (Geyid *et al*., 2005). Other studies carried out have shown that herbal extracts are generally effective against *Candida albicans* (Pramila *et al*., 2012; Hussein *et al*., 2014).

2.2.5 *Enterococcus faecalis*

Enterococcus faecalis is a Gram-positive bacteria that is a commensal as well as an opportunistic pathogen (Murray, 1990). The bacteria belongs to the genus of lactic acid bacteria of phylum firmicutes and when observed under the microscope they are seen to occur in pairs or in short chains (Maurya *et al*., 2012). They have been increasingly becoming nosocomial pathogens over the past decade (Dupre *et al*., 2003; Van – Kerchaven *et al*., 2004). They are known to cause clinical infections such as urinary tract infection, bacteremia, bacterial endocarditis, diverticulitis and even meningitis (Ryan *et al*., 2004). Herbal plant extracts from some of the previous studies carried out have shown antimicrobial activity against that *Enterococcus faecalis.*

2.2.6 *Shigella flexineri*

Shigella is a genus of Gram-negative, rod-shaped facultative bacteria responsible for shigellosis (Kotloff *et al*., 1999). Only a few cells of the bacteria can cause infections and its clinical manifestations include exothermic reactions, diarrhoea, abdominal pain and sometimes vomiting (Eilers *et al*., 2010). Shigellosis epidemic tends to occur in developing countries due to poor sanitation and the transmission rate from one person to another is more frequent especially when it's

due to water or food contamination (Qu *et al*., 2012). Shigellosis is responsible for most of the diarrhoea episodes and causes approximately over one million deaths annually (Kotloff *et al*., 1999). Over time the increase in the use of conventionally produced antimicrobials against *Shigella flexineri* has led to the development of resistance (Bennish *et al*., 1992). The increase in resistance has led to the need for an alternative to the antimicrobials produced (Palpasa e*t al*., 2011). This has led to the extensive study of extracts from medicinal plants with antimicrobial activities (Palpasa *et al*., 2011). Some of the medicinal plants extracts have been known to contain polyphenols such as flavonoids that have shown antimicrobial activity against pathogenic bacterial organisms (Cowan, 1999).

2.3 Phytochemicals in medicinal plants

Secondary plant metabolites (Phytochemicals) have been extensively investigated as a source of medicinal agents (Krishnaraju, 2005). Plants can synthesize and accumulate a great variety of phytochemicals in their cells including saponins, tannins, flavonoids, cyanogenic, phenolic compounds, lignins, lignans, alkaloids and glycosides (Okwu, 2004). Plants also have a great potency of antimicrobial activity due to the presence of phenolic compounds and essential oils (Aboaba and Efuwape, 2001). Medicinal plants have been known to produce an array of phytochemicals with recognized antibacterial activity belonging to chemical structural classes: phenolic, terpenoids, alkaloids, lectins, polypeptides, and polyacetylenes but the most bioactive constituents are alkaloids, tannins, flavonoids, and phenolic compounds (Hill, 1952). The screening of plant extracts

and plant products for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotic prototypes (Afolayan, 2003). Numerous studies have identified compounds within herbal plants that are effective antibiotics (Afolayan, 2003). Some of the commonly used traditional remedies have already produced compounds that are effective against antibiotic-resistant strains of bacteria (Kone *et al*., 2004).

2.3.1 Tannins

Tannin is astringent vegetable product found in a wide range of plants parts ranging from the barks, roots, seeds, fruits, leaves, galls and roots (Ramakrishnan, 2006). They occur naturally in plants and are water soluble phenolic compounds of the higher molecular weight of about 500 - 3000 containing phenolic hydroxyl groups that make them to effectively cross-link with proteins and other macromolecules (Ramakrishnan, 2006).

Tannins are generally found in plants and they are thought to function as chemical defenses against pathogens and herbivores (Gedir *et al*., 2005). They have been commercially used primarily in the preservation of leather, making glue stains and mordant (Kanth *et al*., 2009). It has also been used in the vegetable industry in different concentration in pickling process to provide protection against bacteria, mold, and yeasts (Andrade *et al*., 2005). Antimicrobial activity of tannins has been tested in various fields of medicine providing positive results such as antioxidant activities, anticarcinogenic activities and antimutagenic properties

(Lopes *et al*., 1999). Tannins have been used in inhibiting the growth of many fungi, yeasts, bacteria and viruses (Chung *et al*., 1998). Studies carried out have shown that tannins have antibacterial activity (Akiyama *et al*., 2001). Some of the bioactive compounds of tannins such as catechin and pyrogallol found in vegetable tannins have been found to be toxic to microorganisms (Cowan, 1999). Tannins have been found not only effective against pathogenic microbes but also have a significant value as a cytotoxic and an antitumor agent (Josh *et al*., 2013).

2.3.2 Flavonoids

Flavonoids or bioflavonoids are secondary metabolites of plants that chemically have a general structure of 15 carbon skeleton consisting of two phenyl rings and a heterocyclic ring (Mc Naught, 1997). There are over 500 groups of flavonoids that have been characterized from various plants according to their chemical structure (Ververidis *et al*., 2007). They are usually subdivided into anthoxanthins, flavanones, flavanols, flavans, and anthocyanidin (Zhao *et al*., 2012). In plants they are responsible for floral pigmentation, ultraviolet ray's filtration in higher plants and symbiotic nitrogen fixation (Galoetti *et al*., 2008). They are also known to have inhibitory activities against organisms that cause plant diseases for example *Fusarium oxysporum* (Galoetti *et al*., 2008). Flavonoids have been known to possess antimicrobial activity against bacterial, fungal and viral microorganisms (Cowan, 1999). They are usually known for their antimicrobial activity of inhibiting the synthesis of the nucleic acids, tampering with the integrity of the cytoplasmic membrane function and the energy

metabolism process (Cushnie and Lamb, 2005). Flavonoids from some medicinal plants have been found to inhibit the synthesis of the nucleic acids, cause permeability of the inner bacterial membrane and a dissipation of the membrane potential of Gram negative and Gram positive bacteria (Cushnie and Lamb, 2005). Some of the bioactive components that have been isolated from flavonoids have been found to contain antifungal, antibacterial and insecticidal activities (Abdel *et al*., 2013). Previous studies carried out have shown that when mixed with antibiotics they have synergistic activity and suppress many pathogenic microorganisms in numerous in vitro and in vivo studies (Cushnie and Lamb, 2011; Manner *et al*., 2013). Additional in vivo studies have shown that flavonoids can be used as pharmaceutical drugs for bacterial infections or through the dietary intake to offer protection against infection (Zamora *et al*., 2012).

2.3.3 Alkaloids

They are a group of naturally occurring compounds that contain nitrogen and can be neutral or have weakly acidic properties (Mc Naught, 1997). They may also sometimes contain oxygen, Sulphur, more rarely other elements such as chlorine, bromine, and phosphorus (Schardl *et al*, 2007). They are mainly secondary metabolites of plants but can also be produced by a variety of organisms including bacteria, fungi, and animals (Kittakoop *et al*., 2014). They dissolve in water poorly but readily dissolve in organic solvents (Shi *et al*., 2104). They are divided into five major groups namely: true alkaloids (contain nitrogen in heterocyclic and originate from amino acids), proto alkaloids, polyamine alkaloids, peptide

and cyclopeptides alkaloids and pseudoalkaloids (Faulkner *et al*., 2006). They have a wide range of pharmacological activities such as antiasthma, antimalarial, anticancer, cholinomimetic, vasodilatory, antiamyhyrithic, analgesic, antibacterial and antihyperglycemic activities (Cushnie and Lamb, 2014). Some alkaloids have been known to possess psychotropic and stimulant activities and have been used as recreational drugs and entheogenic rituals (Blankenship *et al*., 2005). Alkaloids have great antimicrobial activity against bacterial pathogens such as *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus* and *Pseudomonas aureginosa* (Maatalah *et al*., 2012).

Some of the bioactive components of alkaloids such as morphine and cordine have been found to be active not only against bacterial and fungal pathogens but also trypanosomes and plasmodia (Freiburghaus *et al*., 1996; Omulokoli *et al*., 1997). Some of the Alkaloids found in dietary food materials have also been found to contain microbiocidal and antidiarrheal effect in the small intestines where they show the ability to intercalate with the microbial genetic material (Ghoshal *et al.*, 1996; Phillipson and Niell, 1997). Other studies carried out on alkaloids extracted from a variety of medicinal plants in Nigeria showed a great antimicrobial activity against both Gram-negative and Gram-negative bacteria and also showed great antifungal activity (Garba and Okeniyi, 2012).

2.3.4 Saponins

They are a class of chemical compounds found in various plant species and they are amphipathic glycoside grouped structurally by having one or more hydrophilic glycosides moieties combined with lipophilic triterpene (Hostettmann and Martson, 1995). In plants, saponins are known to provide protection against microbes and fungi (Riguera, 1997). Saponins have been used by a wide range of commercial therapeutic claims for natural products whereby in organismal or human benefit are often based on preliminary biochemical and cell biology studies (Skene and Phillip, 2006). Saponins are also considered as one of the natural antimicrobial products that make up the defense system of the plants and some can be beneficial rather than harmful to animals (Rupasighe *et al*., 2003; Hubert *et al*., 2005).

There has been evidence of the presence of saponins in traditional medicine preparations where the administration is through oral means that is expected to lead to the hydrolysis of glycosides from terpenoids (Asl *et al*., 2008). Studies carried out have shown medicinal plant extracts fractions rich in saponins are effective against microorganisms such as *Escherichia coli, Salmonella typhi*, *Aeromonas hydrophilia* and other fungal pathogens such as *Candida albicans* (Deshpande *et al*., 2013). Saponins antimicrobial activity is attributed mainly to its capability of lysing microorganism's membranes rather than the surface tension of the extracellular medium (Asl, 2008). Apart from antimicrobial

activity, saponins have shown other biological properties with its cytotoxic activity on cancer or tumor cells being considered the most important one (Yokosuka and Mimaki, 2009). Other plants are known to produce steroidal saponins for example cholestane glycosides which are known to have a broad spectrum of biological activity such as cytotoxic activity, antifungal, antibacterial and in vivo antitumor activities (Li *et al*., 2012).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Plants sampling

The plants were randomly collected in densely populated areas in the Kenyatta University arboretum. The plants were then placed on a table and the leaves selected from the plants using the following criteria: those with no dead leaves, those with no flowers and of the same height. The plant material was randomly sampled in densely populated areas where; twenty samples of *Tagetes minuta*, ten samples of *Vernonia lasiopus*, five samples of *Aloe secundiflora* and five samples of *Bulbine frutescens* plants based on the criteria were collected. Voucher specimens were prepared and deposited in the University herbarium in Plant Sciences Department for future reference. The plants were brought to the laboratory and thoroughly washed in running water to remove debris and dust particles and then rinsed using distilled water and finally air dried.

3.2 Preparation of plant extract

The air dried leaves from the plants were ground into powder and 500 grams were soaked in 750 millilitres of Analar grade (AR) methanol in a conical flask for 72 hours, placed in a Gallenkamp shaker rotating at 65 revolutions per minute. The contents were homogenized and filtered using Whatman filter paper no. 1. The filtrate was poured into a round bottom flask and concentrated using a Buchi Rotavapor R-200 yielding 2.8 grams of *Bulbine frutescens*, 3.1 grams of *Aloe secundiflora*, 2.6 grams of *Tagetes minuta* and 2.1 gram of *Vernonia lasiopus*.

The extracts were then stored in a labelled amber glasses bottle slightly opened where they were further left to dry bottle at room temperature away from light and heat in a laminar flow before being used for antimicrobial efficacy test.

3.3 Preparation of Media

The media used were Muller Hinton agar and Potato dextrose agar (Sharau®) were prepared according to commercially given instructions.

3.3.1 Preparation of Potato dextrose agar

39 milligram of potato dextrose agar powder was added into one litre of distilled water in a flat-bottomed conical flask. Boiling while mixing was done so as to dissolve the potato dextrose agar powder. The flask was then tightly closed using cotton wool and further covered with aluminum foil. The mixture was autoclaved for 15 minutes at 121 degree celsius after which it was left to cool down to room temperature. 40mg of tetracycline was added to inhibit bacterial growth and the media stirred before dispensing in Petri dishes. The media was poured in the Petri dishes in a laminar flow to give uniform depth of 3-4 millimetres. The Petri dishes were left to cool and after which they were placed in a sterile plastic bags and stored at a temperature of 2-8 degree celsius before use.

3.3.2 Preparation of Muller-Hinton agar

38 milligram of Muller-Hinton agar powder was added into one litre of distilled water in a flat-bottomed conical flask. The mixture was heated with frequent

agitation and boiled for one minute to completely dissolve the media. The flask was then tightly closed using cotton wool and further covered with aluminum foil. The mixture was autoclaved for 15 minutes at 121 degree celsius after which it was left to cool down to room temperature. The media was poured in the Petri dishes in a laminar flow to give uniform depth of 3-4 millimetres. The Petri dishes containing the media were then placed in a sterile plastic bags and stored at a temperature of 2-8 degree celsius before use.

3.4 Preparation of susceptibility test discs

Discs of 6 milliliters were prepared from Whatman no.1 filter paper. This was done by punching the filter papers using a paper punch. The discs prepared filled four McCartney bottles. The discs were then sterilized by autoclaving at 121-degree celsius for 15 minutes after which the autoclave was left to cool before removing the McCartney bottles containing the discs. The discs were dried in hot air oven at 50 degrees celsius to remove moisture (Arunkumar *et al*., 2009).

The discs used for the antimicrobial activity were impregnated with formulated stock solution of the plant leaf extracts of 1000µg/ml of *Tagetes minuta, Aloe secundiflora*, *Bulbine frutescens* and *Vernonia lasiopus*. This was done by; taking the sterile discs and the stock solutions into the laminar flow. A forceps used for picking the discs was first sterilized using a spirit lamp and left to cool. The forceps were then used in picking the sterilized discs and placing them in the stock solutions of the plants leaf extracts. The forceps were sterilized after every

pick. The disc was then left to stay in the plant leaf extracts stock solution for two hours. The discs were then removed and placed in sterile Petri dish in a laminar flow and left to dry for 30 minutes. The discs impregnated with leaf extracts from each plant were then picked by sterilized forceps and stored in a sterilized McCartney bottle and stored in a refrigerator at a temperature of 4-8 degree celsius before being used for the antimicrobial susceptibility test.

3.5 Test bacterial organisms

The test microorganisms used in the study were clinical isolates of *Escherichia coli*, *Salmonella typhi*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Shigella flexineri*, *Enterococcus faecalis* and *Candida albicans*. The microorganisms were isolated from samples collected from first-time patients at Kenyatta University Health Centre Laboratory, Nairobi. The samples were collected from patients who showed symptoms associated with enteric bacterial infections such as fever, abdominal pain, diarrhoea, vomiting and have no known drug resistance.

Isolation, morphological identification, and biochemical test were carried out in Microbiology laboratory, Department of Microbiology. The samples for isolating enteric bacteria were from faecal material, sterile water was used in cleaning infected wounds to get a sample for isolation of *Staphylococcus aureus* and high vaginal swab for isolation of *Candida albicans.* The collected samples were used in isolation and identification by streaking of the samples collected on selective media for characteristic morphological identification based on the type of colonies

formed. The morphologically identified microorganisms were then subjected to a biochemical test for identification up to biochemical level.

3.5.1 Isolation and Morphological identification

The faecal material collected for the isolation and morphological identification of enteric bacteria was first mixed with distilled sterile water. The mixture was serially diluted up to 10^{-6}. A wire loop was sterilized by heating and the left to cool. It was then dipped into the serially diluted sample of 10^{-6} and streaked on selective and differential media in Petri dishes known to support the growth of each of the test microorganism used. The Petri dishes were then tightly closed using a parafilm and incubated for 24 hours at 37 degrees celsius. The plates were then removed and the bacteria`s identified according to their morphological characteristics.

Escherichia coli was isolated by streaking the sample on Eosin methylene blue and MacConkey agar. The Petri dish tightly closed used parafilm and incubated at 37 degrees celsius for 24 hours. The plate was then later observed for growth. The growth of metallic green colonies on Eosin methylene blue agar and pink to brick red colonies on MacConkey agar with or without a zone of precipitated bile is a morphological growth characteristic of *Escherichia coli*.

Salmonella typhi was isolated by streaking sample on *Salmonella-Shigella* agar and Hektoen agar. The Petri dish tightly closed used parafilm and incubated at 37 degrees celsius for 24 hours. The plate was then later observed for growth. The growth of colonies with or without black centres on *Salmonella-Shigella* agar and blue-green colonies with black centres on Hektoen agar is a morphological growth characteristic of *Salmonella typhi*. *Shigella flexineri* was isolated by streaking sample on *Salmonella-Shigella* agar. The Petri dish tightly closed used parafilm and incubated at 37 degrees celsius for 24 hours. The plate was then later observed for growth. The growth of transparent and translucent colonies on *Salmonella-Shigella* agar is a morphological growth characteristic of *Shigella flexineri*.

Enterococcus faecalis was isolated by streaking the sample on Columbia agar with 5% sheep blood. The Petri dish tightly closed used parafilm and incubated at 37 degrees celsius for 24 hours. The plate was then later observed for growth. The growth of diplococcus colonies with gamma hemolysis on Columbia agar with 5% sheep blood is a morphological growth characteristic of *Enterococcus faecalis*.

Staphylococcus aureus was isolated by streaking the diluted sample from clean infected wounds on Mannitol agar. The Petri dish tightly closed used parafilm and incubated at 37 degrees celsius for 24 hours. The plate was then later observed for growth. The growth of yellow colonies with yellow zones on Mannitol salt agar is a morphological growth characteristic of *Staphylococcus aureus*.

Candida albicans was isolated by streaking the diluted sample from high vaginal swabs on Sabourauds agar containing tetracycline to prevent bacterial growth. The Petri dish tightly closed used parafilm and incubated at 37 degrees celsius for 24 hours. The plate was then later observed for growth. The growth of white to cream colonies, smooth, glabrous, yeast-like colonies on Sabourauds agar is a morphological growth characteristic of *Candida albicans*.

3.5.2 Biochemical identification

The biochemical test carried out was based on the capability of the isolated and morphologically identified test microorganisms to cause fermentation of sugars and oxidation. The biochemical tests carried out were; citrate test, urease test, nitrate test, gelatin test, hydrogen sulphide gas test, arabinose test, fructose test, glucose test, inositol test, lactose test, maltose test, mannitol test, mannose test, raffinose test, sucrose test and sorbitol test. The Medias used to carry out the test were prepared according to the manufacturer instructions and poured into nestler tubes.

Durham tubes were inserted to media containing broth and later observed for gas production. If there was gas production the result was termed as positive and no gas production as negative. The tubes were then closed and autoclaved at 121-degree Celsius for 15 minutes and left to cool before being stored in a refrigerator before use. One millilitre inoculum of the isolated test microbes from a diluted sample of 10^{-6} was introduced to the media and incubated at 37 degrees celsius and observation did after 24 hours. The tubes containing solid media were observed for colour change. Where colour change occurred the results were termed as positive and no colour change as negative using standards (http://www.microbiologyinfo.com/biochemical test, 15th June 2014).

Table 3.1: Standard for biochemical tests on isolated microorganisms

Biochemical test	**Microorganisms**					
	E.coli	*S.typhi*	*S.aureus*	*S.flexineri*	*E.faecalis*	*C.albicans*
Citrate	-	-	+	-	-	+
Urease	-	-	+	-	-	-
Nitrate	+	n/a	+	n/a	+	-
Gelatin	-	-	+	n/a	n/a	n/a
H_2S production	-	+	-	-	-	n/a
Arabinose	+	-	n/a	n/a	-	+
Fructose	-	n/a	+	n/a	+	n/a
Glucose	+	+	+	-	+	+
Inositol	-	-	n/a	n/a	n/a	+
Lactose	+	-	+	-	+	-
Maltose	n/a	-	+	n/a	+	+
Mannitol	+	+	+	+	+	+
Mannose	n/a	+	+	n/a	+	n/a
Raffinose	n/a	-	-	n/a	-	-
Sucrose	n/a	-	+	-	+	+
Sorbitol	+	+	n/a	-	+	+

Key: (+) - Positive, (-) - Negative, (n/a) - Not applicable

3.5.3 Maintenance of the bacterial and fungal cultures

The cultures of the clinical isolates of the test microorganisms were maintained on agar slants. The agar slants were prepared by making 250 millilitres of nutrient agar and potato Dextrose agar prepared according to their commercial instruction. The solution of the Medias was divided into bijou bottles at equal amount of 10 millilitres. The bijou bottles were then closed and placed in an autoclave and sterilized for about 15 minutes at 121 degrees celsius. The autoclave was left to cool before the bijou bottles containing the media are removed. The bottle was then placed on a wedge in a laminar flow slanting at around 45 degrees and left to cool forming agar slants. The bacterial microorganisms were streaked on nutrient agar slant whereas *Candida albicans* was streaked on potato dextrose agar slant. The streaked agar slants were placed in an incubator at 37 degrees celsius and observed for growth after every 24 hours. The test microorganism was subsequently subcultured after every 48 hours to maintain their viability.

3.6 Antimicrobial susceptibility testing

The microorganisms used (*Escherichia coli, Salmonella typhi, Enterococcus faecalis, Staphylococcus aureus, Shigella flexineri, Enterococcus faecalis* and *Candida albicans*) were concentrated by comparing it with a 0.5 McFarland standard. All the plant extracts were dissolved in 4% dimethyl sulphoxide (DMSO) to form a stock solution of 1000µg/ml.

The stock solution concentration of each extract was formulated using the formula below:

$$\frac{\mathbf{S}\times\mathbf{M}}{\mathbf{Q}} = \mathbf{C}$$

Where: **S** - Solute weight (milligrams), **Q** - Dilution solvent (milliliters), **M** - Conversion units to micrograms, **C -** Concentration (µg/ml).

The discs prepared from the Whatman filter paper no.1 were impregnated with different the plant leaf extracts (*Tagetes minuta*, *Aloe secundiflora*, *Vernonia lasiopus* and *Bulbine frutescens*) from the highest concentration of 1000µg/ml to the lowest concentration of 1µg/ml by serially halving the concentration in subsequent dilutions using and stored in McCartney bottles away from light (Mangoma *et al*., 2010).

The antimicrobial efficacy test was carried out using Kirby-Bauer method (Newall *et al*., 1996). Muller Hinton agar (Sharau®) was used for bacterial test microorganisms and potato dextrose agar for *Candida albicans* in the spread plate technique where the clinical isolates were spread using sterilized cotton wool swabs. They were exposed to extracts impregnated discs in milligrams per microliter from *Aloe secundiflora*, *Tagetes minuta*, *Vernonia lasiopus* and *Bulbine frutescens*. The discs were placed with equal distance between them on agar plates inoculated with the bacterial pathogens and *Candida albicans*.

Positive control standard discs containing ciprofloxacin (5µg/ml) was used for the bacteria's *Escherichia coli*, *Salmonella typhi*, *Enterococcus faecalis*, *Shigella flexineri*; vancomycin (3µg/ml) for *Staphylococcus aureus*, and fluconazole (15µg/ml) for *Candida albicans*. A negative control of discs impregnated with 4% Dimethyl sulphoxide and 100% Analar (AR) methanol were also used. The Petri dishes were incubated at 37-degree celsius for 24 hours, after which they were removed and observed to see if there was the formation of any zones of inhibition by the extracts from the plant against the test microbes. The experiment was carried in duplicates and the diameter of zones of inhibition formed was measured and their average determined.

Minimal inhibitory concentration (MIC) was determine using the broth tube method according to Eloff *et al*., 1998) whereby: 100µl of 250mg/ml of methanol extract was added to 100µl of sterile bacteriological peptone in the first well of the 96 well microplate and mixed well with a micropipette. 100µl of this dilution was transferred subsequently to wells two folding each dilution of the original extract. This was done to the extracts of *Aloe secundiflora*, *Bulbine frutescens*, *Vernonia lasiopus*, and *Tagetes minuta*. An inoculum of 100µl (0.5 McFarland standard) of overnight clinical cultures of ; *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Shigella flexineri*, *Enterococcus faecalis* and fungus *Candida albicans* were added in each of the wells. Triplicate of each microplate was made and the procedure repeated for each of the test organisms. The plates

were then incubated at 37-degree celsius for 24 hours. After incubation 40µl of 0.2 mg/µl of INT was added in each of the wells and the plates examined after an additional 60 minutes of incubation. Growth was indicated by a red colour (conversion of INT to formazan). The lowest concentration at which the colour was apparently invisible as compared to the next dilution was taken as the minimum inhibitory concentration (Rabe *et al*., 2002). Minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) was determined according to Rabe *et al*., 2002 whereby: 100µl of the suspension was taken from microplate wells that demonstrated no growth and inoculated on agar plates. The plates were incubated at 37 degrees celsius for 24 hours. In the case where there was no bacterial growth and also not greater than the minimum inhibitory concentration was used to determine the maximum bacterial concentration and maximum fungicidal concentration.

3.7 Combined effect test

The combinations were determined using the permutation formula of P=N! / (N-R)! Producing six combinations namely: *Aloe secundiflora* and *Tagetes minuta* **(AT)**, *Aloe secundiflora* and *Vernonia lasiopus* **(AV)**, *Bulbine frutescens* and *Tagetes minuta* **(BT)**, *Bulbine frutescens* and *Vernonia lasiopus* **(BV)**, *Aloe secundiflora* and *Bulbine frutescens* **(AB)** and *Vernonia lasiopus* and *Tagetes minuta* **(VT)**. The combined effect was determined by using the plants leaf extracts using the concentrations of 1000µg/ml of each extract in making a combination the ratio of 1:1. The combined plant leaf extracts were used against

the test microorganisms in replicates and the zones of inhibition measured. The average zones of inhibition formed when the extracts were used in combinations against the test microbes were compared with the zones of inhibition when they are used singly.

3.8 Qualitative phytochemical analysis

The presence of saponins, tannins, flavonoids and alkaloids in the crude extract were determined according to the method defined by Congesta *et al* (2005).

3.8.1Tannins

Each of the extracts was weighed to 0.5mg and dissolved in 1 ml of distilled water. Filtration was carried out after 2ml of $FeCl_3$ was added. If there was the presence of a blue or black precipitate then it indicated the presence of tannins.

3.8.2 Flavonoids

Each of the extracts was weighed to 0.5mg and dissolved in 1 ml of ethanol and filtered. 2ml of 1% HCl and magnesium ribbon was added to the filtrate. If there was the formation of a pink or red colour it indicated the presence flavonoids.

3.8.3 Alkaloids

Each of the extracts was weighed to 0.5mg and dissolved in 1ml of methanol and filtered. 1% HCL was added to the filtrate and the solution heated. Mayor`s reagent was added dropwise and if there was the formation of any colored precipitate it indicated the presence of alkaloids.

3.8.4 Saponins

Each of the extracts was weighed to 0.5mg and dissolved in 1 ml of methanol and filtered. Distilled water was added and shaking done for a few minutes. If there was persistence frothing then it indicated the presence of saponins.

3.9 Statistical analysis

The data collected was exported to Microsoft excel spreadsheet where descriptive statistics were carried out. The data was analyzed using SAS version 9.1 whereby; ANOVA (one way) was carried out to show statistical difference using the varying zones of inhibition between the test microbes exposed to plant leaf extracts from *Tagetes minuta*, *Aloe secundiflora*, *Vernonia lasiopus* and *Bulbine frutescens*. Two-way ANOVA was also carried out to determine if there was any interaction between the plant extracts and test microorganism with $P \leq 0.05$ considered significant. The tests were further subjected to a tukey`s post hoc test to find the difference between the means.

CHAPTER FOUR

RESULTS

All the bacterial pathogens (*Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Shigella flexineri* and *Enterococcus faecalis*) and *Candida albicans* were tested against plant extracts of the concentration of 1000µg/ml impregnated on discs. The bacterial pathogens and *Candida albicans* cultures of 0.5 McFarland standard were used for the efficacy test.

4.1 Efficacy test of the plant extracts on the bacterial pathogens and *Candida albicans*

All the bacterial pathogens (*Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Shigella flexineri* and *Enterococcus faecalis*) and fungus *Candida albicans* were exposed to the plant extract in replicates and they showed varied antimicrobial activity (Table 4.1). *Aloe secundiflora* extract produced the largest average zone of inhibition of 16.67±2.58mm against *Escherichia coli* when compared to the other plant extracts (Table 4.1).

The average zones of inhibition formed by *Tagetes minuta* and *Aloe secundiflora* extracts were significantly different to those formed by *Vernonia lasiopus* ($P<0.05$; Table 4.1). Moreover, the average zones of inhibition formed by ciprofloxacin, methanol and DMSO (negative control) were significantly different to those formed by the plant extract ($P<0.05$; Table 4.1). *Tagetes minuta* extract

produced the largest average zone of inhibition of 17.17±2.48mm against *Salmonella typhi* when compared to the other plant extracts (Table 4.1). The average zone of inhibition formed by an extract from *Vernonia lasiopus* was significantly different to those of *Tagetes minuta* ($P<0.05$; Table 4.1). Moreover, the average zones of inhibition formed by ciprofloxacin, methanol and DMSO (negative control) were significantly different to those formed by the plant extract ($P<0.05$; Table 4.1). *Tagetes minuta* extract produced the largest average zone of inhibition of 16.67±3.44mm against *Staphylococcus aureus* when compared to other plant extracts (Table 4.1). The average zones of inhibition formed by the plant extracts were not significantly different from each other ($P>0.05$; Table 4.1).

However, the average zone of inhibition formed by the plant extracts were significantly different to those formed by vancomycin, methanol and DMSO (negative control) were significantly different to those formed by the plant extract ($P<0.05$; Table 4.1). *Bulbine frutesens* extract produced the largest average zone of inhibition of 19.50±1.05mm against *Shigella flexineri* when compared to other plant extracts (Table 4.1). The average zones of inhibition formed by the plant extracts were not significantly different from each other ($P>0.05$; Table 4.1). However, the average zones of inhibition formed by ciprofloxacin, methanol and DMSO (negative control) were significantly different to those formed by the plant extract ($P<0.05$, Table 4.1). *Tagetes minuta* extract produced the largest average zones of inhibition of 18.67±1.03mm against *Enterococcus faecalis* when

compared to other plant extracts (Table 4.1). The average zones of inhibition formed by all the plant extracts were not significantly different ($P>0.05$; Table 4.1). However, the average zones of inhibition formed by ciprofloxacin (positive control), methanol and DMSO (negative control) were significantly different to those formed by the plant extract ($P<0.05$; Table 4.1). *Vernonia lasiopus* extract produced the largest zone of inhibition of 20.17±2.71mm against *Candida albicans* when compared to other plant extracts (Table 4.1). The average zone of inhibitions formed by *Tagetes minuta* and *Vernonia lasiopus* were significantly different from each other ($P<0.05$; Table 4.1). All the average zone of inhibition formed by the plant extracts were significantly different to those formed by fluconazole, methanol and DMSO (negative control) ($P<0.05$; Table 4.1).

Table 4.1: Average zones of inhibition in millimetres when plant extracts are used singly against bacterial pathogens and *Candida albicans*

Plant extracts	**Test microorganisms**					
	E. coli	***S. typhi***	***S.aureus***	***S. flexineri***	***E. faecalis***	***C. Albicans***
Tagetes minuta	16.50±1.87^{c}	17.17±2.48^{c}	16.67±3.44^{c}	19.00±1.41^{bc}	18.67±1.03^{c}	15.17±2.71^{d}
Aloe secundiflora	16.67±2.58^{c}	16.50±1.87^{cd}	14.17±2.93^{c}	18.17±1.47^{c}	17.67±1.63^{c}	17.00±2.10^{cd}
Bulbine frutescens	13.33±1.75^{cd}	15.33±2.73^{cd}	11.83±2.48^{c}	19.50±1.05^{bc}	18.50±1.05^{c}	17.83±1.72^{cd}
Vernonia lasiopus	12.33±2.58^{d}	13.17±1.84^{d}	13.00±2.61^{c}	18.17±1.47^{c}	18.00±0.89^{c}	20.17±2.71^{c}
Controls	***E. coli***	***S. typhi***	***S.aureus***	***S. flexineri***	***E. faecalis***	***C. Albicans***
Antibiotics	21.67±2.42^{b}	25.67±1.63^{b}	24.83±3.54^{b}	21.50±2.07^{b}	21.67±2.66^{b}	27.17±0.98^{b}
Methanol	28.67±2.34^{a}	30.17±2.71^{a}	31.33±2.94^{a}	31.67±2.88^{a}	33.50±2.56^{a}	31.00±3.20^{a}
4%DMSO	0.00±0.00^{e}	0.00±0.00^{e}	0.00±0.00^{d}	0.00±0.00^{d}	0.00±0.00^{d}	0.00±0.00^{e}
P value	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

The value of average zones of inhibition ± standard error after one-way ANOVA followed by Tukey`s HSD test. A value followed by the same superscript within the same column are not significantly different (P>0.05).

Key: DMSO - Dimethyl sulphoxide, Plant extracts concentration (1000µg/ml) Antibiotics standard discs of; Ciprofloxacin (5µg/ml) for Gram negative bacteria, Vancomycin (3µg/ml) for Gram positive bacteria, Fluconazole (15µg/ml) for *Candida albicans.*

Table 4.2: Interactions between the plants extract and test microorganism when used singly

Extract	**Zone of inhibition±SEM**
Aloe secundiflora	16.69±0.40^{cd}
Bulbine frutescens	16.06±0.56^{d}
Tagetes minuta	17.19±0.42^{c}
Vernonia lasiopus	15.81±0.61^{d}
Controls	**Zone of inhibition±SEM**
Antibiotics	23.5±0.43^{b}
Methanol	31.17±0.34^{a}
DMSO	0.00±0.00^{e}
Test microorganisms	**Zone of inhibition±SEM**
Candida albicans	18.31±1.45^{a}
Escherichia coli	15.26±1.25^{c}
Enterococcus faecalis	18.26±1.41^{a}
Salmonella typhi	16.60±1.37^{b}
Shigella flexineri	18.69±1.45^{a}
Staphylococcus aureus	16.10±1.51^{bc}
P values **of the main factors and their interactions**	
Extract	<0.001
Test microorganisms	<0.001
Extract*Test microorganisms	<0.001

The value of average zones of inhibition ± standard error of mean (SEM) after two-way ANOVA followed by Tukey`s HSD test. A value followed by the same superscript within the same column are not significantly different (P>0.05).
Key: Antibiotics (ciprofloxacin, vancomycin, and fluconazole), DMSO - dimethyl sulphoxide

The average zone of inhibition formed by the plants extracts against all the test microorganism were significantly different to those formed by antibiotics, methanol and DMSO (P<0.05; Table 4.2). Moreover, zones formed by *Tagetes minuta* extract against all the test microorganism was significantly different to

those formed by *Vernonia lasiopus* and *Bulbine frutescens* (P<0.05; Table 4.2). However, it was not significantly different to zones formed by *Aloe secundiflora* (P>0.05; Table 4.2). The average zones of inhibition formed by the plant extracts against *Enterococcus faecalis*, *Candida albicans*, and *Shigella flexineri* were not significantly different from each other (P>0.05; Table 4.2). However, they were significantly different to those formed by *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus* (P<0.05; Table 4.2). The interaction between the plant extracts and test microorganisms were significant (P<0.05; Table 4.2).

Table 4.3: Minimum inhibitory concentration in micrograms/milliliter of plant extracts against bacterial pathogens and *Candida albicans*

Plant extracts	**Test microorganisms**					
	E.coli	***S.typhi***	***S.aureus***	***S.flexineri***	***E.faecalis***	***C.albicans***
Tagetes minuta	8.7	6.1	8.9	7.4	5.1	6.2
Aloe secundiflora	9.1	5.5	10.2	3.7	7.0	8.1
Bulbine frutescens	12.5	8.8	10.4	3.2	6.5	6.9
Vernonia lasiopus	10.0	5.6	12.2	3.3	3.9	4.0
Antibiotics	5.0	5.0	3.0	5.0	5.0	15.0

Key: Antibiotics; Ciprofloxacin (5µg/ml) - Gram-negative bacteria, Vancomycin (3µg/ml) - Gram-positive bacteria, Fluconazole (15µg/ml) - *Candida albicans*)

Tagetes minuta extract was more active against *Escherichia coli* when compared to the other extracts. However, the antibiotic used as a positive control (Ciprofloxacin) was more active against *Escherichia coli* when compared to all the plant extracts (Table 4.3). *Aloe secundiflora* extract had the highest antimicrobial activity against *Salmonella typhi* when compared to other plant extracts although ciprofloxacin was more active against *Salmonella typhi* when compared to all the extracts (Table 4.3).

Tagetes minuta extracts were more active against *Staphylococcus aureus* at low concentration when compared to the other plant extracts. The positive control antibiotic (Vancomycin) used against *Staphylococcus aureus*, showed to be more active in low concentration when compared to all the extracts (Table 4.3). *Bulbine frutescens* was more active against *Shigella flexineri* at low concentration when compared to the other plant extracts. Furthermore, the level of concentration of *Bulbine frutescens* extract was lower when compared to the used standard antibiotic (Ciprofloxacin) against *Shigella flexineri* (Table 4.3).

The plant extract from *Vernonia lasiopus* was more active at low concentration against *Enterococcus faecalis* when compared to the other plant extracts. Its concentration was lower when compared to the used standard antibiotic (Ciprofloxacin) (Table 4.3). All the plant extracts were more active against *Candida albicans* as compared to the used standard antibiotic (Fluconazole). However, *Vernonia lasiopus* was the most active against *Candida albicans* in low concentration among the used plant extracts (Table 4.3).

Table 4.4: Minimum bactericidal concentration and minimum fungicidal concentration in micrograms/milliliter of plant extracts against bacterial pathogens and *Candida albicans*

Plant extracts	**Test microorganisms**					
	E.coli	***S.typhi***	***S.aureus***	***S.flexineri***	***E.faecalis***	***C.albicans***
Tagetes minuta	10	8.2	10	12.6	6.3	8.7
Aloe secundiflora	10.4	7.3	12.9	8.0	9.7	9.0
Bulbine frutescens	14.0	10.9	13.9	6.2	9.1	8.0
Vernonia lasiopus	11.5	7.5	14.2	7.1	5.0	5.5

Tagetes minuta extract was bactericidal in low concentration against *Escherichia coli* when compared to the other extracts (Table 4.4). The plant extract from *Aloe secundiflora* was bactericidal against *Salmonella typhi* at low concentration when compared to other plant extracts (Table 4.4). When used against *Staphylococcus aureus* the extract from *Tagetes minuta* was bactericidal at low concentration when compared to the other plant extracts (Table 4.4). *Bulbine frutescens* plant extract was bactericidal against *Shigella flexineri* at low concentration when compared to the other plant extracts (Table 4.4). When used against *Enterococcus faecalis* and *Candida albicans*, *Vernonia lasiopus* was bactericidal and fungicidal respectively at low concentrations as compared to the other plant extracts (Table 4.4).

4.2 Combined effect of the plant extracts on the bacterial pathogens and *Candida albicans*

The plant extracts showed to be both more effective and less effective in some instances against all the test microorganisms when used in combinations. *Vernonia lasiopus* and *Tagetes minuta* plant extract combinations formed the largest average zone of inhibition of 16.67±1.37mm against *Escherichia coli* when compared to the other plant extract combinations (Table 4.5). The average zones of inhibition formed by the plant extracts combinations were not significantly different ($P>0.05$; Table 4.5). However, they were significantly different to those formed by ciprofloxacin, methanol and DMSO (negative control) ($P<0.05$; Table 4.5).

Bulbine frutescens and *Vernonia lasiopus*; *Bulbine frutescens* and *Tagetes minuta* plant extract combinations formed the largest average zones of inhibition of 16.67±2.58mm and 16.67±2.26mm against *Salmonella typhi* when compared to the other plant extract combinations (Table 4.5). The average zones of inhibition formed by all the plant extracts combinations were not significantly different ($P>0.05$; Table 4.5). However, the average zones of inhibition formed by ciprofloxacin, methanol and DMSO (negative control) were significantly different to those formed by the plant extract ($P<0.05$; Table 4.5). *Bulbine frutescens* and *Vernonia lasiopus* plant extract combination formed the largest average zone of inhibition of 15.00±2.28mm against *Staphylococcus aureus* when compared to

other plant extract combinations (Table 4.5). The average zone of inhibition formed by all the plant extracts combinations were not significantly different ($P>0.05$; Table 4.5). However, the average zones of inhibition formed by vancomycin, methanol and DMSO (negative control) were significantly different to those formed by the plant extract ($P<0.05$; Table 4.5). *Aloe secundiflora* and *Bulbine frutescens*; *Aloe secundiflora* and *Tagetes minuta* plant extract combinations formed the largest average zones of inhibition of 18.67±1.03mm and 18.67±0.24mm against *Shigella flexineri* when compared to other plant extracts combinations (Table 4.5). The average zone of inhibition formed by the plant extracts combinations was not significantly different to those formed by ciprofloxacin (positive control) ($P>0.05$; Table 4.5).

However, methanol and DMSO (negative control) were significantly different to those formed by the plant extract and ciprofloxacin ($P<0.05$; Table 4.5). *Aloe secundiflora* and *Bulbine frutescens* plant extract combination produced the largest average zone of inhibition of 18.00±1.80mm against *Enterococcus faecalis* when compared to the other plant extracts (Table 4.5). The zones formed by the plant extracts were not significantly different ($P>0.05$; Table 4.5). Moreover, *Aloe secundiflora* and *Bulbine frutescens* plant extract combination formed zones that were not significantly different to those formed by ciprofloxacin (positive control) ($P>0.05$; Table 4.5). However, the zones of inhibition formed by methanol and DMSO (negative control) were significantly different to those formed by the plant

extract combinations and ciprofloxacin ($P<0.05$; Table 4.5). *Bulbine frutescens* and *Tagetes minuta* plant extract combination formed the largest average zone of inhibition of 16.83±1.47mm against *Candida albicans* when compared to the other plant extract combinations (Table 4.5). The average zones of inhibition formed by *Aloe secundiflora* and *Tagetes minuta* extract were significantly different when compared to the other plant extract combinations; ($P<0.05$; Table 4.5). Moreover, it was also significantly different from fluconazole (positive control), methanol and DMSO (negative control) ($P<0.05$; Table 4.5).

Table 4.5: Average zone of inhibition in millimetres when extracts are combinations against the bacterial pathogens and *Candida albicans*.

Plant extracts	Test microorganisms					
	E. coli	***S. typhi***	***S.aureus***	***S. flexineri***	***E. faecalis***	***C.albicans***
AB	16.33 ± 1.97^{c}	14.83 ± 2.14^{c}	14.17 ± 2.32^{c}	18.67 ± 1.03^{bc}	18.00 ± 1.80^{bc}	14.17 ± 2.14^{b}
AV	15.33 ± 2.16^{c}	15.00 ± 3.03^{c}	14.33 ± 1.86^{c}	18.16 ± 1.47^{c}	17.67 ± 1.75^{c}	14.67 ± 2.42^{b}
AT	17.33 ± 1.86^{c}	14.50 ± 2.43^{c}	14.50 ± 1.04^{c}	18.67 ± 0.24^{bc}	17.67 ± 1.51^{c}	8.67 ± 1.86^{c}
BV	16.33 ± 1.75^{c}	16.67 ± 2.58^{c}	15.00 ± 2.28^{c}	17.67 ± 1.63^{c}	17.50 ± 1.64^{c}	13.50 ± 1.52^{b}
BT	15.83 ± 2.32^{c}	16.67 ± 2.26^{c}	14.17 ± 2.17^{c}	17.83 ± 1.47^{c}	16.83 ± 1.47^{c}	16.83 ± 1.47^{b}
VT	16.67 ± 1.37^{c}	14.83 ± 2.64^{c}	14.50 ± 2.17^{c}	18.16 ± 1.47^{c}	16.17 ± 3.19^{c}	14.17 ± 2.99^{b}
Antibiotics	21.67 ± 2.42^{b}	25.67 ± 1.63^{b}	24.83 ± 3.54^{b}	21.50 ± 2.07^{b}	21.67 ± 2.66^{b}	27.17 ± 0.98^{a}
Methanol	28.67 ± 2.34^{a}	30.17 ± 2.71^{a}	31.67 ± 2.88^{a}	31.67 ± 2.88^{a}	33.50 ± 2.26^{a}	30.33 ± 3.20^{a}
4%DMSO	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}
P values	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

The value of average zones of inhibition ± standard deviation after one-way ANOVA followed by Tukey`s HSD test. A value followed by the same superscript within the same column are not significantly different (P>0.05).

Key: AB - *Aloe* + *Bulbine*; **AV** - *Aloe* + *Vernonia*; **AT** - *Aloe* +*Tagetes*; **BV** - *Bulbine* + *Vernonia*; **BT** - *Bulbine* + *Tagetes*; **VT** - *Vernonia* + *Tagetes*; ± Standard error; DMSO – Dimethyl sulphoxide; Antibiotics standard discs of; Ciprofloxacin (5µg/ml), Vancomycin (3µg/ml) and Fluconazole (15µg/ml); 7mm - 10mm Resistant; 11mm - 20mm Intermediate; 21mm - 30mm Sensitive.

Table 4.6: Interactions between the plants extract and test microorganism when used in combinations

Extract	**Zone of inhibition±SEM**
AB	16.03±0.43[c]
AT	15.22±0.63[c]
AV	15.86±0.42[c]
BT	16.28±0.37[c]
BV	16.11±0.39[c]
VT	15.75±0.44[c]
Controls	**Zone of inhibition±SEM**
Antibiotics	23.50±0.43[b]
Methanol	30.83±0.34[a]
DMSO	0.00±0.00[e]
Test microorganisms	**Zone of inhibition±SEM**
Candida albicans	15.56±1.20[b]
Escherichia coli	16.20±0.96[b]
Enterococcus faecalis	17.59±1.11[a]
Salmonella typhi	16.28±1.08[b]
Shigella flexineri	18.35±1.13[a]
Staphylococcus aureus	15.74±1.10[b]
***P values* of the main factors and their interactions**	
Extract	<0.001
Test microorganisms	<0.001
Extract*Test microorganisms	<0.001

The value of average zones of inhibition ± standard error of mean (SEM) after two-way ANOVA followed by Tukey`s HSD test. A value followed by the same superscript within the same column are not significantly different (P>0.05).
Key: Antibiotics (ciprofloxacin, vancomycin, and fluconazole), DMSO - dimethyl sulphoxide.

The average zones of inhibition formed by the plant extracts combinations when used against the test microorganisms were not significantly different (P>0.05; Table 4.6). However, they were significantly different to those formed by

antibiotics, methanol and DMSO ($P<0.05$; Table 4.6). The average zones of inhibition formed by the test microorganisms; *Enterococcus faecalis* and *Shigella flexineri* were not significantly different ($P>0.05$; Table 4.6). However, they were significantly different to those formed by the other test microorganism ($P<0.05$; Table 4.6).

4.3 Qualitative phytochemical analysis

Table 4.7: Qualitative phytochemical tests on the plant extracts

Name of test	**Plants leaf extracts**			
	T. minuta	*A. secundiflora*	*B. frutescens*	*V. lasiopus*
Saponins test	+	+	+	+
Tannins test	+	+	+	+
Alkaloids test	+	+	+	+
Flavonoids test	+	+	+	+

Key: (+) present

The plant leaf extracts from *Tagetes minuta*, *Aloe secundiflora*, *Bulbine frutescens* and *Vernonia lasiopus* were qualitatively tested for the presence of phytochemicals. All the plant extracts were found to contain saponins, tannins, alkaloids, and flavonoids (Table 4.7).

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

The increase of antimicrobial resistance to many available antimicrobial agents has led the need for the invention of new drugs. The use of plant extracts to test for antimicrobial activity has been brought forward as one of the ways of achieving this goal. The plants used in the study have been said to be of medicinal value. This study evaluated the use of the plants in treating selected bacterial pathogens (*Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Shigella flexineri* and *Enterococcus faecalis*) and fungus *Candida albicans* and test if they are effective or not when used singly and in combinations. Furthermore, qualitative analysis to test for the presence of the phytochemicals was also done.

This is because phytochemicals have been said to be responsible to some of the antimicrobial activity by extracts from plants with medicinal value. From the results, the extracts from the plants were found to have antimicrobial activity when used singly or in combination. However, some of the extracts showed activity when used singly and also in combinations while the vice versa was also true. The extracts have shown both increased and decreased antimicrobial activity when used in combinations.

5.1.1 Antimicrobial activity

Medicinal plant extracts from various studies have shown that plants from the similar genera with *Aloe secundiflora* have shown to have antimicrobial activity. In this study, the antimicrobial activity of methanol extracts from medicinal plants was tested against test bacterial pathogens and fungus *Candida albicans*. It was interesting to note that the plant extracts from *Aloe secundiflora* showed antimicrobial activity against all the test microorganisms. Findings from the study were similar to those obtained in Nigeria by Agarry *et al*. (2005) from activities against medicinal plants. The plant extract from *Aloe secundiflora* had antimicrobial activity against tested bacterial pathogens; *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Shigella flexineri* and *Enterococcus faecalis* and fungus *Candida albicans*.

The findings were similar to those obtained in a study carried out on Gram negative and Gram positive bacteria by Kaithawas *et al*. (2008) in India and Robson (1982) in England; on *Escherichia coli* and Agarry *et al*. (2005) in Nigeria; on *Staphylococcus aureus* and *Candida albicans* who found out that, extracts from *Aloe secundiflora* had antimicrobial activity against the test microorganisms. *Aloe secundiflora* also showed antimicrobial activity which was significant at $P<0.05$ and the zones of inhibition formed $\geq$ 12.00mm. These findings were also similar to those obtained in a previously carried out study in Kenya (Mariita *et al*., 2011) who found out that the methanol extracts of *Aloe*

secundiflora along the lake region in Kenya to be effective against bacterial pathogens such as *Escherichia coli, Salmonella typhi, and Staphylococcus aureus* among others. The study also showed that methanol extracts of *Aloe secundiflora* showed a great antimicrobial activity significant at $P<0.05$ producing zones of inhibition of ≥9.00mm against bacterial pathogens which included, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi* and a fungus *Candida albicans*. This could probably be due to, the use of same part of the plant (leaves) and most likely the same age. Msoffe and Mbilu (2009) in Tanzania, also found out that extracts from *Aloe secundiflora* had antimicrobial activity against *Candida albicans*. This may be attributed to the use of the same part of the plant.

The plant extracts from *Tagetes minuta* showed antimicrobial activity against the tested microorganism; *Escherichia coli, Salmonella typhi*, *Staphylococcus aureus*, *Shigella flexineri*, *Enterococcus faecalis* and *Candida albicans*. The antimicrobial activity was significantly higher against *Enterococcus faecalis* compared to *Staphylococcus aureus*. From the study, the plant extract from *Tagetes minuta* formed a zone of inhibition ≥17.00mm against all test microorganism. The findings were similar to those obtained from previously carried out in India by Panwar and Bhatt (2014), who found out that extracts from *Tagetes minuta* produced zone on inhibition ≥17.00mm against *Staphylococcus aureus* which is comparable to the one obtained in this study. This may be due to the same method of extraction and testing. Moreover, the plant used could be of the same age. The

findings in this study showed that the minimum inhibitory concentration against these test microorganisms ranged from 3 to 13µg/ml. The average zones of inhibition formed were ≥ 17.00mm and the standard antibiotic used (Ciprofloxacin) produced zones of inhibition ≥ 20.00mm. The findings were similar to a previously carried out study in Pakistan by Tahir and Khan (2012) who found out that extracts from *Tagetes minuta* had antimicrobial activity against *Salmonella typhi*, *Escherichia coli*, and *Staphylococcus aureus*. Furthermore, the minimum inhibitory concentration against these test microorganisms ranged from 4 to 100µg/ml, the average zones of inhibition formed were ≥ 17.00mm and the standard antibiotic used (Ciprofloxacin) produced zones of inhibition ≥ 20.00mm. This means that if the concentration of *Tagetes minuta* could be standardized, it could used as an alternative therapy for Ciprofloxacin, against the tested organisms.

Bulbine frutescens extract showed antimicrobial activity against all the tested microorganisms. The extract antimicrobial activity was significantly higher against *Shigella flexineri* to *Escherichia coli*. From the findings in the study, the minimum inhibitory concentration of *Bulbine frutescens* against *Staphylococcus aureus* was 10.4 µg/ml. This finding was contrary to the one in South Africa by Coopoosamy (2011) who found out that the minimum inhibitory concentration against *Staphylococcus aureus* was 2.0 µg/ml which was low when compared to the one obtained in the study. Furthermore, Coopoosamy (2011) found out that,

extract from *Bulbine frutescens* had no antimicrobial activity against *Escherichia coli*. This differences could be due to the different geographical and environmental conditions during the growth of the plant, the method of extraction used and the age of the plant. In the study, *Bulbine frutesens* showed antifungal activity against *Candida albicans* producing a zone of inhibition ≥18.00mm. The findings were similar to those obtained from previously carried out studies in South Africa by Fennel *et al*. (2004); Stafford *et al*. (2005) who found out that, extracts from *Bulbine frutescens* were active against *Candida* spp and *Candida albicans producing zones of inhibition* ≥10.00mm. Furthermore, they indicated inhibition between 41-50mm very high, 31-40mm high, 21-30mm medium, 11-20mm low in fungal species of *Candida* sp, *Candida albicans* included. The similarity in the activity could be associated with plant's age and the methods of extraction and testing.

The extract from *Vernonia lasiopus* showed antimicrobial activity against tested microorganisms. The extract had a significantly higher antimicrobial activity against *Candida albicans* compared to *Escherichia coli*. In this study, when the plant extract from *Vernonia lasiopus* was used against *Escherichia coli* and *Staphylococcus aureus* the average zone of inhibition formed were ≥ 12.00mm and ≥ 13.00mm respectively. These findings concurred with those of previously obtained in Kenya by Kareru *et al*. (2008) who found out that leaf extracts from *Vernonia lasiopus* had antimicrobial activity against *Escherichia coli*. However,

the findings were contrary to a study carried out in Kenya by Kareru *et al*. (2008) who found out that, *Escherichia coli* and *Staphylococcus aureus* was resistant (≤6.5mm). This could be due to the difference in; the age of the plant, the environmental condition in which the plant grew and the aqueous method of extraction used in the study.

5.1.2 Combined effect of the plant extracts

When plant extracts were used in combinations they showed diverse antimicrobial activity against the tested microorganisms. When the plant extracts were used in combination against *Escherichia coli*, they showed a pronounced antimicrobial activity as compared to when each of them is used separately against it. *Bulbine frutescens* and *Vernonia lasiopus* produced small average zones of inhibition when each of them is used separately against the microorganism.

However, the combinations of the plant extracts of; *Aloe secundiflora* and *Bulbine frutescens*, *Aloe secundiflora* and *Vernonia lasiopus*, *Bulbine frutescens* and *Vernonia lasiopus*, *Bulbine frutescens* and *Tagetes minuta*, produced large average zones of inhibition against *Escherichia coli*. These study concurred with those of a previously done study in Brazil by Nascimento *et al*. (2000); Alzoreky and Nakahara (2003) in Japan who found out that when the plant extracts are used in combination against *Escherichia coli*, there is an increase in their antomicrobial activity. This could be due to the same method of extraction and testing.

The use of plant extracts in combinations against *Salmonella typhi* of; *Bulbine frutescens* and *Vernonia lasiopus*, *Aloe secundiflora* and *Vernonia lasiopus* produced large average zones of inhibition against *Salmonella typhi* compared to when used separately against the same microorganism. The findings showed that, combining of *Bulbine frutescens*, *Aloe secundiflora* and *Vernonia lasiopus* with the other plant extracts enhanced their antimicrobial activity against *Salmonella typhi*. The findings were similar to a previously carried out study in the United States of America by Cutter (2000); Anjeza and Mandal (2012) in India who found out that combining of the plant extracts increased their antimicrobial activity against *Salmonella typhi*. This could be due to the use of the same part of the plant. Moreover, the plants used could be of the same age.

The use of plant extracts in combinations against *Staphylococcus aureus* significantly increased the antimicrobial activity as compared to when each of them is used separately. The combinations of ; *Bulbine frutescens* and *Vernonia lasiopus*, *Aloe secundiflora* and *Tagetes minuta*, *Bulbine frutescens* and *Tagetes minuta* showed enhanced antimicrobial activity as by producing large average zones of inhibition compared to when; *Bulbine frutescens*, *Aloe secundiflora,* and *Vernonia lasiopus* were used singly against *Staphylococcus aureus*. The findings were similar to a previously carried out study in the Japan by Alzoreky and Nakahara (2003); Adawan and Mhanna (2008) in Palestine who found out that combining of the plant extracts and increased their antimicrobial activity against

Staphylococcus aureus. Furthermore, Adwan and Mhanna (2008) found out that their combination with conventionally available antibiotics also increased their antimicrobial activity. This means that if the concentration of the combined extracts can be standardized, it can be used in combination therapy with antibiotics against *Staphylococcus aureus*.

Shigella flexineri showed either an increase or a decrease in antimicrobial activity when exposed to the plant extracts combinations. When each of the plant extracts was used separately against *Shigella flexineri* they showed pronounced antimicrobial activity with *Bulbine frutescens* extract producing the largest average zone of inhibition when compared to extracts from other plants. However, the combining of *Bulbine frutescens* with; *Tagetes minuta*, *Aloe secundiflora*, and *Vernonia lasiopus* each separately against *Shigella flexineri* showed decreased antimicrobial activity by producing small average zones of inhibition as compared to combinations formed using *Tagetes minuta*, *Aloe secundiflora* and *Vernonia lasiopus*. The findings concurred with a previously carried out study in India by Chanda and Rakholiya (2011) and Barkarnga *et al*. (2015) in Cameroon who found out that combining of the plant extracts showed both an increase and decrease in their antimicrobial activity against *Shigella flexineri.* This similarity in activity could be associated with the method of extraction and testing, the plant's age and the part of the plant used.

When the combinations of the plant extracts were used against *Enterococcus faecalis*, they showed a decrease in antimicrobial activity as compared to when used separately against it. From the findings of the study, plant extracts from; *Tagetes minuta*, *Aloe secundiflora*, *Bulbine frutescens* and *Vernonia lasiopus* showed pronounced antimicrobial activity by producing large average zones of inhibition when each of them is used separately against *Enterococcus faecalis*. Plant extracts combinations from; *Aloe secundiflora* and *Vernonia lasiopus*, *Aloe secundiflora* and *Tagetes minuta*, *Bulbine frutescens* and *Tagetes minuta*, *Vernonia lasiopus* and *Tagetes minuta*, *Bulbine frutescens* and *Vernonia lasiopus*, showed decreased antimicrobial activity as compared to when each of them is used separately against *Enterococcus faecalis*. The findings were similar to a previously carried out study in India by Chanda and Rakholiya (2011) and Olajuyigbe and Afolayan (2012) in South Africa who found out that combining of the plant extracts showed decreased antimicrobial activity against *Enterococcus faecalis*. This could probably be due to, the use of the same part of the plant (leaves) and most likely of the same age.

Candida albicans showed either an increase or decrease in antimicrobial activity with the latter being more pronounced when the plant extracts were used in combinations. The plant extracts had pronounced antimicrobial activity when each of them was used separately against *Candida albicans* with *Vernonia lasiopus* being the most active by producing the largest average zone of inhibition

as compared to others. Plant extracts combination of *Bulbine frutescens* and *Tagetes minuta* showed increased antimicrobial activity by producing a larger average zone of inhibition against *Candida albicans* as compared to when *Tagetes minuta* was used separately against it. The findings were similar to a previously carried out study in Cameroon by Bakarnga *et al*. (2015) who found out that combining of the plant extracts showed increased antimicrobial activity against *Candida albicans*. However, the findings in the study also showed that the combined extracts had a decrease in antimicrobial activity with the combination of *Aloe secundiflora* and *Tagetes minuta* showing dismal antimicrobial activity by producing the smallest average zone of inhibition when compared to others. The findings concurred with a previously carried out study in Palestine by Adwan *et al*. (2011) who found out that combining of the plant extracts showed decreased antimicrobial activity against *Candida albicans*. This similarity in the activity could be due to the plant's age and the methods of extraction and testing.

5.1.3 Phytochemicals

The extract from *Aloe secundiflora* showed that the plant contained pharmacologically active components. The extract contained flavonoids, saponins, alkaloids and tannins which may be responsible for the antimicrobial activity. Similar studies previously carried out have shown that some of the pharmacologically active components have antimicrobial activity. These findings concurred with those obtained in a previously carried out study in Kenya by Mariita e*t al*. (2011) who after qualitative analysis of phytochemical components

of *Aloe secundiflora* extract used against *Escherichia coli, Salmonella typhi, Staphylococcus aureus* and *Candida albicans* from plant collected along the Kenya lake region found it to contained tannins, saponins, flavonoids, and alkaloids. Furthermore, similar findings were obtained in a study carried out in India by Arankumar and Methuselvan (2009) who confirmed the presence of flavonoids, saponins, tannins and alkaloids in *Aloe* extract when used against *Staphylococcus aureus* and *Escherichia coli*. Qualitative analysis for the presence of phytochemicals in *Tagetes minuta* extract showed the presence of flavonoids, saponins, alkaloids and tannins. This pharmacologically active components might be responsible *Tagetes minuta* extract antimicrobial activity against the test microorganisms.

The study findings were similar to those obtained in a study carried out in Pakistan by Tahir and Khan (2012) and in Argentina by Tereschuk (1997) who also confirmed the presence of phytochemicals flavonoids, saponins, tannins and alkaloids in *Tagetes minuta* extract used against Gram positive and Gram negative bacteria. The extract from *Bulbine frutescens* contained pharmacologically active compounds namely saponins, tannins, alkaloids, and saponins which could be responsible for antimicrobial activity. These study concurred with those of a previously done study in South Africa by Coopoosamy (2011) who found out that when the plant extract from *Bulbine frutescens* was used against *Staphylococcus aureus* and *Escherichia coli* and qualitative analysis of phytochemicals showed

the presence of alkaloids, saponins, tannins, and flavonoids. However, the findings were contrary to those obtained in previously carried out a study in South Africa by Van Staden *et al*. (1994) who found out that, the extract from *Bulbine frutescens* did not contain any of the four phytochemicals. This could be due to diverse plant metabolites associated with a geographical and ecological difference from where the plant was obtained and also the age of the plant used. The extracts from *Vernonia lasiopus* had active pharmacological compound; flavonoids, saponins, tannins and alkaloids which could be responsible for the antimicrobial activity.

These findings were similar to those of a previously carried out study by Kareru *et al*. (2008) who found out that, extract from *Vernonia lasiopus* contained alkaloids, flavonoids, saponins, and tannins when used against Gram positive and Gram negative bacteria (*Escherichia coli* and *Staphylococcus aureus*). Ayoola *et al*. (2008) in Nigeria also found out that extract from the plant from the family *Vernonia* contained flavonoids, alkaloids, tannins, and saponins. However, the findings were contrary to those obtained in a study carried out in Southwestern region in Nigeria by Ibrahim *et al*. (2012) who found out the extract from *Vernonia lasiopus* did not contain alkaloids. This difference could be associated with the geographical and environmental factors of the area from which the plant was collected.

5.2 Conclusion

- The plant extracts showed antimicrobial activity when used against the bacterial pathogens and fungus *Candida albicans*.
- Increase and decrease in antimicrobial activity was also observed when the plant extracts were combined and used against the test microorganism.
- There was the presence of phytochemicals in the plant extracts; saponins alkaloids, tannins, and flavonoids.

5.3 Recommendations

- The plants leaf extracts can be used in the formulation of a drug against the bacterial microorganisms and fungus *Candida albicans* only after scientific validation of their safety.
- The combining of the plants leaf extracts can aid in achieving a greater antimicrobial activity against the test microorganisms and other pathogenic microbes only after scientific validation of the components in the extracts responsible for the increasing antimicrobial activity.
- There is need to elucidate phytochemical components present in the extracts which might be responsible for the antimicrobial activity.

5.4 Suggestions for further studies

- Determine the possible mechanism of antimicrobial action of the extracts.
- Determination of individual contribution of each plant extracts towards combined effect.
- Identify and quantitatively isolate individual phytochemical components in the extracts.

REFERENCE

Abdel Ghani, S.B., Mugisha, P.J., Wilcox, J.C., Gado, E.A., Medu, E.O., Lamb, A.J., and Brown, R.C. (2013). Convenient one-pot synthesis of chromone derivatives and their antifungal and antibacterial evaluation. *Journal of Synthetic Communications*, 43(11), 1549-1556.

Aboaba, O., and Efuwape, B.M. (2001). Antibacterial properties of some Nigerian species. *Journal of Biochemical and Biophysical Research Communications*, 13, 183-188.

Acocks, J.P.H. (1988). Veld types of South Africa. *Memoirs of the Botanical Survey of South Africa*, (57):100-146.

Adwan, G., and Mhanna, M. (2008). Synergistic effects of plant extracts and antibiotics on Staphylococcus aureus strains isolated from clinical specimens. *Middle-East Journal of Scientific Research*, 3(3), 134-139.

Adwan, G., Salameh, Y., and Adwan, K. (2011). Effect of ethanolic extract of *Ecballium elaterium* against *Staphylococcus aureus* and *Candida albicans*. *Asian Pacific Journal of Tropical Biomedicine*, 1(6), 456-460.

Afolayan, A.J. (2003). Extracts from the shoots of *Arctotis arctotoides* inhibit the growth of bacteria and fungi. *Journal of Pharmaceutical Biology*, 41(1), 22-25.

Agarry, O.O., and Olaleye, M.T. (2005). Comparative antimicrobial activities of *Aloe vera* gel and leaf. *African journal of Biotechnology*, 4(12), 1410-1413.

Akinyele, B.O., and Odiyi, A.C. (2007). Comparative study of the vegetative morphology and the existing taxonomic status of *Aloe vera* L. *Journal of Plant Sciences*, 2(5), 558-563.

Akiyama, H., Fujii, K., Yamasaki, O., Oono, T., and Iwatsuki, K. (2001). Antibacterial action of several tannins against *Staphylococcus aureus*. *Journal of Antimicrobial Chemotherapy*, 48(4), 487-491.

Akram, M., Shahid, M., and Khan, A.U. (2007). Etiology and antibiotic resistance patterns of community-acquired urinary tract infections in JNMC Hospital Aligarh, India. *Annals of Clinical Microbiology and Antimicrobials*, 6(1), 1- 10.

Alzoreky, N.S., and Nakahara, K. (2003). Antibacterial activity of extracts from some edible plants commonly consumed in Asia. *International Journal of Food Microbiology*, 80(3), 223-230.

Andrade, R.G., Dalvi, L.T., Silva, J.M.C., Lopes, G.K., Alonso, A., and Hermes-Lima, M. (2005). The antioxidant effect of tannic acid on the in vitro copper-mediated formation of free radicals. *Archives of Biochemistry and Biophysics*, 437(1), 1-9.

Anjeza C. and Mandal S. (2012). Synergistic or additive antimicrobial activities of Indian spice and herbal extracts against pathogenic, probiotic and food-sp. *International Food Resources Journal*. 19(3):1185-1191.

Ao, C., Li, A., Elzaawely, A.A., Xuan, T.D., and Tawata, S. (2008). Evaluation of antioxidant and antibacterial activities of *Ficus microcarpa* L. fil. Extract. *Journal of Food Control*, 19(10), 940-948.

Arendrup, M.C., Fuursted, K., Gahrn-Hansen, B., Jensen, I.M., Knudsen, J.D., Lundgren, B., and Tvede, M. (2005). Seminational surveillance of fungemia in Denmark: notably high rates of fungemia and numbers of isolates with reduced azole susceptibility. *Journal of Clinical Microbiology*, 43(9), 4434-4440.

Arunkumar, S., and Muthuselvam, M. (2009). Analysis of phytochemical constituents and antimicrobial activities of *Aloe vera* L. against clinical pathogens. *World Journal of Agricultural Sciences*, 5(5), 572-576.

Asl, M.N., and Hosseinzadeh, H. (2008). Review of pharmacological effects of *Glycyrrhiza* sp. and its bioactive compounds. *Journal of Phytotherapy Research*, 22(6), 709-724.

Atherton, P. (1997). *The essential Aloe vera the actions and evidence*. (2nd Edt). Amazon press, United Kingdom. p. 23-25.

Ayoola, G.A., Coker, H.A., Adesegun, S.A., Adepoju-Bello, A.A., Obaweya, K., Ezennia, E.C., and Atangbayila, T.O. (2008). Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria. *Tropical Journal of Pharmaceutical Research*, 7(3), 1019-1024.

Bakarnga Via I., Yande, H. K., Kouipou, R.M. T., Kanko, M.M., Kammalac T.N., and Boyom, F.F. (2015). Effect of Combined Extracts from Different Plant Parts of *Annona senegalensis* on Antibacterial and Antifungal Activities. *International Journal of Pharmacognosy and Phytochemical Research*, 8(1); 162-166.

Bennish, M.L., Salam, M.A., Hossain, M.A., Myaux, J., Khan, E.H., Chakraborty, J., and Ronsmans, C. (1992). Antimicrobial resistance of *Shigella* isolates in Bangladesh, 1983 - 1990: increasing frequency of strains multiply resistant to ampicillin, trimethoprim-sulfamethoxazole, and nalidixic acid. *Journal of Clinical Infectious Diseases*, 14(5), 1055-1060.

Benthsath, A., Rusznyak, S.T., and Szent-Györgyi, A. (1937). "Vitamin P". *Nature Journals*, 139(3512):326-327.

Blankenship, J.D., Houseknecht, J.B., Pal, S., Bush, L.P., Grossman, R.B., and Schardl, C.L. (2005). Biosynthetic precursors of fungal pyrrolizidines, the loline alkaloids. *Journal of Chemistry and Biochemistry*, 6(6), 1016-1022.

Block, G., Patterson, B., and Subar, A. (1992). Fruit, vegetables, and cancer prevention: a review of the epidemiological evidence. *Journal of Nutrition and Cancer*, 18(1), 1-29.

Braga, L.C., Leite, A.A., Xavier, K.G., Takahashi, J.A., Bemquerer, M.P., Chartone-Souza, E., and Nascimento, A.M. (2005). The synergic interaction between pomegranate extract and antibiotics against *Staphylococcus aureus*. *Canadian Journal of Microbiology*, 51(7), 541-547.

Broussalis, A.M., Ferraro, G.E., Martino, V.S., Pinzón, R., Coussio, J.D., and Alvarez, J.C. (1999). Argentine plants as a potential source of insecticidal compounds. *Journal of Ethnopharmacology*, 67(2), 219-223.

Buckle, G.C., Walker, C.L., and Black, R.E. (2012). Typhoid fever and paratyphoid fever: Systematic review to estimate global morbidity and mortality for 2010. *Journal of Global Health*, 2(1), 10398-10401.

Chanda, S., and Rakholiya, K. (2011). Combination therapy: Synergism between natural plant extracts and antibiotics against infectious diseases. *Microbiology Book Series*, 520-529.

Chung, K.T., Wong, T.Y., Wei, C.I., Huang, Y.W., and Lin, Y. (1998). Tannins and human health: a review. *Critical Reviews in Food Science and Nutrition*, 38(6), 421-464.

Cock, I.E. (2008). Antimicrobial activities of *Aloe barbadensis* miller leaf gel components. *Journal of Microbiology*, (4), 223-235.

Congesta W.T.C. (2005). Preliminary screening of some folklore medicinal plants from a Preliminary screening of some folklore medicinal plants from

Western India for potential antimicrobial activity eastern India for potential antimicrobial activity. *Indian Journal of Pharmacology*, 37(6), 408-409.

Coopoosamy, R.M. (2011). Traditional information and antibacterial activity of four *Bulbine* species (Wolf). *African Journal of Biotechnology*, 10(2), 118-220.

Coopoosamy, R.M., and Magwa, M.L. (2007). Traditional use, antibacterial activity and antifungal activity of crude extract of *Aloe excelsa*. *African Journal of Biotechnology*, 6(20): 8-10.

Coopoosamy, R.M., Magwa, M.L., and Mayekiso, B. (2000). Proceedings: Science and Society University of Fort Hare, Bhisho, Eastern Cape, South Africa.

Cowan, M.M. (1999). Plant products as antimicrobial agents. *Journal of Clinical Microbiology Reviews*, 12(4), 564-582.

Crellin, J.K., and Philpott, J. (1990). A reference guide to medicinal plants: Herbal medicine past and present (p. 551). Durham: Duke University Press.

Cushnie, T.T. and Lamb, A.J. (2014). Alkaloids: an overview of their antibacterial, antibiotic-enhancing and antivirulence activities. *International Journal of antimicrobial agents*, 44(5), 377-386.

Cushnie, T.T., and Lamb, A.J. (2005). Antimicrobial activity of flavonoids. *International Journal of Antimicrobial Agents*, 26(5), 343-356.

Cushnie, T.T., and Lamb, A.J. (2011). Recent advances in understanding the antibacterial properties of flavonoids. *International Journal of Antimicrobial Agents*, 38(2), 99-107.

Cutter, C.N. (2000). Antimicrobial effect of herb extracts against *Escherichia coli* O157: H7, Listeria monocytogenes, and *Salmonella typhimurium* associated with beef. *Journal of Food Protection*, 63(5), 601-607.

Da Silva, J.B., Temponi, V.D.S., Gasparetto, C.M., Fabri, R.L., Aragão, D.M.D. O., Pinto, N.D.C.C., and Alves, M.S. (2013). *Vernonia condensata* Baker (Asteraceae): A promising source of antioxidants. *Journal of Oxidative Medicine and Cellular Longevity*, Article ID 698018.

Das, S., Anjeza, C., and Mandal, S. (2012). Synergistic or additive antimicrobial activities of Indian spice and herbal extracts against pathogenic, probiotic and food-spoiler micro-organisms. *International Food Research Journal*, 19(3), 1185-1191.

Dasgupta, N., Ranjan, S., Saha, P., Jain, R., Malhotra, S., and Arabi Mohamed Saleh, M. A. (2012). Antibacterial activity of leaf extract of Mexican marigold (*Tagetes erecta*) against different gram positive and gram negative bacterial strains. *Journal of Pharmaceutical Research*, 5(8), 4201-4203.

Deshpande, S., Kewatkar, S., and Paithankar, V. (2013). Antimicrobial activity of Saponins rich fraction of *Cassia auriculata Linn* against various microbial strains. *International Current Pharmaceutical Journal*, 2(4), 85-87.

Duprè, I., Zanetti, S., Schito, A.M., Fadda, G., and Sechi, L.A. (2003). The incidence of virulence determinants in clinical *Enterococcus faecium* and *Enterococcus faecalis* isolates collected in Sardinia (Italy). *Journal of Medical Microbiology*, 52(6), 491-498.

Eilers, B., Mayer-Scholl, A., Walker, T., Tang, C., Weinrauch, Y., and Zychlinsky, A. (2010). Neutrophil antimicrobial proteins enhance *Shigella flexneri* adhesion and invasion. *Journal of Cellular Microbiology*, 12(8), 1134-1143.

Eloff, J. N. (1998). A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. *Planta Medica*, 64(8), 711-713.

Eshun, K., and He, Q. (2004). *Aloe vera*: a valuable ingredient for the food, pharmaceutical and cosmetic industries - a review. *Critical Reviews in Food Science and Nutrition*, 44(2), 91-96.

Espinel-Ingroff, A., Canton, E., Peman, J., Rinaldi, M.G., and Fothergill, A.W. (2009). Comparison of 24-hour and 48-hour voriconazole MICs as determined by the Clinical and Laboratory Standards Institute broth microdilution method (M27-A3 document) in three laboratories: results obtained with 2,162 clinical isolates of *Candida* sp. and other yeasts. *Journal of Clinical Microbiology*, 47(9), 2766-2771.

Everett, T. H. (1982). The New York botanical garden illustrated encyclopedia of horticulture (Vol. 10). Taylor & Francis.

Fabricant, D.S., and Farnsworth, N.R. (2001). The value of plants used in traditional medicine for drug discovery. *Journal of Environmental Health Perspectives*, 109(Suppl 1), 65-69.

Facon, M.J., and Skura, B.J. (1996). Antibacterial activity of lactoferricin, lysozyme and EDTA against *Salmonella enteritidis*. *International Dairy Journal*, 6(3), 303-313.

Faulkner, J.R., Hussaini, S.R., Blankenship, J.D., Pal, S., Branan, B.M., Grossman, R.B., and Schardl, C.L. (2006). On the sequence of bond formation in loline alkaloid biosynthesis. *Journal of Chemistry and Biochemistry*, 7(7), 1078-1088.

Fennell, C.W., Lindsey, K.L., McGaw, L.J., Sparg, S.G., Stafford, G.I., Elgorashi, E.E., and Van Staden, J. (2004). Assessing African medicinal plants for efficacy and safety:pharmacological screening and toxicology. *Journal of Ethnopharmacology*, 94(2), 205-217.

Ferro, V.A., Bradbury, F., Cameron, P., Shakir, E., Rahman, S.R., and Stimson, W.H. (2003). In vitro susceptibilities of *Shigella flexneri* and *Streptococcus pyogenes* to the inner gel of *Aloe barbadensis* Miller. *Journal of Antimicrobial agents and chemotherapy*, 47(3), 1137-1139.

Freiburghaus, F., Kaminsky, R., Nkunya, M.H.H., and Brun, R. (1996). Evaluation of African medicinal plants for their in vitro trypanocidal activity. *Journal of Ethnopharmacology*, 55(1), 1-11.

Fullerton, M., Khatiwada, J., Johnson, J.U., Davis, S., and Williams, L.L. (2011). Determination of the antimicrobial activity of sorrel (*Hibiscus sabdariffa*) on *Escherichia coli* O157: H7 isolated from food, veterinary, and clinical samples. *Journal of Medicinal Food*, 14(9), 950-956.

Galeotti, F., Barile, E., Curir, P., Dolci, M., and Lanzotti, V. (2008). Flavonoids from carnation (*Dianthus caryophyllus*) and their antifungal activity. *Journal of Phytochemistry Letters*, 1(1), 44-48.

Garba, S., and Okeniyi, S.O. (2012). Antimicrobial activities of total alkaloids extracted from some Nigerian medicinal plants. *Journal of Microbiology and Antimicrobial Agents*, 4(3), 60-63.

García-Sosa, K., Villarreal-Alvarez, N., Lübben, P., and Peña-Rodríguez, M.L. (2006). Chrysophanol, an antimicrobial anthraquinone from the root extract of *Colubrina greggii*. *Journal of Mexican Chemotherapy Society*, 50(2), 76-78.

Gedir, J.V., Sporns, P., and Hudson, R.J. (2005). Extraction of condensed tannins from cervid feed and faeces and quantification using a radial diffusion assay. *Journal of Chemical Ecology*, 31(12), 2761-2773.

Ghoshal, S., Prasad, B.K., and Lakshmi, V. (1996). The antiamoebic activity of *Piper longum* fruits against *Entamoeba histolytica in vitro* and *in vivo*. *Journal of Ethnopharmacology*, 50(3), 167-170.

Green, M.M., Singer, J.M., Sutherland, D.J., and Hibben, C.R. (1991). Larvicidal activity of *Tagetes minuta* (marigold) toward *Aedes aegypti*. *Journal of the American Mosquito Control Association*, 7(2), 282-286.

Hanafy, M.S., Shalaby, S.M., El-Fouly, M.A., Abd, E.A.M., and Soliman, F.A. (1994). Effect of garlic on lead contents in chicken tissues. *DTW. Deutsche Tierarztliche Wochenschrift*, 101(4), 157-158.

Hendricks, L., and Wright, N. (1979). Diagnosis of cutaneous leishmaniasis by in vitro cultivation of saline aspirates in Schneider's *Drosophila* Medium. *The American Journal of Tropical Medicine and Hygiene*, 28(6), 962-964.

Hill, A.F. (1952). Economic Botany: *A Textbook of Useful Plants and Plants Products* (No. SB103. H54 1937).

Holzmuller, P., Sereno, D., Cavaleyra, M., Mangot, I., Daulouede, S., Vincendeau, P., and Lemesre, J.L. (2002). Nitric oxide-mediated proteasome-dependent oligonucleosomal DNA fragmentation in *Leishmania amazonensis* amastigotes. *Journal of Infection and immunity*, 70(7), 3727-3735.

http://www.traffic.org/medicinal-plants (30th May 2014)

http://www.microbiologyinfo.com/biochemical test (15th June 2014)

Husein, A.I., Ali-Shtayeh, M.S., Jamous, R.M., Zaitoun, S.Y.A., Jondi, W.J., and Zatar, N.A.A. (2014). Antimicrobial activities of six plants used in Traditional Arabic Palestinian Herbal Medicine. *African Journal of Microbiology Research*, 8(38), 3501-3507.

Ibarra, J.A., and Steele-Mortimer, O. (2009). *Salmonella*-the ultimate insider. *Salmonella* virulence factors that modulate intracellular survival. *Journal of Cellular Microbiology*, 11(11), 1579-1586.

Ibrahim, H.A., Imam, I.A., Bello, A.M., Umar, U., Muhammad, S., and Abdullahi, S.A. (2012). The potential of Nigerian medicinal plants as antimalarial agent: A review. *International Journal of Science and Technology*, 2(8), 600-605.

Ijeh, I.I., and Ejike, C.E. (2011). Current perspectives on the medicinal potential of *Vernonia amygdalina Del*. *Journal of Medicinal Plants Research*. 5(7):1051 - 1061.

Induli, M., Cheloti, M., Wasuna, A., Wekesa, I., Wanjohi, J. M., Byamukama, R., and Yenesew, A. (2012). Naphthoquinones from the roots of *Aloe secundiflora*. *Journal of Phytochemistry Letters*, 5(3), 506-509.

Joshi, N.U.P.U.R., Bhatt, S.H.A.S.H.A.N.K., Dhyani, S., and Nain, J.Y.O.T.I. (2013). Phytochemical screening of secondary metabolites of *Argemone mexicana* Linn. Flowers. *International Journal of Current Pharmaceutical Research*, 5(2), 144-147.

Kaingu, F., Kibor, A., Waihenya, R., Shivairo, R., and Mungai, L. (2013). Efficacy of *Aloe secundiflora* crude extracts on *Ascaridia galli* in vitro. *Journal of Sustainable Agriculture Research*, 2(2), 45-49.

Kaithwas, G., Kumar, A., Pandey, H., Acharya, A. K., Singh, M., Bhatia, D., and Mukerjee, A. (2008). Investigation of comparative antimicrobial activity of *Aloe vera* gel and juice. *Pharmacologyonline*, 1, 239-243.

Kambizi, L., and Afolayan, A.J. (2001). An ethnobotanical study of plants used for the treatment of sexually transmitted diseases (njovhera) in Guruve District, Zimbabwe. *Journal of Ethnopharmacology*, 77(1), 5-9.

Kanth, S.V., Venba, R., Madhan, B., Chandrababu, N.K., and Sadulla, S. (2009). Cleaner tanning practices for tannery pollution abatement: the role of enzymes in eco-friendly vegetable tanning. *Journal of Cleaner Production*, 17(5), 507-515.

Kaper, J.B., Nataro, J.P., and Mobley, H.L. (2004). Pathogenic *Escherichia coli*. *Journal of Nature Reviews Microbiology*, 2(2), 123-140.

Kareru, P.G., Gachanja, A.N., Keriko, J.M., and Kenji, G.M. (2008). Antimicrobial activity of some medicinal plants used by herbalists in the eastern province, Kenya. *African Journal of Traditional, Complementary and alternative medicines*, 5(1), 51-55.

Keeley, S.C., Forsman, Z.H., and Chan, R. (2007). A phylogeny of the "evil tribe" (Vernonieae: Compositae) reveals Old/New World long distance dispersal: Support from separate and combined congruent data sets (trnL-F, ndhF, ITS). *Molecular Phylogenetics and Evolution*, 44(1), 89-103.

Kelmanson, J.E., Jäger, A.K., and van Staden, J. (2000). Zulu medicinal plants with antibacterial activity. *Journal of Ethnopharmacology*, 69(3), 241-246.

Kigondu, E.V., Rukunga, G.M., Keriko, J.M., Tonui, W.K., Gathirwa, J.W., Kirira, P.G., and Ndiege, I.O. (2009). Antiparasitic activity and cytotoxicity of selected medicinal plants from Kenya. *Journal of Ethnopharmacology*, 123(3), 504-509.

Kinyamasyo, E.M., Mwagandi, C.L., Kaingu, F.B., and Gicharu, G.K. (2014). In Vitro Analysis of Antibacterial and Antifungal Potency of Tissue Cultured and Indigenous *Aloe Secundiflora* Plant Extracts. *Journal of Agriculture and Environmental Sciences*, 3(2), 53-64.

Kittakoop, P., Mahidol, C., and Ruchirawat, S. (2014). Alkaloids as important scaffolds in therapeutic drugs for the treatments of cancer, tuberculosis, and smoking cessation. *Journal of Current Topics in Medicinal Chemistry*, 14(2), 239-252.

Kokwaro, J. O. (2009). *Medicinal plants of east Africa*. University of Nairobi Press.

Kotloff, K.L., Winickoff, J.P., Ivanoff, B., Clemens, J.D., Swerdlow, D.L., Sansonetti, P.J., and Levine, M.M. (1999). Global burden of *Shigella* infections: implications for vaccine development and implementation of control strategies. *Bulletin of the World Health Organization*, 77(8), 651-666.

Koul, J.L., Koul, S., Singh, C., Taneja, S.C., Shanmugavel, M., Kampasi, H., and Qazi, G.N. (2003). In vitro cytotoxic elemanolides from *Vernonia lasiopus*. *Planta Medica*, 69(2), 164-166.

Krishnaraju, A.V., Rao, T.V., Sundararaju, D., Vanisree, M., Tsay, H.S., and Subbaraju, G.V. (2005). Assessment of bioactivity of Indian medicinal plants using brine shrimp (*Artemia salina*) lethality assay. *International Journal for Applied Sciences Engineering*, 3(2), 125-34.

Kwon, Y.I., Apostolidis, E., Labbe, R.G., and Shetty, K. (2007). Inhibition of *Staphylococcus aureus* by phenolic phytochemicals of selected clonal herbs species of *Lamiaceae* family and likely mode of action through proline oxidation. *Journal of Food Biotechnology*, 21(1), 71-89.

Li, R., Wang, M.Y., and Li, X.B. (2012). Chemical constituents and biological activities of genus Hosta (Liliaceae). *Journal of Medicinal Plants Research*, 6(14), 2704-2713.

Lichterman, B.L. (2004). Book: aspirin: the story of a wonder drug. *BMJ: British Medical Journal*, 329(7479), 1404-1408.

Lopes, G.K., Schulman, H.M., and Hermes-Lima, M. (1999). Polyphenol tannic acid inhibits hydroxyl radical formation from Fenton reaction by complexing ferrous ions. *Biochimica et Biophysica Acta (BBA)-General Subjects*, 1472(1), 142-152.

López, M.L., Bonzani, N.E., and Zygadlo, J.A. (2008). Allelopathic potential of *Tagetes minuta* terpenes by a chemical, anatomical and phytotoxic approach. *Biochemical Systematics and Ecology*, 36(12), 882-890.

Maatalah, M.B., Bouzidi, N.K., Bellahouel, S., Merah, B., Fortas, Z., Soulimani, R., and Derdour, A. (2012). Antimicrobial activity of the alkaloids and saponin extracts of *Anabasis articulate*. *Journal of Biotechnology Pharmaceutical Research*, 3(3), 54-57.

Mandel, M.J., and Silhavy, T.J. (2005). Starvation for different nutrients in *Escherichia coli* results in differential modulation of RpoS levels and stability. *Journal of Bacteriology*, *187*(2), 434-442.

Mangoma, N., Saidi, B., and Mbanga, J. (2010). An evaluation of the antimicrobial activities of *Aloe barbadensis*: *A. chabaudii* and *A. arborescens* leaf extracts used in folklore veterinary medicine in Zimbabwe. *Journal of Animal and Veterinary Advances*. 9(23):2918-2923.

Manner, S., Skogman, M., Goeres, D., Vuorela, P., and Fallarero, A. (2013). Systematic exploration of natural and synthetic flavonoids for the inhibition of *Staphylococcus aureus* biofilms. *International Journal of Molecular Sciences*, 14(10), 19434-19451.

Mariita, R.M., Orodho, J.A., Okemo, P.O., Kirimuhuzya, C., Otieno, J.N., and Magadula, J.J. (2011). Methanolic extracts of *Aloe secundiflora* Engl. inhibits in vitro growth of tuberculosis and diarrhea-causing bacteria. *Journal of Pharmacognosy Research*, 3(2), 90-95.

Maurya, A., Chauhan, P., Mishra, A., and Pandey, A.K. (2012). Surface functionalization of TiO2 with plant extracts and their combined antimicrobial activities against *E. faecalis* and *E. coli*. *Journal of Research Updates in Polymer Science*, 1(1), 39-43.

McNaught, A.D. (1997). Compendium of chemical terminology (**Vol. 1669**). Oxford: Blackwell Science.

Mohanta, B., Chakraborty, A., Sudarshan, M., Dutta, R., and Baruah, M. (2003). Elemental profile in some common medicinal plants of India. Its correlation with traditional therapeutic usage. *Journal of Radioanalytical and Nuclear Chemistry*, 258(1), 175-179.

Msoffe, P., and Mbilu, Z. (2009). The efficacy of a crude extract of *Aloe secundiflora* on *Candida albicans. African Journal of Traditional, Complementary and Alternative Medicines*, 6(4), 592-595.

Murray, B. E. (1990). The life and times of the *Enterococcus*. *Journal of Clinical Microbiology Reviews*, 3(1), 46-65.

Mwale, M., Bhebhe, E., Chimonyo, M., and Halimani, T.E. (2005). Use of herbal plants in poultry health management in the Mushagashe small-scale commercial farming area in Zimbabwe. *International Journal of Applied Research in Veterinary Medicine*, 3(2), 163-170.

Nascimento, G.G., Locatelli, J., Freitas, P.C., and Silva, G.L. (2000). Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. *Brazilian Journal of Microbiology*, 31(4), 247-256.

Newall, C.A., Anderson, L.A., and Phillipson, J.D. (1996). Herbal medicines. The Pharmaceutical Press London, p. 25.

Nickerson, E.K., West, T.E., Day, N.P., and Peacock, S.J. (2009). *Staphylococcus aureus* disease and drug resistance in resource-limited countries in South and East Asia. *The Lancet Infectious Diseases*, 9(2), 130-135.

Okwu, D.E. (2004). Phytochemicals and vitamin content of indigenous spices of South Eastern Nigeria. *Journal for Sustainable Agriculture Environment*, 6(1), 30-37.

Olajuyigbe, O.O., and Afolayan, A.J. (2012). Synergistic interactions of methanolic extract of *Acacia mearnsii* De Wild. with antibiotics against bacteria of clinical relevance. *International Journal of Molecular Sciences*, 13(7), 8915-8932.

Omulokoli, E., Khan, B., and Chhabra, S.C. (1997). Antiplasmodial activity of four Kenyan medicinal plants. *Journal of Ethnopharmacology*, 56(2), 133-137.

Palpasa, K., Pankaj, B., Sarala, M., and Gokarna, R.G. (2011). Antimicrobial resistance surveillance on some bacterial pathogens in Nepal: A technical cooperation. *Journal of Infectious Diseases in Developing Countries*, 5, 163-168.

Panwar, D., and Bhatt, R.P. (2014). Antibacterial activity of *Tagetes minuta* l. against *Staphylococcus aureus* and *Streptococcus pyrogenes. Journal of Pharmaceutical Biology*, 4(2), 109-118.

Park, Y.I., and Jo, T.H. (2006). The perspective of industrial application of *Aloe vera*. In *New Perspectives on Aloe* (pp. 191-200). Springer U.S.A.

Pérez-Vázquez, M., Vindel, A., Marcos, C., Oteo, J., Cuevas, O., Trincado, P., and Campos, J. (2009). The spread of invasive Spanish *Staphylococcus aureus* spa-type t067 associated with a high prevalence of the aminoglycoside-modifying enzyme gene ant (4′)-Ia and the efflux pump genes msrA/msrB. *Journal of Antimicrobial Chemotherapy*, 63(1), 21-31.

Pfaller, M.A., Messer, S.A., Hollis, R.J., Boyken, L., Tendolkar, S., Kroeger, J., and Diekema, D.J. (2009). Variation in susceptibility of bloodstream isolates of *Candida glabrata* to fluconazole according to patient age and geographic location in the United States in 2001 to 2007. *Journal of Clinical Microbiology*, 47(10), 3185-3190.

Phillipson, J.D., and O Neill, M.J. (1989). New leads to the treatment of protozoal infections based on natural product molecules. *Acta Pharmaceutica Nordica*, 1(3), 131-144.

Piccaglia, R., Marotti, M., Pesenti, M., Mattarelli, P., and Biavati, B. (1997). Chemical composition and antimicrobial activity *of Tagetes erecta and Tagetes patula,* in Essential Oils. *Journal of Basic and Applied Research.* p. 49 - 51.

Pramila, D.M., Xavier, R., Marimuthu, K., Kathiresan, S., Khoo, M.L., Senthilkumar, M., and Sreeramanan, S. (2012). Phytochemical analysis and antimicrobial potential of methanolic leaf extract of peppermint (*Mentha piperita*: Lamiaceae). *Journal of Medicinal Plant Research*, 6(2), 331-335.

Qu, F., Bao, C., Chen, S., Cui, E., Guo, T., Wang, H., and Mao, Y. (2012). Genotypes and antimicrobial profiles of *Shigella sonnei* isolate from diarrheal patients circulating in Beijing between 2002 and 2007. *Diagnostic Microbiology and Infectious Disease*, 74(2), 166-170.

Rabe, T., Mullholland, D., and Van Staden, J. (2002). Isolation and identification of antibacterial compounds from *Vernonia colorata* leave. *Journal of Ethnopharmacology*, 80(1), 91-94.

Ramakrishnan, K., Selvi, S.R., and Shubha, R. (2006). Tannin and its analytical techniques. *Indian Journal of Chemical Engineering*, 48(2), 88.

Ramesh, P., and Okigbo, R.N. (2008). Effects of plants and medicinal plant combinations as anti-infectives. *African Journal of Pharmacy and Pharmacology*, 2(7), 130-135.

Reynolds, T., and Dweck, A.C. (1999). *Aloe vera* leaf gel: a review update. *Journal of Ethnopharmacology*, 68(1), 3-37.

Riguera, R. (1997). Isolating bioactive compounds from marine organisms. *Journal of Marine Biotechnology*, 5, 187-193.

Rivas, J.D. (1991). Reversed-phase high-performance liquid chromatographic separation of lutein and lutein fatty acid esters from marigold flower petal powder. *Journal of Chromatography A*, 464, 442-447.

Robson, M.C., Heggers, J.P., and Hagstrom Jr, W.J. (1982). Myth, magic, witchcraft, or fact? *Aloe vera* revisited. *Journal of Burn Care & Research*, 3(3), 154-163.

Rupasinghe, H.V., Jackson, C.J.C., Poysa, V., Di Berardo, C., Bewley, J.D., and Jenkinson, J. (2003). Soyasapogenol A and B distribution in soybean (Glycine max L. Merr.) in relation to seed physiology, genetic variability, and growing location. *Journal of Agricultural and Food Chemistry*, 51(20), 5888-5894.

Ryan, K.J., and Ray, C.G. (2004). *Medical Microbiology* (pp. 362-368). McGraw-Hill.

Sardi, J.C.O., Almeida, A.M.F., and Giannini, M.J.S.M. (2011). New antimicrobial therapies used against fungi present in subgingival sites; a brief review. *Archives of Oral Biology*, 56(10), 951-959.

Schardl, C.L., Grossman, R.B., Nagabhyru, P., Faulkner, J.R., and Mallik, U.P. (2007). Loline alkaloids: currencies of mutualism. *Journal of Phytochemistry*, 68(7), 980-996.

Servet, Ç.E.T.E., Arslan, F., and Yasar, A. (2005). Investigation of antimicrobial effects against some microorganisms of *Aloe vera* and *Nerium oleander* also an examination of the effects on the xanthine oxidase activity in liver tissue treated with cyclosporin. *Gazi University Journal of Science*, 18(3), 375-380.

Shahzadi, I., Hassan, A., Khan, U.W., and Shah, M.M. (2010). Evaluating biological activities of the seed extracts from *Tagetes minuta* L. found in Northern Pakistan. *Journal of Medicinal Plants Research*, 4(20), 2108-2112.

Shan, B., Cai, Y.Z., Brooks, J.D., and Corke, H. (2007). The in vitro antibacterial activity of dietary spice and medicinal herb extracts. *International Journal of Food Microbiology*, 117(1), 112-119.

Shao, L.C., Sheng, C.Q., and Zhang, W.N. (2007). Recent advances in the study of an antifungal lead compound with new chemical scaffolds. *Acta Pharmaceutica Sinica*, 42(11), 1129-1136.

Skene, C.D., and Sutton, P. (2006). Saponin-adjuvanted particulate vaccines for clinical use. *Methods*, 40(1), 53-59.

Soule, J A. (1993). *Tagetes minuta*: A potential new herb from South America. *New Crops. Wiley*, New York, 649-654.

Soule, J.A. (1994). Infrageneric systematics of *Tagetes*. In *Compositae: systematics. Proceedings of the International Compositae Conference, Kew*, **Vol. 1**, pp. 435-443.

Srivastava, J., Lambert, J., and Vietmeyer, N. (2005). Medicinal plants: An expanding role in from Western India for potential antimicrobial activity. *Indian Journal of Pharmacology*, 37, 406 - 409.

Stafford, G.I., Jager, A.K., and Van Staden, J. (2005). Effect of storage on the chemical composition and biological activity of several popular South African medicinal plants. *Journal of Ethnopharmacology*, 97(1), 107-115.

Tahir, L., and Khan, N. (2012). Antibacterial potential of crude leaf, fruit and flower extracts of *Tagetes Minuta* L. *Journal of Public Health and Biological Sciences*, 1, 70-74.

Talmadge, J., Chavez, J., Jacobs, L., Munger, C., Chinnah, T., Chow, J.T., and Yates, K. (2004). Fractionation of *Aloe vera* L. inner gel, purification and molecular profiling of activity. *International Journal of Immunopharmacology*, 4(14), 1757-1773.

Tereschuk, M.L., Riera, M.V., Castro, G.R., and Abdala, L.R. (1997). Antimicrobial activity of flavonoids from leaves of *Tagetes minuta*. *Journal of Ethnopharmacology*, 56(3), 227-232.

Toyang, N.J., and Verpoorte, R. (2013). A review of the medicinal potentials of plants of the genus *Vernonia* (Asteraceae). *Journal of Ethnopharmacology*, 146(3), 681-723.

Van Staden, L.F., and Drewes, S.E. (1994). Knipholone from *Bulbine latifolia* and *Bulbine frutescens*. *Journal of Phytochemistry*, 35(3), 685-686.

Van Wyk, B.E. (2008). A broad review of commercially important southern African medicinal plants. *Journal of Ethnopharmacology*, 119(3), 342-355.

Van Wyk, B.E., and Gericke, N. (2000). People's plants: A guide to useful plants of Southern Africa. Briza Publications.

Van Wyk, B.E., Oudtshoorn, B.V., and Gericke, N. (1997). Medicinal Plants of South Africa. Briza.

Vankerckhoven, V., Van Autgaerden, T., Vael, C., Lammens, C., Chapelle, S., Rossi, R., and Goossens, H. (2004). Development of a multiplex PCR for the detection of asa1, gelE, cylA, esp, and hyl genes in *Enterococci* and survey for virulence determinants among European hospital isolates of *Enterococcus faecium*. *Journal of Clinical Microbiology*, 42(10), 4473-4479.

Ververidis, F., Trantas, E., Douglas, C., Vollmer, G., Kretzschmar, G., and Panopoulos, N. (2007). Biotechnology of flavonoids and other phenylpropanoid-derived natural products. Part I: Chemical diversity, impacts on plant biology and human health. *Journal of Biotechnology*, 2(10), 1214-1234.

Waihenya, R.K., Mtambo, M.M.A., and Nkwengulila, G. (2002). Evaluation of the efficacy of the crude extract of *Aloe secundiflora* in chickens experimentally infected with Newcastle disease virus. *Journal of Ethnopharmacology*, 79(3), 299-304.

Watson, K.G., and Holden, D.W. (2010). Dynamics of growth and dissemination of *Salmonella* in vivo. *Journal of Cellular Microbiology*, 12(10), 1389-1397.

White, T.C., Marr, K.A., and Bowden, R.A. (1998). Clinical, cellular, and molecular factors that contribute to antifungal drug resistance. *Journal of Clinical Microbiology Reviews*, 11(2), 382-402.

WHO (1999). WHO *monographs on selected medicinal plants*, world health organization, Geneva. **Vol.1** .ISBN 9241545178.

Wu, Y.W., Ouyang, J., Xiao, X.H., Gao, W.Y., and Liu, Y. (2006). Antimicrobial properties and toxicity of anthraquinones by microcalorimetric bioassay. *Chinese Journal of Chemistry*, 24(1), 45-50.

Yakubu, M.T., and Afolayan, A.J. (2009). Effect of aqueous extract of *Bulbine natalensis* (Baker) stems on the sexual behaviour of male rats. *International Journal of Andrology*, 32(6), 629-636.

Yokosuka, A., and Mimaki, Y. (2009). Steroidal saponins from the whole plants of *Agave utahensis* and their cytotoxic activity. *Journal of Phytochemistry*, 70(6), 807-815.

Zamora-Ros, R., Agudo, A., Luján-Barroso, L., Romieu, I., Ferrari, P., Knaze, V., and Sánchez-Cantalejo, E. (2012). Dietary flavonoid and lignan intake and gastric adenocarcinoma risk in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. *The American Journal of Clinical Nutrition*, 96(6), 1398-1408.

Zhao, D.Q., Han, C.X., Ge, J.T., and Tao, J. (2012). Isolation of a UDP-glucose: Flavonoid 5-O-glucosyltransferase gene and expression analysis of anthocyanin biosynthetic genes in herbaceous peony (*Paeonia lactiflora* Pall.). *Electronic Journal of Biotechnology*, 15(6), 9-9.

Ziarlarimi, A., Irani, M., Gharahveysi, S., and Rahmani, Z. (2013). Investigation of antibacterial effects of garlic (*Allium sativum*), mint (Menthe spp.) and onion (*Allium cepa*) herbal extracts on *Escherichia coli* isolated from broiler chickens. *African Journal of Biotechnology*, 10(50), 10320-10322.