EVALUATION OF ANTIMICROBIAL ACTIVITY, TOXICITY AND PHYTOCHEMICAL SCREENING OF SELECTED MEDICINAL PLANTS OF LOSHO, NAROK COUNTY, KENYA.

By

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DECLARATION

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DEDICATION

I dedicate this project to the best dad in the world Patrick Chalo Mutiso, my late mum, my siblings Jaelyn, Richard, my sweetheart (Ngina), Okito, Tyso, Mbuvi, Damaris and my entire friends who kept me in their prayers for their invaluable support and motivation during this study.

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LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA: Analysis of Variance

AST: Antimicrobial Susceptibility Test

ATTC: American Type Culture Collection

DCM : Duncan Mutiso Chalo

DMC: Dichloromethane

DMSO: Dimethylsulphoxide

ENT: Ear nose and throat infection

EASL: European Association for the Study of the Liver

KEMRI: Kenya Medical Research Institute

LC_{50}: Median Lethal Concentration (concentration required to kill 50% of a population)

MRSA: Methicilin resistant *Staphylococcus aureus*

NAI: University of Nairobi Herbarium

NIAD: National Institute of Allergy and Infectious Disease

SPSS: Statistical Packages for Social Scientists

STDs: Sexually Transmitted Diseases.

TM: Traditional Medicine

USD: United States Dollar

WHO: World Health Organization

ABSTRACT

In Kenya, microbial infections are a major cause of morbidity. Antibiotic effectiveness is threatened by increasing resistance of pathogenic microbes against most available drugs as new pathogens continue to emerge. Currently, herbal remedies offer hope considering they are readily and cheaply available. The objective of this study was to investigate antimicrobial activity, brine shrimp lethality and phytochemical composition of crude extracts of four selected plants namely *Schrebera alata* (Oleaceae), *Ormocarpum kirkii* (Papilionoideae), *Helichrysum forskahlii* (Asteraceae) and *Cussonia holstii* (Araliaceae) that are medicinally used by herbalists from Losho, Narok County Kenya for treatment of ear, nose and throat infections, gastrointestinal disorders and skin ailments. Qualitative antimicrobial susceptibility test against five microorganisms, methicillin resistant *Staphylococcus aureus, Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa and Candida albicans* was investigated using agar diffusion methods to generate inhibition zones and data accrued analyzed using Analysis of variance. Minimum inhibitory concentrations were determined by broth microdilution method. Toxicity of the extracts was investigated using brine shrimp lethality assay. Median lethal concentration fifty was determined by analysis of data using Finney's computer program. Phytochemical screening for flavanoids, sterols, alkaloids, tannins, quinones and terpenoids and saponins was determined using standard procedures. It was observed that the organic crude extracts of *H. forskahlii* had the highest inhibition zone against methicillin resistant *Staphylococcus aureus* of 19.5 and 18.5 mm in agar well and agar disk diffusion respectively. In addition, organic extracts of *H. forskahlii* revealed the highest antifungal inhibition zone of 8.5 mm in agar well diffusion. Minimum inhibitory concentrations values varied from 15.625 to 250mg/ml.Organic crude extracts of *Helichrysum forskahlii* and *Cussonia holstii* were found to be highly toxic with lethal concentration of 0.009 mg/ml. All the plant crude extracts contained flavanoids, sterols, alkaloids, tannins, quinones and terpenoids. Saponins were present in all the plant extracts screened except organic extracts of *H. forskahlii*. This study provides the first record of antimicrobial activity, toxicity and phytochemical composition of *S.alata* and *C.holstii*. The study has shown that *H. forskahlii* and *O.kirkii* possess promising antimicrobial activity against microbes of health importance and could lead to the isolation of novel, safe and efficacious

antimicrobial compounds. Further research should be carried on *O. kirkii* and *S. alata* to isolate and characterize the compounds responsible for the observed activity.

Key words: Medicinal plants; Antimicrobial activity; Brine shrimp lethality assay; phytochemical composition; Losho; Narok County; Kenya.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Microbial infections remain a threat to millions of lives globally (Kalita *et al.,* 2012).Currently there is an increased problem of antibiotic resistance due to microbial persistence (Kitonde *et al.,* 2013). The rapid rise in microbial resistance to synthetic drugs has urged formulation of new antimicrobial agents and evaluation of the efficacy of natural plant products as a substitute for chemical antimicrobial agents (Pandian *et al*., 2006). Traditional medicine is either the mainstay of health care delivery or serves as a complement to it across the world (WHO 2008). The World Health Organization 2008 estimates that up to 80% of the population in some developing countries use traditional medicine. Traditional medicine has been recognized as a part of primary health care program in many African countries. In Kenya, rich pharmacopoeia systems have been documented for communities such as the Maasai, Gusii, Luo, Abaluhyia and the Kikuyu (Kokwaro 2009).

According to WHO (2002) and Pandey *et al.*, (2011), a medicinal plant is that which contains substances that can be used for therapeutic purpose or which are precursors of chemo-pharmaceuticals semi synthetic new drugs. Phytochemicals offer unique platform for structural diversity and biological functionality which is indispensable for drug discovery. Plants have an almost limitless ability to synthesize secondary metabolites which may have both a defensive role against herbivores, pathogen attack and interplay competition. Moreover these metabolites act as attractant for pollinators or symbionts. Many natural occurring compounds in plants possess antimicrobial function and serve as antimicrobial agents (Kalita *et al*., 2012).

In Kenya, like other African countries, traditional medicine is practiced in the treatment of sexually transmitted diseases, eye infections, skin related problems, wounds, gastrointestinal diseases, measles and snake bites among others (Njoroge and Bussmann, 2007;Kokwaro 2009;Odhiambo *et al*., 2010).Microbial infections such as tuberculosis, candidiasis, cryptococcosis and salmonellosis have been on the increase in recent past, partially due to HIV/AIDS pandemic (Mwitari *et al.,* 2013). At the same time, antibiotics are becoming less and less effective against microbial illnesses due to emergence of drug-resistant bacteria.

Natural products of higher plants may give new sources of antimicrobial agents with possibly novel mechanisms of action (Bhalodia and Shukla, 2011).

According to Kokwaro (2009), overdose by patient due to imprecise nature of diagnosis is worldwide. Toxic effects have been attributed to certain active principles found in plants (Nguta *et al*., 2011).

The Maasai of Losho who are mainly pastoralists not only depend on plants for food, fuel, wood but also for medicine in rituals and ceremonies (Karehed and Odhult, 1997). However, many of these plants have not been investigated for phytochemical composition, antimicrobial and toxicity activities. Therefore, this study aimed to evaluate the antimicrobial activity, brine shrimp lethality and phytochemical composition of crude extracts from *Schrebera alata*, *Ormocarpum kirkii*, *Helichrysum forskahlii* and *Cussonia holstii.* These plants are majorly used in traditional medicine for treatment of skin diseases, gastrointestinal tract diseases and respiratory problems.

1.2 Problem statement

Antibiotic resistance has become a global concern due to an increasing incidence of multiple resistances in human pathogenic microorganisms in recent years, largely due to indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases (Adenisa *et al*. 2000).Drugs from plant sources are key way towards addressing the problem. In addition, synthetic antibiotics are not only expensive, but also have side effects in the treatment of infectious diseases (Kone *et al*., 2004).

In Kenya, many communities especially from the rural areas still rely on herbal remedies. Currently, there is little information regarding literature on the use of medicinal plants in Kenya (Kigen *et al*., 2013). Several drugs have been derived directly or indirectly from plants including digoxin, taxol, vinblastine, nabilone and artemesin (Cragg and Newman, 2005).

1.3 Justification of study

The basis of choosing Losho as the study site is that the people in this area are primarily Maasai living in a traditional pastoral culture and they still cherish the use of herbal remedies despite the presence of a Losho

Dispensary. *Schrebera alata, Omorcarpum kirkii, Cussonia holstii* and *Helichrysum forskahlii* are commonly used medicinal plants for microbial infections in Losho and no phytochemical composition, antimicrobial and toxicity activities have been documented or reported in literature. The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens. There is a constant need to search for cheap, new and effective antimicrobial agents and plants turn out to be a significant source of therapeutics and many potent and powerful drugs. Therefore, this research is necessary as a means of providing alternative antimicrobial medicines to overcome the constant and increasing drug resistance.

There is an urgent need to document information on Kenyan traditional medicine because there are genuine concerns that this knowledge may be completely lost. There should also be development of a legal framework to regulate the herbal practices in Kenya and incorporating herbal practices in mainstream health services as it has been successfully done in parts of Asia.

1.4 Objectives

1.4.1 Main Objective

To evaluate *in vitro* antimicrobial, toxicity and phytochemical composition of crude extracts of four selected medicinal plants traditionally used in Losho, Narok County, Kenya.

1.4.2. Specific objectives

i. To evaluate the antimicrobial potential of the crude plant extracts against *Pseudomonas aeruginosa, Escherichia coli,* methicillin resistant *Staphylococcus aureus* (MRSA), *Bacillus cereus* and *Candida albicans*
ii. To determine the acute toxicity of the crude plant extracts using brine shrimp (*Artemia salina*) lethality assay.
iii. To investigate the presence of major phytochemical constituents in the crude plant extracts.

1.5 Hypothesis

Null Hypothesis: Crude extracts of medicinal plants don’t have antimicrobial properties and are not safe.

Alternative Hypothesis: Crude extracts of medicinal plants have antimicrobial properties and are safe.

CHAPTER TWO

LITERATURE REVIEW

2.1 Significance and prospects of herbal remedies/medicine

Since time immemorial man has used plants to treat various human and livestock ailments (Kokwaro, 2009).These medicines initially took the form of crude drugs such as tinctures, teas, poultices, powders, and other herbal formulations (Balick and Cox, 1997; Balunas and Kinghorn, 2005).The use of local medicinal plants varies from species to species and from disease to disease, from place to place, from tribe to tribe and even from person to person (Kokwaro 1993). There are also many similarities in plant utilization (Kokwaro, 2009) .Several parts of plants such as stem, leaves, roots, tubers, flowers and seeds are utilized during preparation of plant drugs mainly through decoction.

About 20% of patients, who seek conventional medical care, first consult traditional healers (De Jong 1991). Traditional medicine therefore is a significant source of healthcare for many Africans, (De Jong 1991; WHO 2002; Boer *et al.,* 2005). In Kenya, 90% of the population has used medicinal plants at least once for various health conditions (Chirchir *et al*., 2006).

Herbal medicines are deep rooted in Maasai life (Sindiga, 1994).The importance of medicinal plants among the Maasai can be seen in the name, *olchani,* which is used both as a general name for all plants as well as for medicine (Maundu *et al*., 2001).The Maasai attribute most illness to the effect of pollutants that block or inhibit digestion. These pollutants can include "polluted" food, contact with sick people and witch craft. In most cases these treatment of illness involve herbal purgatives to cleanse the patient (Bussmann *et al*., 2006).

Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well-being. Quinine is an example of a secondary product present in plants with medicinal benefits. This alkaloid occurs naturally in the bark of *Cinchona officinalis* and is useful in the treatment of malaria (Achan *et al.,* 2011).

Nevertheless, secondary metabolites have antimicrobial properties. Some of the plants used in African traditional medicine have been investigated as sources of antibiotics, anti-tumour agents and other useful substances (Kokwaro, 1993)

Today, herbal remedies offer a real hope for discovery of new antibiotics (Panghal *et al.*, 2010),since very few plant species have been studied so far for their pharmaceutical potential, there is enormous potential for finding more compounds (Gurib-Fakim, 2005). For example, there is encouraging news by Australian researchers on a new compound SBEL1, derived from a Chinese herb and also reported to be in Taiwan, has since time immemorial been used as a traditional remedy for sore throat and inflammatory conditions (EASL, 2014).Today, rationally designed polyherbal formulation is being developed as option for multi-target therapeutic and prophylactic applications (Gurib-Fakim, 2005).

Drug companies generate more than USD 100 million each year from the sale of drugs from natural products in USA (Onaga, 2001).

2.2 Microbial infections and health effects

Bacterial infections are prevalent due to various factors such as the HIV/AIDS pandemic, poor hygiene, overcrowding and resistance to convectional antimicrobials (Adenisa *et al*., 2000) and without urgent coordinated action, the world is heading towards a post-antibiotic era (WHO, 2014).

According to healthcare experts, infectious diseases caused by microbes are responsible for more deaths worldwide than any other single cause (NIAID, 2009).Plants are a possible source of antimicrobial agents (Adenisa *et al*., 2000). The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens (Bandow *et al.*, 2003). Bacterial and fungal pathogens have evolved numerous defense mechanisms against antimicrobial agents, and resistance to conventional therapeutics is rapidly increasing. The increasing antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity (Colombo and Bosisio 1996; Scazzocchio *et al.*, 2001).

It is estimated that among the traditional medicinal plants, less than 10% have undergone pharmacological evaluation (Marimuthu *et al*., 2011).There is therefore a need to carry out more research on traditional

medicinal plants to acquire new antimicrobial agents from plants as an alternative source to the available antibiotics as they may be effective against resistant pathogens of plants and animals to avoid the threat of post biotic era (Marimuthu *et al.,* 2011).

There are several reports in the literature regarding the antimicrobial activity of crude extracts prepared from plants. Kalita *et al*., 2012 reports antibacterial and antifungal potentiality of *Centella asiatica,Nerium indicum and Cuscuta reflexa*; Bulb extracts of *Gladiolus dalenii* have antifungal activity (Odhiambo *et al*., 2010). *Vernonia glabra* have antimicrobial activity (Kitonde *et al*., 2013).Infections associated with bacterial pathogens are among some of the indications treated using traditional remedies in Kenya (Njoroge and Bussmann 2007).

2.2.1. *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a Gram-negative bacterium commonly found in soil and ground water. It rarely affects healthy people and most community-acquired infections are associated with prolonged contact with contaminated water. Although *P. aeruginosa* is an opportunistic pathogen it can cause a wide range of infections, particularly among immunocompromised people (HIV or cancer patients) and persons with severe burns, diabetes mellitus or cystic fibrosis (Nicasio *et al*., 2008).

These microbes have low antibiotic susceptibility attributable to a concerted action of multidrug efflux pumps with chromosomally encoded antibiotic resistance genes and the low permeability of the bacterial cellular envelopes thus becoming resistance to a wide range of antibiotic (Poole, 2004). Also *P. aeruginosa* easily develops acquired resistance either by mutation in chromosomally encoded genes or by the horizontal gene transfer of antibiotic resistance determinants. It is naturally resistant to penicillin and related beta-lactam antibiotics (Li *et al.,* 2000).

2.2.2 *Escherichia coli*

Escherichia coli is a gram negative bacteria mostly harmless that form part of the natural microbial flora of healthy human or animal intestinal tract. However, some *E. coli* strains are pathogenic, meaning they can cause illness, either diarrhoea or illness outside of the intestinal tract. *E. coli* antibiotic resistance is a growing problem due to overuse of antibiotics in humans and use of antibiotics as growth promoters in

animal feeds (Johnson *et al.,* 2006). These bacteria often carry multiple drug-resistance plasmids, and under stress, readily transfer those plasmids to other species hence important reservoir of transferable antibiotic resistance (Salyers *et al.,* 2004).

2.2.3 Methicillin resistant *Staphylococcus aureus* (MRSA)

Staphylococcus aureus is a gram- positive bacteria frequently found in the human respiratory tract and skin (Kanafani and Fowler, 2006).Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of hospital-acquired infections that are becoming increasingly difficult to combat because of emerging resistance to all current antibiotic classes. The evolutionary origins of MRSA are poorly understood, no rational nomenclature (Enright *et al.,* 2011).MRSA is any strain of *Staphylococcus aureus* that has developed resistance to beta-lactam antibiotics, Aminoglycosides, Quinolones, Clindamycin and Erythromycin through the process of natural selection (Turnidge *et al*., 2002).

2.2.4 *Bacillus cereus*

As *B. cereus* is found in soil, raw plant foods such as rice, potatoes, peas, beans and spices are common sources of *B. cereus* (Wijnands 2008). It is a Gram-positive, rod-shaped, endospore forming, facultative aerobic bacteria that produces toxins (Vilain *et al.,* 2006).These toxins can cause two types of illness: one type characterized by diarrhoea and the other by nausea and vomiting (Wijnands *et al.,* 2006).

In addition, *B. cereus* is an opportunistic human pathogen and is occasionally associated with infections, causing periodontal diseases and other more serious infections (Hill *et al*., 2006). Immunocompromised patients are susceptible to this bacterium and may cause endocarditis, meningitis, pneumonia and endophthalmitis. Its potential to cause systemic infections is of current public health and biomedical concerns (Dufrenne *et al.,* 2006).

2.2.5 *Candida albicans*

Candida albicans is a diploid fungus that grows both as yeast and filamentous cells and a causal agent of opportunistic oral and genital infections in humans (Marchese *et al*., 2007).*Candida* is the major opportunistic pathogen in immune compromised patients; over 90% of HIV-infected individuals develop

oral candidiasis (Feigal *et al*., 1991). It is also a major etiological agent of oral candidiasis (Hazen *et al*., 1995) especially in diabetic mellitus patients due to high sugar concentrations (Mubarak *et al.,* 2013).*C. albicans* is reported to be resistant against fluconazole, flucytosine, and intraconazole whereas resistance to Amphotecirin B is rare (Marchese *et al*., 2007).

2.3 Significance of toxicity studies

Although plants have been extensively used, specific evaluation on toxicity has not been done and could lead to serious complications (Rahman *et al.*, 1995). Less than 10% of herbal products in the world market are standardized to known active components (Sahoo and Manchikanti, 2013).

Toxicological studies constitute an essential part of the effort in developing a herbal medicine into a drug product and its aim is to identify adverse effects and to determine limits of exposure level at which such effects can occur (Wu *et al*.,2008).An equally important objective of toxicity testing is the detection of toxic plant extracts or compounds derived thereof in the early (pre-clinical) and late (clinical) stages of drug discovery and development from plant sources (Ifeoma and Oluwakanyinsola, 2013).

There have been reports in the literature that many herbal medicine preparations are potentially toxic and some are even carcinogenic (Nagarisichia *et al*., 2012). For example, aristolochic acid derived from *Aristolochia* spp. is associated with the development of nephropathy and urothelial cancer (Stiborova and Schmeiser, 2002).

In order to study the toxicity of the medicinal plants, brine shrimp lethality bioassay can be performed which is based on the ability to kill laboratory cultured brine shrimp (*Artemia salina*). Brine shrimp lethality assay has proven to be a convenient system for monitoring biological activities of plant species that are used in traditional medicine. The lethal concentration for 50% (LC_{50}) mortality after 24 hrs of exposure is determined as a measure of toxicity of the extract (Nguta *et al.,* 2011). The assay is considered a useful tool for preliminary assessment of toxicity because it is easy, cheap and small amount of extracts are utilized. Nguta *et al.,* (2013) also reported that this method is rapid, reliable and convenient as an in-house bioassay tool and brine shrimp larvae can be stored for long periods of time without losing viability.

2.4 Phytochemical screening.

Phytochemicals are very useful in pharmaceutical industries (Stafford and Warren, 1993).In addition, they are biodegradable and renewable (Kubo and Taniguchi, 1993).More than 25% of modern medicines are directly or indirectly derived from plants (Okoro *et al.,* 2010).

Medicinal plants are an important source of traditional drugs, modern medicines, folk medicines, nutraceuticals, pharmaceutical intermediates and entities for synthetic drugs since plant extracts contain many medicinal metabolites such as alkaloids, glycosides, terpenoids, flavanoids and lignins (Tiwar *et al*., 2011).Some basic compounds associated with plants in Kenya and reported to have medicinal value are: oils, alkaloids and anthraquinones (Kokwaro, 1993).

Medicinal plant materials have been successfully used for treatment of fungal and bacterial infection in humans (Akinyosoye and Oladummoye, 2000), suggesting that plant materials may also possess antifungal and antibacterial constituents which are useful in controlling plant diseases (Amadioha, 1998).

The herbal plants commonly used by the Maasai community in Kajiado district are rich in phytochemicals and minerals (Onyango *et al*., 2014).A preliminary phytochemical literature on bioactivity of some Maasai plant species, antibacterial species included *Acacia nilotica* (whole plant), *Asparagus africanus* (root), *Ricinis communis* (whole plant) and *Solanum incanum* (fruit) (Karehed and Odhult, 1997).

2.5 Description, traditional use and chemical constituents of the four plant species.

2.5.1 *Schrebera alata* (Hochst.) Welw. (Oleaceae)

Schrebera alata is a quick-growing evergreen tree or shrub, 4–15 m high, with a greyish or light brown bark (Figure1). The leaves are opposite and pinnately compound with few leaflet pairs and a single terminal one. The petiole and rachis are narrowly winged. The leaves are shiny dark green above, paler beneath and smooth or velvety when young. The flowers are trumpet-shaped and white to pink, with reddish brown hairs near the mouth of the corolla tube. They are sweet-scented (the fragrance is stronger in the evening)

and arranged in terminal clusters up to 110 mm long. The fruits are pear-shaped and shiny green, turning brown in maturity and becoming woody. They split into two halves when ripe and contain about 8 papery, winged seeds. The seeds are dispersed by wind (Beentje, 1994.

Ethnobotanical studies in Samburu show that the root bark of *S. alata* is pounded or chewed as treatment for candidiasis and toothache (Nanyingi *et al.,* 2008). Pounded roots, twigs and leaves are chewed to treat tooth complaints and as antitussive, and in water they are applied as a wash to ulcers. Bark is also chewed to treat toothache, and bark decoctions are applied as anodyne. Leaves are chewed to treat tonsillitis, pharyngitis and headache, and leaf decoctions are administered as a vapour bath for treatment of headache and taken to treat colds, cough, fever, and as emetic, oxytocic and tonic anaesthetic (Kokwaro 2009).

Iridoid glycosides are taxonomic markers of Oleaceae family (Jensen 2002). *Schrebera swietenioides* root have been reported to contain alkaloids, steroids, saponins and glycosides (Manda *et al.,* 2009)

Figure 1 *:* *Schrebera alata* (photo by Duncan M. Chalo)

2.5.2 *Ormocarpum kirkii* (Taub.) Engl. (Fabaceae, Papilionoideae)

Shrub or small tree (Figure 2), bark is dark brown, fissured, corky; young branches often with white hairs. Leaves clustered on dwarf spur branchlets, imparipinnate with 7-15 leaflets; leaflets elliptic-oblong, 3-11 mm long, under surface often with white hairs and densely covered with very small black dots; apex mucronate. Flowers in 1-4-flowered clusters, mauve-pink to violet (Figure 3). Fruit a very distinct small pod, densely covered in long, stiff golden hairs, resembling a caterpillar. Found in stony places and on rocky hillsides in hot dry areas (Agnew and Agnew 1994).

Dried roots are powdered and applied locally for bone setting and also boiled taken for bilharziasis (Moshi 2005). The leaves of *Ormocarpum trichorcarpum* are used for treating stomach-related ailments in traditional medicine and antibacterial and antioxidant activities of the leaves have been reported (Chukwujekwu *et al.*, 2013). Isoflavanones, & bisdihydrocoumarins have been isolated from *Ormocarpum kirkii* (Xu *et al.*, 2012;Dhooghle *et al.*, 2010).*Ormocarpum cochinchinense* leaf extracts have been reported to contain alkaloids, cardiac glycosides, flavanoids and saponins (Pazhanisamy and Ebenezer 2013).

Figure 2: *Ormocarpum kirkii* (photo by Duncan M. Chalo)

Figure 3: *Ormocarpum kirkii* leaves (photo by Duncan M. Chalo)

2.5.3 *Helichrysum forskahlii* (J.F. Gmel.) Hilliard & B.L. Burttv (Asteraceae)

Showing yellow inflorescence perennial herb or shrub, 20–100 cm high; stem woody, much branched, the branches terete, tomentose but glabrescent, usually densely beset with leaves and often with marcescent leaves as well (Figure 4 and 5). Leaves narrowly lanceolate or linear, sessile, 0.4–2.5(–5.5) cm long, 0.1–0.8 cm wide, base half-amplexicaul, margins entire and revolute, apex acute, sparsely to densely tomentose. Capitula 2–4.5 mm long, heterogamous, in dense (sub-) globose cymes arranged in terminal leafy corymbs, all axes tomentose; stalks of individual capitula to 1.5 mm long; receptacle fimbriae to 1 mm; phyllaries pale green, pale yellow or silvery yellow with darker apices, 4–5-seriate, ovate to oblong, 1–3.5 mm long, the outermost smallest with some indument near the base, the inner glabrous and sparsely glandular, apex obtuse or eroded. Florets pale yellow, becoming orange when dead; outer florets 7–20, tube filiform, 2.2–2.6 mm long, lobes 0.2–0.4 mm long and glandular, style 2.4–2.8 mm long; inner florets 2–10, tube cylindrical to narrowly infundibuliform, 1.8–2.4 mm long, lobes 0.3–0.5 mm long, glandular, anthers 0.6–1 mm long, style 2.5–2.9 mm long. Achenes ellipsoid, 0.4–0.7 mm long, glabrous and papillose; pappus 1.5–2.5 mm long, barbellate, caducous in groups (Beentje, 1994).

Several *Helichrysum* species have been used as diuretics, anti inflammatory and anti-allergic. Chemical studies on *Helichrysum* species have been carried out by many investigators and the presence of flavonoids, phloroglucinols

a-pyrones,couma-rins and terpenoid compounds has been reported (Al-Rehaily *et al.,* 2008). *Helichrysum* species have been found to have antimicrobial activity (Farah *et al*., 2008).

In Rwanda and Burundi, many species of the *Helichrysum* genus (Asteraceae) are used in traditional folk medicine for treating diarrhoeal diseases. *Helichrysum forskahlii, Helichrysum panduratum* and *Helichrysum odoratissimum and* the essential oil from *Helichrysum forskahlii* has antibacterial activity (Kajangwe *et al.,* 2008).Oleanolic acid has also been isolated from the whole plant (Al-Rehaily *et al.,* 2008).

Figure 4: *Helichrysum forskhalii* (photo by Duncan M. Chalo)

Figure 5: *Helichrysum forskahlii* inflorensence (photo by Duncan M. Chalo).

2.5.4 *Cussonia holstii Harms* ex Engl (Araliaceae).

A tree up to 20 m tall with fissured bark, juvenile leaves simple, ± deeply palmately lobed, the adult ones digitately compound with petiole up to c. 40 cm long; leaflets 3–7, ovate, hairy to glabrous, (5–) 6–13(–18) x 3–7(–9) cm, acuminate at the apex, cuneate to cordate and slightly asymmetric at the base, with serrate or crenate margin; petiolules up to 6 cm long(Figure 7 and 8). Flowers sessile, in spikes up to 12(–15) cm long, spikes often many together; bracts subtending flowers scale-like, 1 mm long. Fruit subglobose, 3–6 mm long(Agnew and Agnew 1994). Upon biological screening, a substantial antitrichomonas activity was found in the dichloromethane extract of the bark of *Cussonia holstii* (He *et al.,* 2003).

A detailed chromatographic study using centrifugal partition chromatography revealed pentacyclic triterpenoid (He *et al*.,2003).Triterpene glycosides, ursolic and Hederagenic have been isolated from leaves

of *Cussonia paniculata* (Dovgii *et al.,* 2005).The stem bark of *Cussonia bancoensis* has been reported to contain triterpenoid saponins (Tapondjon *et al*., 2003).

The hollow trunk is used locally to make bee hives, and the wood is white, soft and used for making doors by local people. A decoction of the bark is used by the Masai and Meru tribes for expulsion of the placenta after birth-giving (He *et al.,* 2003).

Figure 6: *Cussonia holstii* (photo by Duncan M.Chalo)

Figure 7: *Cussonia holstii* aerial part (photo by Duncan M.Chalo)

CHAPTER THREE

MATERIALS AND METHODS

3.1. Collection of plant materials

Four plants selected based on their ethno medicinal usage were collected from Losho, Narok County, Kenya with the help of herbalists. The bark stems of *Schrebera alata* and *Cussonia holstii* were peeled; aerial parts of *Ormocarpum kirkii* were cut and chopped into pieces while the whole plant of *Helichrysum forskahlii* (herb) was uprooted. The plant parts were stuffed in a polythene bag which was placed in a cooler box and transported to Nairobi where they were thoroughly washed with running tap water and dried at room temperature for six weeks there after ground into a fine powder using an electric mill.

Plants specimens were collected in duplicate; one specimen was used for preliminary identification in the field as previously described (Agnew and Agnew 1994) while the other was pressed and carried to the University of Nairobi Herbarium for authentication and further compared with the available permanent prepared herbarium collections.

Table 1: Voucher specimens and plants parts collected from Losho Narok County

Voucher specimen Number	**Plant species**	**Family**	**Part Collected**
DMC2014/001	*Schrebera alata* (Hochst.)Welw.	Oleaceae	Bark
DMC2014/002	*Omorcarpum kirkii* (Taub.) Engl.	Fabaceae	Aerial part
DMC2014/003	*Cussonia holstii* Harms ex Engl.	Araliaceae	Bark
DMC2014/004	*Helichrysum forskahlii* (J.F. Gmel.) Hilliard & B.L. Burttv.	Asteraceae	Whole plant

3.2. Preparation of crude extracts

3.2.1. Preparation of organic extracts.

Dichloromethane/methanol (1:1) was used to extract 50g of grounded material by cold solvent percolation according to standard extraction methods .The powdered plant material was mixed thoroughly with the solvent, left to stand for 24 hrs, and decanted (this was repeated twice). The filtrates were pooled and filtered using a Buchner funnel. After evaporation of the solvents at 40ºC to obtain crude extracts, dichloromethane and methanol extracts were obtained. These were then stored in airtight containers at 4ºC awaiting bioassay and phytochemical screening (Odhiambo *et al*. 2014).

3.2.2 Preparation of aqueous extracts

The ground materials were extracted by cold maceration method. Fifty grams of ground plant material were extracted with distilled water (500 ml) to obtain aqueous extracts. The aqueous extracts were filtered and the filtrate kept in a deep freezer then lyophilized (freeze dried) resulting to a dry powder and then stored in airtight containers at 4°C awaiting bioassays and phytochemical screening (Odhiambo *et al*., 2014).

3.3 Media preparation

3.3.1 Mueller Hinton Agar

Fifty eight grams of MHA medium was suspended in 1000 ml distilled water. Heating to boiling to dissolve the medium completely was done followed by sterilization by autoclaving at 15lbs pressure (121°C) for 15 minutes. Mixing was done well before pouring (Bauer *et al*., 1996).

3.3.2 Sabouraud Dextrose Agar

Sixty five grams of the medium were suspended in one liter of purified water and heated with frequent agitation and boil for one minute to completely dissolve the medium then autoclaved at 121°C for 15 minutes. The prepared media was stored at 8-15°C (Murray *et al*., 2003).

3.4 Source of microorganisms.

The microorganisms methicillin resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa* were obtained from KEMRI, Centre for Microbiology Research (CMR) while *Bacillus cereus, Escherichia coli, and Candida albicans* were from the department of Public health, Pharmacology and Toxicology, University of Nairobi.

Table 2: List of microbes tested in the study

Name of microbe	Microbe type	Gram strain type	Details of strain used
Bacillus cereus	Bacteria	Gram Positive	ATCC 11778
MRSA	Bacteria	Gram Positive	ATCC 1385
Pseudomonas aeruginosa	Bacteria	Gram Negative	ATCC 27823
Escherichia coli	Bacteria	Gram Negative	ATCC 25922
Candida albicans	Fungus		ATCC10231

3.5. Sub culturing of test microorganisms.

Sub culturing of the test strains was done. Bacteria were grown in Muller–Hinton agar for 18hrs and fungus in Sabouraud Dextrose agar for 48hrs to obtain freshly growing strains. The microbial suspensions were standardized according to the Clinical and Laboratory Standards Institute procedures (CLSI, 2009 for bacteria) and (CLSI, 2008 for fungi) with sterile saline to turbidity equivalent to 0.5 McFarland (approximately 1.5×10^8 CFU/ml for bacteria and 1.5×10^6 CFU/ml for *Candida* sp.) and stored at 4^oC until used during antimicrobial test.

3.6. Antimicrobial susceptibility testing

Agar diffusion methods according to National Committee for Clinical Laboratory Standards (CLSI 2009) procedures were used to evaluate antimicrobial activities of the crude extracts. Twenty ml of sterile Muller-Hinton Agar and Sabouraud Dextrose Agar was poured into sterile petri plates and allowed to set. An inoculum suspension was swabbed uniformly to solidified 20 ml Mueller-Hinton Agar (MHA) for bacteria and Sabouraud Dextrose Agar (SDA) for fungi and the inoculum was allowed to dry for 5 min. Three concentrations (400, 200, 100 mg/ml) of each

test extract (organic extracts and aqueous extracts of *S.alata, C.holstii, O.kirkii, H. forskahlii*) were prepared for susceptibility testing using 1% DMSO for organic extracts and distilled water for aqueous extracts.

3.6.1 Agar well diffusion

Holes of 10 mm in diameter were made in the seeded agar using sterile cork borer.100 µl of the test extracts was introduced into the wells using microtiter-pipette and allowed to stand on the bench for 1h for proper diffuse into agar and thereafter incubated at 24hrs at 37° C. Microbial growth was determined by measuring the diameter of zone of inhibition in millimetres (mm). For each microbial strain, controls were maintained where pure solvents were used instead of the crude extracts (Parekh and Chanda, 2007). The experiment was performed in triplicates under sterile condition and the mean values obtained.

3.6.2 Disc diffusion

Commercially prepared sterile discs of 6 mm in diameter were impregnated with 100 µl of each crude extract dried and placed aseptically onto plates inoculated with 1ml overnight growth test microorganism. Bacterial cultures and fungal culture were incubated at 24hrs at 37° C for bacteria and 37 ° C for 72hrs for fungi. Chloramphenicol 30µg/ml (for bacteria) and Amphotericin B 30 µg/ml (for fungi) were used as positive controls while discs with diluting solvents only were used as negative controls. Each extract was tested in triplicate under sterile conditions. Microbial growth was determined by measuring the diameter zone of inhibition in millimeters (Kitonde *et al*.,2013).

3.7 Determination of Minimum Inhibitory Concentration (MIC)

Broth micro dilution method was used to determine minimum inhibitory concentration for the active crude extracts against the test microorganisms. The procedures was done as recommended by the National Committee for Clinical Laboratory Standards now Clinical Laboratory Standard Institute (CLSI) (Ferraro, 2003). 0.5 mL of 24 h culture of test organisms (10^7 CFU/mL) adjusted to McFarland turbidity standard 0.5 McFarland (approximately 1.5×10^8 CFU/ml for bacteria and

1.5×10^6CFU/ml for *Candida* sp. were incubated in serial dilution 250mg/ml, 125mg/ml, 62.5mg/ml, 31.25mg/ml, 15.625mg/ml. Incubation was done for 24hrs at 37 $^{\circ}$ C. The least concentration of the plant extract that did not permit any visible growth of the inoculated test microorganism in broth culture as indicated by lack of turbidity was regarded as visual MIC in each case (Michael *et al.,* 2003). Tubes inoculated with microbes alone and media alone served as control. All the experiments were done in triplicates and results were recorded.

3.8 Determination of acute toxicity of crude extracts

The acute toxicity assay was performed using brine shrimp *nauplii* based on Meyer method (Nguta *et al*., 2013). Artificial sea water was prepared by dissolving 38 grams of sea salt in 1litre of distilled water. A tank measuring 14 cm by 9 cm and 5 cm having two unequal compartment chambers with several holes on the divider was used for hatching. The chambers were filled with artificial sea water. Brine shrimp eggs were placed in the larger compartment and yeast was added to act as food for the *nauplii*. The larger compartment was then covered with dark background paper while the smaller compartment was illuminated. The incubation was done at room temperature (23-29^{o}C) for 48h to allow hatching and *nauplii* were collected in the illuminated section.

Various concentrations of the crude extract in sea water were used: 10, 100, and 1000 µg/ml in testing toxicity. A stock solution of 10,000µg/ml for each crude extract was prepared. For the aqueous extracts, the stock solution of 10, 000µg/ml was prepared by dissolving 0.5g of the crude extract in 10 ml of sea water while for organic extracts; 0.1 g of each sample was first dissolved in 1% DMSO then further diluted using artificial water to 10 ml to make stock solution.

10 brine shrimp larvae were drawn from the hatching tank using Pasteur pipettes and placed in each vial. The volume of artificial sea water in each vial containing 10 Brine shrimp salina was increased to 5ml for vials of 10 and 100 µg/ml of the plant extracts while for 1,000 µg/ml; it was topped to 4.5 ml. Using micropipettes, 0.5 ml, 0.05 ml and 0.005 ml were transferred from the stock solution to the vials containing 5mls artificial sea water to make experimental solutions

containing 1000 µg/ml, 100µg/ml and 10 µg/ml respectively (Table 3).Control experiments were done using artificial sea water and DMSO for organic extract and artificial sea water only in the case of aqueous extract (Wanyoike *et al.,* 2004). Three replicates for the three serial dilutions of different crude extracts and the control were performed. Surviving *nauplii* were counted after 24hr using a magnifying glass and the average mortality at each concentration was determined as it was essential for estimation of $LC_{50.}$

Table 3: Brine shrimp bioassay set up for each plant extract

Vials	**Volume of Artificial sea water (ml)**	**No of Brine shrimp larvae**	**Volume of stock solution (ml)**	**Concentration (µg/ml)**	**Nature of experiment**	**Final volume in the vial (ml)**
1	4.5	10	0.5	1,000	Trial	5
2	4.5	10	0.5	1,000	Repeat	5
3	4.5	10	0.5	1,000	Repeat	5
4	5	10	0.05	100	Trial	5
5	5	10	0.05	100	Repeat	5
6	5	10	0.05	100	Repeat	5
7	5	10	0.005	10	Trial	5
8	5	10	0.005	10	Repeat	5
9	5	10	0.005	10	Repeat	5
10	5	10	0	0	Control	5
11	5	10	0	0	Control	5
12	5	10	0	0	Control	5

3.9 Qualitative phytochemical screening of crude extracts

In order to identify some classes of the secondary metabolites in these plants, qualitative chemical tests were conducted on all the crude plant extracts. Identification was based on a characteristic colour change of precipitate or foam development. Alkaloids, flavanoids, saponins, tannins, glycosides, quinones and terpenoids were detected using standard methods (Trease and Evans, 2002) as illustrated in Table 4.

Table 4: Detection for phytochemicals

Phytochemical Test	Detection
Test for Saponins Foam test	1g of each extract was shaken with distilled water in a test tube for 15mins.Frothing which persist on warming was taken as preliminary evidence for the presence of saponins.
Test for Sterols Salkowaski test	1g of each extract was dissolved in 2 ml of chloroform and 2ml of concentrated sulphuric acid was added from the side of the test tube. Test tube was shaken for few minutes. The development of red color in chloroform layer indicated the presence of sterols.
Test for Alkaloids Dragendorff's test	1 g of each extract was dissolved into 5 ml of hydrochloric acid (1.5% v/v) and filtered. These filtrates were then used for testing alkaloids. Dragendorff's reagent was added into 2ml of filtrate. Formation of orange-brown precipitate indicated the presence of alkaloid
Test for Tannins	1g of each extract was stirred with 10ml of distilled water, filtered and 1ml of 5% ferric chloride was added to the filtrate. A blue black, green or blue-green precipitate was taken as evidence for the presence of tannins.
Test for Flavanoids	In a test tube with 1 g of each extract, a few drops of dilute sodium hydroxide(NaOH) was added and shaken. An intense yellow colour was produced in the plant extract which becomes colorless on addition of few drops of dilute acid indicates the presence of flavanoids.
Test for Quinones	1g of extract was shaken with 1ml of concentrated sulphuric acid(H_2SO_4). Formation of red colour shows the presence of quinones.
Test for Terpenoids	5g of each extract was mixed with 2 ml of chloroform. 3ml of concentrated sulphuric acid (H_2SO_4) was then added to form a layer. A reddish brown precipitate colouration at the interface formed indicated the presence of terpenoids.

3.10. 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. The lethal concentration (LC_{50}), 95% confidence interval of the selected plants was determined using the Finney (1971) computer program.

CHAPTER FOUR

RESULTS

4.1. Yields of extracts from test plants

The resulting dry powders were weighed and expressed as percentages. Organic extracts yielded a higher percentage in comparison to the water extracts except for *C.holstii* (Table 5).

Table 5: Percentage yields of extracted crude plant extracts

Plant species	**Part used**	**Solvent**	**Extraction type**	**% yield to weight of dry powered plant**
Ormocarpum kirkii	Aerial part	Water	Freeze drying	3.79
		Dichloromethane-Methanol (1:1)	Rotary evaporation	3.94
Schrebera alata	Bark	Water	Freeze drying	9.35
		Dichloromethane-Methanol (1:1)	Rotary evaporation	13.23
Cussonia holstii	Bark	Water	Freeze drying	4.76
		Dichloromethane-Methanol (1:1)	Rotary evaporation	4.47
Helichrysum forskahlii	Whole plant	Water	Freeze drying	6.32
		Dichloromethane-Methanol (1:1)	Rotary evaporation	10.74

Percentage yields of crude extract (% yields) = extracted weights/initial weights x 100 (All weights in grams)

4.2. Antimicrobial activity of the crude extracts against selected microorganisms

4.2.1. Antimicrobial activity of the crude extracts on MRSA

Crude extracts of *O. kirkii* and *H. forskahlii* had inhibition zones of 15mm at 100 mg/ml in agar well diffusion and disc diffusion (Figure 8 and 12).At 200mg/ml only organic extracts of the four plants were active against this microbe in both agar well and disk diffusion(Figure 9 and 14). Effective growth inhibition was noted at higher concentrations of each extracts (Figure 10 and 13). Of all the aqueous extracts tested in both agar well and disk diffusion, only *H. forskahlii was* active against this tested bacteria at 100,200,400 mg/ml concentration (Figure 11). In both agar well diffusion and disk diffusion, among the tested plants extracts, only organic extracts were active against MRSA at all the concentrations tested (Figure 15 and 16). The growth inhibition of MRSA by the various extracts of the four selected plants were significantly ($P \leq 0.05$) different from each other in both agar well and disk diffusion methods (Table 6).The significance levels of comparison of all but *H. forskahlii* organic plant extracts at the tested were all less than 0.05 hence the extracts had growth inhibitions of MRSA which were significantly different from that of the positive control at $P \leq 0.05$.However, organic extracts of *H. forskahlii* at 400mg/ml showed no significant difference to the positive control at $P \geq 0.05$ in both agar well and disk diffusion.

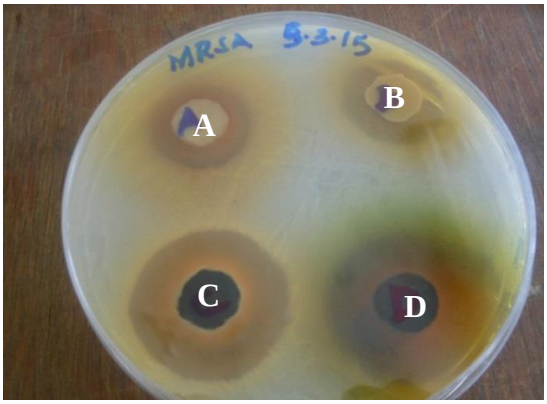

Figure 8: Antibacterial activity of organic extracts (100mg/ml) in a plate of MRSA in agar well diffusion method

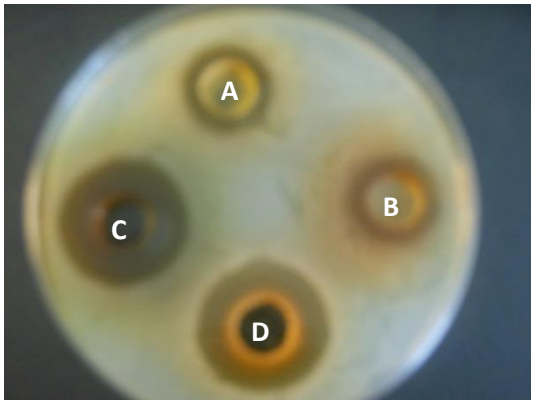

Figure 9: Antibacterial activity of organic extracts (200mg/ml) in a plate of MRSA in agar well diffusion method

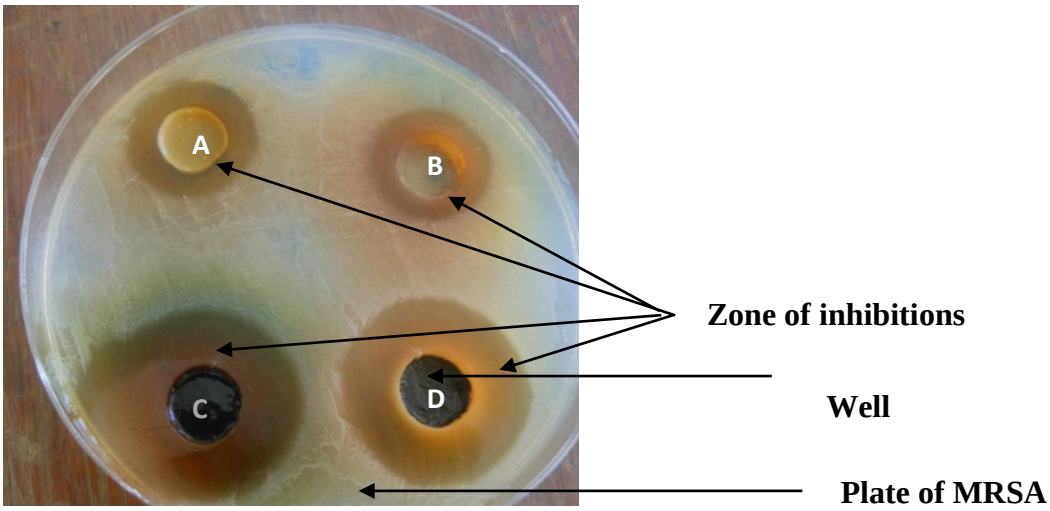

Figure 10: Antibacterial activity of organic extracts (400mg/ml) in a plate of MRSA in agar well diffusion method

Key: A: organic *Cussonia holstii* B: organic *Schrebera alata* C: organic *Omorcarpum kirkii* D: organic *Helichrysum forskahlii*

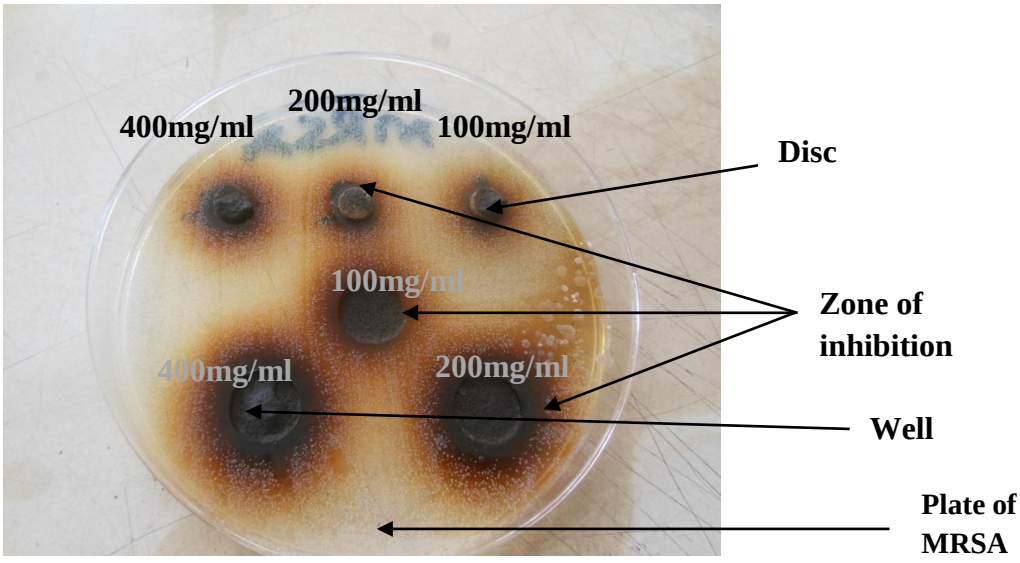

Figure 11: Antibacterial activity of aqueous extracts of *H. forskahlii* in a plate of MRSA in agar well and disk diffusion method at various concentrations.

Figure 12: Antibacterial activity of organic extracts (100mg/ml) in a plate of MRSA in agar disc diffusion method

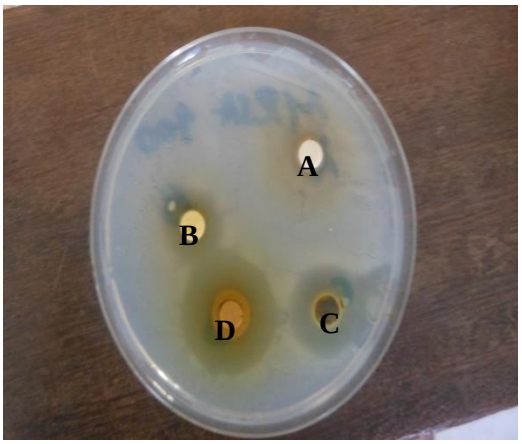

Figure 13: Antibacterial activity of organic extracts (400mg/ml) in a plate of MRSA in agar disc diffusion method

Key: A: organic *Cussonia holstii* B: organic *Schrebera alata* C: organic *Omorcarpum kirkii* D: organic *Helichrysum forskahlii*

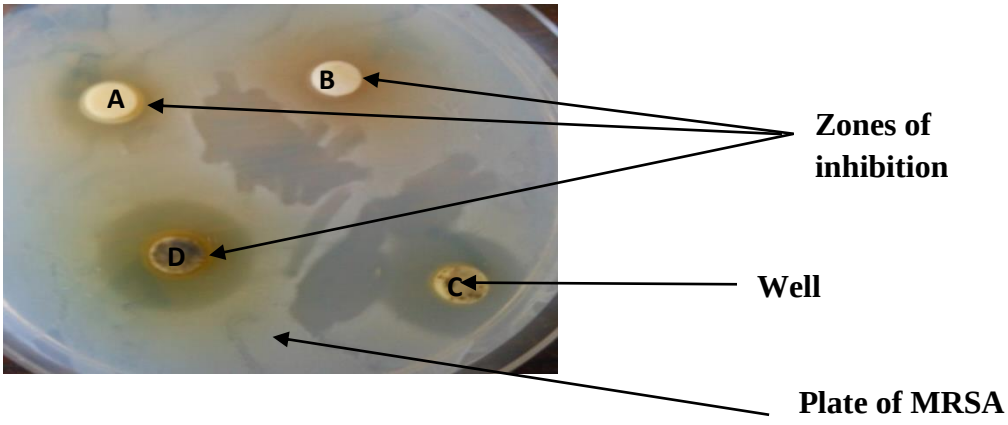

Figure 14: Antibacterial activity of organic extracts (200mg/ml) in a plate of MRSA in agar disc diffusion method**.**

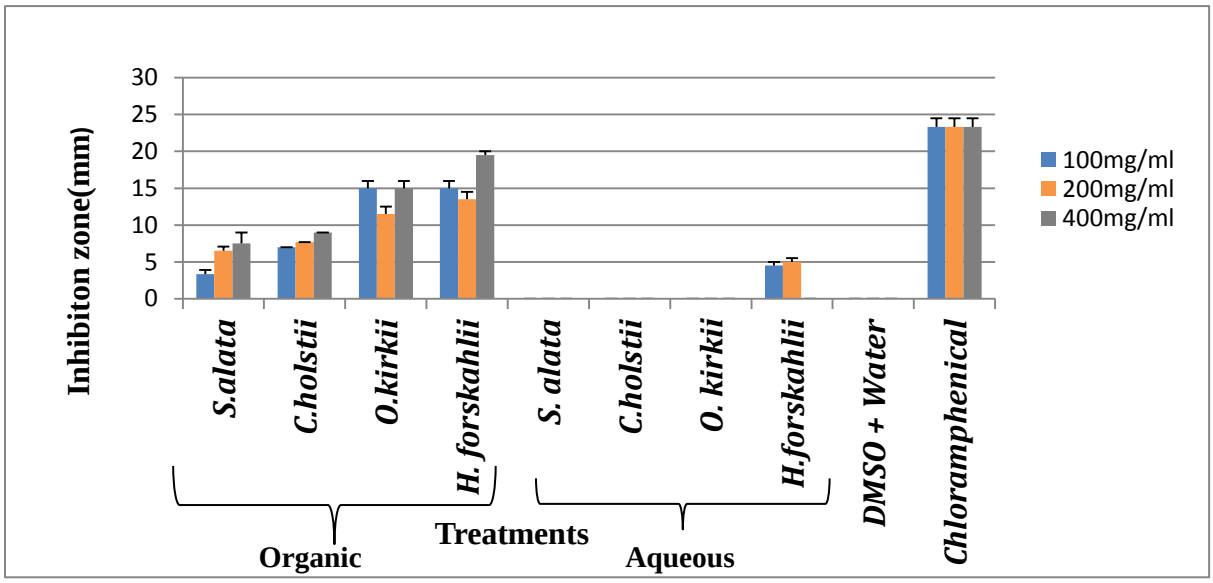

Figure 15: Growth inhibition of the crude extracts on MRSA in agar disc diffusion**.**

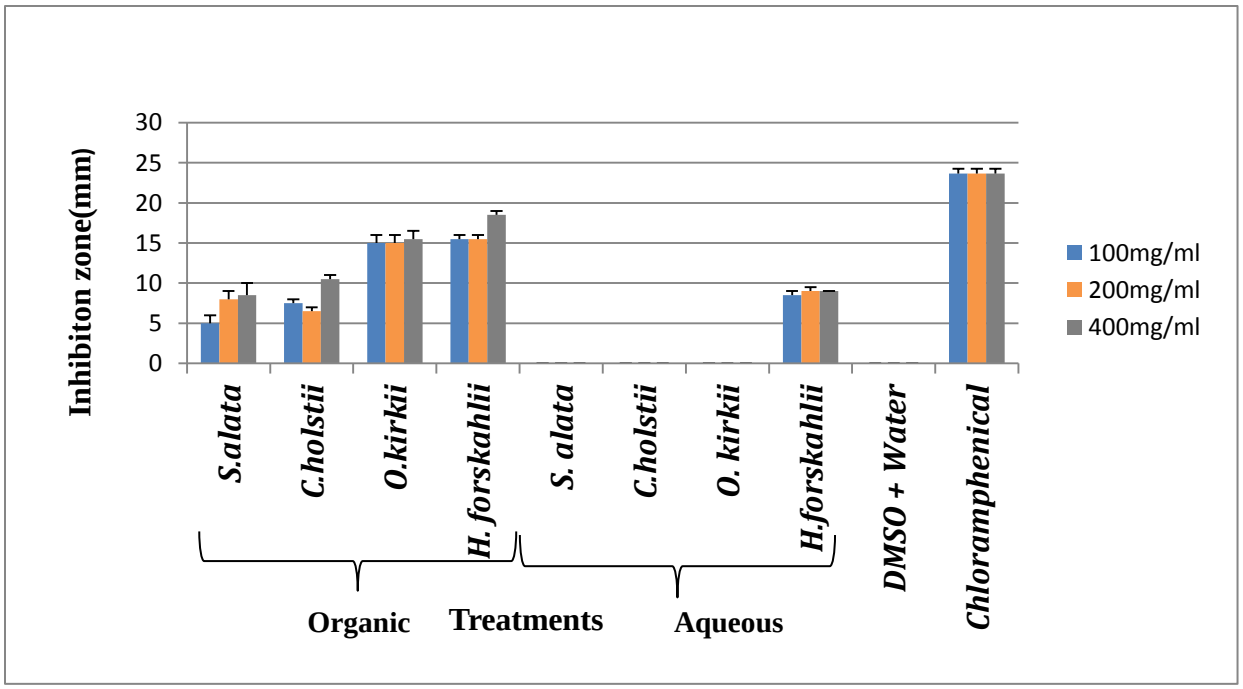

Figure 16: Growth inhibition of the crude extracts on MRSA in agar well diffusion.

Table 6: Dependent Variable: MRSA inhibition at different concentrations by various extracts

Method	Concentration in mg/ml		
	100	200	400
Disc diffusion	F=519.27	F=325.75	F=466.22
	P=0.00	P=0.00	P=0.00
Agar well diffusion	F=663.63	F=516.84	F=469.74
	P=0.00	P=0.00	P=0.00

4.2.2. Antimicrobial activity of the crude extracts on *P. aeruginosa*

H. forskahlii organic extracts had a diameter of 6mm in agar disc diffusion at 200mg/ml (Figure 17). Organic extracts of *S. alata* showed antibacterial activity at 400mg/ml in both agar well and disc diffusion method (Figure 18 and 20). Among the aqueous extracts tested, only *Omorcarpum kirkii* showed antibacterial activity against this bacterial strain at 400mg/ml in both experiments with an inhibition zone of 10mm (Figure 19).Of all the tested extracts, *H. forskahlii* organic extracts had the highest inhibition diameter of 10mm in agar well (Figure 21). Organic extracts of *C.holstii*, *O. kirkii* were not active against this microbe. In addition, *H. forskahlii* organic extracts had an inhibition zone of 5.5mm at 400 mg/ml in agar well diffusion (Figure 22). Growth inhibitions of *P. aeruginosa* by the various extracts of the four selected plants in both experiments were found to be significantly different from each other at 95% confidence intervals ($P \leq 0.05$)(Table 7). The significance levels of all the plants compared with the positive control at 400, 200, and 100 mg/ml were all less than 0.05 showing that all the extracts had growth inhibitions of *P. aeruginosa* which were significantly different from that of chloramphenicol which was used as a positive control.

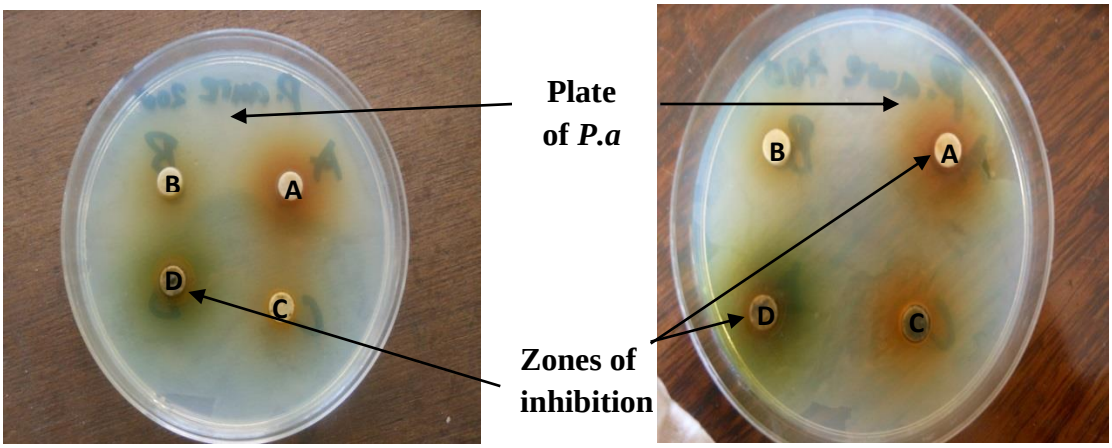

Figure 17: Antibacterial activity of organic extracts (200mg/ml) in a plate of *P. aeruginosa* in agar disc diffusion method

Figure 18: Antibacterial activity of organic extracts (400mg/ml) in a plate of *P. aeruginosa* in agar disc diffusion method

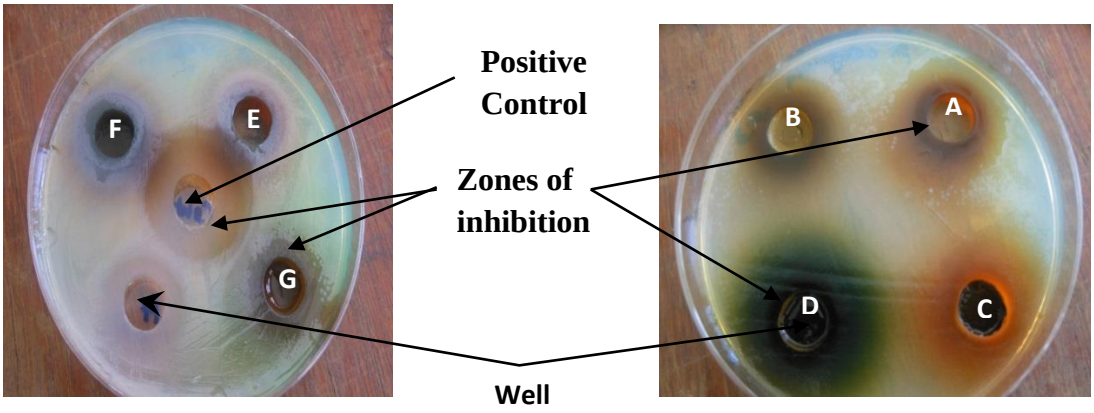

Figure 19: Antibacterial activity of aqueous extracts (400mg/ml) in a plate of *P. aeruginosa* in agar well diffusion method

Figure 20: Antibacterial activity of organic extracts (400mg/ml) in a plate of *P. aeruginosa* in agar well diffusion method

Key: A: Organic *Cussonia holstii* B: organic *Schrebera alata* C: organic *Omorcarpum kirkii* D: organic *Helichrysum forskahlii:* E: aqueous *Cussonia holstii* F: aqueous *Schrebera alata* G: aqueous P.a- *Pseudomonas aureginosa : G:* aqueous *Omorcarpum kirkii*

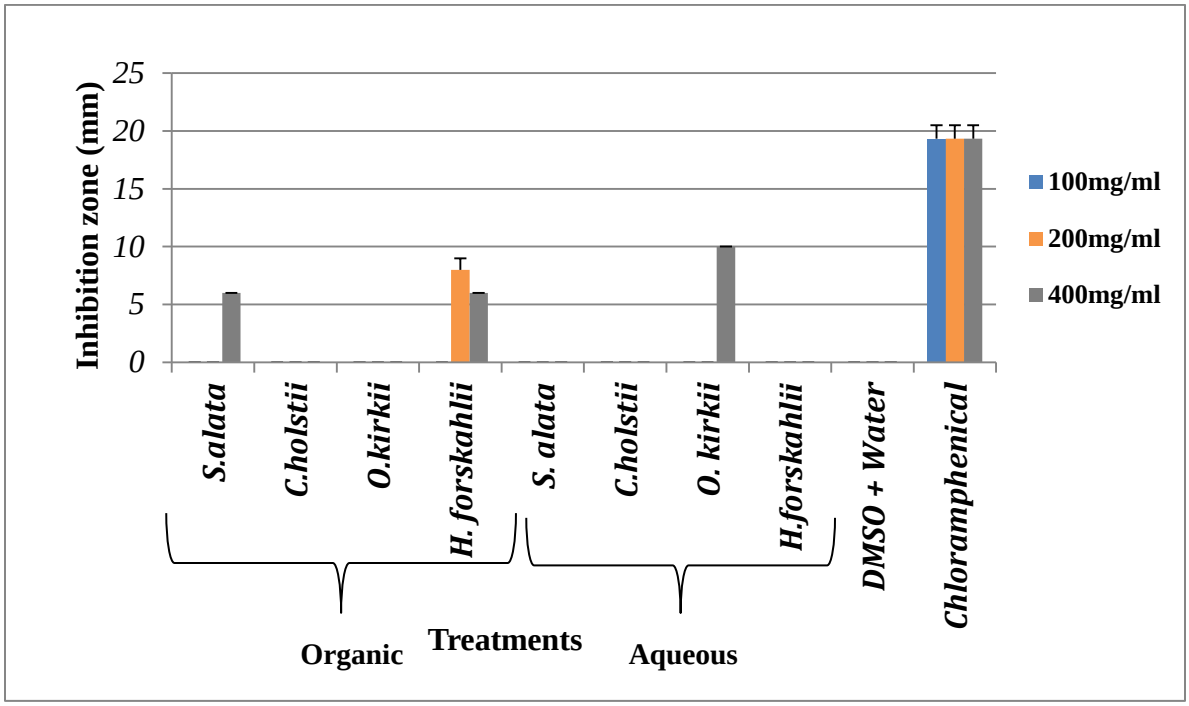

Figure 21 : Growth inhibition of the crude extracts on *P. aeruginosa* in agar disc diffusion

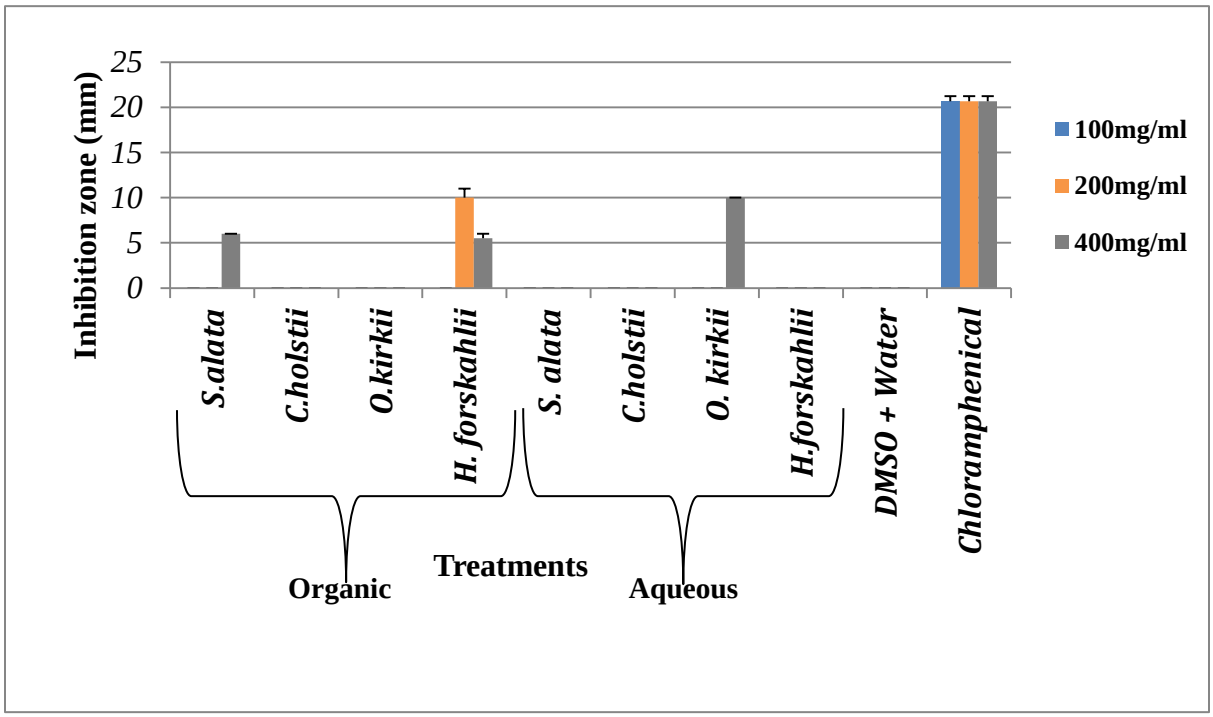

Figure 22: Growth inhibition of the crude extracts on *P. aeruginosa* in agar well diffusion

Table 7: Dependent Variable: *P. aeruginosa* inhibition at different concentrations by various extracts

Method	Concentration in mg/ml		
	100	200	400
Disc diffusion	-	F=1.07	F=2.38
	-	P=0.00	P=0.00
Agar well diffusion	-	F=1.08	F=2.38
	-	P=0.00	P=0.00

4.2.3. Antimicrobial activity of the crude extracts on *Bacillus cereus*

Only organic extract of *H. forskahlii* showed antibacterial activity against this gram positive bacteria with an inhibition zone of 6 and 7mm in disk diffusion at 200 and 400mg/ml respectively (Figure 23 and 24) whereas in agar well diffusion at the same concentrations inhibition zone of 4.5mm and 5mm respectively were showed by the same extracts (Figure 25 and 26). The positive control had an inhibition diameter of 19mm against this microorganism. All the tested extracts showed no activity at 100mg/ml in both agar well and disk diffusion methods (Figure 28 and 29). The growth inhibitions of *B. cereus* by the various extracts in both experiments were significantly different at ($P \leq 0.05$) (Table 8). Their significance levels of comparison of all the plants with the positive control at concentrations; 400 and 200ml were all less than 0.05 hence all the extracts had growth inhibitions of *B. cereus* which were significantly different from that of the positive control at $P \leq 0.05$ in both agar well and disk diffusion methods.

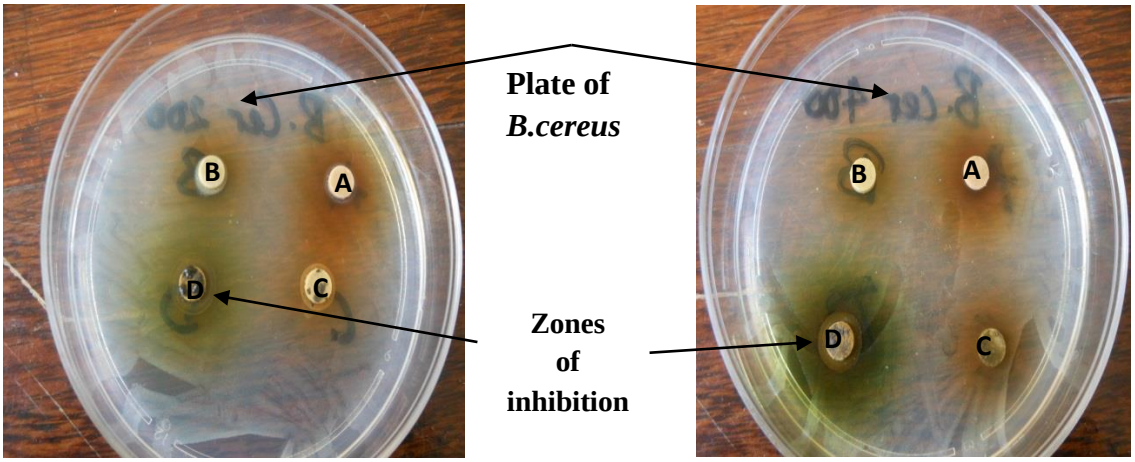

Figure 23: Antibacterial activity of organic extracts D (200mg/ml) in a plate of *B.cereus* in agar disk diffusion method

Figure 24: Antibacterial activity of organic extracts D (400mg/ml) in a plate of *B.cereus* in agar disk diffusion method

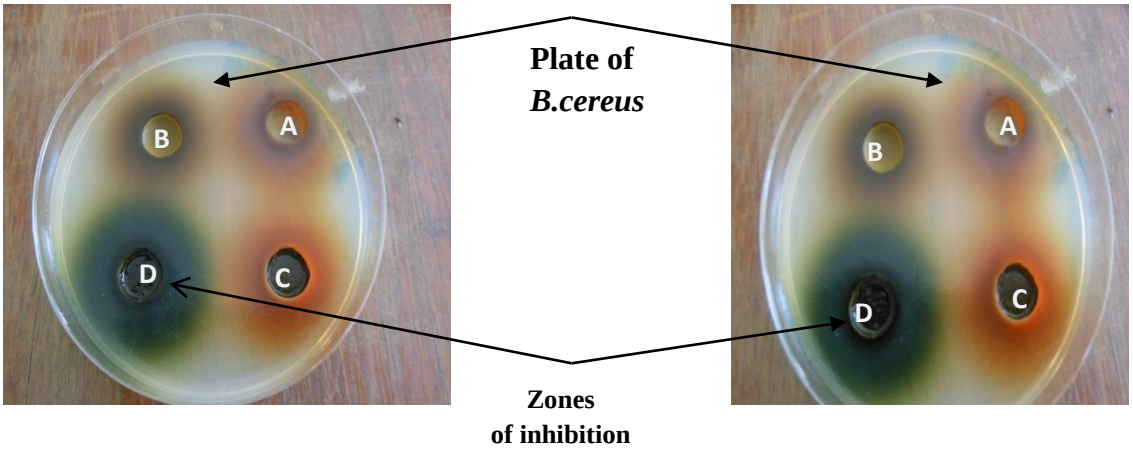

Figure 25: Antibacterial activity of organic extracts D (200mg/ml) in a plate of *B.cereus* in agar disk diffusion method

Figure 26: Antibacterial activity of organic extracts D (400mg/ml) in a plate of *B.cereus* in agar disk diffusion method

Key: A: organic *Cussonia holstii* B: organic *Schrebera alata* C: organic *Omorcarpum kirkii* D: organic *Helichrysum forskahlii*

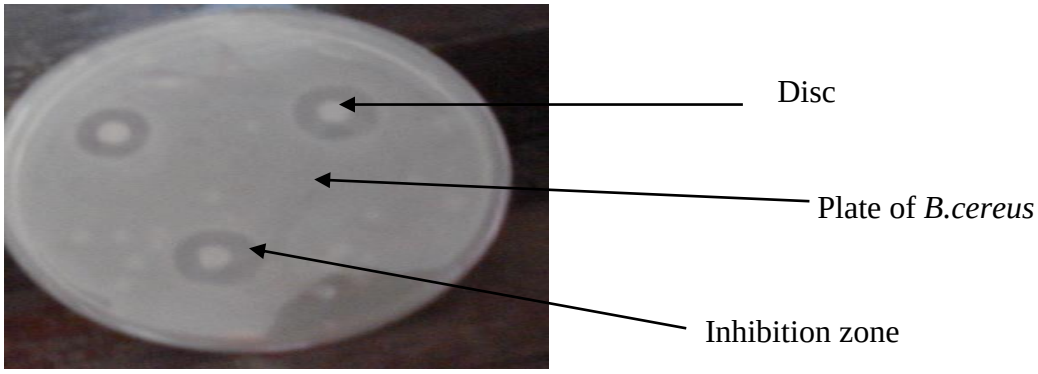

Figure 27: Antibacterial activity of chloramphenicol in a plate of *B.cereus* in agar diffusion method

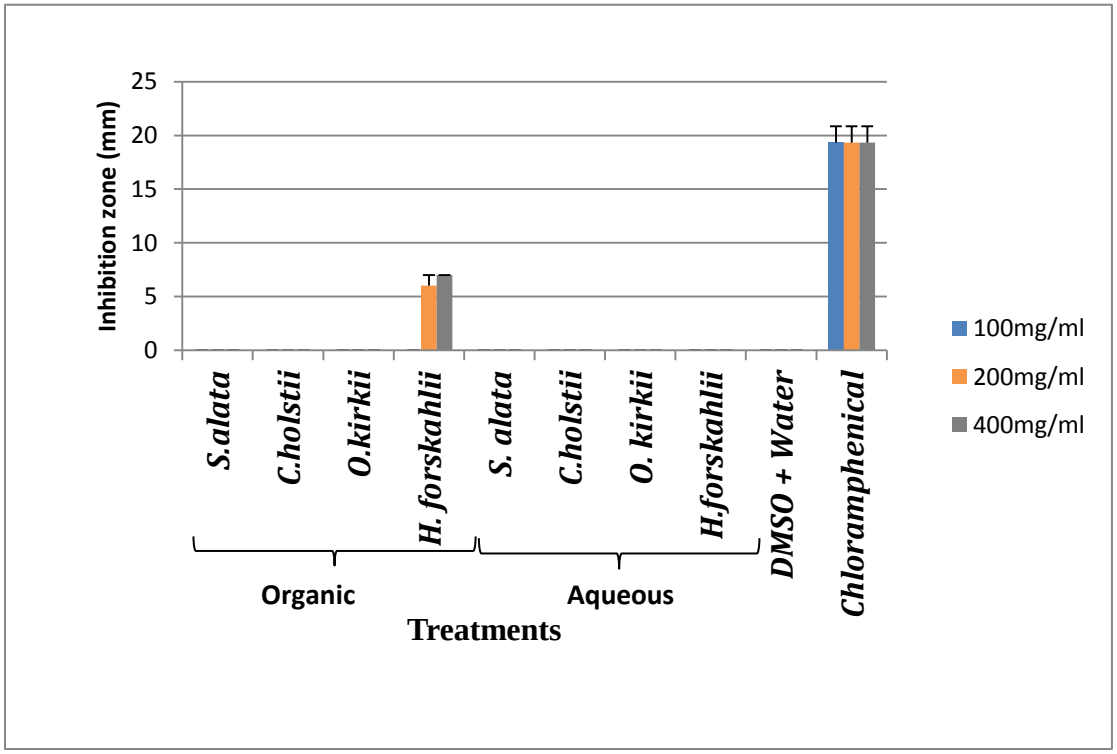

Figure 28: Growth inhibition of the crude extracts on *B.cereus* in disc diffusion**.**

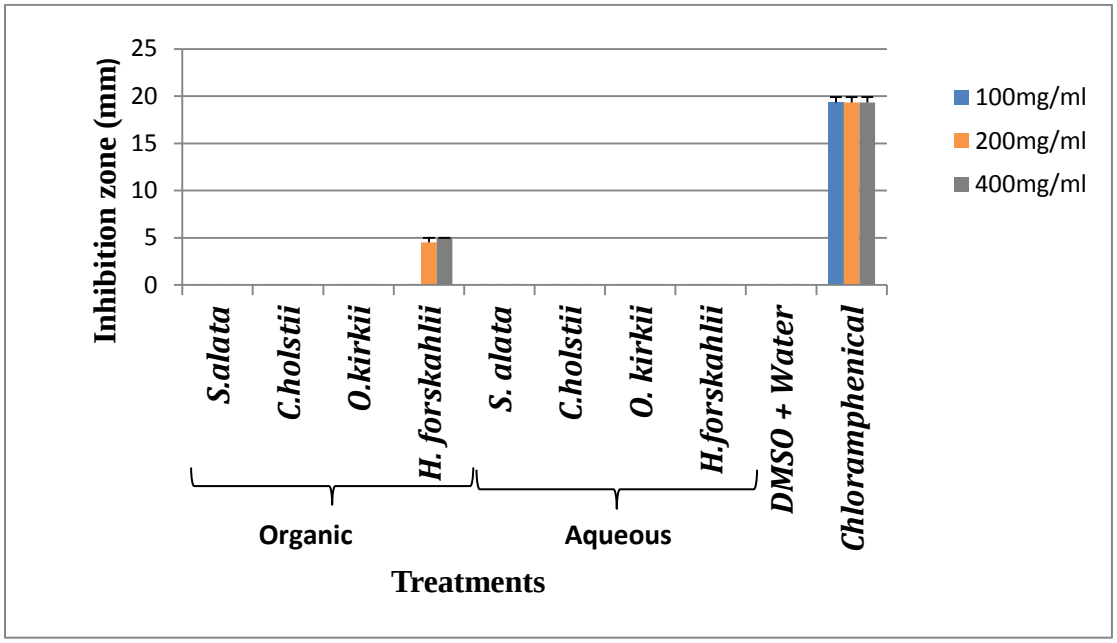

Figure 29: Growth inhibition of the crude extracts on *B.cereus* in agar well diffusion.

Table 8: Dependent Variable: *B.cereus* inhibition at different concentrations by various extracts

Method	Concentration in mg/ml		
	100	200	400
Disc diffusion	-	F=345.60	F=504.91
	-	P=0.00	P=0.00
Agar well diffusion	-	F=1.93	F=3.40
	-	P=0.00	P=0.00

4.2.3. Antimicrobial activity of the crude extracts on *E. coli*

Only organic extracts of *H. forskahlii* were active against this gram negative bacterial strain at 200 and 400mg/ml in disc diffusion method (Figure 30 and 31). In addition of all the tested extracts only organic extracts of *H. forskahlii* were active at 400mg/ml (Figure 32). Inhibition zones 6mm were noted at 400mg/ml in both agar well and disc diffusion (Figure 33 and 34) .Growth inhibitions of *E. coli* by the various extracts of the four selected plants in both methods

were found to be significantly different from each other at 95% confidence intervals (P≤0.05)(Table 9).The significance levels of all the plants compared with the positive control at 400, 200, and 100 mg/ml were all less than 0.05 showing that all the extracts had growth inhibitions of *E. coli* which were significantly different from that of Chloramphenicol which was used as a positive control in both agar well and disk diffusion.

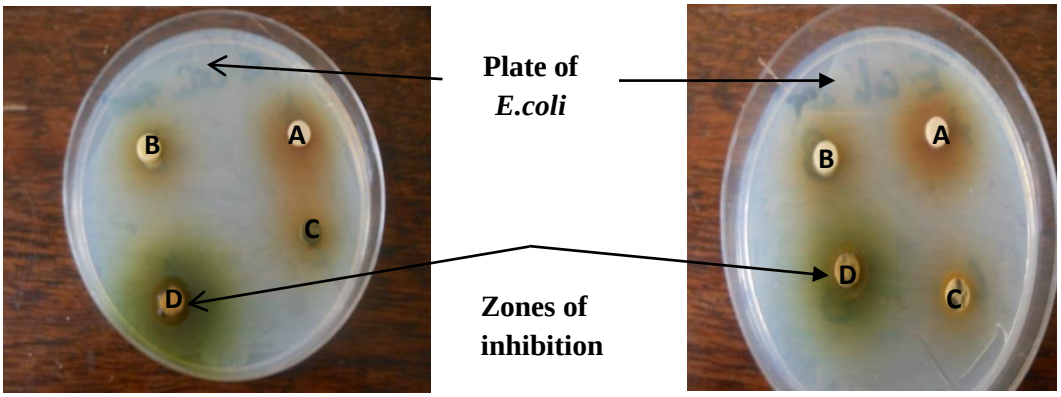

Figure 30: Antibacterial activity of organic extracts D (400mg/ml) in a plate of *E. coli* in agar disk diffusion method

Figure 31: Antibacterial activity of organic extracts D (200mg/ml) in a plate of *E. coli* in agar disk diffusion method

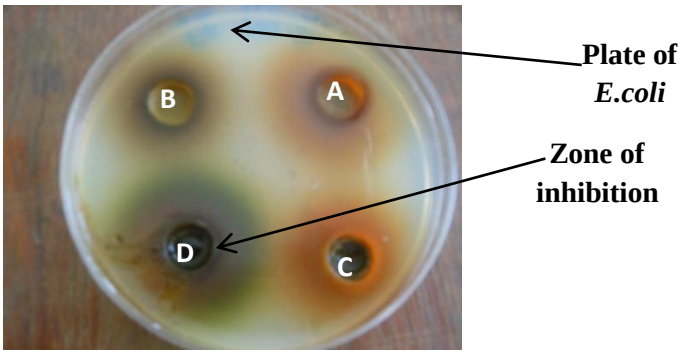

Figure 32: Antibacterial activity of organic extracts D (400mg/ml) in a plate of *E. coli* in agar well diffusion method

Key: A: organic *Cussonia holstii* B: organic *Schrebera alata* C: organic *Omorcarpum kirkii* D: organic *Helichrysum forskahlii*

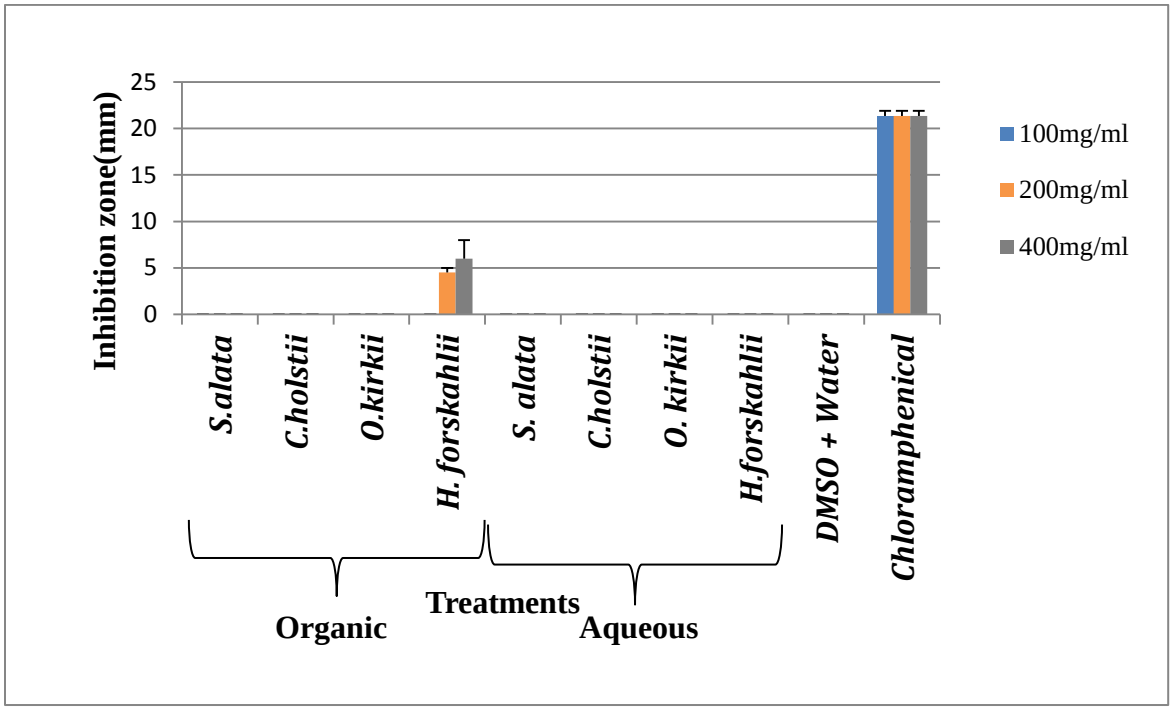

Figure 33: Growth inhibition of the crude extracts against *E .coli* in agar disc diffusion**.**

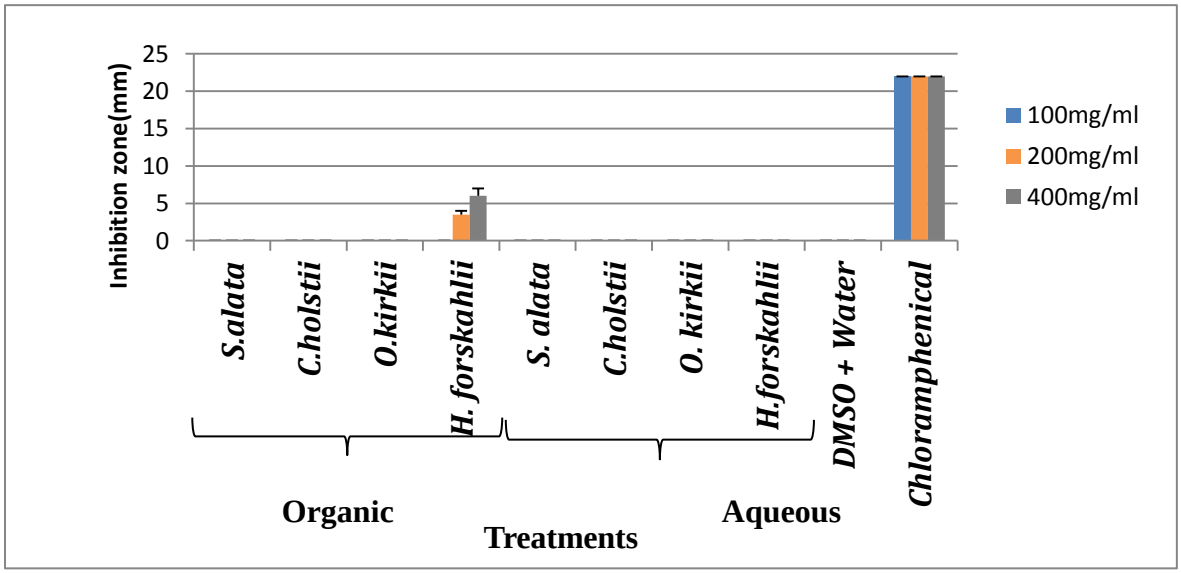

Figure 34: Growth inhibition of the crude extracts on *E. coli* in agar well diffusion

Table 9: Dependent Variable: *E.coli* inhibition at different concentrations by various extracts

Method	Concentration in mg/ml		
	100	200	400
Disc diffusion	-	F=2.33	F=320.31
	-	P=0.00	P=0.00
Agar well diffusion	-	F=5.75	F=1.47
	-	P=0.00	P=0.00

4.2.4. Antifungal activity of the crude extracts on *Candida albicans*

In agar disc method, *H. forskahlii* had antifungal activiry at 200 and 400mg/ml (Figures 35 and 36). More so, inhibition zones were noted in organic extracts of *H. forskahlii* at 400mg/ in agar well diffusion (Figure 37). Amphotericin B (positive control) had an inhibition diameter of 16mm against *C.albicans* (Figure 38). In addition, at 400mg/ml, organic extracts of *S. alata, C. holstii,* were active against this fungi strain (Figures 39 and 40). The highest antifungal activity with an inhibition zone value of 8.5mm was observed in organic extracts of *H. forskahlii* in agar well diffusion. It was evident that growth inhibition of *C. albicans* by the various extracts from the four plants was significantly different from each other in both experiments. ($P \leq 0.05$)(Table 10). Comparison of growth inhibition by all the plants with the positive control at 400, 200 and 100 mg/ml proved that all the extracts had growth inhibitions of *C. albicans* which were significantly different from that Ampotericin B used as a positive control in both agar well and disk diffusion.

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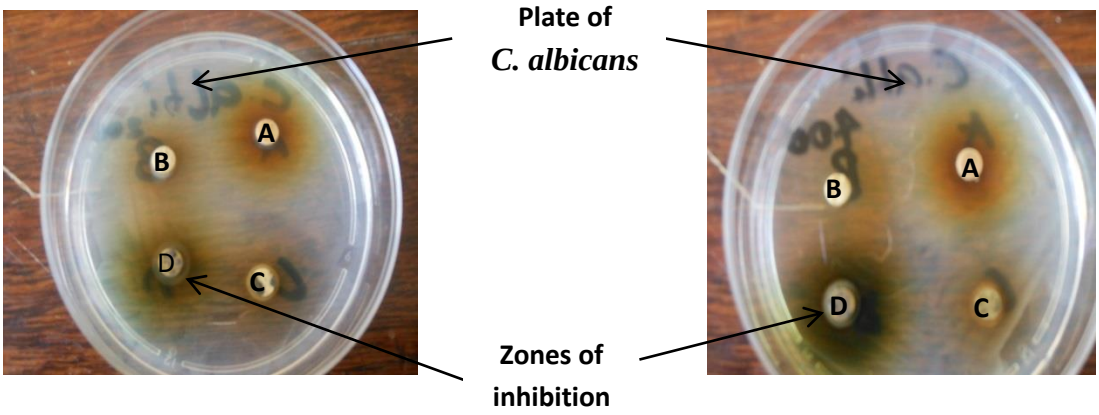


Figure 35: Antibacterial activity of organic extracts (200mg/ml) in a plate of *C. albicans* in agar disc diffusion method

Figure 36: Antibacterial activity of organic extracts (400mg/ml) in a plate of *C. albicans* in agar disc diffusion method

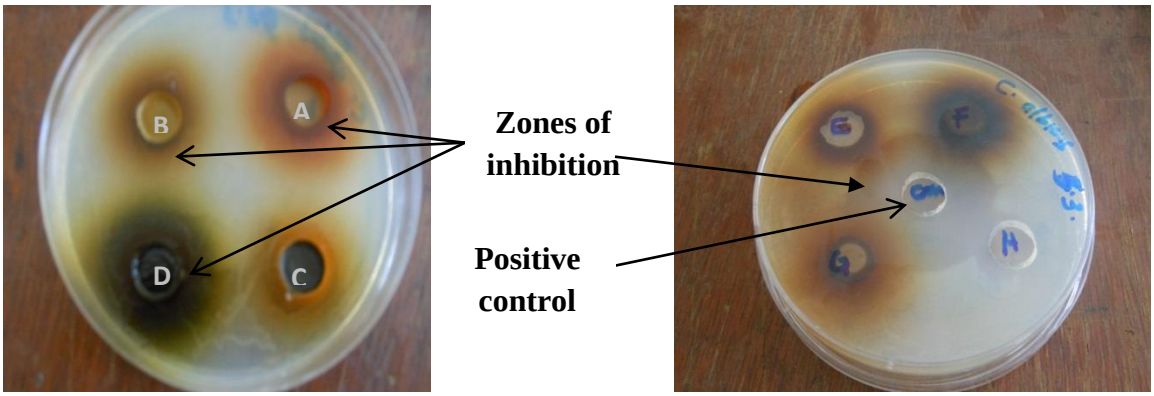


Figure 37: Antibacterial activity of organic extracts (400mg/ml) in a plate of *C. albicans* in agar well diffusion method

Figure 38: Antibacterial activity of organic extracts (400mg/ml) in a plate of *C. albicans* in agar well diffusion method

Key: A: organic *Cussonia holstii* B: organic *Schrebera alata* C: organic *Omorcarpum kirkii* D: organic *Helichrysum forskahlii*

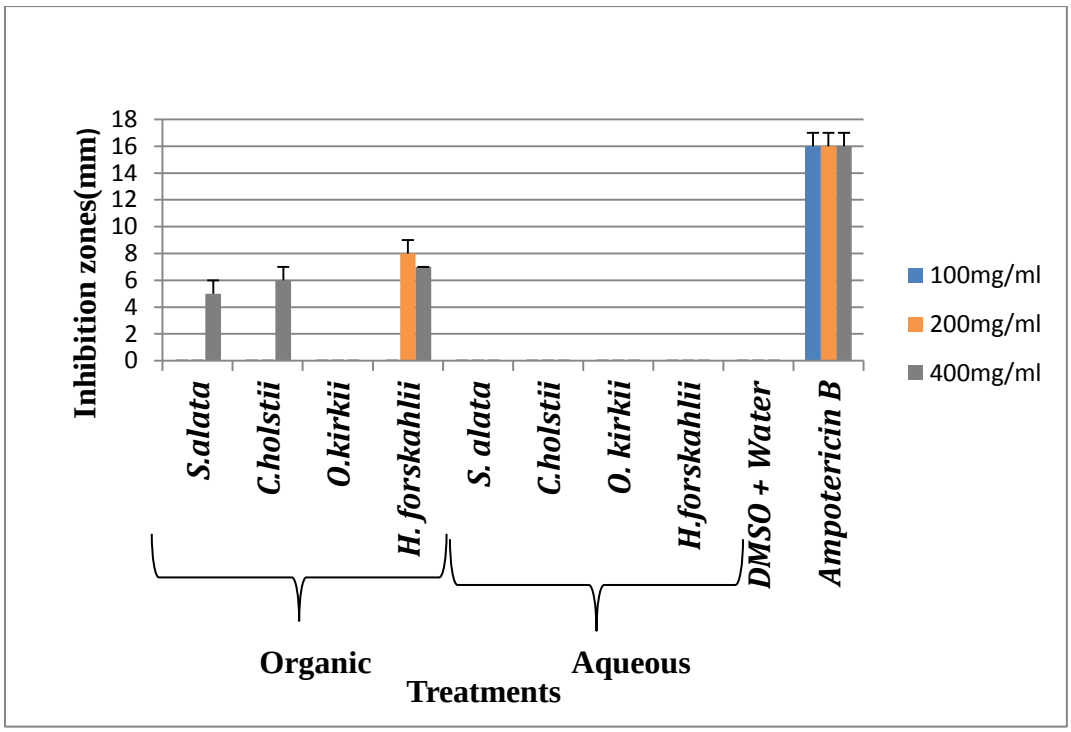


Figure 39: Growth inhibition of the crude extracts on *C. albicans* in disc diffusion

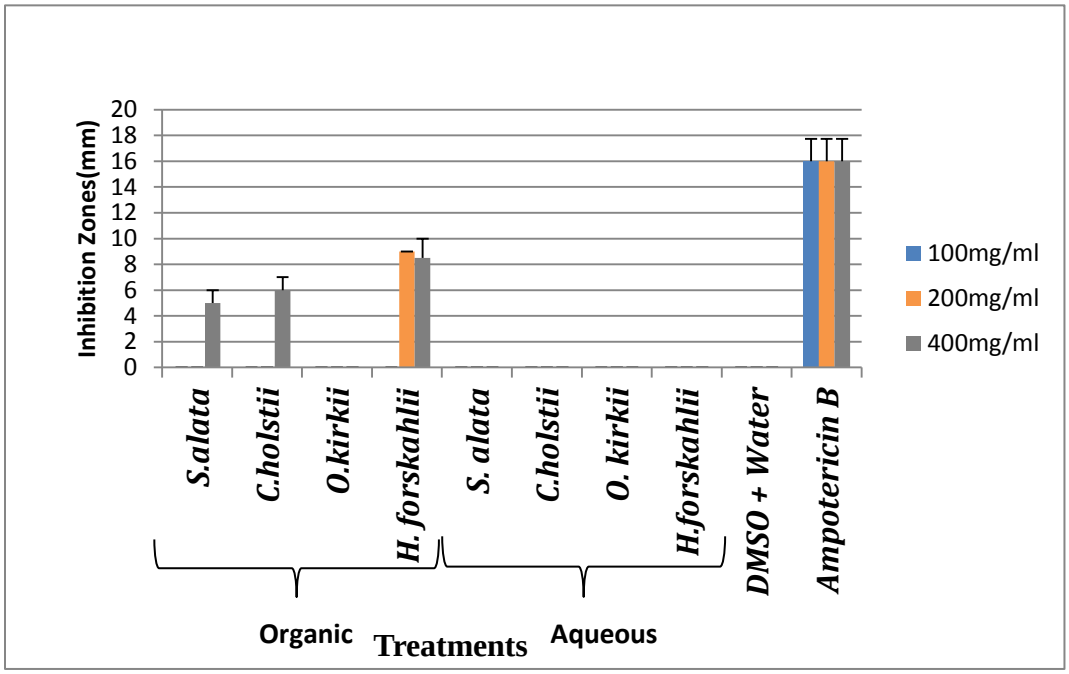


Figure 40: Growth inhibition of the crude extracts on *C. albicans* in agar well diffusion

Table 10: Dependent Variable: *C. ablicans* inhibition at different concentrations by various extracts

Method	Concentration in mg/ml		
	100	200	400
Disc diffusion	-	F=437.33	F=278.22
	-	P=0.00	P=0.00
Agar well diffusion	-	F=305.00	F=121.02
	-	P=0.00	P=0.00

4.3. Minimum inhibitory concentration (MIC) of plant extracts against the test microorganism.

Results of minimum inhibitory concentration (MIC) of plant extracts against the test microorganisms were as shown in Table 11. MIC values varied with plant samples from 250 to 15.625 mg/ml. The MIC values of the test extracts also varied against different test pathogens. The results obtained from these assay revealed that MRSA was the most sensitive bacteria at the lower MIC value of 15.625mg/ml by the largest number of crude extracts.

Table 11: Minimum inhibitory concentration (MIC) in mg/ml

Plant	Solvent	*E.coli*	*P.aeruginosa*	MRSA	*B.cereus*	*C.albicans*
O. kirkii	Organic	-	-	31.25	-	-
	Aqueous	-	250	-	-	-
S. alata	Organic	-	15.625	15.625		62.5
	Aqueous	-	-		-	-
C. holstii	Organic	-	-	15.625	-	62.5
	Aqueous	-	-	-	-	-
H. forskahlii	Organic	62.5	31.25	15.625	31.25	31.25
	Aqueous	-	-	15.625	-	-

4.4. Toxicity of the crude plant extracts on brine shrimp larvae.

The average mortality in the three concentrations (1 mg/ml, 0.1 mg/ml, and 0.01 mg/ml) for each plant was fed into Finney computer program estimate (LC_{50}) of the crude plant extracts. The results are summarised in Table 12 and 13.

Evaluation of toxicity was based on Nguta *et al.*, 2011, where LC_{50} ranging between 0-0.1 mg/ml implied high toxicity, LC_{50} between 0.1-0.5 mg/ml implied moderate toxicity, LC_{50} between 0.5-1 mg/ml implied weakly toxicity and LC_{50} over 1 mg/ml implied non-toxic nature of the extract.Aqueous crude plant extracts of *O.kirkii* (0.412 mg/ml) and *S. alata* (0.317 mg/ml) were found to be moderate toxic whereas aqueous extract of *C. holstii*(0.544 mg/ml) had weakly toxicity while aqueous extracts of *H. forskahlii* were non toxic with LC_{50} of 1.206mg/ml. Organic crude extracts of *H. forskahlii* and *C. holstii* were found to be highly toxic(0.009 mg/ml) while organic extracts of *O.kirkii* (0.207 mg/ml) and *S. alata* (0.399 mg/ml)were found to be moderate toxic

Table 12: Mortality data of aqueous extracts in mg/ml

Aqueous plants extracts	Concentration (mg/ml)	Mean ± SD	$LC_{50\ mg/ml}$
O.kirkii	0.01	1.67±1.53	0.42
	0.1	4.00±2.00	
	1	9.00±1.00	
H.forskhalii	0.01	0	1.21
	0.1	0	
	1	5.67±1.53	
C.holstii	0.01	0	0.54
	0.1	1.00±0.00	
	1	9.00±1.00	
S.alata	0.01	0	0.32
	0.1	4.33±2.08	
	1	10.00±0.00	

Table 13: Mortality data of organic extracts in mg/ml

Organic plants extracts	Concentration (mg/ml)	Mean± SD	$LC_{50\ mg/ml}$
O.kirkii	0.01	2.33±1.53	0.21
	0.1	8.33±1.53	
	1	9.67±0.58	
H.forskhalii	0.01	10.00±0.00	. 0.01
	0.1	10.00±0.00	
	1	10.00±0.00	
C.holstii	0.01	10.00±0.00	0.01
	0.1	10.00±0.00	
	1	10.00±0.00	
S.alata	0.01	3.67±5.51	0.40
	0.1	2.20±2.00	
	1	7.33±4.62	

4.5. Phytochemical constituents of the crude plant extracts.

The plants extracts showed a positive test for the presence of flavanoids, sterols, alkaloids, tannins, quinones and terpenoids (Figure 42, 43, 44, 45 and Table 14). Saponins were present in all the extracts screened except organic extracts of *H. forskahlii* (Figure 41).

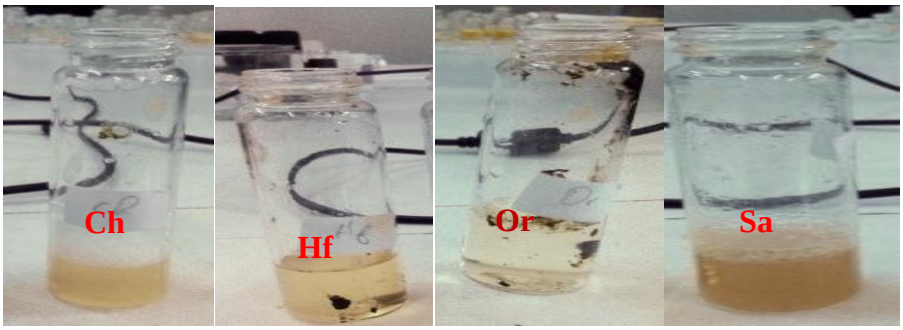
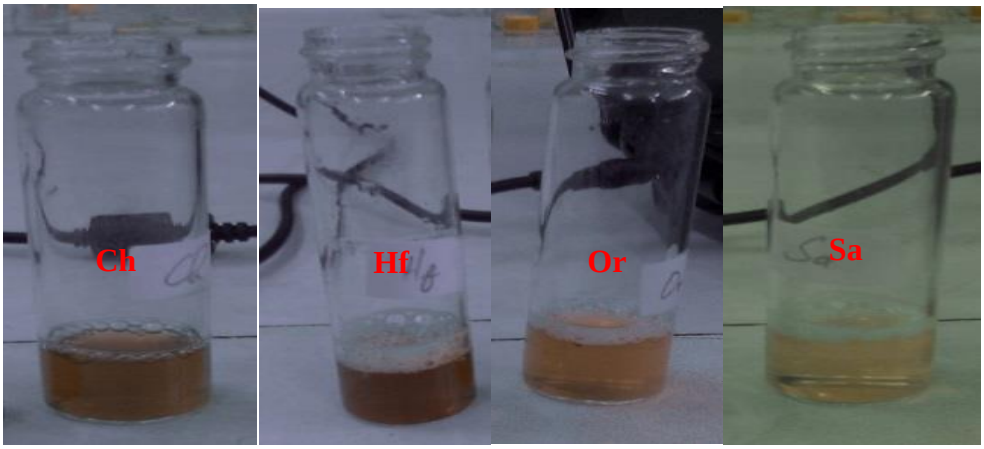


Figure 41: A photograph showing presence of saponins in organic extracts

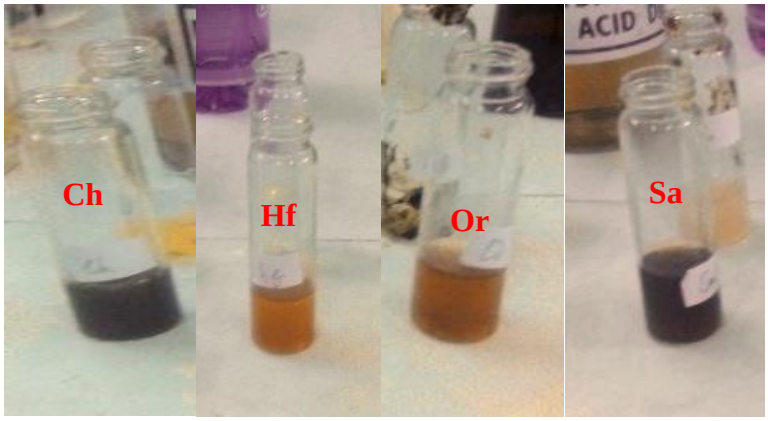
Sa indicates moderate presence of saponins **Key**: Ch: organic *Cussonia holstii* Sa: organic *Schrebera alata* Or: organic *Omorcarpum kirkii* Hf: organic *Helichrysum forskahlii*



Hf indicates strong presence of saponins

Figure 42: A photograph showing presence of saponins in aqueous extracts

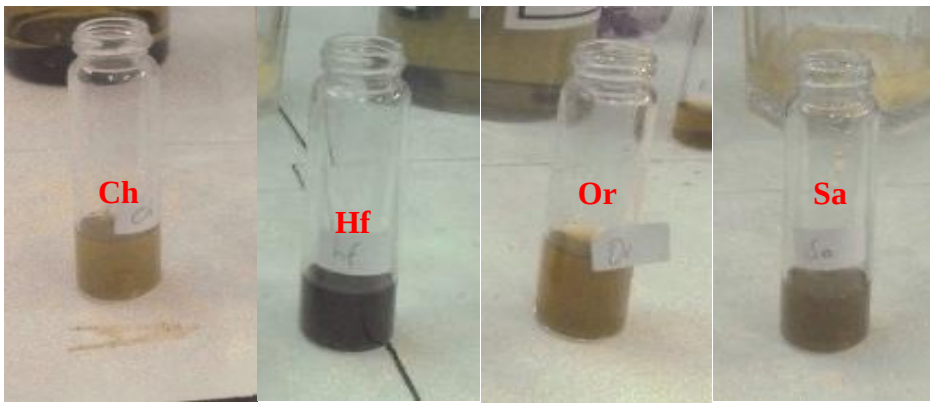
Key: Ch: aqueous *Cussonia holstii* Sa: aqueous *Schrebera alata* Or: aqueous *Omorcarpum kirkii* Hf: aqueous *Helichrysum forskahlii*



The dark green /deep blue in Ch and Sa indicates strong presence of tannins

Figure 43: A photograph showing presence of tannin in organic extract

Key: Ch: organic *Cussonia holstii* Sa: organic *Schrebera alata* Or: organic *Omorcarpum kirkii* Hf: organic *Helichrysum forskahlii*



The dark green /deep blue in Hf indicates strong presence of tannins

Figure 44: A photograph showing presence of tannin in aqueous extracts

Key: Ch: aqueous *Cussonia holstii* Sa: aqueous *Schrebera alata* Or: aqueous *Omorcarpum kirkii* Hf: aqueous *Helichrysum forskahlii*

Table 14: Relative abundance of detected phytochemical in crude plant extracts

Plant	Crude extracts	Sterols	Alkaloids	Saponins	Flavanoids	Tannins	Quinones	Terpenoids
O. kirkii	Organic	++	+	+	+++	+	+++	+++
	Aqueous	+++	++	++	+++	+	+++	+++
S. alata	Organic	+++	+++	++	+++	+++	+++	+++
	Aqueous	+++	+	++	+++	++	+++	+++
C.holstii	Organic	+++	++	+++	+++	+++	++	+++
	Aqueous	+++	+	+	+++	+	+++	+
H.forskahlii	Organic	+++	++	-	+++	+	++	+++
	Aqueous	+++	++	+++	+++	+++	+++	++

Key: +++: strong presence, ++: moderate presence, +weak presence, -: not detected

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Percentage yield of the plant extracts

The total amount of crude extract obtained with the different solvents shows that methanol: DCM (1:1) was quantitatively the best solvent for extraction in all the plants in contrast to distilled water. Organic extracts of *O.kirki , S.alata* and *H.forskahlii* had higher yields than there corresponding aqueous extracts. However aqueous extracts of *C.holstii* had higher yields than of organic extracts of the same plant. The efficiency of methanol in the extraction of phytochemicals has been reported in other studies (Ezekiel *et al.,* 2009). These results seem to be consistent with others confirming methanol as a good solvent for extraction of bioactive compounds from plants as it gave the highest yield in three of the plants studied. More so Dichloromethane extracts less polar compounds while methanol extracts more polar compounds

5.2 Antimicrobial activity of plant extracts

In the present study, *in vitro* antimicrobial activities of four selected medicinal plants evaluated against two gram positive bacteria (MRSA, *B. cereus*), two gram negative bacteria (*E. coli, P. aeruginosa*) and a fungus (*C. albicans*) revealed that they possess potential antimicrobial substances against a majority of the tested microorganisms.

The tested plant extracts were more active against gram positive bacteria compared to gram negative. The most sensitive bacterium was MRSA which was inhibited by the organic crude extracts of all the selected plants. Generally, Gram negative bacteria are more resistant than Gram positive bacteria(Nurul *et al.,* 2010;Darah *et al.,* 2011;Nor *et al.,* 2012).The higher sensitivity of Gram-positive bacteria (MRSA) could be due to the exposure of the outer peptidoglycan layer (Korir *et al*., 2012) while Gram-negative bacteria bear an extra outer membrane (OM) which includes the asymmetric distribution of the lipids with phospholipids and lipopolysaccharide (LPS) located in the inner and outer leaflets, respectively can act as additional barrier which hinders the movement of foreign substance into the cell(Pages *et al.,* 2008).

These results of both agar diffusion methods were found not to differ significantly from each other (P>0.05).This in agreement with Parekh and Chanda (2007).More so, the principle of the agar well diffusion is the same as that of the agar disk diffusion method (Ncube *et al.*, 2008).

5.2.1 Antibacterial activity of organic plant extracts

From this study, it is clear that the dichloromethane: methanol (1:1) solvent extracts of all the plants tested were more potent than their corresponding aqueous extracts against all the tested microbes. This observation is of particular interest, given that traditionally, the preparation of herbal remedy is often with water. This might have resulted from the lower of solubility of the active constituents in aqueous solution. Cowan (1999) indicated that aqueous extraction could be ineffective because water soluble compounds might interrupt the antimicrobial effect. In addition, antimicrobial phytochemicals are soluble in moderate polar solvents. Clarkson *et al*., (2004) explained that the inactivity of water extracts may have been because they (extracts) were not prepared according to the traditional methods, which in some cases involved boiling for several hours.

All organic plant extracts tested showed antibacterial activity against most bacteria employed in this study. Among the tested plant extracts only organic extract from *H.forskhalii* at 400mg/ml against MRSA had similar activity as the positive control (P>0.05) .Investigation in this study also revealed that the sampled plants differ in their activities against the tested pathogens. Results obtained also clearly indicated that antibacterial activity varied with the species of the plant and the solvents used for extraction In addition, all the extracts exhibited concentration dependent activity at tested concentrations; higher activity was observed at high concentration (400 mg/ml) in both agar well and disk diffusion method.

Organic *H.forskhalii* extracts showed significant antimicrobial activity against the tested pathogenic organisms. Antibacterial activity of *H. forskahlii* demonstrated a broad spectrum as compared to other sampled plants in the study and justifies its use in the treatment of a wide range of diseases such as of stomach and diarrhoea (Kajangwe *et al.*, 2008) The largest inhibition zones of the tested plant extracts were recorded in agar well (19.5mm) and disk diffusion (18.5mm) by organic extract of this plant at 400mg/ml. More so, only the above diameters had

no significant difference in activity as Chloramphenicol (P>0.05). Noteworthy inhibition diameters were showed against *Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa* at 200 and 400mg/ml by organic extracts of the same plant. Organic *H.forskhalii* extracts showed antibacterial activity against all the tested microorganisms.This antibacterial activity of *H.forskhalii* is in agreement with (Kajangwe *et al.,* 2008*;* Al-Rehaily *et al.,* 2008).In the present study, the organic crude extracts of *S. alata* showed inhibitory activity against MRSA and *P. aeruginosa.* Against MRSA, *S. alata* recorded inhibition zone at 100,200,400 mg/ml in both agar well and disk diffusion. Organic extracts of *S.alata* showed antibacterial activity only at 400mg/ml against *P. aeruginosa.*Sensitivity of *S. alata* to MRSA and *P. aeruginosa* showed activity similar to another species of the same genus *S. swietenioides* powdered leaf extracts which was found to exhibit potent inhibitory activity against *Staphylococcus aureus* (Mahida and Mohan 2007; Niranjan *et al*., 2010). More so, Nanyingi *et al.,* 2008 reports the use of this plant in Samburu for candidiasis and toothache. Organic extracts of *C. holstii* were active against MRSA in both agar well and disc diffusion methods. Antibacterial activity of this plant against MRSA was observed at all the tested concentrations. The highest antibacterial inhibition activity (10mm) was showed in agar well diffusion at 400mg/ml. This may explain why *Cussonia* species are used in African traditional medicine for several diseases such as pain, inflammation, traditional management of ear, nose and throat (ENT) diseases, gastro-intestinal problems, malaria and sexually transmitted diseases (Njoroge & Bussmann, 2007) , De Villiers *et al.,* 2010).However, this study was not in agreement with other members of the same genus *C.spicata.,C.paniculata, C.arborea* methanolic extracts which show activity against *E.coli and P.aureginosa* (De Villiers *et al.,* 2010).Organic extracts of *O. Kirkii* were only active against MRSA .This is in line with another species of the same genus *Omorcarpum trichocarpum* which showed antibacterial activity (Chukwujekwu *et al*., 2013, Pazhanisamy and Ebenezer 2013).

5.2.2 Antibacterial activity of aqueous plant extracts

Aqueous extracts of *S. alata* and *C.holstii* had no antimicrobial activity against all the tested microbes, wheareas aqueous extracts of *H.forskhalii* had activity in all concentration 100,200,400mg/ml in both agar well and disc diffusion. This observation is agreement with Kajangwe *et al.,* 2008*;* Al-Rehaily *et al.,* 2008 showing this plant is a potential antimicrobial

source. Aqueous extracts of *O. kirkii* were active against only gram negative bacteria, *P. aeruginosa* and this was in line with another species of the same genus *Omorcarpum trichocarpum* which showed antibacterial activity (Chukwujekwu *et al*., 2013, Pazhanisamy *et al*., 2013).

5.2.3 Antifungal activity of plant extracts

Antifungal activity also varied with the species of the plant and the solvents used for extraction. In addition, all the tested extracts exhibited concentration dependent activity at tested concentrations; higher activity was observed at high concentration (400 mg/ml) in both agar well and disk diffusion method. *C.albicans* was not sensitive to any aqueous extract but showed antifungal activity in organic extracts of *S. alata*, *C. holstii* and *H. forskahlii*.

The highest antifungal activity with an inhibition zone value of 8.5mm was observed in organic extracts of *Helichrysum forskahlii* in agar well diffusion at 400mg/ml with an MIC value of 31.25mg/ml. This study was in agreement with another member of the same species *H. italicum* essential oil which shows demelanizing activity against *Aspergillus niger* (Milos *et al*., 2014). Among the *C. holstii* extracts tested for antifungal activity, only the organic extracts were active against *C. albicans* in both agar well and disc diffusion methods with an MIC of 62.5mg/ml. This is in agreement with another member of the same genus *C. bancoensis* which reports antifungal activity of methanol, ethyl acetate and petroleum ether extracts of the stem bark against the same microorganism and minimum inhibitory concentrations ranged from 0.625 mg/mL to 2.5mg/mL (Mireku *et al*., 2014). More so, Njoroge and Bussman 2006 that *C.holstii* is used for traditional management of ear, nose and throat (ENT) diseases in central Kenya.*O.kirkii* extracts had no antifungal activity which was against Maregesi *et al*., 2008 that shows hexane root extracts of *O.kirkii* against *C. albicans* had an MIC of 1mg/ml .Antifungal activity of *Schrebera alata* was reported for the first time in this study though in Samburu , studies show that the root & bark of this plant is pounded or chewed as treatment for candidiasis and toothache (Nanyingi *et al.,* 2008).

5.3 Minimum Inhibitory Concentration

Interestingly, MIC values of less than 100mg/ml were observed in broth micro dilutions but not in AST, and this could be due to microdilution method providing a potentially useful technique for determining MICs increased sensitivity for small quantities of extracts which is important if the antimicrobial is scarce as is the case for many natural products (Ncube *et al.,* 2008). More so, dilution testing methods may be quantitative (MIC), in addition to qualitative (susceptible, intermediate and resistant) whereas disk methods are only qualitative method (Miller, 2005)

At lower concentrations, organic and water *H. forskahlii* extracts were more active against MRSA as shown by MIC value of 15.625 mg/ml when compared to other test microbes. However, Kajangwe *et al.,* (2008) showed the essential oil from *Helichrysum forskahlii* presents activity against *E.coli* (MIC between 0.2 and 0.8mg/ml) which varied with study MIC value (62.5 mg/ml) against the same bacterium strain. *Schrebera alata* recorded an MIC value of 15.625mg/ml against *P. aeruginosa* and MRSA which was in line with another member of the same genus *S. swietenioides* against *Staphylococcus aureus* (Mahida and Mohan 2007).Activity of *C.holstii* against the MRSA recorded an MIC value of 15.625 was in line with De Villiers *et al.,* 2010, Mireku *et al*., 2014 that some other species of the same genus against *E.coli* and *P.aureginosa*. MIC value (31.25mg/ml) was revealed by organic extracts of *O.kirkii* against MRSA in was in line with Maragesi *et al*., 2008. However, lack of antibacterial activity showed by the same plant against *B.cereus* from this study was not in agreement with Maregesi *et al*., 2008 earlier report on MeOH and n-Hexane root extracts of the same species exhibited antibacterial activity against this microorganism at 0.625 and 0.250 mg/ml.

5.4 Brimp shrimp test

Aqueous extracts of *H. forskahlii* were active against MRSA and exhibited non-toxicity effect on brine shrimps with LC_{50} of 1.207 mg/ml, this support inherent selectivity of the plant extracts for treatment of bacterial infections. Notably, aqueous extracts in most cases are the ones used by traditional practitioners. Therefore the concentration of this plant crude extract at the employed concentrations was safe hence justifying continued use of this plant on traditional medication.

McLaughlin *et al.,* 1998 outlined that a plant with less toxicity and equally good concentration of active phytochemicals makes it a good plant for use in traditional medicine compared to one with high toxicity and less concentration of active phytochemicals.

The moderate toxic effect of *O. Kirkii* extracts was observed. Aqueous crude plant extracts of *O. kirkii* (0.411mg/ml) while organic extracts of *O. kirkii* (0.207mg/ml).This study reports for the first time the toxicity of this plant. However, it is not in line with another member of same genus *Ormorcapum trichorcarpum* (0.072mg/ml) which *is* toxic (Moshi *et al.,* 2006).Mbaya (1976), reported that the bark of *Schrebera alata* possesses some toxicity due to its effects on the liver. Mbaya's results are in agreement with results of this studies which show moderate toxicity inorganic *S.alata* (0.399mg/ml) and aqueous *S.alata* (0.317mg/ml). *C.holstii* organic extracts revealed high toxicity (0.009mg/ml).This was similar to Adepado *et al.,* 2008 on *Cussonia paniculata*, a member of the same genus showed that the plant caused 80% mortality on rats. However, aqueous extracts of this plant demonstrated weak toxicity (0.543mg/ml). Extracts of the plants from organic solvents could have shown higher toxicity due to trace amounts of the solvents still in them. Practically, it needs thorough 'washing' to completely remove the solvents.

5.5 Phytochemical screening

Phytochemical screening of bioactive constituents showed that the extracts were rich in secondary metabolites. All the plants extracts demonstrated presence of flavanoids, sterols, alkaloids, tannins, quinones and terpenoids. The higher number of phytochemicals in organic extracts on this study probably explained their comparatively better antimicrobial potential. Phytochemical screening of extracts *H. forskahlii* revealed the presences of flavonoids sterols, alkaloids, tannins, quinones and terpenoids in both water and aqueous extracts while saponins were absent in organic extracts of this plant. Flavanoids and terpenoids phyto constituents had earlier been documented in stem bark of *H. forskahlii* (Kajangwe *et al.,* 2008, Al-Rehaily *et al.,* 2008).

Although *Ormocarpum kirkii* had low antimicrobial activity, it was found to be richer in secondary metabolites such as flavonoids, alkaloids, tannins, quinones and terpenoids saponins in all the extracts. Other researchers have isolated from *O. kirkii*, a series of known flavonoids

and biflavonoids, as well as chamaejasmin, biliquiritigenin, and isovitexin naringenin (Xu *et al*., 2011; Dhoogle *et al*., 2010).Phytochemical analysis of *C. holstii* revealed the presence of saponins, flavanoids, alkaloids, tannins, quinones and terpenoids. Pentacyclic triterpenoid, i.e., hederagenin has been documented before for its antitrichomonas activity (He *et al*., 2011).*S.alata* extracts exhibited flavonoids, alkaloids, tannins, quinones and terpenoids saponins in all the extracts. Phytochemicals of this plant were first recorded in this study. However, antibacterial alkaloids presence in another member of the same genus *Schrebera swietenioides* by Niranjan *et al.,* 2010 were in agreement with this study. Presence of secondary metabolites by this plant may attribute to the detected biological activities (Odhiambo *et al.,* 2014). Flavanoids, saponins, tannins and terpenoids, and have been reported to have antimicrobial activity and are used in treatment against cough diarrhea (Talib and Mahasneh, 2011; Khan *et al.,* 2012).Cytotoxicity, antiviral and antimicrobial activities of alkaloids and flavanoids have also been reported (Ozcelik *et al.*, 2011). Antibacterial activity and cytotoxic effects of steroids have been documented in their usage as arrow poisons (Doughari, 2006). The presence of antibacterial substances in the higher plants is well established (Bhalodia and Shukla 2011). Plants have provided a source of inspiration for novel drug compounds as plants derived medicines have made significant contribution towards human health.

5.6. Conclusions

Bioactivity of organic and aqueous extracts of *S. alata, O. kirkii*, *C. holstii* and *H. forskahlii* against bacterial and fungal strains is noteworthy, with only organic extracts of *H. forskahlii* exhibiting the highest antimicrobial activity similar to positive control (Chloramphenicol) against MRSA. Generally, flavanoids, sterols, alkaloids, tannins, quinones and terpenoids were present in all the four plant species tested positive for saponins except for organic extracts *of H. forskahlii* which lacked saponins. Organic crude extracts of *H. forskahlii* and *C. holstii* were found to be highly toxic (0.009mg/ml). It is also evident from the study that only aqueous *H.forskhalii* of the evaluated crude extracts was non-toxic to *Artemia Salina*, ascertaining value to the selected medicinal plant for continued use in ethno medicine. Among the studied plant extracts, methanol extracts of *H.forskhalii* had an overriding toxicity over other plant extracts. From this study *H. forskahlii* is a better source of antimicrobial agents hence can be of interest in

the development of new chemotherapeutic drugs. This study reports for the first time antimicrobial activity and toxicity of *Schrebera alata* and *Cussonia holstii.*

5.7 Recommendations

- Bioassay guided fractionation, isolation, purification and structure elucidations of active secondary metabolites.
- Antimicrobial investigations of isolated metabolites.
- Use of other solvents which may lead to improvement in yield and biological activity
- Identify other phytochemicals giving rise to antimicrobial activity
- Same plants(at more or less concentration) against different other microbes be investigated
- Further evaluation for *in vivo* toxicity can be carried out of the most active crude extracts.

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APPENDICES

Appendix 1: Toxicity of the test extracts on Brine shrimp

Aqueous extract

	Ormocarpum kirkii							
Conc	1000µg/ml		100µg/ml		10µg/ml		0µg/ml	
	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead
	2	8	6	4	7	3	10	0
	1	9	4	6	8	2	10	0
	0	10	8	2	10	0	10	0
Average Mortality		9		4		1.66		0

	Helichrysum forsakahlli							
Conc	1000µg/ml		100µg/ml		10µg/ml		0µg/ml	
	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead
	4	6	10	0	10	0	10	0
	3	7	10	0	10	0	10	0
	6	4	10	0	10	0	10	0
Average Mortality		5.66		0		0		0

	Cussonia holstii							
Conc	1000µg/ml		100µg/ml		10µg/ml		0µg/ml	
	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead
	0	10	9	1	10	0	10	0
	1	9	9	1	10	0	10	0
	2	8	9	1	10	0	10	0
Average Mortality		9		1		0		0

	Schrebera alata							
Conc	1000µg/ml		100µg/ml		10µg/ml		0µg/ml	
	Alive	Dead	Alive	Dead	Alive	Dead	Alive	De0ad
	0	10	8	2	10	0	10	0
	0	10	4	6	10	0	10	0
	0	10	5	5	10	0	10	0
Average Mortality		10		4.33		0		0

Organic extracts

	Ormocarpum kirkii							
Conc	1000µg/ml		100µg/ml		10µg/ml		0µg/ml	
	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead
	1	9	0	10	9	1	10	0
	0	10	2	8	8	2	10	0
	0	10	3	7	6	4	10	0
Average Mortality		9.66		8.33		2.33		0

	Helichrysum forsakahlli							
Conc	1000µg/ml		100µg/ml		10µg/ml		0µg/ml	
	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead
	0	10	0	10	0	10	10	0
	0	10	0	10	0	10	10	0
	0	10	0	10	0	10	10	0
Average Mortality		10		10		10		0

	Cussonia holstii							
Conc	1000µg/ml		100µg/ml		10µg/ml		0µg/ml	
	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead
	0	10	0	10	0	10	10	0
	0	10	0	10	0	10	10	0
	0	10	0	10	0	10	10	0
Average Mortality		10		10		10		0

	Schrebera alata							
Conc	1000µg/ml		100µg/ml		10µg/ml		0µg/ml	
	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead
	0	10	6	4	9	1	10	0
	0	10	10	0	10	0	10	0
	0	10	8	2	8	2	10	0
Average Mortality		10		2		1		0

APPENDIX 2: MULTIPLE COMPARISONS

Agar disc MRSA inhibition at 100mg/ml

(I) Plant extracts	(J) Plant extracts	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
A	Chloramphenical	-20.0000*	.51099	.000	-21.5055	-18.4945
B	Chloramphenical	-16.3333*	.51099	.000	-17.8389	-14.8278
C	Chloramphenical	-8.3333*	.51099	.000	-9.8389	-6.8278
D	Chloramphenical	-8.3333*	.51099	.000	-9.8389	-6.8278
E	Chloramphenical	-23.3333*	.51099	.000	-24.8389	-21.8278
F	Chloramphenical	-23.3333*	.51099	.000	-24.8389	-21.8278
G	Chloramphenical	-23.3333*	.51099	.000	-24.8389	-21.8278
H	Chloramphenical	-18.8333*	.51099	.000	-20.3389	-17.3278
DMSO + Water	Chloramphenical	-23.3333*	.51099	.000	-24.8389	-21.8278

Agar well MRSA inhibition at 100mg/ml

(I) Plant extracts	(J) Plant extracts	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
A	Chloramphenical	-20.0000*	.51099	.000	-21.5055	-18.4945
B	Chloramphenical	-16.3333*	.51099	.000	-17.8389	-14.8278
C	Chloramphenical	-8.3333*	.51099	.000	-9.8389	-6.8278
D	Chloramphenical	-8.3333*	.51099	.000	-9.8389	-6.8278
E	Chloramphenical	-23.3333*	.51099	.000	-24.8389	-21.8278
F	Chloramphenical	-23.3333*	.51099	.000	-24.8389	-21.8278
G	Chloramphenical	-23.3333*	.51099	.000	-24.8389	-21.8278
H	Chloramphenical	-18.8333*	.51099	.000	-20.3389	-17.3278
DMSO + Water	Chloramphenical	-23.3333*	.51099	.000	-24.8389	-21.8278

Agar well MRSA inhibition at 200mg/ml

(I) Plant extracts	(J) Plant extracts	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
A	Chloramphenicol	-15.6667*	.52175	**.000**	-17.2039	-14.1294
B	Chloramphenicol	-17.1667*	.52175	**.000**	-18.7039	-15.6294
C	Chloramphenicol	-7.1667*	.52175	**.000**	-8.7039	-5.6294
D	Chloramphenicol	-8.1667*	.52175	**.000**	-9.7039	-6.6294
E	Chloramphenicol	-23.6667*	.52175	**.000**	-25.2039	-22.1294
F	Chloramphenicol	-23.6667*	.52175	**.000**	-25.2039	-22.1294
G	Chloramphenicol	-23.6667*	.52175	**.000**	-25.2039	-22.1294
G	Chloramphenicol	-14.6667*	.52175	**.000**	-16.2039	-13.1294
DMSO + WATER	Chloramphenicol	-23.6667*	.52175	**.000**	-25.2039	-22.1294

Agar disc MRSA inhibition at 200mg/ml

(I) Plant extracts	(J) Plant extracts	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
A	Chloramphenical	-16.8333*	.60093	.000	-18.6038	-15.0628
B	Chloramphenical	-15.6667*	.60093	.000	-17.4372	-13.8962
C	Chloramphenical	-11.8333*	.60093	.000	-13.6038	-10.0628
D	Chloramphenical	-9.8333*	.60093	.000	-11.6038	-8.0628
E	Chloramphenical	-23.3333*	.60093	.000	-25.1038	-21.5628
F	Chloramphenical	-23.3333*	.60093	.000	-25.1038	-21.5628
G	Chloramphenical	-23.3333*	.60093	.000	-25.1038	-21.5628
H	Chloramphenical	-18.3333*	.60093	.000	-20.1038	-16.5628
DMSO + Water	Chloramphenical	-23.3333*	.60093	.000	-25.1038	-21.5628

Agar well MRSA inhibition at 400mg/ml

(I) Plant extracts	(J) Plant extracts	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
A	Chloramphenicol	-15.1667*	.53748	.000	-16.7503	-13.5831
B	Chloramphenicol	-13.1667*	.53748	.000	-14.7503	-11.5831
C	Chloramphenicol	-7.6667*	.53748	.000	-8.2503	-5.0831
D	Chloramphenicol	-5.1667*	.53748	**.072**	-6.7503	-3.5831
E	Chloramphenicol	-23.6667*	.53748	.000	-25.2503	-22.0831
F	Chloramphenicol	-14.1667*	.53748	.000	-15.7503	-12.5831
G	Chloramphenicol	-23.6667*	.53748	.000	-25.2503	-22.0831
H	Chloramphenicol	-14.6667*	.53748	.000	-16.2503	-13.0831
DMSO + WATER	Chloramphenicol	-23.6667*	.53748	.000	-25.2503	-22.0831

Agar disc MRSA inhibition at 400mg/ml

(I) Plant extracts	(J) Plant extracts	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
A	Chloramphenicol	-15.8333*	.56765	.000	-17.5058	-14.1609
B	Chloramphenicol	-14.3333*	.56765	.000	-16.0058	-12.6609
C	Chloramphenicol	-8.3333*	.56765	.000	-10.0058	-6.6609
D	Chloramphenicol	-3.8333*	.56765	**.061**	-5.5058	-2.1609
E	Chloramphenicol	-23.3333*	.56765	.000	-25.0058	-21.6609
F	Chloramphenicol	-13.3333*	.56765	.000	-15.0058	-11.6609
G	Chloramphenicol	-23.3333*	.56765	.000	-25.0058	-21.6609
H	Chloramphenicol	-23.3333*	.56765	.000	-25.0058	-21.6609
DMSO + WATER	Chloramphenicol	-23.3333*	.56765	.000	-25.0058	-21.6609

Agar disc *P.aureginosa* at 200mg/ml

(I) Plant extracts	(J) Plant extracts	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
A	Chloramphenical	-19.3333*	.39441	.000	-20.4954	-18.1713
B	Chloramphenical	-19.3333*	.39441	.000	-20.4954	-18.1713
C	Chloramphenical	-19.3333*	.39441	.000	-20.4954	-18.1713
D	Chloramphenical	-11.3333*	.39441	.000	-12.4954	-10.1713
E	Chloramphenical	-19.3333*	.39441	.000	-20.4954	-18.1713
F	Chloramphenical	-19.3333*	.39441	.000	-20.4954	-18.1713
G	Chloramphenical	-19.3333*	.39441	.000	-20.4954	-18.1713
H	Chloramphenical	-19.3333*	.39441	.000	-20.4954	-18.1713
DMSO + Water	Chloramphenical	-19.3333*	.39441	.000	-20.4954	-18.1713

Agar well *P.aureginosa* at 200mg/ml

(I) Plant extracts	(J) Plant extracts	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
A	Chloramphenicol	-20.6667*	.29814	.000	-21.5451	-19.7883
B	Chloramphenicol	-20.6667*	.29814	.000	-21.5451	-19.7883
C	Chloramphenicol	-20.6667*	.29814	.000	-21.5451	-19.7883
D	Chloramphenicol	-10.6667*	.29814	.000	-11.5451	-9.7883
E	Chloramphenicol	-20.6667*	.29814	.000	-21.5451	-19.7883
F	Chloramphenicol	-20.6667*	.29814	.000	-21.5451	-19.7883
G	Chloramphenicol	-20.6667*	.29814	.000	-21.5451	-19.7883
G	Chloramphenicol	-20.6667*	.29814	.000	-21.5451	-19.7883
DMSO + WATER	Chloramphenicol	-20.6667*	.29814	.000	-21.5451	-19.7883

Agar disc *P.aureginosa* at 400mg/ml

(I) Plant extracts	(J) Plant extracts	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
A	Chloramphenicol	-13.3333*	.29814	.000	-14.2117	-12.4549
B	Chloramphenicol	-19.3333*	.29814	.000	-20.2117	-18.4549
C	Chloramphenicol	-19.3333*	.29814	.000	-20.2117	-18.4549
D	Chloramphenicol	-19.3333*	.29814	.000	-20.2117	-18.4549
E	Chloramphenicol	-19.3333*	.29814	.000	-20.2117	-18.4549
F	Chloramphenicol	-19.3333*	.29814	.000	-20.2117	-18.4549
G	Chloramphenicol	-19.3333*	.29814	.000	-20.2117	-18.4549
H	Chloramphenicol	-19.3333*	.29814	.000	-20.2117	-18.4549
DMSO + WATER	Chloramphenicol	-19.3333*	.29814	.000	-20.2117	-18.4549

Agar well Agar disc *P.aureginosa* at 400mg/ml

(I) Plant extracts	(J) Plant extracts	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
A	Chloramphenicol	-14.6667*	.19720	.000	-15.2477	-14.0856
B	Chloramphenicol	-20.6667*	.19720	.000	-21.2477	-20.0856
C	Chloramphenicol	-20.6667*	.19720	.000	-21.2477	-20.0856
D	Chloramphenicol	-15.1667*	.19720	.000	-15.7477	-14.5856
E	Chloramphenicol	-20.6667*	.19720	.000	-21.2477	-20.0856
F	Chloramphenicol	-20.6667*	.19720	.000	-21.2477	-20.0856
G	Chloramphenicol	-10.6667*	.19720	.000	-11.2477	-10.0856
H	Chloramphenicol	-20.6667*	.19720	.000	-21.2477	-20.0856
DMSO + WATER	Chloramphenicol	-20.6667*	.19720	.000	-21.2477	-20.0856

Agar disc *B.cereus* at 200mg/ml

(I) Plant extracts	(J) Plant extracts	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
A	Chloramphenicol	-19.3333*	.39441	.000	-20.4954	-18.1713
B	Chloramphenicol	-19.3333*	.39441	.000	-20.4954	-18.1713
C	Chloramphenicol	-19.3333*	.39441	.000	-20.4954	-18.1713
D	Chloramphenicol	-12.3333*	.39441	.000	-13.4954	-11.1713
E	Chloramphenicol	-19.3333*	.39441	.000	-20.4954	-18.1713
F	Chloramphenicol	-19.3333*	.39441	.000	-20.4954	-18.1713
G	Chloramphenicol	-19.3333*	.39441	.000	-20.4954	-18.1713
H	Chloramphenicol	-19.3333*	.39441	.000	-20.4954	-18.1713
DMSO + WATER	Chloramphenicol	-19.3333*	.39441	.000	-20.4954	-18.1713

Agar well *B.cereus* at 200mg/ml

(I) Plant extracts	(J) Plant extracts	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
A	Chloramphenicol	-19.3333*	.19720	.000	-19.9144	-18.7523
B	Chloramphenicol	-19.3333*	.19720	.000	-19.9144	-18.7523
C	Chloramphenicol	-19.3333*	.19720	.000	-19.9144	-18.7523
D	Chloramphenicol	-14.8333*	.19720	.000	-15.4144	-14.2523
E	Chloramphenicol	-19.3333*	.19720	.000	-19.9144	-18.7523
F	Chloramphenicol	-19.3333*	.19720	.000	-19.9144	-18.7523
G	Chloramphenicol	-19.3333*	.19720	.000	-19.9144	-18.7523
G	Chloramphenicol	-19.3333*	.19720	.000	-19.9144	-18.7523
DMSO + WATER	Chloramphenicol	-19.3333*	.19720	.000	-19.9144	-18.7523

Agar well Multiple *B.cereus* at 400mg/ml

(I) Plant extracts	(J) Plant extracts	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
A	Chloramphenicol	-19.3333*	.14907	.000	-19.7725	-18.8941
B	Chloramphenicol	-19.3333*	.14907	.000	-19.7725	-18.8941
C	Chloramphenicol	-19.3333*	.14907	.000	-19.7725	-18.8941
D	Chloramphenicol	-14.3333*	.14907	.000	-14.7725	-13.8941
E	Chloramphenicol	-19.3333*	.14907	.000	-19.7725	-18.8941
F	Chloramphenicol	-19.3333*	.14907	.000	-19.7725	-18.8941
G	Chloramphenicol	-19.3333*	.14907	.000	-19.7725	-18.8941
H	Chloramphenicol	-19.3333*	.14907	.000	-19.7725	-18.8941
DMSO + WATER	Chloramphenicol	-19.3333*	.14907	.000	-19.7725	-18.8941

Agar disc *B.cereus* 400mg/ml

(I) Plant extracts	(J) Plant extracts	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
A	Chloramphenicol	-19.3333*	.39441	.000	-20.4954	-18.1713
B	Chloramphenicol	-19.3333*	.39441	.000	-20.4954	-18.1713
C	Chloramphenicol	-19.3333*	.39441	.000	-20.4954	-18.1713
D	Chloramphenicol	-12.3333*	.39441	.000	-13.4954	-11.1713
E	Chloramphenicol	-19.3333*	.39441	.000	-20.4954	-18.1713
F	Chloramphenicol	-19.3333*	.39441	.000	-20.4954	-18.1713
G	Chloramphenicol	-19.3333*	.39441	.000	-20.4954	-18.1713
H	Chloramphenicol	-19.3333*	.39441	.000	-20.4954	-18.1713
DMSO + WATER	Chloramphenicol	-19.3333*	.39441	.000	-20.4954	-18.1713

Agar disc *E.coli* 200mg/ml

(I) Plant extracts	(J) Plant extracts	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
A	Chloramphenical	-21.3333*	.19720	.000	-21.9144	-20.7523
B	Chloramphenical	-21.3333*	.19720	.000	-21.9144	-20.7523
C	Chloramphenical	-21.3333*	.19720	.000	-21.9144	-20.7523
D	Chloramphenical	-16.8333*	.19720	.000	-17.4144	-16.2523
E	Chloramphenical	-21.3333*	.19720	.000	-21.9144	-20.7523
F	Chloramphenical	-21.3333*	.19720	.000	-21.9144	-20.7523
G	Chloramphenical	-21.3333*	.19720	.000	-21.9144	-20.7523
H	Chloramphenical	-21.3333*	.19720	.000	-21.9144	-20.7523
DMSO + Water	Chloramphenical	-21.3333*	.19720	.000	-21.9144	-20.7523

Agar well *E.coli* 200mg/ml

(I) Plant extracts	(J) Plant extracts	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
A	Chloramphenicol	-22.0000*	.12910	.000	-22.3804	-21.6196
B	Chloramphenicol	-22.0000*	.12910	.000	-22.3804	-21.6196
C	Chloramphenicol	-22.0000*	.12910	.000	-22.3804	-21.6196
D	Chloramphenicol	-18.5000*	.12910	.000	-18.8804	-18.1196
E	Chloramphenicol	-22.0000*	.12910	.000	-22.3804	-21.6196
F	Chloramphenicol	-22.0000*	.12910	.000	-22.3804	-21.6196
G	Chloramphenicol	-22.0000*	.12910	.000	-22.3804	-21.6196
G	Chloramphenicol	-22.0000*	.12910	.000	-22.3804	-21.6196
DMSO + WATER	Chloramphenicol	-22.0000*	.12910	.000	-22.3804	-21.6196

Agar disc *E.coli* at 400mg/ml

(I) Plant extracts	(J) Plant extracts	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
A	Chloramphenicol	-21.3333*	.53748	.000	-22.9169	-19.7497
B	Chloramphenicol	-21.3333*	.53748	.000	-22.9169	-19.7497
C	Chloramphenicol	-21.3333*	.53748	.000	-22.9169	-19.7497
D	Chloramphenicol	-15.3333*	.53748	.000	-16.9169	-13.7497
E	Chloramphenicol	-21.3333*	.53748	.000	-22.9169	-19.7497
F	Chloramphenicol	-21.3333*	.53748	.000	-22.9169	-19.7497
G	Chloramphenicol	-21.3333*	.53748	.000	-22.9169	-19.7497
H	Chloramphenicol	-21.3333*	.53748	.000	-22.9169	-19.7497
DMSO + WATER	Chloramphenicol	-21.3333*	.53748	.000	-22.9169	-19.7497

Agar well *E.coli* 400mg/ml

(I) Plant extracts	(J) Plant extracts	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
A	Chloramphenicol	-22.0000*	.25820	.000	-22.7607	-21.2393
B	Chloramphenicol	-22.0000*	.25820	.000	-22.7607	-21.2393
C	Chloramphenicol	-22.0000*	.25820	.000	-22.7607	-21.2393
D	Chloramphenicol	-16.0000*	.25820	.000	-16.7607	-15.2393
E	Chloramphenicol	-22.0000*	.25820	.000	-22.7607	-21.2393
F	Chloramphenicol	-22.0000*	.25820	.000	-22.7607	-21.2393
G	Chloramphenicol	-22.0000*	.25820	.000	-22.7607	-21.2393
H	Chloramphenicol	-22.0000*	.25820	.000	-22.7607	-21.2393
DMSO + WATER	Chloramphenicol	-22.0000*	.25820	.000	-22.7607	-21.2393

Agar disk *C.albicans* at 200mg/ml

(I) Plant extracts	(J) Plant extracts	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
A	Ampotericin B	-16.0000*	.36515	.000	-17.0758	-14.9242
B	Ampotericin B	-16.0000*	.36515	.000	-17.0758	-14.9242
C	Ampotericin B	-16.0000*	.36515	.000	-17.0758	-14.9242
D	Ampotericin B	-8.0000*	.36515	.000	-9.0758	-6.9242
E	Ampotericin B	-16.0000*	.36515	.000	-17.0758	-14.9242
F	Ampotericin B	-16.0000*	.36515	.000	-17.0758	-14.9242
G	Ampotericin B	-16.0000*	.36515	.000	-17.0758	-14.9242
H	Ampotericin B	-16.0000*	.36515	.000	-17.0758	-14.9242
DMSO + WATER	Ampotericin B	-16.0000*	.36515	.000	-17.0758	-14.9242

Agar well C.albicans at 200mg/ml

(I) Plant extracts	(J) Plant extracts	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
A	Ampotericin B	-16.0000*	.44721	.000	-17.3176	-14.6824
B	Ampotericin B	-16.0000*	.44721	.000	-17.3176	-14.6824
C	Ampotericin B	-16.0000*	.44721	.000	-17.3176	-14.6824
D	Ampotericin B	-7.0000*	.44721	.000	-8.3176	-5.6824
E	Ampotericin B	-16.0000*	.44721	.000	-17.3176	-14.6824
F	Ampotericin B	-16.0000*	.44721	.000	-17.3176	-14.6824
G	Ampotericin B	-16.0000*	.44721	.000	-17.3176	-14.6824
H	Ampotericin B	-16.0000*	.44721	.000	-17.3176	-14.6824
DMSO + WATER	Ampotericin B	-16.0000*	.44721	.000	-17.3176	-14.6824

Agar disk *C.albicans* at 400mg/ml

(I) Plant extracts	(J) Plant extracts	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
A	Ampotericin B	-11.0000*	.44721	.000	-12.3176	-9.6824
B	Ampotericin B	-10.0000*	.44721	.000	-11.3176	-8.6824
C	Ampotericin B	-16.0000*	.44721	.000	-17.3176	-14.6824
D	Ampotericin B	-9.0000*	.44721	.000	-10.3176	-7.6824
E	Ampotericin B	-16.0000*	.44721	.000	-17.3176	-14.6824
F	Ampotericin B	-16.0000*	.44721	.000	-17.3176	-14.6824
G	Ampotericin B	-16.0000*	.44721	.000	-17.3176	-14.6824
H	Ampotericin B	-16.0000*	.44721	.000	-17.3176	-14.6824
DMSO + WATER	Ampotericin B	-16.0000*	.44721	.000	-17.3176	-14.6824

*. The mean difference is significant at the .05 level.

Agar well *C.albicans* 400mg/ml

(I) Plant extracts	(J) Plant extracts	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
A	Ampotericin B	-11.0000*	.69522	.000	-13.0483	-8.9517
B	Ampotericin B	-10.0000*	.69522	.000	-12.0483	-7.9517
C	Ampotericin B	-16.0000*	.69522	.000	-18.0483	-13.9517
D	Ampotericin B	-7.5000*	.69522	.000	-9.5483	-5.4517
E	Ampotericin B	-16.0000*	.69522	.000	-18.0483	-13.9517
F	Ampotericin B	-16.0000*	.69522	.000	-18.0483	-13.9517
G	Ampotericin B	-16.0000*	.69522	.000	-18.0483	-13.9517
H	Ampotericin B	-16.0000*	.69522	.000	-18.0483	-13.9517
DMSO + WATER	Ampotericin B	-16.0000*	.69522	.000	-18.0483	-13.9517

*. The mean difference is significant at the .05 level.

APPENDIX 3: PAIRED T TEST

Inhibitions at 400 mg/ml

Paired Samples descriptive statistics					
		Mean	N (A to H)	Std. Deviation	Std. Error Mean
Pair 1	Inhibition by MRSA_Agar well	9.0000	8	6.59004	2.32993
	Inhibition by MRSA_Disc Diffusion	7.6250	8	7.34239	2.59593
Pair 2	Inhibition by *B.cereus*_Agar well	.6250	8	1.76777	.62500
	Inhibition by *B.cereus*_Disc Diffusion	.8750	8	2.47487	.87500
Pair 3	Inhibition by *E.coli*_Agar well	.7500[a]	8	2.12132	.75000
	Inhibition by *E.coli*_Disc Diffusion	.7500[a]	8	2.12132	.75000
Pair 4	Inhibition by *P.aeruginosa*_Agar well	2.6875	8	3.93644	1.39174
	Inhibition by *P.aeruginosa*_Disc Diffusion	.7500	8	2.12132	.75000
Pair 5	Inhibition by *C.albicans*_Agar well	2.4375	8	3.49936	1.23721
	Inhibition by *C.albicans*_Disc Diffusion	2.2500	8	3.15096	1.11403

N= 8 extracts (A to H)

Comparison of the two methods using growth inhibitions at 400 mg/ml

Paired Samples Test									
		Paired Differences							
	Comparison of the two methods	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference		t	df	Sig. (2-tailed)
					Lower	Upper			
Pair 1	Inhibition by MRSA_Agar well - Inhibition by MRSA_Disc Diffusion	1.37500	3.19318	1.12896	-1.29457	4.04457	1.218	7	**.263**
Pair 2	Inhibition by *B.cereus*_Agar well - Inhibition by *B.cereus*_Disc Diffusion	-.25000	.70711	.25000	-.84116	.34116	-1.000	7	**.351**
Pair 3	Inhibition by *E.coli*_Agar well - Inhibition by *E.coli*_Disc Diffusion	.12500	.35355	.12500	-.17058	.42058	1.000	7	**.351**
Pair 4	Inhibition by *P.aeruginosa*_Agar well - Inhibition by *P.aeruginosa*_Disc Diffusion	1.93750	3.78378	1.33777	-1.22582	5.10082	1.448	7	**.191**
Pair 5	Inhibition by *C.albicans*_Agar well - Inhibition by *C.albicans*_Disc Diffusion	.18750	.53033	.18750	-.25587	.63087	1.000	7	**.351**

All the probability levels (significance levels) obtained were > 0.05 (see the last column) implying that growth inhibitions obtained using the two methods were not significantly different. Hence the two methods did not differ significantly.

Inhibitions at 200 mg/ml

Paired Samples descriptive Statistics					
		Mean	N(A to H)	Std. Deviation	Std. Error Mean
Pair 1	Inhibition by MRSA_Agar well	6.8125	8	6.50789	2.30089
	Inhibition by MRSA_Disc Diffusion	5.5208	8	5.30082	1.87412
Pair 2	Inhibition by *B.cereus*_Agar well	.5625	8	1.59099	.56250
	Inhibition by *B.cereus*_Disc Diffusion	.7500	8	2.12132	.75000
Pair 3	Inhibition by *E.coli*_Agar well	.4375	8	1.23744	.43750
	Inhibition by *E.coli*_Disc Diffusion	.5625	8	1.59099	.56250
Pair 4	Inhibition by *P.aeruginosa*_Agar well	1.2500	8	3.53553	1.25000
	Inhibition by *P.aeruginosa*_Disc Diffusion	1.0000	8	2.82843	1.00000
Pair 5	Inhibition by *C.albicans*_Agar well	1.1250	8	3.18198	1.12500
	Inhibition by C.albicans_Disc Diffusion	1.0000	8	2.82843	1.00000

A. Comparison of the two methods using growth inhibitions at 200 mg/ml

Paired Samples Test

	Comparison of the methods	Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Inhibition by MRSA_Agar well - Inhibition by MRSA_Disc Diffusion	1.29166	1.93701	.68484	-.32772	2.91104	1.886	7	**.101**
Pair 2	Inhibition by *B.cereus*_Agar well - Inhibition by *B.cereus*_Disc Diffusion	-.18750	.53033	.18750	-.63087	.25587	-1.000	7	**.351**
Pair 3	Inhibition by *E.coli*_Agar well - Inhibition by *E.coli*_Disc Diffusion	-.12500	.35355	.12500	-.42058	.17058	-1.000	7	**.351**
Pair 4	Inhibition by *P.aeruginosa*_Agar well - Inhibition by *P.aeruginosa*_Disc Diffusion	.25000	.70711	.25000	-.34116	.84116	1.000	7	**.351**

Paired Samples Test

	Comparison of the methods	Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Inhibition by MRSA_Agar well - Inhibition by MRSA_Disc Diffusion	1.29166	1.93701	.68484	-.32772	2.91104	1.886	7	**.101**
Pair 2	Inhibition by *B.cereus*_Agar well - Inhibition by *B.cereus*_Disc Diffusion	-.18750	.53033	.18750	-.63087	.25587	-1.000	7	**.351**
Pair 3	Inhibition by *E.coli*_Agar well - Inhibition by *E.coli*_Disc Diffusion	-.12500	.35355	.12500	-.42058	.17058	-1.000	7	**.351**
Pair 4	Inhibition by *P.aeruginosa*_Agar well - Inhibition by *P.aeruginosa*_Disc Diffusion	.25000	.70711	.25000	-.34116	.84116	1.000	7	**.351**
Pair 5	Inhibition by *C.albicans*_Agar well - Inhibition by *C.albicans*_Disc Diffusion	.12500	.35355	.12500	-.17058	.42058	1.000	7	**.351**