ANTIMICROBIAL ACTIVITY, TOXICITY AND PHYTOCHEMICAL ANALYSIS OF FOUR MEDICINAL PLANTS TRADITIONALLY USED IN MSAMBWENI DISTRICT, KENYA

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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 family members.

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LIST OF ABBREVIATIONS

ANOVA: Analysis of Variance

ATCC: American Type Culture Collection

BSLB: Brine Shrimp Lethality Bioassay

CMR: Centre for Microbiology Research

CLSI: Clinical and Laboratory Standards Institute

$CHCl_3$: Chloroform

DMSO Dimethylsulphoxide

DPHPT: Department of Public Health, Pharmacology and Toxicology

H_2SO_4: Sulphuric acid

KEMRI: Kenya Medical Research Institute

LC_{50}: Lethal Concentration 50

MIC: Minimum Inhibitory Concentration

MRSA: Methicillin Resistant *Staphylococcus aureus*

MeOH: Methanol

SPSS: Statistical Package for the Social Sciences

TLC: Thin Layer Chromatography

UoN: University of Nairobi

UV : Ultra Violet Light

WHO: World Health Organization

ABSTRACT

Introduction: Antimicrobial resistance to currently utilized drugs has caused major challenges in both the agricultural and medical sectors in the past years. This resistance has not only hampered the treatment of microbial diseases, but has also increased the cost of treatment which is especially burdening third world countries. Therefore, there is need to find alternative methods of curbing the infectious microorganisms.

Methodology: The current study was designed to evaluate the antimicrobial activity, toxicity and phytochemical composition of organic and aqueous crude extracts from each plant were were evaluated for their *in vitro* antimicrobial activity against methicillin resistant *Staphylococcus aureus (*MRSA*)*, *Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa* and *Candida albicans* using broth dilution and disc diffusion methods. Toxicity was determined using Brine-shrimp larvae assay while the presence of flavonoids, alkaloids, saponins and sesquiterpene lactones was determined using Thin Layer Chromatography (TLC). Analysis of variance (ANOVA) was used to analyse the disc diffusion results. Finney's computer programme was used to determine the LDC_{50} of each extract.

Results: It was observed that the organic crude extracts of all the tested plants except *L. cornuta* showed activity against four micro-organisms and at concentrations lower than the aqueous crude extracts. None of the crude extracts elicited any inhibition against *E. coli. Adansonia digitata* and *G. trichocarpa* had LC_{50}>1000 µg/ml and were shown to be non-toxic to Brine shrimp larvae unlike those of *Z. chalybeum* and *L. cornuta* which both had LC_{50}<500 ug/ml and were considered to be toxic. Phytochemical screening of the crude extracts showed that alkaloids, flavonoids, sesquiterpene lactones and saponins were present in the four plants under investigation.

Conclusion: This study provides the first record of antimicrobial activity, toxicity and phytochemical composition of *G. trichocarpa*. The study has shown that *A. digitata* and *Z. chalybeum* possess promising antimicrobial activity against microbes of health importance and could lead to the isolation of new and effective antimicrobial compounds. Further research should be carried on *Z. chalybeum* and *A. digitata* to isolate and characterize the compounds responsible for the observed activity.

Key words: Antimicrobial activity, *Adansonia digitata*, *Launaea cornuta, Zanthoxylum chalybeum, Grewia trichocarpa*, Brine shrimp lethality test, Phytochemical analysis,

CHAPTER ONE: INTRODUCTION

A two-fold increase in microbial resistance against antibiotics has developed over the last forty years in both medical and livestock sector (Daboor and Haroon, 2012). Microbial infections have caused a big burden of diseases and bacteria are listed in the first position among common microorganisms responsible for opportunistic diseases associated with HIV/AIDS (Rathee *et al*., 2012). Increased antibiotic resistance has become a global concern, coupled with the problem of microbial persistence, thus highlighting the need to develop novel microbial drugs that are not only active against drug resistant microbes, but more importantly, kill persistent micro-organisms and shorten the length of treatment (Mariita *et al*., 2010). People in various parts of Africa have since time immemorial have used plant extracts to treat themselves together with their livestock whenever they became sick or injured in their environment (Kokwaro, 2009). According to World Health Organisation (2001), 80% of African and Asian populations depend on traditional medicine particulary plant biodiversity for primary health care because plant derived medicines are relatively cheaper and safer compared to the synthetic alternatives. Many commercial drugs used in modern medicine are derived from plants following ethno-botanical and ethno-medical knowledge (Arokiyaraj *et al*., 2012). Medicinal plants have bioactive compounds which treat various ailments caused by microorganisms. These compounds may have evolved in plants as self defence against pests and pathogens to help plants to establish themselves in their environment (Sukumaran *et al*., 2011).

The World Health Organisation (WHO, 2001) has advocated the use of traditional medicine as a safe remedy for ailments of microbial and non-microbial origin. Plant derived secondary metabolites such as alkaloids, flavonoids, phenolics, saponins, and tannins protect plants from invaders such as fungi, bacteria, viruses, and nematodes. It is estimated that among the traditional medicinal plants, only 12,000 or less than10% have undergone pharmacological

evaluation (Marimuthu *et al*., 2011). It is therefore possible that many plants not yet validated may be the source of drugs that may be effective against resistant pathogens to avoid the threat of post biotic era (Marimuthu *et al.,* 2011). The importance of a country's diverse medicinal plants lies not only in its chemotherapeutic value in traditional medicine but also in its potential in drug discovery and this prompts a need to research on Kenyan medicinal plants (Vital and Rivera, 2011). Traditional medicine is widely practiced in Kenya and about 400 plant species have been recorded to be used in traditional remedies (Kokwaro, 2009). In the rural areas, reliance on traditional medicine is high and is attributed to both economic and cultural factors (Aketch, 1992).

Plants have toxic effects on livestock and humans, such toxicity may lead to death of an animal or humans consuming the plant (Hood, 2009). In traditional medicine, there is likelihood to overdose the patient due to imprecise nature of diagnosis and dosage not only in Kenya but worldwide. This is not only unique to traditional medicine but can also occur in modern medicine (Kokwaro, 2009). Toxicity is attributed to certain active principles found in plants; these chemical substances interact with living systems and affect normal processes. All chemicals can cause harm to organisms at some level of exposure. Toxicity tests are important so as to determine the lethality of drugs and to determine the harmless concentration of drugs for consumption (Hood, 2009). Biological activity of plants is attributed to the class and concentration of phytochemical constituents which makes some plant extracts exhibit a variety of activities (Wang *et al*., 2010). Phytochemical screening of plant extracts especially those which have been used in traditional medicine is therefore essential so as to identify phytochemical constituents in the plants that are responsible for a given bioactivity. The current study investigated the antimicrobial activity, toxicity and phytochemical composition of *Adansonia digitata, Zanthoxylum chalybeum, Launaea cornuta and Grewia trichocarpa* traditionally used in Msambweni ethnomedicine.

CHAPTER TWO: LITERATURE REVIEW

2.1. Microbial infections and their health effects

Microbial infections have caused a big burden of diseases and bacteria are listed in the first position among common microorganisms responsible for opportunistic diseases associated with HIV/AIDS (Rathee *et al*., 2012).In developing countries, bacterial infections are prevalent due to factors such as poor hygiene, sanitation and overcrowding in the living conditions (Rosoanaivo and Ratsimamanga-Urveg, 1993). Throughout history, infectious diseases have been a major threat to human and animal health and a prominent cause of morbidity and mortality, WHO, (2003). Increased antibiotic resistance has become a global concern, coupled with the problem of microbial persistence, thus highlighting the need to develop novel microbial drugs that are not only active against drug resistant microbes, but more importantly, kill persistent micro-organisms and shorten the length of treatment. Apart from toxicity, lengthy therapy also creates poor patient compliance (Mariita *et al*., 2010).

2.2. Characteristics of Various Microbial Pathogens

2.2.1. Methicillin (ATCCMP-2) resistant *Staphylococcus aureus* (MRSA)

Staphylococcus aureus is a facultative anaerobic Gram positive bacterium belonging to the family Staphylococcaceae and is frequently found in the human respiratory tract and on the skin. It is a non motile and non spore forming bacteria that appears in grape-like clusters with large, yellow colonies and reproduces asexually by binary fission (Nizet and Bradley, 2011). It is a versatile organism and a source of a wide range of infectious diseases in humans such as life-threatening deep seated infections like bacteremia, pneumonia and meningitis (Kanafani and Fowler Jr., 2006). In burn patients, wound sepsis remains a major cause of death due to colonisation with methicillin resistant *S. aureus* on the surface of burn wounds which may go on to cause systemic infections and other serious clinical complications. *S. aureus* resistance

against methicillin was first reported in 1960s soon after introduction of penicillinase-resistant ß-lactam antibiotics methicillin (Cook *et al*., 1998). In addition to their resistance against all ß-lactam antibiotic, methicillin resistant *S.aureus* strains are also resistant to several other classes of antibiotics including the Aminoglycosides, Quinolones, Clindamycin and Erythromycin. There are several mechanisms responsible for the resistance, the major one being production of penicillin-binding protein *2a* encoded by *mec*A gene, two additional mechanisms are hyper-production of penicillinase and modified affinity of usual penicillin-binding proteins (Durmaz *et al.,* 1997).

2.2.2. *Bacillus cereus*

B. cereus is a Gram positive, endospore-forming, facultative, rod shaped motile bacterium (Pirtijärvi *et al*., 1999). It is found widely distributed in nature including mucous membrane and faeces of healthy humans (Williams, 1986). *B. cereus* causes food poisoning of two types characterized by abdominal pain and diarrhea for 8-16 hours or by nausea and vomiting 1-5 hrs after ingestion of contaminated food (Asano *et al*., 1997; Gilbert, 1979). Infection of central nervous system is rare but it is associated with immunosuppression and central nervous system invasive devices (Drobniewski, 1993). Some *B. cereus* isolates according to Waber *et al*. (1988) are susceptible *in vitro* to vancomycin, gentamicin, clindamycin, erythromycin and chloramphenicol.

2.2.3. *Escherichia coli*

It is a facultative anaerobic, Gram- negative, non- spore forming, rod-shaped bacterium that can be a commensal or a deadly pathogen in the lower intestine of warm blooded organisms (Kaper *et al*., 2004). Enteropathogenic *E. coli* causes watery diarrhea which in serious cases resemble classic cholera among infants (Cravioto *et al*., 1991). According to Čížek *et al*.

(2008), some isolates are resistant to chloramphenicol, sulphonamide and trimethoprin-sulphomethoxale combination.

2.2.4. *Pseudomonas aeruginosa*

It is a Gram- negative bacterium occurring as short chains, pairs or singly with an optimum growth temperature of 37^0c. It is widely found in moist environment with low nutrient and ion concentration (Bennik *et al*., 1999). *Pseudomonas aeruginosa* is an opportunistic pathogen causing serious infections to already damaged tissues such as burns or to immunosuppressed individuals such as cancer and HIV/AIDS patients (McCarthy, 1990; Soltan,1998). *P. aeruginosa* is one of the most resistant microbes encountered in clinical laboratories (Shimeld and Rodgers, 1999).

Some strains such as metallo-beta-lactamase producing are reported to be resistant to all antibiotics except colistin (Edelstein *et al*., 2013).

2.2.5. *Candida albicans*

Candida albicans is a diploid, dimorphic, unicellular yeast causing opportunistic oral (Thrush disease) and genital (Candidiasis) infections in humans (Pesti *et al*., 2012). Oral infection caused by *C. albicans* is higher in diabetic mellitus patients due to high sugar concentrations (Mubarak *et al*., 2013). It is believed to be one of the fungi that play a role in sexually transmitted diseases especially in immune-compromised and HIV/AIDS patients (

et al., 2011).

Studies have shown that oral candidiasis, mostly commonly characterized by development of oral thrush, is the most frequent AIDS-associated opportunistic infection, with up to 90% of HIV-infected individuals suffering at least one episode during the course of their disease

(Vazquez, 1999). *C. albicans* is reported to be resistant against Fluconazole, Flucytosine, and intraconazole whereas resistance to AmphotericinB is rare (Marchese *et al*., 2007).

2.3. Antibiotic resistance and its socio-economic impacts

Millions of lives have been saved due to substantial reduction in mortality achieved with antibiotic therapy. However, the antibiotic effectiveness almost all the available antibiotics is being threatened by the rising resistance of microorganisms The major cause of this resistance is the over-use and misuse of antibiotics which is exerting undue selective pressure on microorganisms (Paphitou, 2013). This has made infections more difficult to treat which is contributing to the high morbidity and mortality of previously treatable infections (Byarugaba, 2004).

Infections caused by antibiotic resistant organisms are more likely to prolong hospitalisation, increase the risk of death and require more toxic and expensive antibiotics (Graf and Martin, 2000). In addition, as the prevalence of multidrug resistant organisms increase, these additional costs will become a greater threat to the local, regional and national medical care systems, many of which are already struggling to survive (DiazGranados *et al*., 2008). The pharmaceutical industry finds it increasingly difficult to keep pace with the antimicrobial resistance and is no longer reliable in bringing novel and more effective drugs to the market (Graf and Martin 2000).

2.4. Traditional medicine in Africa

The use of natural products with therapeutic properties is as ancient as human civilization and for a long time, mineral, plant and animal products were the main source of drugs in Africa (Maryam *et al*., 2010). About 80% of the worlds people rely on traditional plant based medicine (UNEP, 2010). It is estimated that about 75% population in Kenya seeks health care

among traditional healers (Sandiga *et al*., 1995). Traditional medicine is widely practiced in Kenya, and has been documented by various ethnomedical surveys (Miaron *et al*., 2004; Kareru *et al.*, 2007).

The high cost of important conventional drugs and/ or inaccessibility to western health care facilities has led to over-reliance on traditional medicine since it is affordable and available to people. On the other hand, even when western health facilities are available, traditional medicine is viewed as an efficient and an acceptable system from a cultural perspective (Munguti, 1997). Infections associated with bacterial and fungal pathogens are among some of the indications treated using traditional remedies in Kenya (Njoroge and Bussmann, 2007).

2.5. Conventional antibiotics and the problem of microbial resistance

Conventional antibiotics refer to the synthetic chemicals used as bactericides and fungicides. Antibiotics act in various ways such as; inhibition of cell membrane functions, inhibition of protein synthesis, inhibition of nucleic acid synthesis and inhibition of cell wall synthesis (Kalayou *et al*., 2012). Since their discovery, antimicrobial drugs have proved remarkably effective for the control of bacterial infections. However, it was soon evident that bacterial pathogens were unlikely to surrender unconditionally, because some pathogens rapidly become resistant to many of the first discovered effective drugs (Cowan, 1999).

Due to indiscriminate use of antimicrobial drugs, microorganisms have developed resistance to many antibiotics and that has created immense clinical problems in the treatment of infectious diseases (Davis, 1994). In addition, antibiotics are associated with adverse effects on host, which include depletion of beneficial gut and mucosal microorganisms, immunosuppression, hypersensitivity and allergic reactions. The drug resistant bacteria have further complicated the treatment of infectious diseases in immunocompromised, AIDS and cancer patients especially in the case of nosocomial infections (McGaw *et al*., 2001). There is not only the lost of

effectiveness of antibiotics against multidrug resistant microorganisms, but also global problem for the lost of budget for treating infectious diseases (Ahmad and Beg, 2001).

The emergence of antimicrobial resistance has its roots in the use of antimicrobials in animals and the subsequent transfer of resistance genes and bacteria among animals, animal products and the environment (McEwen and Fedorka-Cray, 2002). Extra-chromosomal genes were found responsible for these antimicrobial resistant phenotypes that may impart resistance to an entire antimicrobial class. These resistance genes have been associated with plasmids which are large, transferable, extra-chromosomal DNA elements. Other DNA mobile elements, such as transposons and integrons, are present on plasmids. These DNA mobile elements transmit genetic determinants for antimicrobial resistance mechanisms and may cause rapid dissemination of resistance genes among different bacteria (Toutou *et al*., 2004). The emergence of multiresistant bacteria to antimicrobial drugs has increased the need for new antibiotics or modifications of older antibiotics (Tollefson and Miller, 2000).

2.6. Plant derived antimicrobials

The antimicrobial compounds from plants may inhibit bacterial growth by different mechanisms than those presently used antimicrobials and may have a significant clinical value in treatment of resistant microbial strains (Shankar *et al*., 2010). Extracts isolated from several plants have been reported to have biological activity such as antimicrobial, anti-inflammatory and antioxidant activities (Yusuf *et al*., 2001).

Phenolic compounds are one of the most diverse groups of secondary metabolites found in edible plants. They are found in a wide variety of fruits, vegetables, nuts, seeds, stems and flowers as well as tea, wine, propolis and honey, and represent a common constituent of the human diet. In nature they are involved in plant growth and reproduction, provide resistance from pathogens and predators and protect crops from disease and pre-harvest seed germination

(Ross and Kasum 2002). There are different classes of polyphenols known as tannins, lignins and flavonoids. Each class of polyphenols possesses chemical characteristics that set them apart from one another.

Flavonoids are the most widely occurring polyphenol and are present in almost every form of human consumed vegetation. Dietary flavonoids have attracted interest because they have a variety of beneficial biological properties, which may play an important role in the maintenance of human health. Flavonoids are potent antioxidants, free radical scavengers and metal chelators; they inhibit lipid peroxidation and exhibit various physiological activities including anti-inflammatory, anti-allergic, anti-carcinogenic, anti-hypertensive, anti-arthritic and antimicrobial activities. Consumption of phenol-rich beverages, fruit and vegetables has commonly been associated with reduction of the risk of cardiovascular diseases in epidemiological studies (Whiting 2001). Flavonoids have been found to be the most abundant polyphenols. The biosynthesis of flavonoids is stimulated by sunlight (ultraviolet radiation), so higher concentrations of flavonoids can typically be found in the outer most layers of fruits and vegetables (i.e. the skins). Flavonoids can be divided into six subclasses according to the degree of oxidation of the oxygen heterocycle: flavones, flavonols, isoflavones, anthocyanins, flavanones and flavonols (catechins and anthocyanidins).

Extraction of polyphenols can be performed using a solvent like water, hot water, methanol, methanol/formic acid, methanol/water/acetic or formic acid etc. Therefore, the total polyphenol amounts detected from the same plant and their corresponding antioxidant and antimicrobial activities may vary widely, depending on external conditions applied. It was reported that an antimicrobial action of phenolic compounds was related to inactivation of cellular enzymes, which depended on the rate of penetration of the substance into the cell or caused by membrane permeability changes (Schultz *et al*., 1992).

Terpenoids and essential oils are also plant derived antimicrobials. The oils are secondary metabolites which are highly enriched in compounds based on isopropene structure. They are called terpenes. When compounds contain additional elements usually oxygen, they are termed terpenoids (Singh and Handique, 1997). Terpenes or terpenoids are active against bacteria, viruses and protozoa. The mechanism of action of terpenes is not fully known but it is speculated to involve membrane disrupton by lipophilic compounds (Hamed, 2011).

Heterocyclic nitrogen compounds are called alkaloids. The mechanism of action of highly aromatic planar quaternary alkaloids is attributed to their ability to intercalate with DNA of microorganisms (Cowan, 1999).

Lectins and polypeptides are often positively charged and contain disulphide bonds. Their mechanism of action may be the formation of ion channels in the microbial membrane or competitive inhibition of adhesion of microbial proteins to host polysaccharide receptors (Sher, 2004)

2.7. Plants systematics, phytoconstituents and their economic importance

There are several approaches for selecting plant species for biological investigation. Selection of species based on their use in traditional medicine is reported to be more valuable and many drugs that are of plant origin were discovered from plants used in traditional medicine (Cotton, 1996).

2.7.1. *Adansonia digitata* L. (Bombacaceae)

Adansonia digitata, is commonly known as baobab, is a tree that grows in dry parts of Africa. It has leaves in form of five-leaflets, large showy white flowers and the fruit is in form of a pulp. It is a multipurpose tree with leaves eaten as fresh vegetable, dried pulp used to prepare juice and different parts utilised in cure of various diseases. The pulp, leaves and seeds contain

calcium, magnesium, potassium, zinc, iron and vitamins A and C in variable proportions (Assogbadjo *et al,.*2008).

Proanthocyanidins, terpenoids, flavonoids, and sterols have been isolated from *A. digitata* (VanStaden *et al.,* 2012). Compaoré *et al*. (2011) has shown that flavonoids, proanthocyanidins and phenolic compounds are present in the pulp of *A. digitata* which make it a good radical scavenger. Elsewhere the fruits of *A.digitata* have been reported to contain proanthocyanidins as the major compounds (Shahat, 2006). Triterpenes, alkaloids, anthraquinones, saponins, tannins have also been reported to be present in the fruit pulp of *A.digitata* (Ramadan *et al*., 1994: Gbadamosi *et al*., 2011).

The plant has anti-inflammatory, antipyretic, antimicrobial and analgesic activities (Ramadan *et al*, 1994) Powdered leaves have anti-asthmatic and have anti-tension and antihistamine properties. Leaves are also used for other conditions such as diarrhea, dysentery, opthalmia and otitis media. Bark is used as a substitute for quinine to curb high fever and act as prophylactic measure for malaria as well (Sidibe and Williams, 2002). The stem extracts are non-toxic to brine shrimp larvae and aqueous extract has a high anti-malarial activity (Musila *et al*., 2013). In other studies VanStaden *et al*, (2011), *A. digitata* has showed considerable antimicrobial activity against *Bacillus subtilis, S. aureus* and *C. albicans*.

Figure 1: *Adansonia digitata* tree (Bombacaceae)

(Photograph: Magrate Kaigongi in Tharaka-Nithi County)

2.7.2. *Launaea cornuta* (Hocht. ex Oliv. & Hern) C.Jeffrey (Compositae)

L. cornuta is a perennial herb with erect stem, rosette of leaves and a diffuse inflorescence growing in disturbed and artificial grasslands and roadsides. The fresh young leaves and shoots are used as vegetable as the plant contains vitamin C, sodium, potassium, calcium and iron (Katariina, 2000). Lyimo *et al.* (2003) also reported that the leafy vegetable contains vitamin C, iron, fat, calcium and protein. A decoction from the whole plant is used to treat cancer and as a remedy to diabetes (Kareru *et al*., 2007). Leaves and roots of *L. cornuta* are used as antimalarial, antimicrobial and used by the Suba people in Kenya to treat opportunistic diseases associated with HIV/AIDS. They also treat stomach ache and stop blood flow during circumcision (Nagata *et al*., 2011).

The genus *Launaea* is characterized by flavonoids, triterpenes, sesquiterpene lactones, coumarins and steroids (Ali *et al*., 2003). Flavones like apigenin and luteolin are common compounds in the genus. Steroids like stigmasterol, cholesterol, taraxasterol have been identified in *L. nudicaulis*. Flavone glycosides such as apigenin-7-glycoside, luteolin-7-glycoside, luteolin-7-rutinoside and Vitexin have been isolated from the extract of *L. tenuiloba* and *L. resedifolia* an anthocyanin is found in *L. asplenifolia* (Ali *et al*., 2003; Fairouz *et al*., 2010).

Related species that have antimicrobial activity include: *L. cassiniana* Kuntze has nematocidal activity, *L. intybacea* likewise was found to have hepatoprotective activity on paracetamol introduced hepatotoxicity in albino rats (Takate *et al*., 2010) and *L. sonchoides* inhibits the growth of the fungi *Trichoderma hamatum* and *T. viridae* (Abou-Zeid *et al*., 2008).

Various species of *Launaea* such as *L. arborescens*, *L. mucronata, L. nudicaulis* and *L. capitata* contains several types of flavones such as luteolin, apigenin, and flavone glycosides such as apigenin 7-O-glucoside, vitexin, luteolin 7-O-glucoside and luteolin 7-O-rhamnoside (Christian and Vipaporn, 2010). Two isoprenylated flavonoids; asplenitin and asplenetin 5-O-neohesperidoside have been identified in *L. asplenifolia* (Denis and Ragaj, 1996). Roots of *L. mucronata* Muschl, aerial parts of *L. spinosa* Sch and aerial parts and roots of *L. tenuiloba* contain lactucin type guianolides as stated by Christian (2008).

The crude extracts (both organic and aqueous extracts) have moderate antimalarial activity and highly toxic to the Brine-shrimp larvae. They also contain flavonoids, alkaloids and saponins with sesquiterpene lactone being only present in the organic extract (Musila *et al.,* 2013)

Figure 2: A flowering herb of *Launaea cornuta* (Compositae)

(Photograph: Magrate Kaigongi in Tharaka-Nithi County)

2.7.3. *Grewia trichocarpa* Hochst. ex A.Rich (Tiliaceae)

It is an evergreen shrub or small tree growing up to 6m The Leaves are alternate, simple; stipules subulate, 6–8 mm long. The flowers are yellow to orange with superior ovary. The fruit is drupe (Beentje, 1994). It is used in various ways; the bark fibre is used in hut construction and in basketry. The pounded bark is used for soap-making. The woody branches and wood are used for withies, construction, floors of granaries, tool handles, walking sticks, bows and spear- and arrow shafts. It is also used as fuel wood. The aerial parts are used as fodder. The flowers are a source of bee forage. The ripe fruit is eaten (Beentje, 1994). No

available literature on antimicrobial activity, toxicity and phytochemistry reports on *G. trichocarpa* exists.

Grewia trichocarpa is used among the Luo community to clean snake bite wounds in both humans and livestock (Kokwaro, 2009). *Grewia hexaminta,* one cup is taken 3 times daily for 3-4 days among the Msambmweni people of Kenya to treat malaria (Nguta *et al*., 2011) and has Triterpenoid compounds (Raghunathaiyar, 1996). No available reports on the phytoconstituents of *G. trichocarpa* exist.

Figure 3: *Grewia trichocarpa* (Tiliaceae) showing leafy shoot

(Photograph: Magrate Kaigongi in Kilifi County)

2.7.4. *Zanthoxylum chalybeum* Engl. (Rutaceae)

It is a deciduous tree growing up to 12m high with pale grey bark, dark scales and prickles and a rounded crown. It has compound leaves, usually 3-5 pairs of shiny leaflets with a strong citrus smell when crushed. Flowers are sweet scented, inconspicuous, yellowish-green, produced immediately below the leaves at the base of the new branchlets. The fruits are

spherical (Beentje, 1994). It grows in low altitude areas, dry woodlands and savannah grasslands. Leaves are used as fodder and can be brewed to make tea while the bark is a source of durable timber and charcoal (Bamford and Henderson, 2003).

Leaf decoction is rubbed on swelling for the treatment of oedema in kwashiorkor and snake bites, root decoction is drunk for the treatment of chest pain, pneumonia and bark decoction drunk for the treatment of malaria, colds, coughs, dizziness and also chewed for toothache (Kokwaro, 2009). Seed extracts of *Z. chalybeum* has antiviral activity against measles virus (Olila *et al*., 2002). The plant has been used traditionally in the management of diabetes (Keter and Mutiso, 2011). *Z. chalybeum* has shown *in vitro* antiplasmodial activity (Rukunga *et al*., 2009; Nguta *et al*., 2010), antifungal activity against *Penicillium crustosum* and *Saccharomyces cerevisiae* (Taniguchi *et al.,* 1978), antibacterial activity against *Staphylococcus aur*eus (Matu and vanStaden, 2003) and cytotoxic activity against Brine-shrimp larvae (Nguta *et a*l., 2011). *Zanthoxylum. rhifolium* is used in treating headaches, anaemia, jaundice and high blood pressure (Gislaine *et al*., 2012).

Z. chalybeum has quinoline alkaloids (Kato *et al*., 1996) and organic extract has flavonoids, alkaloids, saponins and sesquiterpene lactones (Musila *et al*., 2013).

Figure 4: *Zanthoxylum chalybeum* (Rutaceae) tree

(Photograph: Magrate Kaigongi in Tharaka-Nithi County)

The plants used in this study were selected on the basis of their ethnopharmacological use by the Msambweni community. The preparation for treatment of various ailments in the traditional medicine, chemical constituent and the reported ethnomedical uses for each plant are tabulated below (Table 1).

Table 1: Selected plant species from Msambweni district used in traditional medicine

Family/Species/(Voucher specimen number)	Habit	Part used	Treatment preparation	chemical constituents	Relevant reported ethnomedical uses
Adansonia digitata L. (Bombacaceae) JN01	Tree	Leaf	Decoction	Proanthocyanidins, terpenoids, flavonoids, and sterols have been isolated from *A. digitata* (VanStaden *et al.,* 2012).	Leaf decocton is used as antimalarial, diaphoretic and prophylactic against fevers (Nguta *et al*., 2010)
Grewia trichocarpa Hochst. Ex A. Rich (Tiliaceae) JN02	Shrub	Root	Decoction	No previous reports	Root decoction is used to treat malaria (Nguta *et al*., 2011)
Launaea cornuta (Hocht.ex. Oliv. & Hiern.) C. Jeffrey (Compositae) JN03	Herb	Leaf	Decoction	flavonoids, alkaloids and saponins with sesquiterpene lactone (Musila *et al*., 2013)	Root and leaf decoction used to treat malaria and typhoid respectively (Nguta *et al*., 2010; Kokwaro, 2009)
Zanthoxylum chalybeum. Engl. (Rutaceae) JN04	Tree	Root bark	Decoction	quinoline alkaloids (Kato *et al*.,1996) and organic extract has flavonoids, alkaloids, saponins and sesquiterpene lactones (Musila *et al*., 2013).	Leaf decoction is rubbed on swelling for the treatment of oedema in kwashiorkor and snake bites, root decoction is drunk for the treatment of chest pain, pneumonia and bark decoction drunk for the treatment of malaria, colds, coughs, dizziness and also chewed for toothache (Kokwaro, 2009) bark of the Kenyan plant was reported active against *Bacillus subtilis, Penicillium crustosum* and The *Saccharomyces cerevisiae* (Taniguchi *et al*.,1978)

2.8. Toxicity Studies

Many substances we handle daily are toxic. Different organisms respond differently to the same toxin. Lethal Dose 50 is a term which is used to describe acute toxicity. It refers to lethal

dose which is acutely lethal to 50% of all the organisms in a given experiment. LD_{50} is expressed in mg/kg or ppm (parts per million) (Shankar *et al*., 1980). To meet the criteria of efficacy, safety and quality control like synthetic drug products, the pharmacological, toxicological and phytochemical profiles of the plant extracts have to be scientifically evaluated (Wagner, 1997).

2.8.1. Significance of toxicity studies

The use of plant extracts in treatment of diseases without any standard dosage accompanied with lack of adequate scientific studies has raised concern on their toxicity. Toxicity studies help in assessing the right dosage to be administered without causing health risks in the organisms (Ashafa *et al*, 2012). The lack of precise dosage in traditional medicine is not peculiar as it also occurs in modern medicine due to imprecise diagnosis (Kokwaro, 2009). The toxicity testing using Brine shrimp is cheap, easy, quick and only small amount of the sample to be tested is required. Thus it is an appropriate bioassay of determining the LC_{50} of various extracts (Apu *et al.,* 2010).

Simple bioassays for determination of bioactivities of crude plant extracts are important for the purpose of standardization or quality control of plant botanicals. Due to this Brine shrimp lethality test is a "top bench" procedure useful in natural product chemistry (Jerry and Lingling, 1998). Using Brine shrimp in toxicity studies is economical, easy, uses small amount of test material, inexpensive, simple and requires little chemical training. This test is a convenient probe for preliminary assessment of toxicity of natural products from plant biodiversity, heavy metals and pesticides (Aseer *et al*., 2009).

2.8.2. Brine shrimp lethality assay

The shrimp lethality assay was proposed by Michael (Krishanaraju *et al*., 2005) and later developed (Vanhaecke *et al*., 1981; Sleet and Brendel 1983). It is based on the ability of pharmaceutic compounds to kill laboratory-cultured *Artemia* nauplii brine shrimp. The brine shrimp lethality bioassay (BSLB) has been used extensively in the primary screening of the crude extracts as well as the isolated compounds to evaluate the toxicity towards brine shrimps, which could also provide an indication of possible cytotoxic properties of the test materials (Meyer *et al*., 1982).

Since BLSB is the simple method useful for screening large number of extracts in the drug discovery process. The method allows the use of smaller quantity of the extracts and permits larger number of samples and dilutions within shorter time than using the original test vials (Sam, 1993). Furthermore it has been established that the cytotoxic compounds generally exhibit significant activity in the BSLB, and this assay can be recommended as a guide for the detection of antitumour and pesticidal compounds because of its simplicity and low cost (Mazid *et al*., 2008). This bioassay has also a good correlation with the human solid tumour cell lines. The inhibitory effect of the extract might be due to the toxic compounds present in the active fraction that possess ovicidal and larvicidal properties. The metabolites either affected the embryonic development or slay the eggs. Therefore the cytotoxic effects of the plant extracts enunciate that it can be selected for further cell line assay because there is a correlation between cytotoxicity and activity against the brine shrimp nauplii using extracts (Manilal *et al*., 2009).

2.9. Clinical and Laboratory Standards Institute protocol on evaluation of antimicrobial activity

The test plates are inoculated with microorganism by optimally dipping a sterile cotton swab into a suspension adjusted to turbidity of 0.5 McFarland scale. The dried surface of agar plate is inoculated by streaking the swab over the agar surface. This procedure is repeated twice by rotating the plate approximately 60^0 each time to ensure even distribution of inoculums. The discs are applied on the inoculated agar by gently pressing them down to ensure complete contact with the agar. Whether the discs are applied individually or with a dispensing apparatus, they must be distributed evenly so that they are no closer than 24mm from center to center. Ordinarily, no more than 12 discs in one plate. It is also important to pay attention to how close the discs are to the edge of the plate. The plates should be inverted and placed in an incubator set to 37^0c for 24hours and 30^0c for 48 hours for fungi (CLSI, 2006).

2.10. Interpretation of antimicrobial results

The "susceptible" category implies that isolates are inhibited by the usually achievable concentrations of antimicrobial agent when the recommended dosage is used for the site of infection. The "intermediate" category includes isolates with antimicrobial agent MICs that approach usually attainable blood and tissue levels and for which response rates may be lower than for susceptible isolates. The intermediate category implies clinical efficacy in body sites where the drugs are physiologically concentrated (e.g., quinolones and β-lactams in urine) or when a higher than normal dosage of a drug can be used (e.g., β-lactams). This category also includes a buffer zone, which should prevent small, uncontrolled, technical factors from causing major discrepancies in interpretations, especially for drugs with narrow pharmacotoxicity margins. The "resistant" category implies that isolates are not inhibited by the usually achievable concentrations of the agent with normal dosage schedules, and/or that

demonstrate zone diameters that fall in the range where specific microbial resistance mechanisms (e.g., beta-lactamases) are likely, and clinical efficacy of the agent against the isolate has not been reliably shown in treatment studies. A microorganism for example is said to be resistant against 40µg/ml of gentamicin if the zone of inhibition exhibited is ≤ 12mm, intermediate at 13-14mm and susceptible at ≥ 15mm. The commercially manufactured antibiotics and isolated pure compounds exhibit larger zones of inhibition at low concentrations compared to crude extracts (CLSI, 2006). According to Kitonde *et al*. (2013), the lowest MICs ≤20 mg/ml indicate highest activity, while 31.25-62.5 mg/ml indicate moderate activity, and ≥125 mg/ml indicate low activity

A crude extract with LC_{50} values ranging between 0-500 µg/ml is said to be highly toxic while that with LC_{50} values between 500-1000 µg/ml is termed as moderately toxic. An extract with LC_{50} values above 1000 µg/ml is non toxic (Nguta *et al*., 2011)

2.11. Problem statement

Microbial infections have caused a big burden of diseases. An alarming increase in microbial resistance against conventional antibiotic treatment has developed over the last forty years in both medical and livestock sectors. This alarming increase in the development of resistance to the antibiotics in the clinical practice has led to the incidence of new and re-emergence of infectious diseases making them expensive and difficult to treat. Plants have great potential as antimicrobial agents due to the presence of a wide range of bioactive compounds such as alkaloids, flavonoids, saponins and sesquiterpene lactones. However, since many medicinal plants are unevaluated, it is possible that the active compounds of many plants go undetected while some plants used therapeutically result in poisoning. Evaluation of chemical activity and toxicity of plants extracts is important in determining safe and efficient dosages. This study was carried out to determine the antimicrobial activity, phytochemical and toxicity analysis of;

Zanthoxylum chalybeum, Adansonia digitata, Launaea cornuta and Grewia trichocarpa crude extracts.

2.12. Justification

In Kenya antibiotic resistance has led to significant morbidity and mortality which is a drawback to economic development. Antibiotics are expensive to poor communities in developing countries and most microbes such as methicilin resistant *S. aureus* have become resistant to almost all available drugs. Research into new alternative for such microbes is warranted. The World Health Organisation (WHO, 2001) advocates for traditional medicine as a safer remedy for ailments of microbial and non-microbial origin. Most modern medicines were discovered through study of plants which were used traditionally to treat specific illnesses. In addition, very few medicinal plants have been analyzed chemically and their bioactive constituents are yet to be validated. As a result, knowledge from traditional medicine can be very essential in the development of cheap and effective antibiotics.

2.13. Objectives

2.13.1. Main Objective

To determine *in vitro* antimicrobial activity, toxicity and phytochemical composition of crude extracts from *Zanthoxylum chalybeum , Adansonia digitata, Launaea cornuta* and *Grewia trichocarpa*.

2.13.2. Specific objectives

1. To evaluate the antimicrobial potential of selected aqueous and organic crude plant extracts

2. To determine the acute toxicity of the crude extracts on Brine shrimp larvae

3. To characterise the major phytochemical compounds in the crude plant extracts.

2.13. 3. Null Hypothesis

Crude extracts of *Zanthoxylum chalybeum, Adansonia digitata, Launaea cornuta and Grewia trichocarpa* will have activity against *in vitro* growth of methicilin resistant *Staphylococcu*s *aureus*, *Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa* and *Candida albicans* and will be toxic *to Artemia salina* larvae

CHAPTER THREE: MATERIALS AND METHODS

3.1. Collection of plant materials

The various plants parts depending on the ethnobotanical knowledge on specific plants were collected from Msambweni District in Kenya by Dr. Joseph Nguta of University of Nairobi. The selection of the four plants was done on the basis of their wide usage in treatment of not only microbial infections but also malaria (Nguta et al., 2011) by the Msambweni community. Plants were identified by a taxonomist from the University of Nairobi and voucher specimens deposited in the University of Nairobi herbarium. The plant parts were air dried at room temperature, chopped into small pieces and ground into powder.

Table 2: Plants Collected from Msambweni District, Kenya

Voucher specimen number	**Plant species**	**Family**	**Plant Part Collected**
JN01	*Adansonia digitata* L	Bombacaceae	Stem Bark
JN02	*Grewia trichocarpa* Hochst. Ex A. Rich	Tiliaceae	Roots
JN03	*Launaea-cornuta* (Hocht.ex.Oliv.&Hiern.) C.Jeffrey	Compositae	Leaves
JN04	*Zanthoxylum chalybeum*.Engl.	Rutaceae	Root bark

3.2. Preparation of Crude Extracts

The ground materials were extracted by cold maceration method. 50gm of ground plant material in 500mls of distilled water to obtain aqueous extracts and 50gms in 500mls of chloroform-Methanol mixture (1:1) for 48 hours to obtain organic extracts. The aqueous extracts were filtered and the filtrate kept in a deep freezer then lyophilized (freeze dried) resulting to a dry powder. Organic extracts were filtered and concentrated with a rotary

evaporator to dry powder (Nguta *et al*., 2011). The resulting dry powders were used in antimicrobial, phytochemical screening and toxicity evaluation.

3.3. Microorganism Suspension Standardization

The microorganisms used in the study were obtained from the Department of Public Health, Pharmacology and Toxicology, University of Nairobi and the Centre for Microbiology Research (CMR), Kenya Medical Research Institute (KEMRI). The fungal suspension was standardized according to the Clinical and Laboratory Standards Institute (CLSI, 2008). The yeast was grown on Sabouraud agar for 72 hours and standardized with sterile saline to turbidity equivalent to 0.5 McFarland standards which is approximately $1\text{-}5\times10^{6}$ CFU/ml. Bacteria were grown in Muller–Hinton agar for 24 hours and standardized with sterile saline to turbidity equivalent to 0.5 McFarland scale approximately$1\text{-}2\times10^{8}$CFU/ml (CLSI, 2009) and stored at 4^{o}C. The antibacterial and antifungal activity was determined using disc diffusion test and broth dilution method (Kalayou *et al*., 2012).

Table 3: List of microbes tested in the study

Name of microbe	Microbe and type used	Details of strain used	Source
Bacillus cereus	Gram positive bacteria	ATCC 11778	DPHPT, UoN
Methicilin Resistant *S. aureus* (MRSA)	Gram positive bacteria	Untyped pure clinical isolate collected from CMR- KEMRI	KEMRI
Pseudomonas aeruginosa	Gram negative bacteria	ATCC 27853	DPHPT, UoN
Escherichia coli	Gram negative bacteria	ATCC 25922	DPHPT, UoN
Candida albicans	Fungal yeast	ATCC 10231	DPHPT, UoN

The microbes were selected on the basis of their availability.

3.4. Disc Diffusion Test

The disc diffusion method followed by the Clinical and Laboratory Standards Institute protocol was used to evaluate antimicrobial activities (Kaloyou *et al*., 2012). To determine susceptibility, four concentrations (200 mg/ml, 100 mg/ml, 50 mg/ml and 25 mg/ml of crude extracts) were prepared in dimethylsulfoxide (DMSO) and water for organic and aqueous extracts respectively by dissolving 2g of each extracts in 10mls and serially diluted to make 100mg/ml, 50mg/ml and 25mg/ml. Sterile antibiotic assay discs (Whatman, 6 mm) were impregnated with 100µL of the reconstituted extract and dried completely under sterile conditions in a laminar flow. Each disc was gently pressed down to ensure complete contact with the agar inoculated with 1ml of test microorganisms (bacteria and fungi). Extracts were tested in triplicate. DMSO and water saturated assay discs were used as negative control. Gentamicin and Amphotericin B were used as positive control for bacteria and fungi respectively. The plates were incubated at 37 ^{0}C for 18-24 h for bacteria and 30^{0}C for 72 hrs for fungi. Inhibition zones were recorded as the diameter of growth free zones.

3.5. Determination of minimum inhibitory concentration (MIC)

The extracts that showed antibacterial activity against the selected pathogens were selected for further tests to calculate their MIC by dilution method. This test was performed in sterile 96-well microplates. Microdilution was performed in 96-well microtiter plates with U-shaped wells where the cultures were diluted in Müeller-Hinton broth at a density adjusted to 0.5 McFarland turbidity. Controls with 0.5 ml of only culture medium or others with plant extracts were used in the tests. The wells were filled with 100 µl of sterile H_2O and 100 µl of the plant extracts were added to the wells by serial two fold dilution from the suspension of plant extract stock solution. Each well was inoculated with 100 µl of bacterial and fungal suspensions. The plates were covered, placed in plastic bags to create anaerobic conditions and incubated at

37°C for 24 hrs for bacteria and 30^0C for 72hrs for fungi. This was followed by inoculating the cultures on the agar. In this study, the MIC was the lowest concentration of plant extracts that exhibited no growth of the microorganism (Kalayou *et al*., 2012)

3.6. Acute toxicity testing

The toxicity of the extracts (Both the aqueous and organic) was determined using Brine shrimp-Leech (*Artemia salina L.*) eggs (Wanyoike *et al*., 2004). A hatching tank measuring 14cm by 5cm and 9cm was used. The tank was divided into two unequal compartments by a plastic divider with holes of diameter of 2mm. The tank was filled to the brim using sea water. The sea water was prepared by dissolving 30g of commercial sea salt in a one liter of distilled water. A spoonful of *Artemia salina* eggs and a gram of yeast to act as food for the nauplii were added into the larger compartment of the hatching tank containing the sea water. This was followed by covering the larger compartment to enhance hatching of the eggs by preventing light penetration. This was incubated at room temperature (22-29^0C) for 48hrs to allow hatching of the eggs.

Various concentrations of the crude extract in sea water were used: 10,100 and 1000µg/ml. A stock solution for both the organic and aqueous crude extract of10, 000µg/ml was prepared from which serial dilutions were done to make the three concentrations used in the experiment. The aqueous extracts stock solution of 10,000µg/ml was prepared by dissolving 0.1g of the crude extract in 10 mls of distilled water and the chloroform: MeOH extracts, 0.1g of the sample was first dissolved in dimethylsulphoxide (DMSO) then diluted further using artificial water to 10 mls to make stock solution of 10,000µg/ml. The stock solutions from the crude extracts were filtered using 0.22 µm micro filters under lamina flow hood (Wanyoike *et al*., 2004). The solutions were then put into the pre-marked vials and 10 live brine shrimp *nauplii* were added in 5 ml simulated sea water (Apu *et al*., 2010). Each experiment was replicated

three times. A negative control was prepared with artificial sea water and DMSO only for organic extract and artificial sea water only in the case of aqueous extract (Wanyoike *et al*., 2004). Survivors were counted after 24 hrs using a dissecting microscope. The mortality endpoint of this bioassay was defined as the absence of controlled forward motion during 30 s of observation (Apu *et al*., 2010).

3.7. Phytochemical Screening of Plants Extracts Using Thin Layer Chromatography (TLC)

The crude plant extracts were screened for flavonoids, alkaloids, sesquiterpene lactones and saponins. This was done by dissolving the organic and aqueous extracts in chloroform and methanol (1:1). Using capillary tubes, spots of the dissolved extracts were made on the base line of the aluminium TLC plates. The plates were put in a chamber containing appropriate chromatographic solvent system (mobile phases) specific for the determination of the presence of a given class of phytochemical constituents (Harbourne, 2002)

Table 4: Procedure followed in screening for different chemical compounds

Class of Secondary metabolites	Solvent system for developing TLC plates	Detection
Alkaloids	Dichloromethane: Methanol (85:15) (Harborne, 2002)	Dragendorff's reagent was sprayed on the developed plates. Formation of orange colors indicated the presence of alkaloids. Spraying the plates with sodium nitrate made the orange colors more intense (Harborne, 2002)
Flavonoids	n-hexane: ethyl acetate: acetic acid (6:3:1) (Waksmundzka *et al*., 2008)	At 254 nm, flavonoids appeared as dark blue zones on a yellow background on the developed plates. The intensity of the yellow color in the background increased when ammonia was sprayed on the plates. At 365 nm flavonoids fluoresced yellow, blue or green (Waksmundzka *et al*. 2008).
Saponins	Dichloromethane:Ethyl acetate (9:1) according to Karem *et al*.(2005)	Detection of saponins occurred when plates were sprayed with a mixture of ethanol and H_2SO_4 (9:1) and then heated at 110^0C for 10 minutes. Saponins appeared as black spots (Karem *et al*., 2005)
Sesquiterpene lactones	n- hexane: ethyl acetate (9:1) (Waksmundzka *et al*., 2008).	Detection was done by placing the developed plates in a chamber containing iodine crystals to observe brown spots (Waksmundzka *et al*., 2008). Sesquiterpene lactones were also detected as brown, yellow spots when plates were sprayed with concentrated H_2SO_4 and heated for 5 minutes at 100-110^0C (Harborne, 2002).

3.8. Data analysis

Statistical analysis of antimicrobial activity was done using statistical program for social sciences (statistical analysis software). Using the software, ANOVA was used to determine whether there were significant differences in the mean diameter of inhibition zones in various concentrations. Once the means were found to be different from each other, Dunnett test was then used for multiple comparisons of inhibition to determine whether inhibition arising from the various treatments were different from the inhibition induced by the positive controls. The significance level used in the analysis was 0.05 (Alpha Level $\leq$ 0.05).

In toxicity testing, LD_{50} was determined by Finney's probit analysis. Probit analysis is a specialized regression model of binomial response variable and it has been used to analyse dose-response experiments in a variety of fields. It involves conversion of concentrations into logarithms and the corresponding percentage mortalities into probits. The logarithms of the concentrations are then plotted against the probits to give a straight line graph. This regression line is then used in the determination of LD_{50} (Finney, 1971).

CHAPTER FOUR: RESULTS

4.1 Extraction yields

The resulting dry powders were weighed and expressed as percentages. The ground powders of organic extracts of *A. digitata*, *G. trichocarpa*, *L. cornuta* and *Z. chalybeum* yielded 1.1 %, 1.7 %**,** 2.8 % and 6.3 % respectively**.** The ground powders of aqueous extracts of yielded *A. digitata*, *G. trichocarpa*, *L. cornuta* and *Z. chalybeum* yielded 1.3 %, 1.6 %, 4.1 % and 3.2 % respectively. *Z. chalybeum* organic extract yielded the highest recovery value of 6.3 % while *A. digitata* organic extract had the lowest yield of 1.1 %. (Table 5).

Table 5: Percentage yield of plant extracts using aqueous and organic extraction methods

Plant species	**Part used**	**Extraction type**	**% yield to weight of dry powered plant**
Adansonia digitata	Stem Bark	Organic	1.1
Adansonia digitata	Stem Bark	Aqueous	1.3
Grewia trichocarpa	Root	Organic	1.7
Grewia trichocarpa	Root	Aqueous	1.6
Launnaea cornuta	Leaves	organic	2.8
Launnaea cornuta	Leaves	Aqueous	4.1
Zanthoxyum chalybeum	Root bark	Organic	6.3
Zanthoxyum chalybeum	Root bark	Aqueous	3.2

4.2. Antimicrobial activity

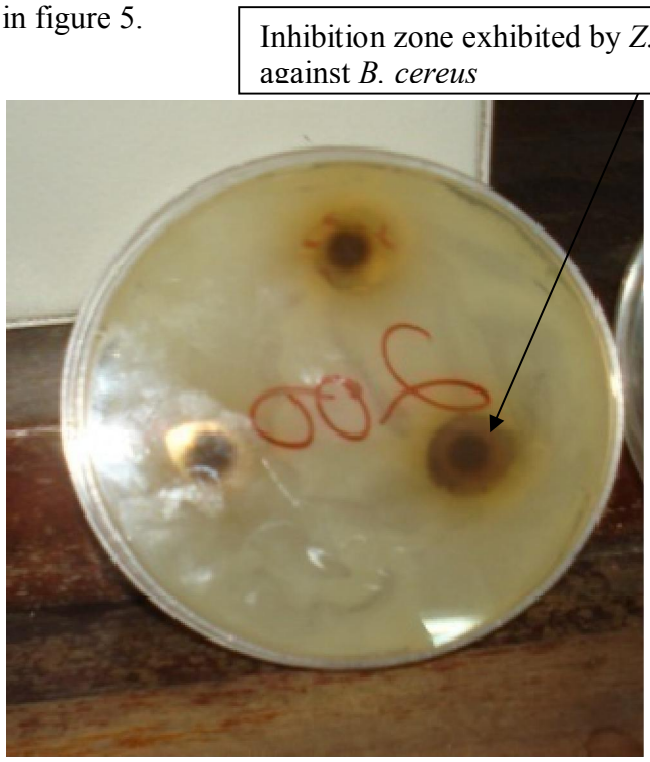
4.2.1. Disc Diffusion Technique

4.2.1.1. Aqueous extracts against *B. cereus*

The aqueous crude extracts of *A. digitata* and *Z. chalybeum* showed antimicrobial activities against *B. cereus* at 200 mg/ml and 100 mg/ml. *A. digitata* showed inhibition zones of 8.67 mm and 7.5 mm at 200 and 100 mg/ml respectively while that of *Z. chalybeum* was 10.5 mm and 7.67 mm at both 200 and 100 mg/ml respectively. The aqueous crude extracts of *G. trichocarpa* and *L. cornuta* did not show any antimicrobial activity against *B. cereus*

4.2.1.2. Organic extracts against *B. cereus*

Organic crude extract of *Z. chalybeum* showed the highest inhibition against *B. cereus* at 200 and 100 mg/ml concentrations with 13.87 mm and 12.167 mm respectively. The growth inhibition (mm) of *Z. chalybeum* organic extract at 200 mg/ml against *B. cereus* did not show much difference from that of the positive control (Gentamicin 0.04 mg/ml), $P \geq 0.05$ as shown in figure 5.



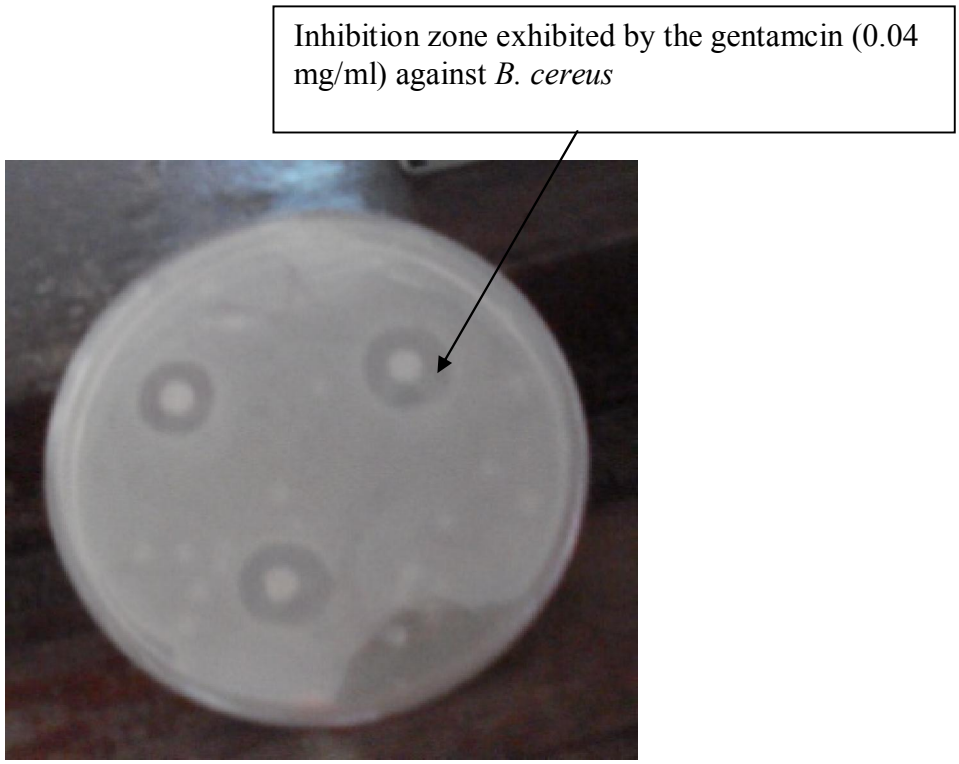


Figure 5: Inhibition zones caused by *Z. chalybeum* at 200 mg/ml and positive control against *B. cereus*

A. digitata organic extract also showed antimicrobial activity against B. cereus at 200 mg/ml and 100 mg/ml with inhibition zones of 8.67 mm and 7.5 mm respectively. *G. trichocarpa* showed antimicrobial activity at 200, 100 and 50 mg/ml with inhibition zones of 11.67, 9.67 and 7.17 mm respectively (Figure 6).

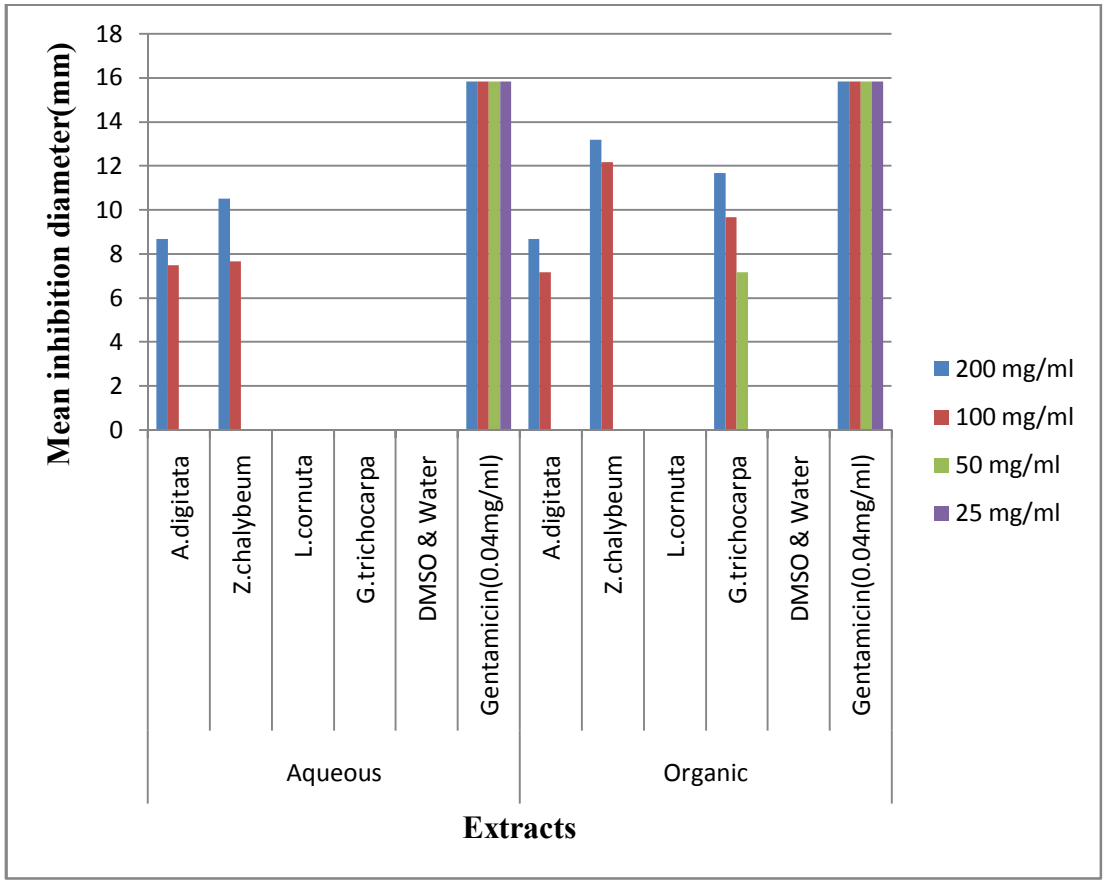


Figure 6: Growth inhibition of *B. cereus* by different concentrations of extracts from the four test plants

4.2.1.3. Aqueous extracts against *P. aeruginosa*

In aqueous extracts of all the tested plants, only *A. digitata* inhibited *P. aeruginosa*. *A. digitata* showed antimicrobial activity 200 mg/ml, 100 mg/ml and 50 mg/ml with inhibition zones of 9.17 mm, 8 mm and 7.5 mm respectively.

4.2.1.4. Organic extracts against *P. aeruginosa*

The organic extracts of *G. trichocarpa* showed the highest levels of inhibition in all concentrations with 10.67, 9.67, 8.83 and 7.33 mm respectively. *Z. chalybeum* showed inhibition zones of 10.5 mm, 9.17 mm, 8 mm and 7.67 mm at 200 mg/ml, 100 mg/ml, 50 mg/ml and 25 mg/ml respectively. *A. digitata* and *L. cornuta* organic extracts showed

antimicrobial activities at 200, 100 and 50 mg/ml with *A. digitata* showing inhibition zones of 9.17 mm, 9 mm and 8.5 mm respectively while *L. cornuta* showed inhibition zones of 10.5 mm, 9.17 mm and 8 mm respectively (figure 7)

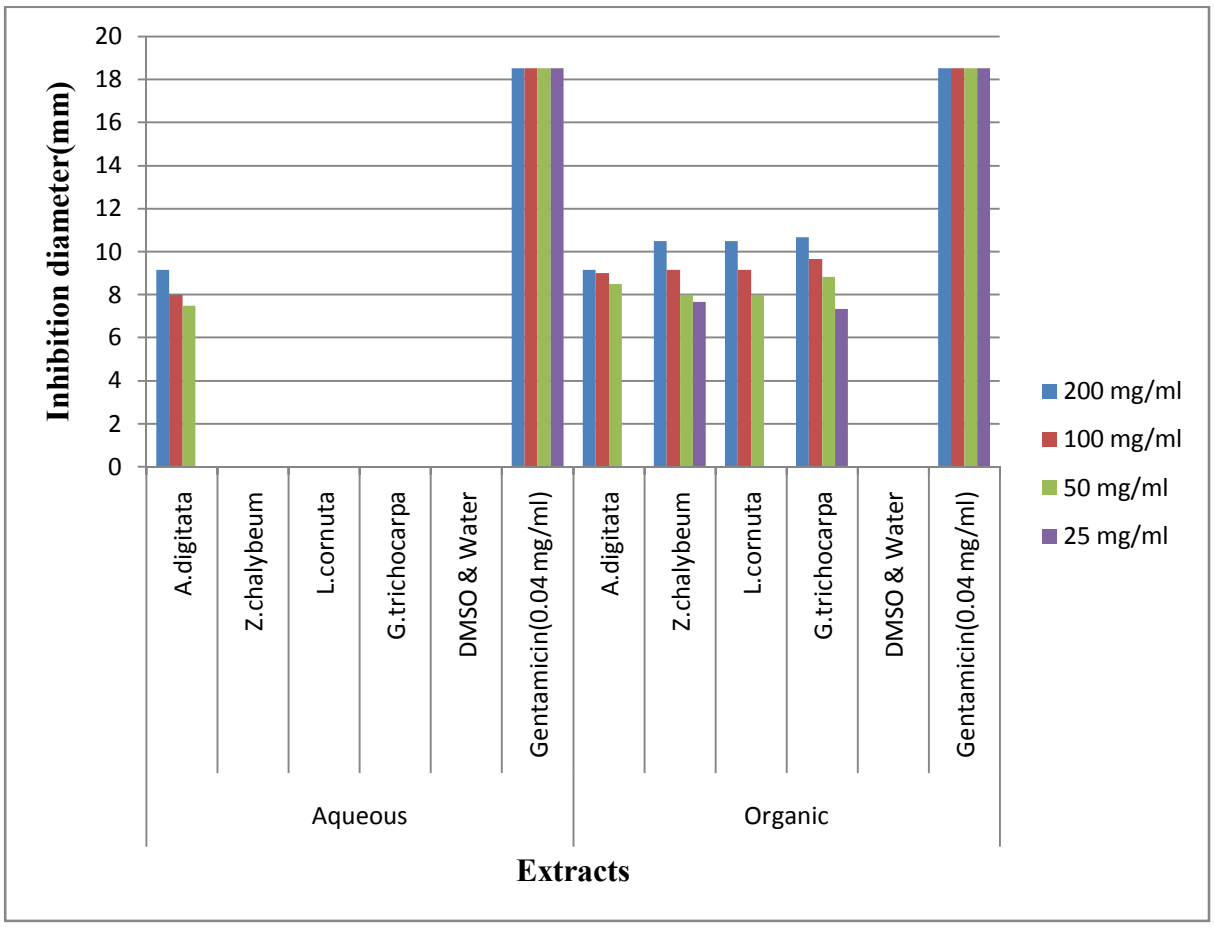


Figure 7: Growth inhibition of *P. aeruginosa* by different concentrations of extracts from the four test plants

4.2.1.5. Aqueous Extracts Against Methicillin Resistant *S. aureus* (MRSA)

In the case of MRSA, none of the aqueous extracts showed any inhibition except *A. digitata* which showed inhibition zones of 9.33 mm and 8.17 mm at 200 mg/ml and 100 mg/ml respectively.

4.2.1.6. Organic Extracts against MRSA

All organic extracts showed some inhibition against MRSA. *A. digitata* organic extract at 200 mg/ml and 100 mg/ml showed highest inhibition zones of 14.33 mm and 12 mm respectively. *A. digitata* organic extract zone of inhibition (mm) at 200 mg/ml against MRSA was not much different from that of the positive control (Gentamicin, 0.04 mg/ml), $P \geq 0.05$. The organic extract of *G. trichocarpa* was the second in terms of activity at 200 mg/ml with 10.33 mm followed by the organic extract of *Z. chalybeum* at 200 mg/ml with 9.33 mm. All the organic extracts showed inhibition at 50 mg/ml while *A. digitata* showed inhibition of 8.33 mm at the lowest concentration 25 mg/ml (Figure 8).

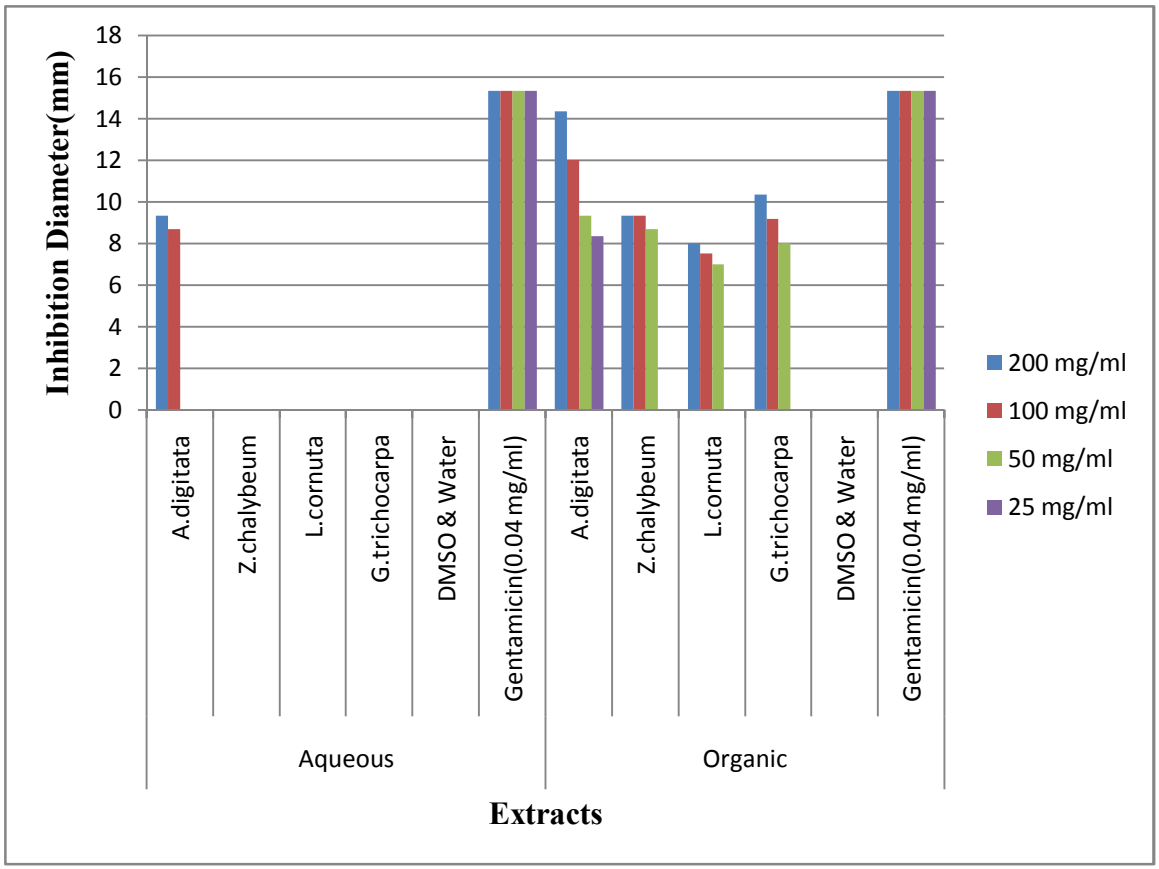


Figure 8: Growth inhibition of MRSA by different concentrations of extracts from the four test plants

4.2.1.7. Aqueous extracts against *C. albicans*

The aqueous extracts of the four plants did not inhibit the growth of *C. albicans* at any of the concentration used.

4.2.1.8. Organic extracts against *C. albicans*

All the organic extracts except for *L. cornuta* showed inhibition against *C. albicans* at 200 and 100 mg/ml. *A. digitata* registered the inhibition of 9.7 mm and 8.3 mm at 200 mg/ml and 100 mg/ml respectively. The inhibition zones caused by *A. digitata* organic extract at 200 mg/ml and AmpotecirinB against *C. albicans* is shown in figure 9.

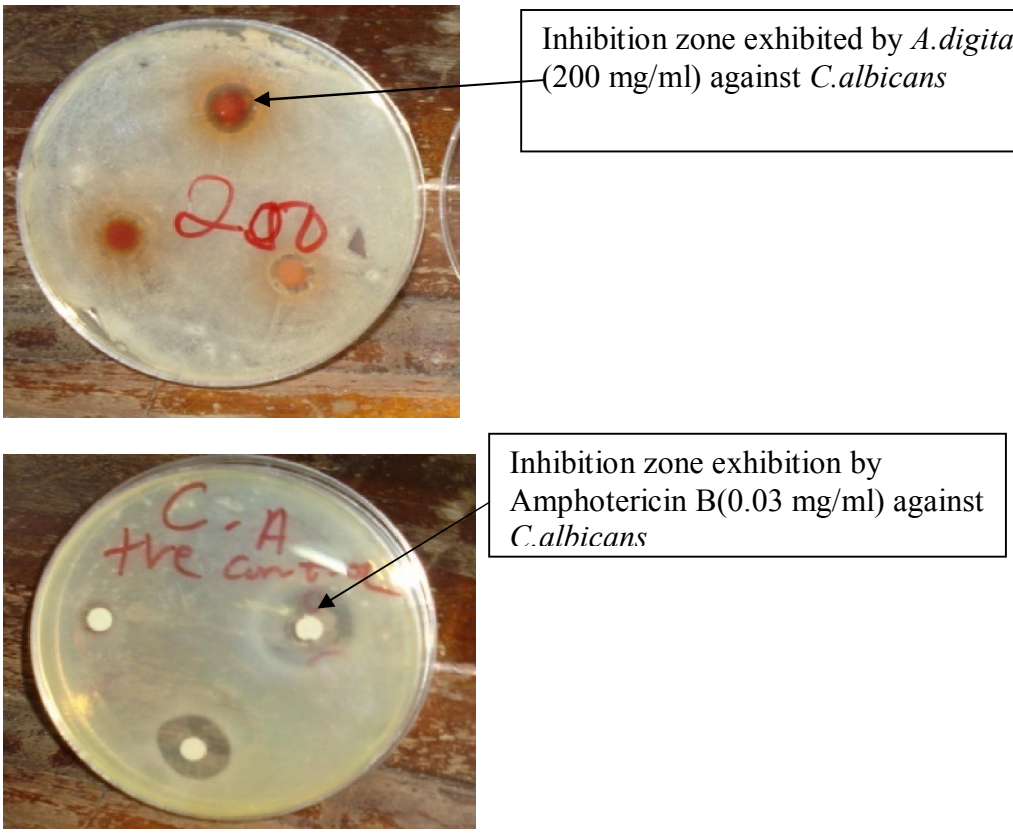


Figure 9: Inhibition zones showing antifungal activity of *A. digitata* at 200 mg/ml and positive control against *C. albicans*

Z. chalybeum at 200 mg/ml and 100 mg/ml showed inhibition of 8.7 mm and 7.5 mm respectively while *G. trichocarpa* showed inhibition of 8.5 mm and 7.83 mm at 200 mg/ml and 100 mg/ml respectively (Figure 10).

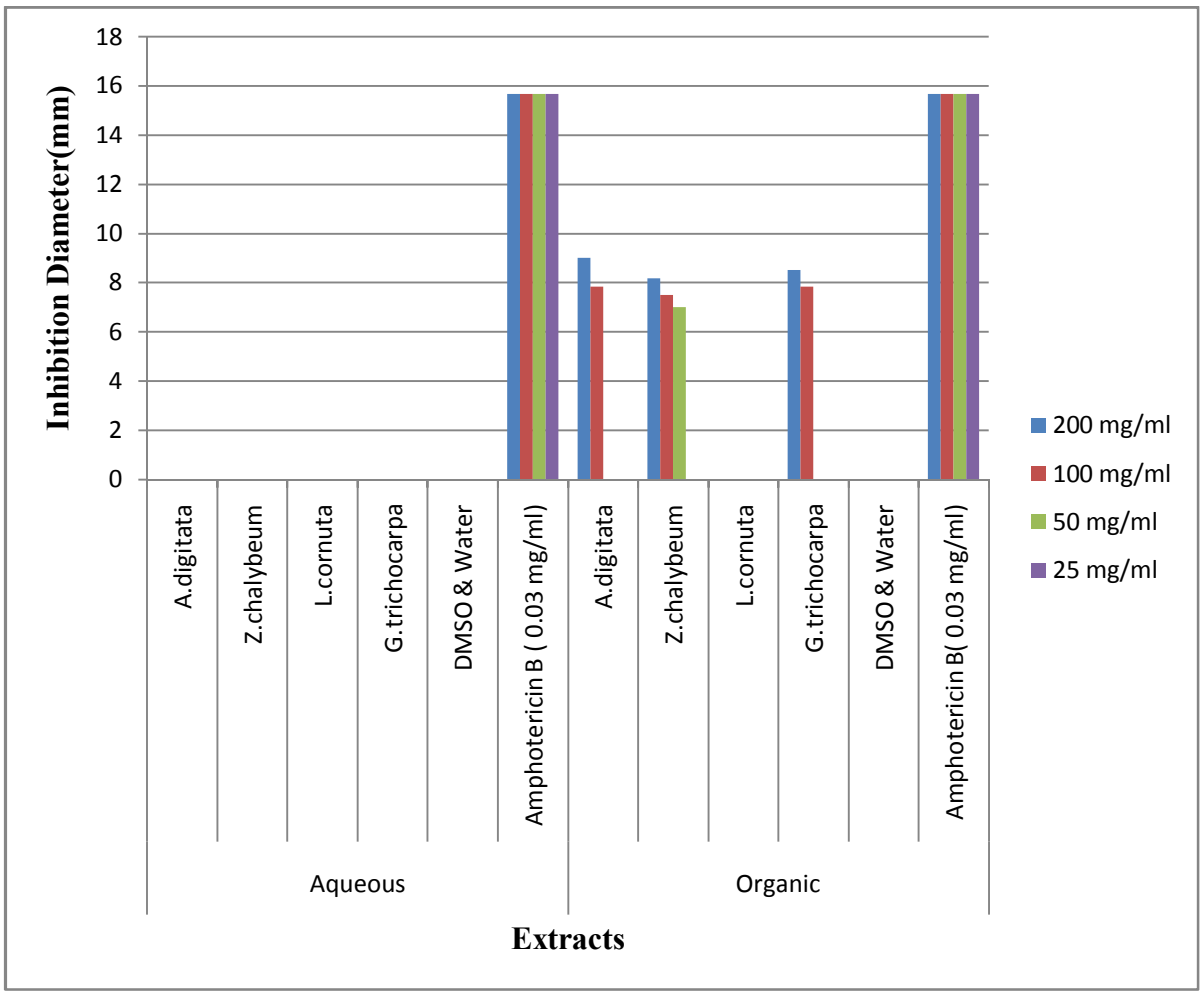


Figure 10: Growth inhibition of *C. albicans* by different concentrations of extracts from the four test plants

4.2.2. Minimum Inhibitory Concentration (MIC) Results

Extracts which at 200 mg/ml did not inhibit the microorganism was assumed to have MIC value greater than 200 mg/ml.

4.2.2.1. Organic Extracts

Minimum inhibitory concentration (MIC) values of the extracts tested against methicillin resistant *S. aureus* (MRSA) shows *A. digitata* organic extract had the highest activity with the lowest MIC of 20 mg/ml. *Z. chalybeum* and *G. trichocarpa* organic extracts had the highest activity against *P. aeruginosa* with MIC of 20 mg/ml. *Z. chalybeum* organic extract exhibited the highest activity against *B. cereus* at 200 mg/ml with an inhibition zone of 13.17 mm, however *G. trichocarpa* organic extract showed the lowest MIC value against *B. cereus*. *Z. chalybeum* organic extract exhibited highest activity against *C. albicans* with a MIC of 40 mg/ml (Table 6).

Table 6: Minimum Inhibitory Concentration (MIC) of Organic Crude Extracts

PLANT NAME	**MIC (mg/ml)**				
	C. albicans	***E. coli***	***P. aeruginosa***	***B. cereus***	**MRSA**
Adansonia digitata	70	> 200	30	90	20
Zanthoxylum chalybeum	40	> 200	20	60	40
Launnaea cornuta	> 200	> 200	40	> 200	40
Grewia trichocarpa	80	> 200	20	40	40

4.2.2.2. Aqueous Extracts

Some aqueous extracts did not show any antimicrobial activity against most of the microbes at any concentration tested and therefore their MIC values were assume to be more than 200 mg/ml. *A digitata* recorded MIC values of 40 mg/ml against *P. aeruginosa*, 90 mg/ml against *B. cereus* and 80 mg/ml against MRSA while *Z. chaybeum* had MIC value of 90 mg/ml against *B. cereus* (Table 7)

Table 7: Minimum Inhibitory Concentration (MIC) of Aqueous Crude Extracts

PLANT NAME	MIC (mg/ml)				
	C. abicans	***E. coli***	***P. aeruginosa***	***B. cereus***	**MRSA**
Adansonia digitata	> 200	> 200	40	90	80
Zanthoxylym chalybeum	> 200	> 200	> 200	90	> 200
Launnaea cornuta	> 200	> 200	> 200	> 200	> 200
Grewia trichocarpa	> 200	> 200	> 200	> 200	> 200

4.3. Brine-Shrimp lethality test

4.3.1. Aqueous Crude Extracts

A. digitata aqueous crude extract had the highest LC_{50} value of 3988 µg/ml followed by *G. trichocarp*a with 1488 µg/ml while *Z. chalybeum* exhibited the least LC_{50} value of 293.5 as shown in table 8

Table 8: LC50 values for various tested aqueous crude extract on Brine-shrimp larvae

	Average mortality at various concentrations				**LC $_{50}$** µg/ml
Plant species	1,000 µg/ml	100 µg/ml	10 µg/ml	0 µg/ml	
Adansonia digitata	1.33	0.33	0	0	3988
Zanthoxylym chalybeum	8	1	0	0	293.5
Launnaea cornuta	6.33	1.67	0.33	0	554.4
Grewia trichocarpa	0.33	0	0	0	1488

4.3.2. Organic Extracts

A. digitata organic crude extract showed the highest LC_{50} value of 5626 followed by *G. trichocarpa* with 5602. *L. cornuta* showed LC_{50} value of 141.3 while *Z. chalybeum* recorded the lowest LC_{50} value of 39.2 (table 9)

Table 9: LC_{50} values for various tested organic crude extract on Brine-shrimp larvae

	Average mortality at various concentrations				**LC $_{50}$**
Plant species	1,000 µg/ml	100 µg/ml	10 µg/ml	0 µg/ml	µg/ml
Adansonia digitata	2.67	1	0	0	5626
Zanthoxylym chalybeum	10	5.67	3	0	39.2
Launnaea cornuta	8.33	4	1.33	0	141.3
Grewia trichocarpa	2.33	0	0	0	5602

4.4. Phytochemical screening of secondary compounds

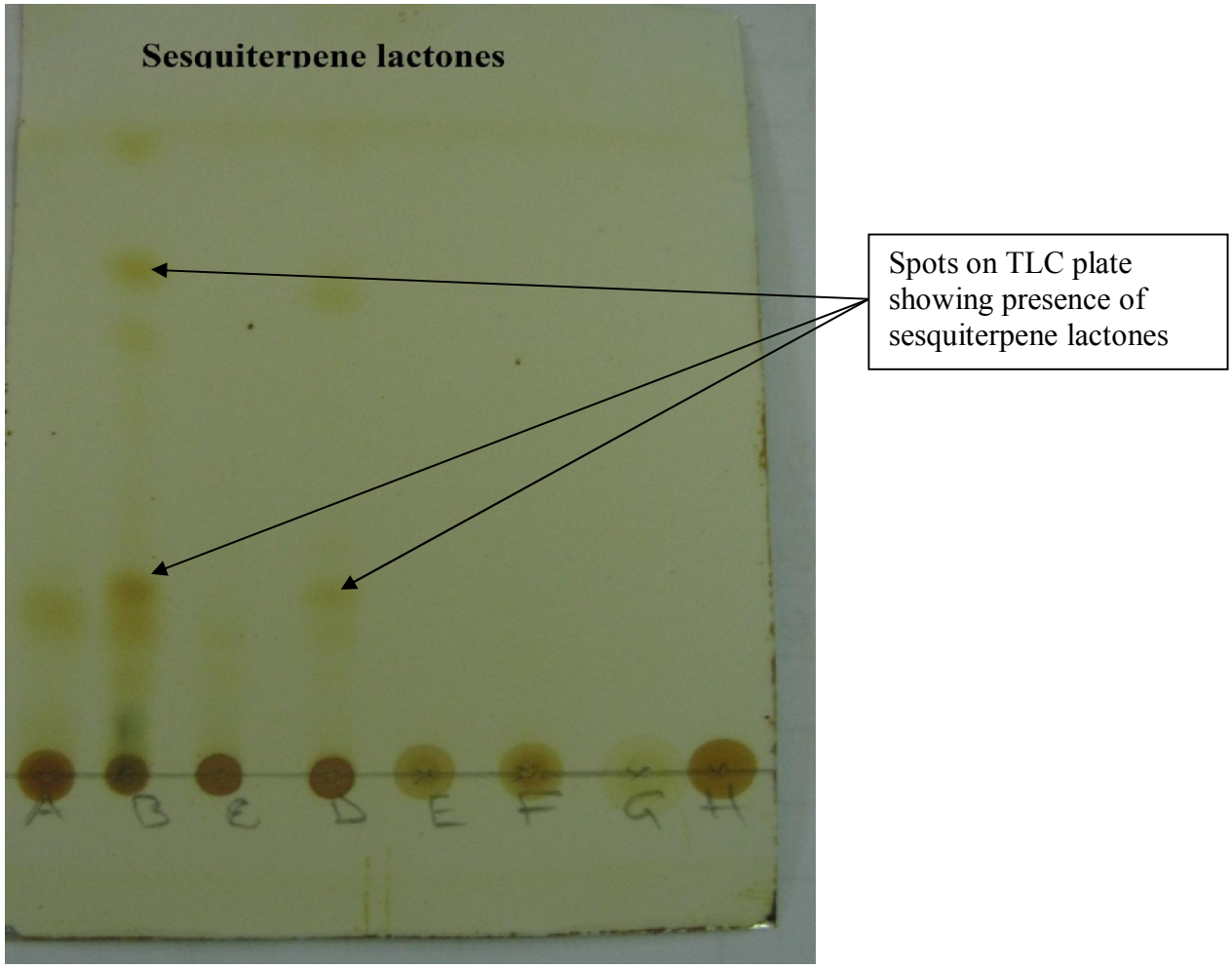


Figure 11: A photograph showing sesquiterpene lactones results of the tested extracts on a TLC plate

A- *A. digitata* organic, B- *L. cornuta* organic, C- *Z. chalybeum* organic, D- *G. trichocarpa* organic, E- *A. digitata* aqueous, F- *L. cornuta* aqueous, G- *Z. chalybeum* aqueous and H- *G. trichocarpa* aqueous

4.4.1. Aqueous Crude Extracts

Alkaloids and flavonoids were present in all the aqueous extracts while saponins were only found in L. cornuta only. Sesquiterpene lactones were absent in all the aqueous extracts (table10)

Table 10: Presence of alkaloids, flavonoids, sesquiterpene- lactones and saponins in aqueous extracts

Extract	alkaloids	flavonoids	Sesqiterpene lactones	saponins
Adansonia digitata	+	+	-	-
Zanthoxylym chalybeum	++	+	-	-
Launnaea cornuta	+	+	-	+
Grewia trichocarpa	+	+	-	-

-…absence
+…presence, the number of (+) shows comparative amounts depending on degree of coloration

(b).Organic Crude Extracts

Alkaloids, flavonoids, Sesquiterpene lactones and saponins were present in all the organic extracts (table 11)

Table 11: Presence of alkaloids, flavonoids, sesquiterpene- lactones and saponins in organic extracts

Extract	alkaloids	flavonoids	Sesqiterpene lactones	saponins
Adansonia digitata	++++	+	+	++
Zanthoxylym chalybeum	+++	+++	+	+++
Launnaea cornuta	+	++	+++	++
Grewia trichocarpa	++	+	++	+

-…absence
+…presence, the number of (+) shows comparative amounts depending on degree of coloration

CHAPTER FIVE: DISCUSSION

The current study was designed to investigate the antimicrobial activity, toxicity and phytochemical screening of four medicinal plants used in Msambweni District, Kenya. Due to the side effects of the current drugs the resistance that pathogenic microorganisms build against antibiotics, much attention has led to study of biologically active compounds isolated from plant species used in herbal medicine (Essawi and Srour, 2000). The results of different studies provide evidence that medicinal plants might indeed be potential sources of new antibacterial agents even against some antibiotic-resistant strains (Kone *et al*., 2004).

From this study, it is clear that the chloroform: methanol (1:1) solvent extracts of all the plants tested were more potent than their corresponding aqueous extracts against all the tested microbes. *A. digitata* organic extract was active against all the microbes except the *E. coli*. The highest activity of *A. digitata* recorded was against MRSA followed by *P. aeruginosa* and *B. cereus*. The high antimicrobial activity of organic extract of *A. digitata* stem bark against MRSA may be used as yardstick to identify novel drugs with the potential to combat bacterial strains resistant to known antibiotics, shorten the length of treatment, increase patient compliance, and avoid overdose which may lead to toxicity or side effects to patients. These results are in agreement with VanStaden *et al*, (2011) who reported that *A. digitata* has considerable antimicrobial activity against *Bacillus subtilis, S. aureus* and *C. albicans*. This signifies that, *A. digitata* extract has potential novel antimicrobial compounds for management of diseases caused by the above microbes.

L*aunnaea cornuta* aqueous extract did not show any inhibition against the tested microbes at any concentration. However, the organic extract showed moderate activity against *P. aeruginosa* and MRSA and failed to inhibit *B. cereus, E. coli* and *C. albicans*. This is in

agreement with findings from Nagata *et al*. (2011) who reported that leaves and roots of *L. cornuta* are used as antimicrobial by the people of Suba District-Kenya particularly those living with HIV/AIDS to manage opportunistic diseases. However, the low activity exhibited by *L. cornuta* in this study may be attributed to the difference in the dose used in this study, the method of extraction of medicinal plants, the method of antimicrobial study, the genetic variation of plant, age of the plant or the environment.

Zanthoxylum chalybeum showed antimicrobial activity with the highest activity being observed against *B. cereus* whereby at 200 mg/ml, there was no much difference in terms of inhibition zones (mm) with that of standard antibiotic (Gentamicin, 0.04 mg/ml) ($P \geq 0.05$). *Z. chalybeum* organic extract also showed high activity against *P. aeruginosa* and moderate activity against MRSA and *C. albicans* but failed to inhibit the growth of *E. coli* at all tested concentrations. These results are in agreement with studies by Adesina's (2005) findings that the ethanol extract of *Z. chalybeum* has antibacterial activity against *B. cereus* and *P. aeruginosa* and antifungal activity against *C. albicans*.

Grewia trichocarpa organic extract ($CHCl_3$: MeOH) showed considerable antimicrobial activity against four of the five tested microbes; MRSA, *B. cereus, P. aeruginosa* and *C. albicans* and failed to show any inhibition against *E. coli.* The aqueous extract of *G. trichocarpa* did not inhibit any of the tested microbes. The organic extract showed high activity against *P. aeruginosa* and moderate activity against *C. albicans*, *B. cereus* and MRSA. This is the first report on antimicrobial activity of *G. trichocarpa*.

Differential sensitivity of microbes to the extracts may be explained by the cell wall composition of the gram positive and gram-negative bacteria. The cell wall of gram negative

bacteria contains the outer membrane and lipid bilayer embedded with proteins and porins. This determines and allows different molecules or ions either into or out of the cell and thus the outer membrane serves as a barrier to the passage of many molecules and hence less sensitive to many extracts (Kalayou *et al*., 2012). This supports why all the extracts did not inhibit the growth of *E. coli* which was one of the gram negative bacteria. The gram positive bacterium has a relatively thick layer of peptidoglycan sheets of interconnected glycan chains made up of polymer which is fully permeable to many substances, and thus sensitive to most extracts, Nester *et al*., (2004). This may explain why the gram positive bacteria MRSA and *B. cereus* in this study were susceptible to most extracts.

Low activity of the crude extracts against *C. albicans* (yeast fungus) compared to the bacteria may also be due to differences in cell wall composition of the organisms. Yeast fungus cell wall contains polysaccharides and proteins (Nester *et al*., 2004). The protein expression on *C. albicans*, function as selective transport system used to expel wastes and compounds that are otherwise deleterious to the cell; a function called efflux which is medically important in that, it allows micro-organisms to oust antimicrobial medications that are made to destroy them and therefore render them resistance, Nester *et al*., (2004). This explanation could be one of the reasons why *C. albicans* was resistant to the tested crude extracts. The observation reported in this study is in agreement with the findings of Masakazu *et al*., (2010), who did research on antifungal drug resistance of *Candida* spp and reported that expression of drug efflux pumps were responsible for the resistance.

The organic extracts were more active than aqueous extracts. Noting that the traditional herbal remedy preparation from these plants is by use of water, it is a paradox that the aqueous extracts were inactive in this study. This may be due to the absence or insufficient and

ineffective concentration of the antimicrobial constituents in the aqueous extracts. These findings are in agreement with the studies of Koua *et al*., (2011) who reported that inactivity of aqueous extracts of *Striga hermonthica* may be due to absence or insufficient and effective concentration of the antimicrobial agents of *S. hermonthica* extracts. It is well known that patients using the traditional herbs take in large amounts of the concoctions and hence may eventually consume sufficient amounts of the curative drugs to elicit healing.

The study on toxicity of *A. digitata* against Brine-shrimp confirmed that both the aqueous and organic extract of *A. digitata* were non- toxic since both extracts had LC_{50} > 1000 µg/ml, an observation that is in agreement with that of Musila *et al*., (2013). *A. digitata* has also been shown to be non toxic on mice from previous studies. For instance, LD_{50} of aqueous extract of the fruit pulp of *A. digitata* on mice was found to be over 8000 µg/ml (Ramadan *et al*., 1994). This corroborates the results obtained in this study on the non toxicity of stem bark of *A. digitata* on Brine shrimp larvae. Non toxicity of *A. digitata* explains why most of the plant parts: seeds, fruit pulps and leaves are consumed as food by many communities. The non toxicity of *A. digitata* stem implies that the plant may be used to inhibit the growth disease causing bacteria and fungi without eliciting any toxicity on the patients.

Effects of *L. cornuta* on Brine shrimp from this study showed that both the aqueous and organic extracts ($CHCl_3$: MeOH) were toxic particularly the ($CHCl_3$: MeOH) ones. Similarly high levels of toxicity of stem bark of *L. cornuta* on Brine shrimp larvae has also been reported by Nguta *et al* .(2011) and Musila *et al*. (2013).

The study on toxicity of *Z. chalybeum* found that both the aqueous and organic ($CHCl_3$: MeOH) extracts of *Z. chalybeum* were highly toxic against brine shrimp larvae. Nguta *et a*l. (2011) while investigating toxicity of aqueous extracts of the leaves, stem bark and root bark of

Zanthoxylum chalybeum on Brine shrimp larvae obtained LC_{50}<500 µg/ml for the three plant parts. This confirms the results obtained in this study and implies that *Z. chalybeum* may not make safe antimicrobial herbal remedies. This calls upon cautious use of the plant through dose adjustment amongst communities using this plant for preparation of herbal decoctions. Similarly, toxicity of *Z. chalybeum* methanolic root bark extract on human normal fetal lung fibroblast cells has also been reported by Kamuhabwa *et al*. (2000).

Toxicity study showed that both the organic and aqueous extracts of *G. trichocarpa* were non toxic to brine shrimp larvae. This is the first report on the toxicity studies of *G. trichocarpa.*
The organic extract of *G. trichocarpa* contained alkaloids, flavonoids, saponins and sesquiterpene lactones while the aqueous extract contained only alkaloids and flavonoids. There is no available report from the literature on the antimicrobial activity, toxicity and phytoconstituents of *Grewia* species except on *Grewia hexaminta* which is said to contain triterpenoid compounds (Raghunathaiyar, 1996). This could be the first time to report on *G. trichocarpa* phytochemistry.

Thin layer chromatography (TLC) study of *A. digitata* stem bark shows that the organic extract contained alkaloids, flavonoids, saponins and sesquiterpene lactones while the aqueous extract lacked both saponins and sesquiterpene lactones and contained alkaloids and flavonoids only. This is in agreement with Musila *et al*. (2013). In addition, *A. digitata* stem bark contains medicinal compounds which are largely classified under saponins, alkaloids and flavonoids such as lupeol acetate, β-sitosterol, scopoletin, friedelin, betullinic acid and adansonin while the fruit pulp of *A.digitata* is rich in procyanidins (Sidibe & Williams, 2002; Shahat, 2006). Alkaloids, flavonoids and sesquitepenes have been reported to be potent plant secondary metabolites with broad spectrum of bioactivities (Mazid *et al*., 2011). The higher antimicrobial

activity of *A. digitata* on MRSA could be attributed to the presence of large amounts alkaloids. Alkaloids are pharmacogenically active basic principles of flowering plants, (Das *et al*., 2010). The observed activity of these compounds in *A. digitata* is in line with that of Karou *et al*. (2006) who demonstrated that the Indoloquinoline alkaloid causes cell lysis and morphological changes of *S. aureus*.

Secondary metabolites screening in this study found that both aqueous and organic ($CHCl_3$: MeOH) extracts of *L. cornuta* contained flavonoids, alkaloids, saponins with sesquiterpene lactones lacking in the aqueous extract. These results are in agreement with (Musila *et al*., 2013). Elsewhere in Ali *et al*. (2003), the genus *Launaea* is characterized for flavonoids, triterpenes, sesquiterpene lactones, coumarins and steroids. Various species of *Launaea* such as, *L. arborescens*, *L. mucronata, L. nudicaulis* and *L. capitata* contain various types of flavones such as luteolin, apigenin and flavone glycosides such as apigenin 7-O-glucoside, vitexin, luteolin 7-O-glucoside and luteolin 7-O-rhamnoside besides others (Christian & Vipaporn, 2010).

Organic extract ($CHCl_3$: MeOH) of *Z. chalybeum* was found to contain alkaloids, flavonoids, saponins and sesquiterpene lactones while the aqueous extract contained only alkaloids and flavonoids. Similar results were obtained by Musila *et al*. (2013). *Z. chalybeum* has been reported to contain alkaloids which have antibacterial and cytotoxic activity (Chrian *et al.*, 2011. *Zanthoxylum* species contains various compounds such as alkaloids, aliphatic and aromatic amides, lignans, coumarins, sesquiterpene lactones and sterols (Cheng *et al*., 2011; He *et al*., 2002).

Biological activity is attributed to the presence of various secondary metabolites in plants (Mazid *et al*., 2011). Not only their presence, but also the quantity of the phytochemical constituents in a given plant extract determines the extent of extracts' bioactivity. In addition, presence of more than one class of secondary metabolites in a given plant extract will also determine the nature and extent of extract's biological activity (Wang *et al*., 2010). The presence of flavonoids in the crude extracts may explain the antimicrobial activity as flavonoids are known to complex with the extracellular and soluble proteins and bacterial cell wall of bacteria. More flavonoids may also disrupt microbial membrane. The sesquiterpene lactones are known to be active against bacteria, protozoa and viruses through membrane disruption. (Cowan, 1999).

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

The organic crude extracts of three plants; *A. digitata*, *Z. chalybeum* and *G. trichocarpa* were active against *B. cereus* and *C. albicans* while all the four plants organic crude extracts were active against *P. aeruginosa* and MRSA. None of the tested crude extracts was active against *E. coli.* The current study shows that *Z. chalybeum* and *A. digitata* inhibit the growth of *B. Fcereus* and MRSA respectively with no significant difference ($P \geq 0.05$) with that of standard antibiotic (Gentamicin). The crude extracts of *A. digitata* and *G. trichocarpa* proved to be non toxic on Brine shrimp larvae with LC_{50}>1000 µg/ml while *Z. chalybeum* and *L. cornuta* were found to be highly toxic with LC_{50}<500 ug/ml.

Flavonoids, alkaloids, saponins and sesquiterpene lactones were present larger amounts in organic extracts of *A. digitata*, *G. trichocarpa*, *L. cornuta* and *Z. chalybeum* compared to their aqueous extracts. Alkaloids and flavonoids were also present in aqueous extracts of *A. digitata, G. trichocarpa*, *L. cornuta* and *Z. chalybeum* while saponins were only present in aqueous extracts of *L. cornuta*. Alkaloids were present in high amount while sesquiterpene lactones were present in low amounts in all the extracts.

Laboratory and clinical studies of these plants are required in order to better understand their antimicrobial activities so as allow the scientific community to recommend their uses as an accessible alternative to synthetic antibiotics. The antimicrobial activity, toxicity and phytochemistry of crude extracts *G. trichocarpa* is being reported for the first time. This could be a start point for further drug research of this species on a wide diversity of microbial pathogens. Though there is no harm in exploitation or threat against the use of these potential plant species, they should be protected and probably domesticated for commercial exploitation in large scale

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APPENDICES

Appendix 1: Growth inhibition of *B. cereus* by aqueous extracts

Growth inhibition of *B.cereus* at 200mg/ml
Dunnett t (2-sided)

(I) Treatments	(J) Treatments	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
A.digitata	Gentamicin(40ug/ml)	-7.16667*	.36004	.000	-8.2112	-6.1221
Z.chalybeum	Gentamicin(40ug/ml)	-5.33333*	.36004	.000	-6.3779	-4.2888
L.cornuta	Gentamicin(40ug/ml)	-15.83333*	.36004	.000	-16.8779	-14.7888
G.trichocarpa	Gentamicin(40ug/ml)	-15.83333*	.36004	.000	-16.8779	-14.7888
DMSO & Water	Gentamicin(40ug/ml)	-15.83333*	.36004	.000	-16.8779	-14.7888

*. The mean difference is significant at the 0.05 level.

Growth inhibition of *B. cereus* at 100mg/ml
Dunnett t (2-sided)

(I) Treatments	(J) Treatments	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
A.digitata	Gentamicin(40ug/ml)	-8.33333*	.31914	.000	-9.2592	-7.4074
Z.chalybeum	Gentamicin(40ug/ml)	-8.16667*	.31914	.000	-9.0926	-7.2408
L.cornuta	Gentamicin(40ug/ml)	-15.83333*	.31914	.000	-16.7592	-14.9074
G.trichocarpa	Gentamicin(40ug/ml)	-15.83333*	.31914	.000	-16.7592	-14.9074
DMSO & Water	Gentamicin(40ug/ml)	-15.83333*	.31914	.000	-16.7592	-14.9074

*. The mean difference is significant at the 0.05 level.

Appendix 2: Growth inhibition of *B. cereus* by organic extracts

Growth inhibition of *B. cereus* at 200 mg/ml
Dunnett t (2-sided)

(I) Treatments	(J) Treatments	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
A. digitata	Gentamicin(40ug/ml)	-1.00000	.43033	.000	-9.4001	-4.9333
Z. chalybeum	Gentamicin(40ug/ml)	-2.16667	.76980	.058	-4.4001	.0667
L. cornuta	Gentamicin(40ug/ml)	-4.00000*	.76980	.001	-6.2334	-1.7666
G. trichocarpa	Gentamicin(40ug/ml)	-15.83333*	.76980	.000	-18.0667	-13.5999
DMSO & Water	Gentamicin(40ug/ml)	-15.83333*	.76980	.000	-18.0667	-13.5999

*. The mean difference is significant at the 0.05 level.

Growth inhibition of *B. cereus* at 100 mg/ml
Dunnett t (2-sided)

(I) Treatments	(J) Treatments	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
A. digitata	Gentamicin(40ug/ml)	-8.66667*	.67358	.000	-10.6209	-6.7125
Z. chalybeum	Gentamicin(40ug/ml)	-3.66667*	.67358	.001	-5.6209	-1.7125
L. cornuta	Gentamicin(40ug/ml)	-15.83333*	.67358	.000	-17.7875	-13.8791
G. trichocarpa	Gentamicin(40ug/ml)	-6.16667*	.67358	.000	-8.1209	-4.2125
DMSO & Water	Gentamicin(40ug/ml)	-15.83333*	.67358	.000	-17.7875	-13.8791

*. The mean difference is significant at the 0.05 level.

Appendix 3: Growth inhibition MRSA by aqueous extracts

Growth inhibition of MRSA at 200 mg/ml
Dunnett t (2-sided)

(I) Treatments	(J) Treatments	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
A. digitata	Gentamicin(40ug/ml)	-7.16667*	0.76980	.000	-2.2485	.2485
Z. chalybeum	Gentamicin(40ug/ml)	-15.33333*	.43033	.000	-16.5818	-14.0848
L. cornuta	Gentamicin(40ug/ml)	-15.33333*	.43033	.000	-16.5818	-14.0848
G. trichocarpa	Gentamicin(40ug/ml)	-15.33333*	.43033	.000	-16.5818	-14.0848
DMSO & Water	Gentamicin(40ug/ml)	-15.33333*	.43033	.000	-16.5818	-14.0848

*. The mean difference is significant at the 0.05 level.

Growth inhibition of MRSA at 100 mg/ml
Dunnett t (2-sided)

(I) Treatments	(J) Treatments	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
A. digitata	Gentamicin(40ug/ml)	-3.33333*	.50918	.000	-4.8106	-1.8561
Z. chalybeum	Gentamicin(40ug/ml)	-15.33333*	.50918	.000	-16.8106	-13.8561
L. cornuta	Gentamicin(40ug/ml)	-15.33333*	.50918	.000	-16.8106	-13.8561
G. trichocarpa	Gentamicin(40ug/ml)	-15.33333*	.50918	.000	-16.8106	-13.8561
DMSO & Water	Gentamicin(40ug/ml)	-15.33333*	.50918	.000	-16.8106	-13.8561

*. The mean difference is significant at the 0.05 level.

Appendix 4: Growth inhibition of MRSA by the organic extracts

Growth inhibition of MRSA at 200 mg/ml
Dunnett t (2-sided)

(I) Treatments	(J) Treatments	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
A. digitata	Gentamicin(0.04/ml)	-5.66667*	.66667	.055	-7.6008	-3.7325
Z. chalybeum	Gentamicin(40ug/ml)	-5.66667*	.66667	.000	-7.6008	-3.7325
L. cornuta	Gentamicin(40ug/ml)	-7.00000*	.66667	.000	-8.9342	-5.0658
G. trichocarpa	Gentamicin(40ug/ml)	-4.66667*	.66667	.000	-6.6008	-2.7325
DMSO & Water	Gentamicin(40ug/ml)	-15.00000*	.66667	.000	-16.9342	-13.0658

*. The mean difference is significant at the 0.05 level.

Growth inhibition of MRSA at 100 mg/ml
Dunnett t (2-sided)

(I) Treatments	(J) Treatments	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
A. digitata	Gentamicin(40ug/ml)	-6.83333*	.44096	.000	-8.1127	-5.5540
Z. chalybeum	Gentamicin(40ug/ml)	-5.66667*	.44096	.000	-6.9460	-4.3873
L. cornuta	Gentamicin(40ug/ml)	-7.50000*	.44096	.000	-8.7793	-6.2207
G. trichocarpa	Gentamicin(40ug/ml)	-5.83333*	.44096	.000	-7.1127	-4.5540
DMSO & Water	Gentamicin(40ug/ml)	-15.00000*	.44096	.000	-16.2793	-13.7207

*. The mean difference is significant at the 0.05 level.

Appendix 5: Growth inhibition of *P. aeruginosa* by aqueous extracts

Growth inhibition of *P. aeruginosa* at 200 mg/ml
Dunnett t (2-sided)

(I) Treatments	(J) Treatments	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
A. digitata	Gentamicin(40ug/ml)	-9.33333*	.50918	.000	-10.8106	-7.8561
Z. chalybeum	Gentamicin(40ug/ml)	-18.50000*	.50918	.000	-19.9772	-17.0228
L. cornuta	Gentamicin(40ug/ml)	-18.50000*	.50918	.000	-19.9772	-17.0228
G. trichocarpa	Gentamicin(40ug/ml)	-18.50000*	.50918	.000	-19.9772	-17.0228
DMSO & Water	Gentamicin(40ug/ml)	-18.50000*	.50918	.000	-19.9772	-17.0228

*. The mean difference is significant at the 0.05 level.

Growth inhibition of *P. auruginosa* at 100 mg/ml
Dunnett t (2-sided)

(I) Treatments	(J) Treatments	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
A. digitata	Gentamicin(40ug/ml)	-10.50000*	.55277	.000	-12.1037	-8.8963
Z. chalybeum	Gentamicin(40ug/ml)	-18.50000*	.55277	.000	-20.1037	-16.8963
L. cornuta	Gentamicin(40ug/ml)	-18.50000*	.55277	.000	-20.1037	-16.8963
G. trichocarpa	Gentamicin(40ug/ml)	-18.50000*	.55277	.000	-20.1037	-16.8963
DMSO & Water	Gentamicin(40ug/ml)	-18.50000*	.55277	.000	-20.1037	-16.8963

*. The mean difference is significant at the 0.05 level.

Appendix 6: Growth inhibition of *P. aeruginosa* by organic extracts

Growth inhibition of *P. auruginosa* at 200 mg/ml
Dunnett t (2-sided)

(I) Treatments	(J) Treatments	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
A. digitata	Gentamicin(40ug/ml)	-9.33333*	.72008	.000	-11.4225	-7.2442
Z. chalybeum	Gentamicin(40ug/ml)	-8.00000*	.72008	.000	-10.0891	-5.9109
L. cornuta	Gentamicin(40ug/ml)	-8.00000*	.72008	.000	-10.0891	-5.9109
G. trichocarpa	Gentamicin(40ug/ml)	-7.83333*	.72008	.000	-9.9225	-5.7442
DMSO & Water	Gentamicin(40ug/ml)	-18.50000*	.72008	.000	-20.5891	-16.4109

*. The mean difference is significant at the 0.05 level.

Growth inhibition of *P. aeruginosa* at 100 mg/ml
Dunnett t (2-sided)

(I) Treatments	(J) Treatments	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
A. digitata	Gentamicin(40ug/ml)	-9.50000*	.60093	.000	-11.2434	-7.7566
Z. chalybeum	Gentamicin(40ug/ml)	-9.33333*	.60093	.000	-11.0768	-7.5899
L. cornuta	Gentamicin(40ug/ml)	-9.33333*	.60093	.000	-11.0768	-7.5899
G. trichocarpa	Gentamicin(40ug/ml)	-8.83333*	.60093	.000	-10.5768	-7.0899
DMSO & Water	Gentamicin(40ug/ml)	-18.50000*	.60093	.000	-20.2434	-16.7566

*. The mean difference is significant at the 0.05 level.

Appendix 7: Growth inhibition of *C. albicans* by organic extracts

Growth inhibition of *C. albicans* at 200 mg/ml
Dunnett t (2-sided)

(I) Treatments	(J) Treatments	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
A. digitata	Amphotericin (30ug/ml)	-6.66667*	.36004	.000	-7.7112	-5.6221
Z. chalybeum	Amphotericin (30ug/ml)	-7.50000*	.36004	.000	-8.5446	-6.4554
L. cornuta	Amphotericin (30ug/ml)	-15.66667*	.36004	.000	-16.7112	-14.6221
G. trichocarpa	Amphotericin (30ug/ml)	-7.16667*	.36004	.000	-8.2112	-6.1221
DMSO & Water	Amphotericin (30ug/ml)	-15.66667*	.36004	.000	-16.7112	-14.6221

*. The mean difference is significant at the 0.05 level.

Growth inhibition of *C. albicans* at 100 mg/ml
Dunnett t (2-sided)

(I) Treatments	(J) Treatments	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
A. digitata	Amphotericin (30ug/ml)	-7.83333*	.28868	.000	-8.6709	-6.9958
Z. chalybeum	Amphotericin (30ug/ml)	-8.16667*	.28868	.000	-9.0042	-7.3291
L. cornuta	Amphotericin (30ug/ml)	-15.66667*	.28868	.000	-16.5042	-14.8291
G. trichocarpa	Amphotericin (30ug/ml)	-7.83333*	.28868	.000	-8.6709	-6.9958
DMSO & Water	Amphotericin (30ug/ml)	-15.66667*	.28868	.000	-16.5042	-14.8291

*. The mean difference is significant at the 0.05 level.

Appendix 8: Brine shrimp lethality raw data

PLANT NAME	**CONCENTRATION (µg/ml)**											
	1000 **(µg/ml)**				100 **(µg/ml)**				10 **(µg/ml)**			
	1st trial	2nd trial	3rd trial	Average mortality	1st trial	2nd trial	3rd trial	Average mortality	1st trial	2nd trial	3rd trial	Average mortality
A. digitata **(aq)**	2	0	2	1.33	0	0	1	0.33	0	0	0	0
A. digitata **(org)**	2	2	4	2.67	1	2	0	1	0	0	0	0
Z. chalybeum **(aq)**	7	8	9	8	2	3	2	2.33	0	0	0	0
Z. chalybeum **(org)**	10	10	10	10	5	6	6	5.67	4	3	2	3
L. cornuta **(aq)**	7	6	6	6.33	2	1	2	1.67	0	0	1	0.33
L. cornuta **(org)**	8	7	10	8.33	2	4	6	4	2	0	2	1.33
G. trichocarpa **(aq)**	1	0	0	0.33	0	0	0	0	0	0	0	0
G. trichocarpa **(org)**	1	3	3	2.33	0	0	0	0	0	0	0	0