ANTIMICROBIAL ACTIVITY, ACUTE TOXICITY AND PHYTOCHEMICAL COMPOSITION OF FOUR MEDICINAL PLANTS TRADITIONALLY USED IN SOTIK SUB- COUNTY, KENYA

By

CHEROTICH JACKUELINE

BED (Science) University of Nairobi

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN PLANT PHYSIOLOGY AND BIOCHEMISTRY AT THE UNIVERSITY OF NAIROBI

MARCH 2015

DECLARATION

This is my original work and has never been presented for a degree in any other university

Miss Cherotich Jackueline (Bed)

School of Biological Sciences.

College of Biological and Physical Sciences.

University of Nairobi

Signed: _____ Date 01/03/2015

This thesis has been submitted with our approval as university supervisors

Supervisors

Dr. Dossaji Saifuddin F., BSc. MSc. PhD.

School of Biological Sciences.

College of Biological and Physical Sciences,

University of Nairobi

Signature: _____ Date 02/03/2015

Dr. Joseph Mwanzia Nguta, BVM. MSc. Ph.D. (UON).

Department of Public Health, Pharmacology and Toxicology.

Faculty of Veterinary Medicine.

College of Agriculture and Veterinary Sciences.

University of Nairobi

Signature: _____ Date 02/03/2015

Prof. Elijah Akunda, BSc. MSc. PhD.

School of Biological Sciences.

College of Biological and Physical Sciences.

University of Nairobi

Signature: _____ Date 11/03/15

DEDICATION

To my Dad Kipterer Langat, my mum Rael Langat, my siblings and my cousin Michael for their invaluable support and motivation during this study.

ACKNOWLEDGEMENT

My sincere appreciation goes to my supervisors. I am much grateful to my 1st supervisor Dr. Dossaji for his guidance, understanding, encouragements and being instrumental throughout this work especially in phytochemical analysis. My gratitude also goes to my second supervisor Dr. Nguta for his advice, criticism and invaluable assistance particularly in toxicity and antimicrobial activity. Many thanks also go to my third supervisor Prof . Akunda for his constant critics and endless advice during this study.

I am thankful to technical staff SBS, Mr. Mutiso for his help in voucher specimen identification, Ndii, Trisa, Wachira and Magrate for their support and guidance in antimicrobial work. My gratitude also goes to Mr. Kenneth Maloba, senior technologist, Department of Public Health, Pharmacology and Toxicology, Faculty of Veterinary medicine, University of Nairobi, for his support in crude plant extraction and preparation procedures. I also express special thanks to my fellow colleague Magrate Kaigongi for her encouragement and technical support.

Sincere thanks to the herbalists of Sotik Sub- county who provided ethnobotanical information and help in collection of voucher specimens and plant materials. My special gratitude also goes to my Dad Kipterer Langat and my mum Rael Langat for their love, profound financial support, advice and prayers. Indeed, it is their great encouragement that contributed enormously to my educational achievements to this level. Deepest gratitude to my sibling for their endless support and encouragement throughout my study. I also thank my primary school teacher Benjamin Milgo for his invaluable moral support, advice and constant follow up in my academics.

Last but not least, I acknowledge the almighty God for giving me good health, endurance, strength and ability to undertake this study.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

A LIST OF ACRONYMS

ANOVA: Analysis of Variance

ATTC: American Type Culture Collection

DMSO: Dimethylsulphoxide

KEMRI: Kenya Medical Research Institute

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

LSD: Least Significances Difference test

MRSA: Methicilin resistant *Staphylococcus aureus*

SPSS: Statistical Packages for Social Scientists

STDs: Sexually Transmitted Diseases.

TLC: Thin Layer Chromatography

TM: Traditional Medicine

WHO: World Health Organization

ABSTRACT

In Kenya, microbial diseases are prevalent and life threatening. Currently, herbal remedies are of interest as considered to be easily available, safe, effective and affordable. This study was designed to investigate antimicrobial activity, toxicity and phytochemical composotion in crude extracts of *Leucas calostachys, Mimusops kummel, Acacia lahai* and *Caesalpinia decapetala*. These plants are majorly used by herbalist from Sotik Sub- County, Kenya for treatment of gastroinstestinal problems. Antimicrobial activity was investigated using disc diffusion method and data analyzed using ANOVA. Minimum inhibitory concentrations (MIC) were determined by broth dilution method. Microorganisms employed were methicillin resistant *Staphylococcus aureus*, *Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa and Candida albicans.* Extracts toxicity was investigated using brine shrimp lethality assay, LD_{-50} was determined by analysis of data using Finney's computer program. Phytochemical screening for flavonoids, alkaloids, saponins and sesquiterpene lactones was determined using Thin layer Chromatography (TLC). Highest antibacterial activity was noted with methanol extract of *A. lahai* on *B. cereus* while acetone extracts of *A. lahai* exhibited the highest antifungal activity. Most active crude extracts had MICS values of 20mg/ml. All the tested extracts had LC_{50} values greater than 1000 µg/ml hence non-toxic on *Artemia salina* larvae. Flavonoids, alkaloids and saponins were present in all the plant species tested. Sesquiterpene lactones were present in *L.calostachy* and *M. kummel*. The results of this study shows that the tested plants have antimicrobial effects and is non-toxic supporting their traditional usage for treatment of microbial infections. Further bioassay of isolated compounds from active crude extracts is recommended.

Key words: Antimicrobial activity, Brine shrimp lethality assay, Medicinal plants, Phytochemical composition, Sotik Sub- County; Kenya.

CHAPTER ONE

1.0 INTRODUCTION

Plants have been recognized as an indispensible source of effective medicinal agents since time immemorial. This has led to isolation of a huge number of novel bioactive constituents from natural plant sources. Plant derived medicines have made large contributions to maintaining human health (El-Astal *et al.*, 2005). In this case, traditional medicinal plants have been considered to be important therapeutic drugs for alleviating various illnesses of both animals and mankind. In Kenya, traditional medicine (TM) is widely practiced as documented by ethnobotanical surveys (Miaron *et al.*, 2004, Kareru *et al.*, 2007, Njoroge and Bussman, 2007, Kokwaro 2009). Chirchir *et al.*, 2006, reported that an estimate of 90% of the population has used medicinal plants at least once for various health conditions. This is due to the significant healing power of the traditional medicinal systems (Adebolu and Oladimeji, 2005).

Infectious diseases caused by microbes are a major health hazards all over the world (Jigna *et al.*, 2007, Sasikumar *et al.*, 2007). Unfortunately, bacterial and fungal pathogens constantly continue to develop defense mechanisms against antimicrobial agents and resistance to conventional drugs. Bandow *et al.*, 2003 reported that the clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbes has led to the screening of several medicinal plants for their potential antimicrobial activity (Colombo and Bosisio 1996; Scazzocchio *et al.*, 2001). Infections associated with bacterial and fungal pathogens are among some of the conditions treated using traditional remedies in Kenya (Njoroge and Bussmann, 2007).

Recently, a lot of effort has been directed towards discovering new antimicrobial compounds from both organic and non-organic sources. Janovska *et al.,* 2003, stated that one of these sources is folk medicine and systematic screening of traditionally used plants could result in the discovery of novel effective antimicrobial compounds.

There are several reports in literature regarding the antimicrobial activity of crude extracts prepared from plants (El-Seedi *et al.,* 2002). The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body (Edeoga *et al.,* 2005). The most important bioactive compounds present in plants are alkaloids, flavonoids, tannins and phenolic compounds (Edeoga *et al.,* 2005) whose antimicrobial properties have been reported.

Folk medicine has been recognized as part of primary health care system in many indigenous communities. World Health Organization (WHO) estimates that 4 billion people (80% of the World's population) use herbal medicines for some aspect of primary health care (WHO, 2002b). However, there is limited scientific evidence from studies done to evaluate the safety and effectiveness of traditional medicinal products (Oreagba *et al.*, 2011). Since scientific investigation and knowledge of the therapeutic potential of traditionally used medicinal plants is limited, there is need for detailed studies to authenticate the reported anecdotal efficacy and safety.

The rapid rise in microbial resistance to conventionally used drugs calls for urgent research on any potential source of new effective antimicrobial agents. This has led to an increased demand for more drugs from plant sources all over the world. This is due to increased awareness on the limited ability of synthetic pharmaceutical products to control major diseases and the need to discover new molecular structures as lead compounds from the plant kingdom (Nair *et al* 2005).

A great interest is in traditional medicine due to belief that herbal medicines are safe, inexpensive and have no adverse side effects (Biesen *et al.*, 2012). Therefore, the current study aimed to evaluate the antimicrobial activity, brine shrimp lethality and phytochemical composition of crude extracts from *Leucas calostachys, Mimusops kummel, Acacia lahai* and *Caesalpinia decapetala*. These plants are majorly used in traditional medicine for treatment of skin diseases, gastrointestinal tract diseases and respiratory problems.

CHAPTER TWO

2.0 LITERATURE REVIEW

2. 1.Traditional medicine and its significance

Traditional medicine (TM) has continued to serve as a source of medicine in both human and livestock especially in Africa since time immemorial. According to WHO 2002, more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. The use of medicinal plants as traditional medicines is well known in rural areas of many developing countries (Sandhu and Heinich, 2005; Gupta *et al.*, 2005). Sotik Sub-county is one of those areas where majority of the rural population rely on TM to a large extent in treatment of diseases such as malaria, asthma, typhoid, sexually transmitted diseases, wounds, boils, ulcers, arthritis, gastrointestinal disorders, heart and kidney problems among others. This is attributed by factors such low cost, wide availability, less undesirable side effects and reliable accessibility of TM.

Among the TM, plant materials act as the main source of folk remedies used by traditional herbalist in their medication. Several parts of plants such as stem, leaves, roots, tubers, flowers and seeds are utilized during preparation of Plant drugs mainly through decoction. Earlier reports indicate that the overall number of medicinal plants is large, and includes both indigenous and introduced plants (Kokwaro, 1993; Kanafani and Fowler 2006). Some of the plants used in African TM have been investigated as source of antibiotics, anti- tumour and other useful substances (Kokwaro, 1993). Therefore TM turns out to be an indispensible and potent source of cure to a wide range of diseases hence investigations into their potential as future pharmaceuticals is a priority.

2.2. Plant products as antimicrobial agents

The beneficial medicinal effects of plant materials typically result from the combination of secondary products present in the plant. Plants are rich in a wide variety of secondary metabolites with antimicrobial properties, such as tannins, terpenoids, alkaloids and flavonoids (Al-Momani *et al.*, 2007; Bisignano *et al.*, 2000; Bouzada *et al.*, 2009; Chakraborty and Brantner, 1999; Cowan, 1999; Lewis and Ausubel, 2006; Setzer *et al.*, 2000; Sohail *et al.*, 2011). For example, flavonoids are known to be synthesized by plants in response to microbial infection (Dixon *et al.*, 1983) while terpenoids are active against fungi (Rana *et al.*, 1997), viruses (Fujioka and Kashiwada 1994), and protozoa (Ghoshal *et al* 1996). Futhermore, many human physiological activities, such as stimulation of phagocytic cells, host-mediated tumor activity, and a wide range of anti-infective actions, have been assigned to tannins (Haslam, 1996).

There are thousands of species of medicinal plants used globally for the cure of different infections (Boer *et al.,* 2005). Owing to the popular use of plants as remedies for many infectious diseases, searches for substances with antimicrobial activity in plants have recently increased. (Betoni *et al.,* 2006).

2.3. Plant products as resistance modifying agents

Plant metabolites act as the major basis for isolation of potentially useful compounds having resistance modifying activities to potential pathogens. Some traditional remedies have already produced compounds that are effective against antibiotic-resistant strains of bacteria (Kone *et al.,* 2004). Such examples include aqueous extracts of tea (*Camellia sinensis*) which have been shown to reverse methicillin resistance in MRSA and also to some extent, penicillin resistance in betalactamase producing *Staphylococcus aureus* (Stapleton *et al.,* 2004). This information indicates the need for further research into traditional health systems (Romero *et al.,* 2005). It

also emphasises on evidence based pharmacological studies with a potential to identification of potent, efficacious and safe pharmaceutical formulations with different mechanisms of action to avert resistance development (Manna and Abalaka, 2000).

2.4.0. Microorganisms and their health effects.

Microbes cause major infectious diseases that represent an important cause of morbidity and death among humans, especially in developing countries (WHO, 2002). These infections are facilitated by presence of poor hygiene, overcrowded population, HIV/AIDS infection and microbial drug resistance. Moreover, new infections caused by microorganism emerge more often while the pharmacological effectiveness of the currently present antimicrobials is being threatened by rising microbial drug resistance. This is increasing healthcare costs, finally causing disease burden, morbidity and mortality (Korir *et al.*, 2012). Herbal medications that are used by rural communities have proven to be able to manage microbial ailments for several generations. Collectively, natural products from higher plants may provide a new source of antimicrobial agents with possibly novel mechanisms of action (Adenisa *et al.*, 2000).

2.4.1. *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a gram-negative, aerobic bacteria and an opportunistic human pathogen. It is capable of causing severe invasive infections in patients with cystic fibrosis, neutropenia, iatrogenic immunosuppression, or disrupted anatomical barriers (Aoki *et al.*, 2004). Indeed, infections with *P. aeruginosa* are problematic for hospitalized patients, particularly those in intensive care units (Karlowsky *et al.*, 2003). *P. aeruginosa* is reported to have low antibiotic susceptibility attributable to a concerted action of multidrug efflux pumps with chromosomally encoded antibiotic resistance genes (Poole, 2004) and the low permeability of the bacterial cellular envelopes thus becoming resistance to a wide range of antibiotics (Li *et al.*, 2000). Also

P. aeruginosa easily develops acquired resistance either by mutation in chromosomally encoded genes or by the horizontal gene transfer of antibiotic resistance determinants and is naturally resistant to penicillin and related beta-lactam antibiotics (Poole, 2004).

2.4.2. *Escherichia coli*

Escherichia coli is a facultative anaerobic bacterium commonly found in the lower intestines of warm-blooded organisms. In humans, *E. coli* is responsible for a variety of intestinal and extra-intestinal infections. The pathogenic *E. coli* harbor different virulence and adhesion factors which allows it to cause specific diseases. Enterotoxigenic, enteroinvasive, enteropathogenic, enterohemorrhagic, verotoxigenic, and enteroaggregative *E. coli* isolates are relevant agents of diarrhea (Croxen and Finlay, 2010), whereas the others are frequent causes of urinary tract infections, abdominal and bloodstream infections in both community and hospitalized patients. *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). This bacterium often carries multiple drug-resistance plasmids, and under stress, readily transfers those plasmids to other species thus important reservoir of transferable antibiotic resistance (Salyers *et al.,* 2004).

2.4.3. Methicillin resistant *Staphylococcus aureus* (MRSA)

Staphylococcus aureus is a gram- positive bacterium frequently found in the human respiratory tract and skin. It is a common cause of minor skin infections, such as pimples, impetigo, boils, cellulitis folliculitis, carbuncles, scalded skin syndrome, and abscesses to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome, bacteremia and sepsis (Kanafani and Fowler Jr., 2006). Methicillin resistant *Staphylococcus aureus* (MRSA) is any strain of *Staphylococcus aureus* that has developed, through the process

of natural selection, resistance to beta-lactam antibiotics, Aminoglycosides, Quinolones, Clindamycin and Erythromycin.

2.4.4. *Bacillus cereus*

Bacillus cereus is a Gram-positive facultative anaerobe and spore-forming bacterium that produces multiple toxins which cause food poisoning in humans. Endospores of *B. cereus* are able to survive in harsh environments because of their thermal stability and ability to be easily transmitted to food through other environments such as soil or water (Kortiranta *et al.,* 2000). *B. cereus* is responsible for both the emetic and the diarrheal food poisoning syndrome due to toxin production (Ceuppens *et al.,* 2011). Additionally, it has been associated with more severe infection such as endophthalmitis and pneumonia (Miller *et al.,* 1997).

2.4.5. *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. The species 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, 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).

2.5. Importance of acute toxicity testing

Traditional medicine has been used since ancient times and has been anecdotally accepted to be safe. However, recent scientific studies have highlighted the toxic, mutagenic and carcinogenic effects of many plants used as traditional medicines (Fenell *et al.*, 2004). Rahman *et al.,* 1996

also highlighted that although traditional medicinal plants have been extensively used; specific evaluation on toxicity has not been done and could lead to serious complications.

According to Awodele *et al.*, 2011, adulterated, poor quality or poisonous herbal preparations are serious threats to patients' safety. Oreagba *et al.*, 2011 also reported that indiscriminate, irresponsible or non-regulated use of several herbal medicines may put users' health at risk of toxicity. Toxicity studies therefore assist to determine the right dosage to be administered without causing health risks in humans (Ashafa *et al*, 2012). Hence in efforts to verify the efficacy of traditional medicine due to their ethno-pharmacological properties, toxicity testing is vital to justify the activity and safety of herbal drugs.

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 (Subbaraju *et al.*, 2005). The lethal concentration for 50% (LC_{50}) mortality after 24 h of exposure is determined as the 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. Furthermore, *Artemia salina* is easy hatching from dry cysts and can be stored long without losing viability (Sorgeloos *et al.*, 1978). They are also favoured by flexibility to nutrient sources, temperature, and salinity tolerance (Nunes *et al.*, 2006).

2.6.0. Botanical description, traditional use and chemical constituents of the four selected plant species

2.6.1 *Leucas calostachys* oliv. (Labiatae)

Figure1. *Leucas calostachys* shrub (Photo courtesy of Cherotich Jackueline at Mosonik hill in Sotik Sub-county)

Leucas calostachys is a perennial shrub with densely hairy stems which is woody towards the base. Leaves are nearly sessile, oblong and are 1 .5–2 cm. long. The plant has many whorls and flowers of approximately 1cm in diameter. It has hairy Calyx which is about 0.5cm long. Corolla is half as long as the calyx with a densely villous upper lip.

Leucas calostachys have been exploitated in the traditional treatment of the various ailments such as wounds, amoeba, heartburns, muscle pull, waterborne diseases, cough, kidney problems, pneumonia, malaria, stomach-ache (Jeruto *et al.*, 2008). *L. calostachys* leaf extracts have been reported to have terpenoids, alkaloids and phenols (Jeruto *et al.*, 2008). No pharmacological action of this plant has ever been documented. However, arrays of other species of Leucas have

undergone significant investigations for their anecdotal efficacy. For example, *L. aspera* extracts have been reported to have anti-inflammatory activity (Saha *et al.*, 1997b), antibacterial and antifungal properties (Mangathayaru *et al.*, 2005), as mosquito repellant and as insecticide. (Kirtikar and Basu, 1990). The extract of *L. zeylanica* was found to exhibit potent inhibitory activity against *Staphylococcus aureus* and *Bacillus subtilis* (Valsara *et al., 1997).*

2.6.2 *Mimusops kummel* Hochsh. Ex A.DC (Sapotaceae)

Figure 2: ***Mimusops kummel*** **branches (Photo courtesy of Cherotich Jackueline at Mosonik hill in Sotik Sub-county)**

Mimusops kummel is a tree of 25–35 m tall .The bark is deeply grooved, dark grey in colour. Young branches are densely red-brown with leaves that are simple and entire. Flowers are bisexual, sepals present in 2 whorls of 4 while corolla is creamy-white. Fruit is an ellipsoid to

ovoid berry of up to 2.5 cm long, orange-red when ripe with a Seed which is reddish brown in colour (Lemmens, 2005).

Decoction of *M. kummel* bark is utilized by traditional herbalist from Sotik Sub-county in treatment of typhoid, stomach ache, dysentery, tooth ache, hard burn, syphilis, menstruation complications and reduce inflammation. According to Lemmens 2005, *M. kummel* roots are used in traditional medicine as a laxative and galactagogue while the seeds are used to treat ascariasis.

Kone *et al* (2011) reported that hydroalcohol crude extract from stem bark of *M. kummel* possess anthelmintic activity against *Schistosoma mansoni* adult worms. Other beneficial pharmacological properties of the genus includes anti-microbial propeties (Ali, 2008)], anti-ulcer (Payal *et al.*, 2003), anti-oxidant, hyperglycemic (Ganu *et al.,* 2010) and antihelminthic (Goutam, 2010) as exhibited by *M. elengi*. Several triterpenoids, steroids, steroidal glycosides, flavonoids and alkaloids have been identified and reported from Mimusops species (Misra *et al*, 1968). Fatty acids, saponins and tannins extracts from different plant parts of the genus have also been reported.

2.6.3 *Acacia lahai* Stead. &Hochst.ex Benth (Fabaceae)

Figure 3: *Acacia lahai* stem & a branch (Photo courtesy of Cherotich Jackueline at Mosonik hill in Sotik Sub-county)

Acacia lahai is a flat-topped tree 3-15 m tall with rough grey-brown bark .Young branches exhibit a brown to blackish-purple colour. Bark is grey-brown and fibrous in texture. Leaves are pinnate, about 4.5mm long. Flowers colour is cream or white. Fruits are brown, straight or falcate with a dehiscent pod and Seeds are obviate, flattened, 6-7 mm long x 5 mm wide (Orwa *et al.* ,2009).

Folk medication of *Acacia lahai* indicates that bark is used for the treatment of skin eruptions in children, clearing toxaemia of pregnancy and bowels and also as an astringent (Hubbard and Milne-Redhead, 1959). Antimicrobial potentials of acacia plant species have been noted such as *A. nilotica* assays of the stem bark extracts confirms the antibacterial activity against *Streptococcus viridans*, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* (Banso, 2009)

Aqueous bark extracts is reported to contain tannins (Hubbard and Milne-Redhead, 1959). Also phytochemical screening of most of plants in genus *acacia* exposed that the plants contain terpenoids, alkaloids, saponins and glycosides for example stem bark of *A. nilotica* (Banso, 2009).

2.6.4 *Caesalpinia decapetala* (Roth) Alston (Caesalpiniaceae)

Figure 4: *Caesalpinia decapetala* stem (Photo courtesy of Cherotich Jackueline at Mosonik hill in Sotik Sub-county)

Caesalpinia decapetala is a robust thorny evergreen climber or a shrub that grows from two to four meters high or climbs to 10 meters or higher. It has sprawling branches that often form large dense impenetratable thickets. The stems are vine with a diameter of up to 4 cm and covered with minute golden-hair and thorns. Leaves are bipinnate, dark green, paler underneath with thorns in spine. Perfect flowers with yellow petals while pods have black seeds and burst open on their own.

Caesalpinia decapetala concoction of roots is used in ethno-medication for treatment of asthma, burns, whooping cough, stomach disorders, tooth ache, polio, inflammation, head ache, as well

as being an anthelmintic and a digestive. The roots of *C. decapetala* are reported to be used in folk medicine to treat bronchitis, prevent colds, and as an antimalarial agent (Wagner *et al* 1999). In Maharashtra and South India, *C. decapetala* bark is used for tanning, as laxative, tonic, carminative and antipyretic (Kiem *et al.,* 1984). Leaves and root of *C. decapetala* act as a purgative and emmenagogue (Guha *et al.,* 1999). Also fruit extracts from this plant have shown inhibitory effect against *Candida albicans* (Kumar *et al.,* 2006).

Earlier scientific investigations on phytochemical constituents have shown that leaves of *C. decapetala* contain cassane diterpenoid, caesaldecan, spathulenol, 4, 5-epoxy-8(14)-caryophyllene, squalene, lupeol, resveratrol, quercetin, astragalin and Stigmasterol (Kiem *et al.,* 1984). Triterpenoids is also reported to be present in leaves (Jiang *et al.,* 2001). Stems of *C. decapetala* shows the presences of phenols like pauferrol (Zhang *et al.,* 2008).

Table 1: Plant species collected from Sotik Sub-county based on traditional reputation for their use as antimicrobial agents

Plant species &Voucher specimen number)	**Vernacular name**	**Habit**	**Part used**	**Treatment preparation**	**Disease(s) treated**
Leucas calostachys (CJ2013001)	Ngechepjat	Shrub	Leafs	Pounding & burning to ash	Kidney problem, pneumonia, stomachache, wounds.
Mimusops kummel (CJ2013002)	Lalwat	Tree	Bark	Decoction	Typhoid, stomachache, toothache, hard burn, syphilis
Acaia lahai (CJ2013003)	Chebitet	Tree	Bark	Decoction	Syphilis, skin diseases, stomachache
Caesalpinia decapetala (CJ2013004)	Chepkomon	Climbing herb	Roots	Decoction	toothache, headache, stomachache

2.7. Problem statement

Microbial diseases have been a life threatening problem for a long time. In recent years, development of multi-drug resistances by pathogenic microbes to most available drugs has exacerbated this burden hence becoming a global concern. This is largely due to indiscriminate use of conventional antimicrobial drugs commonly employed in the treatment of infectious diseases. New illness caused by bacteria and fungi continues to emerge hence becoming difficult to treat and very expensive. New illness also foster frequent utilization of currently available synthetic antibiotics which can lead to toxicity hence becoming life threatening.

A revival of a prolonged steady development of new drugs from plant sources is emphasized by academic and pharmaceutical scientists as a key way towards addressing the problem. The current study aimed on those plants that have been used in the traditional medicinal practices as therapeutics for human and animal diseases. Considering the prominent potentiality of plants as a source of antimicrobial drugs, investi gation on the traditional medicinal plants should be prioritized since only a few have been evaluated pharmacologically. Toxicity evaluation of herbal medicine is also considered to determine their safety. This study therefore sought to evaluate four traditionally used medicinal plants; *Leucas calostachys, Mimusops kummel, Acacia lahai* and *Caesalpinia decapetala* in Sotik ethnomedicine for their antimicrobial activity, toxicity and phytochemical composition.

2.8. Justification

Microbes cause major infectious diseases that are responsible for morbidity and death among humans, especially in developing countries. The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens. This antimicrobial resistance to the drugs is driving up healthcare costs, increasing the severity of disease, morbidity

and mortality. Increasing trend in the emergence of resistance to antimicrobial agents results from low quality drugs manufactured, patient non-compliance, microbial mutations and indiscriminate use of antibiotics. Resistance is also contributed by HIV pandemic which compromises individuals' immune system, rendering subjects susceptible to opportunistic infections.

Hence, there is a constant need to search for cost effective, new biologically and pharmacologically active antimicrobial agents. Recently, great effort to search for remedies for various critical ailments have driven focus on natural products of plant biodiversity where plants have become an essential source of therapeutics and most potent agents employed for medication. Scientists are turning to research for drugs from traditionally used herbal plants as they are considered to have fewer side effects, easily available and cost effective compared to allopathic systems of medicines. Among these traditional medicinal plants, few have been identified and toxicity of their phytochemicasl studied scientifically. Therefore, this research is necessary to validate antimicrobial activity, phyochemical constituents and toxicity of plants under study and can act as a means of providing alternative antimicrobial medicines to overcome the constant and increasing drug resistance.

2.9.0. Objectives of the study

2.9.1 Main objective

To evaluate *in vitro* antimicrobial activity, toxicity and phytochemical composition of crude extracts of selected medicinal plants used in Sotik Sub-county, Kenya.

2.9.2 Specific objectives

i. To evaluate the antimicrobial potential of the crude plant extracts against methicillin resistant *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus cereus, Escherichia coli* and *Candida albicans.*

ii. To determine the acute toxicity of the crude plant extracts using brine shrimp lethality assay.

iii. To characterize the major phytochemical constituents in the crude plant extracts.

2.10. Hypothesis

This study hypothesises that the crude extracts of *Leucas calostachys, Mimusops kummel, Acacia lahai* and *Caesalpinia decapetala*, which are used in traditional medicine, possess phytochemical constituents with antimicrobial effects and are non-toxic to brine shrimp larvae.

CHAPTER THREE

3.0: MATERIALS AND METHODS

3.1. Collection of plant materials

Four plant samples were collected from Mosonik hill, Sotik Sub County, Bomet county of Kenya in the morning with the help of herbalists following ethnopharmacological usage of the plants (leaves of *Leucas calostachys*, barks of *Mimusops kummel*, *Acacia lahai* and roots of *Caesalpinia decapetala)*. The plant parts were thoroughly washed with running tap water and air dried at room temperature for six weeks then they were chopped into small pieces and ground into powder. Voucher specimens were deposited in the University of Nairobi Herbarium after identification by a taxonomist at the School of Biological Sciences, University of Nairobi.

Figure 5: A map of Kenya showing location of Sotik Sub-county

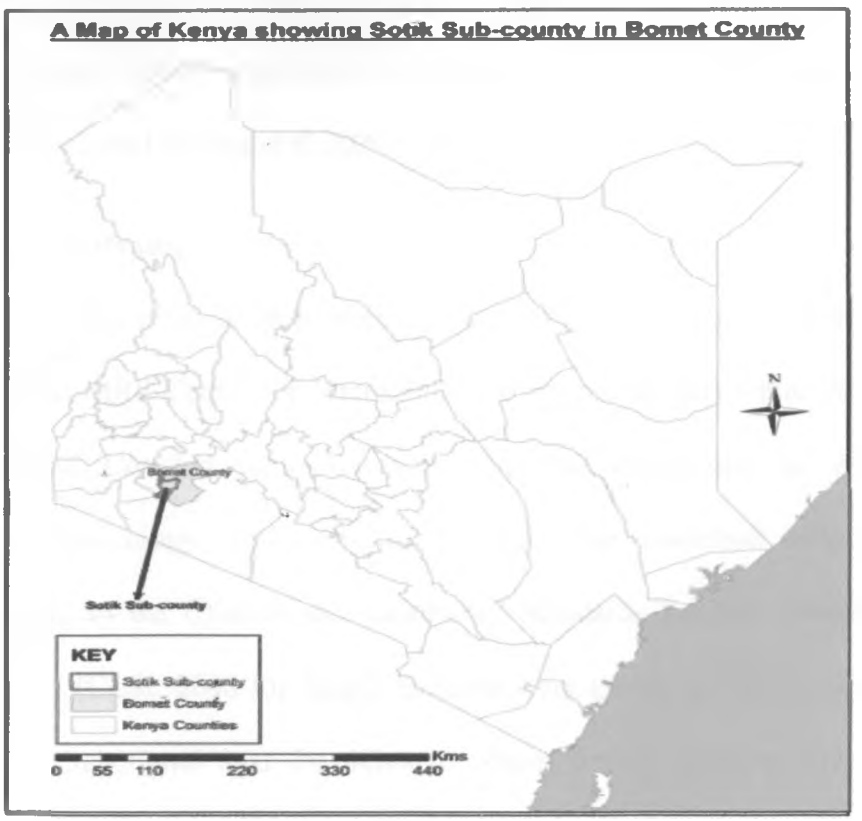


Table 2: Plants collected from Sotik Sub-county

Voucher Specimen number	Plant species	Family	Part collected
CJ2013/001	*Leucas calostachys*	Labiatae	Leaf
CJ2013/002	*Mimusops kummel*	Sapotaceae	Stem bark
CJ2013/003	*Acaia lahai*	Fabaceae	Stem bark
CJ2013/004	*Caesalpinia decapetala*	Caesalpiniaceae	Root

3.2. Preparation of crude extracts

The prepared dried powder (50 grams) of each sample was soaked in 500 ml of distilled water for aqueous extract while acetone and methanol at the same amount (500 ml) was used for organic extracts. Each plant material was extracted by percolation with the respective solvent at the ratio of 1:10 (plant material/solvents), shaken thoroughly and left to stand for 24hrs at room temperature (Korir *et al.,* 2012). The mixtures were then filtered using whatman No. 1 filter paper. Dry crude organic extracts were obtained by concentrating filtrates from organic solvents in vacuum at 45°C by use of a rotary evaporator while water extracts were frozen then lyophilized to dry powder. The dried extracts obtained were weighed then stored in stoppered sample vials at 4°C until used (Sultana *et al.,* 2009).

3.3. Preparation of test strains

Pure cultures of 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. The microbial suspensions were standardized according to the Clinical and Laboratory Standards Institute procedures (CLSI, 2009 for bacteria) and (CLSI, 2008 for fungi). Bacteria were grown in Muller–Hinton agar for 18hrs and fungus in Sabouraud agar for 48hrs to obtain freshly growing strains. Then the

microbial suspensions were standardized with sterile saline to turbidity equivalent to 0.5 McFarland scale (approximately $1\text{-}2\times10^8$ CFU/ml for bacteria and $1\text{-}5\times10^6$ CFU/ml for Candida spp.) and stored at 4°C until used during antimicrobial test.

Table 3: 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	Clinical isolate
Pseudomonas aeruginosa	Bacteria	Gram Negative	ATCC 27823
Escherichia coli	Bacteria	Gram Negative	ATCC 25922
Candida albicans	Fungus		ATCC10231

3.4. Antimicrobial susceptibility testing (Disk diffusion technique)

The disk diffusion method according to National Committee for Clinical Laboratory Standards (CLSI 2009) procedures was used to evaluate antimicrobial activities of the crude extracts. Four concentrations (200, 100, 50 and 25 mg/ml) of each test extract were prepared for susceptibility testing using 1% DMSO for organic extracts and distilled water for aqueous extracts. Filter paper 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 culture was incubated at 37°C for 24 hrs and fungal culture at 30°C for 72 hrs respectively. Gentamicin 40µg/ml (for bacteria) and Amphotericin B 30 µg/ml (for fungi) were used as standards, 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.

3.5. Determination of Minimum Inhibitory Concentration (MIC): Broth micro dilution method.

Broth micro dilution method was used to determine minimum inhibitory concentration (MIC) values for the active crude extracts against the test microorganisms. The procedure was done as recommended by the Clinical Laboratory Standard Institute (CLSI, 2009; Ferraro, 2003). One ml of 24 hrs culture of test organisms (10^7 CFU/mL) adjusted to McFarland turbidity standard was incubated in serial dilution ranging from 10-100 mg/ml of plant extracts .Incubation was done for 24hrs at 37^0 C for bacteria and 30^0C for 72hrs for fungi. 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.

3.6. Acute toxicity determination

The acute toxicity assay was performed using brine shrimp *nauplii* based on Meyer method (Hossain *et al,* 2004; Islam *et al,* 2002; Nguta et al., 2013). Artificial sea water was prepared by dissolving 33 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, a solution of 3.8% sodium chloride. 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 (23-29^0C) 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 0.1g of the crude extract in 10 mls 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 mls 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 mls. Using micropipettes, 0.5 mls, 0.05 mls and 0.005 mls 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. 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 (Table 4). Surviving *nauplii* were counted after 24hrs using a using a magnifying glass (Musila *et al.*, 2013). Median lethal concentrations (LC_{50}) were determined using finney computer program (Nguta *et al*, 2013) by use of probit analysis method described by Finney (Finney, 1971).

Table 4: 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

3.7. Phytochemical determination for secondary metabolites using Thin Layer Chromatography

Phytochemical analysis of organic and aqueous crude plant extracts was done to determine the presences alkaloids, sesquiterpene lactones, flavonoids and saponins following the procedure of Harborne (2002). Extracts were dissolved in chloroform and methanol (1:1 v/v). Phytochemical screening was then perfomed using aluminum TLC plates. The plates were placed in a chamber with appropriate chromatographic solvent system specifically used for determination of the presence of a given class of phytochemical constituents. The developed plates were also sprayed using specific spray reagents for each secondary metabolite as shown in table 5.

Table 5: Determination for flavonoids, alkaloids saponins and sesquiterpene lactones

Class of Secondary metabolites	Solvent system	Detection
Flavonoids	n-hexane: ethyl acetate: acetic acid (6:3:1) (Waksmundzka *et al.*, 2008)	Flavonoids at 254 nm appeared as dark blue zones on a yellow background on the developed plates intensifying when sprayed with ammonia. At 365 nm flavonoids fluoresced yellow, blue or green (Waksmundzka *et al.*, 2008).
Alkaloids	Dichloromethane: Methanol (85:15) (Harborne, 2002)	Dragendorff's reagent was sprayed on the developed plates. Orange colors indicated the presence of alkaloids. Spraying with sodium nitrate intensified the orange colors (Harborne, 2002).
Saponins	Dichloromethane: Ethyl acetate (9:1) according to (Karem *et al.*, 2005)	Saponins appeared as black spots when plates were sprayed with a mixture of ethanol and H_2SO_4 (9:1) and then heated at 110^0C for 10 minutes (Karem *et al.*, 2005).
Sesquiterpene lactones	n-hexane: ethylacetate (9:1) (Waksmundzka *et al.*, 2008)	Brown spots on the developed plates in presence of iodine indicated the presence of sesquiterpene lactones (Waksmundzka *et al.*, 2008). Also detected as brown, yellow spots when plates were sprayed with concentrated H_2SO_4 and heated for 5 minutes at 100-110^0C (Harborne, 2002).

3.8. Data analysis

Statistical analysis of antimicrobial activity was done using SPSS to compare the various extracts from various plants to find out whether the plants exhibited growth inhibition of the various bacteria and fungi differently. Once differences were identified further ANOVA using Least Significance Difference test (LSD) was done to compare the treatments with the positive controls (Amphotericin B and Gentamicin) to find out whether any treatment had any bioactivity

comparable to the positive control. Ms Excel 2007 was used to determine mean inhibition zones while Ms Word 2007 was used to draw tables. The lethal concentration (LC_{50}), 95%confidence interval of the selected plants was determined using the Finney (1971) computer program. The level of significance used in analysis of the data was 0.05 ($p \leq 0.05$).

CHAPTER FOUR

4.0. RESULTS

4.1. Yields of extracts from test plants

Yields of crude extracts of the selected medicinal plants were noted to vary with the plant species and solvent used in the extraction as shown on Table 6.

Table 6: Percentage yields of extracted crude plant extracts

Plant species	Part used	solvent	Extraction type	% yield to weight of dry powered plant
Leucas calostachys	Leafs	Water	Freeze drying	5.16
		Methanol	Rotary evaporation	2.74
		acetone	Rotary evaporation	1.72
Mimusops kummel	Stem	Water	Freeze drying	7.46
		Methanol	Rotary evaporation	3.98
		acetone	Rotary evaporation	3.58
Acacia lahai	Stem	Water	Freeze drying	6.92
		Methanol	Rotary evaporation	4.06
		acetone	Rotary evaporation	3.28
Caesalpinia decapetala	Roots	Water	Freeze drying	5.84
		Methanol	Rotary evaporation	3.16
		acetone	Rotary evaporation	2.34

Percentage yields of crude extract (% yields) = extracted weights/initial weightsx100 (All weights in grams)

4.2. Antimicrobial activity of the crude extracts against selected microorganisms

Methanolic extract of *A. lahai* had the highest antimicrobial activity with inhibition zone diameter of 15 mm compared to the rest of the tested extracts (Table 7). Furthermore, acetone extracts of all the tested plant species showed noteworthy inhibitory effect to most test microorganisms compared to methanol and water extracts (Table 7, 8 & 9) at 200 mg/ml concentrations.

It was observed that growth of *B. cereus* was inhibited by all the methanol, acetone and water extracts of *M. kummel*, *acacia lahai* and *C. decapetala* except that of *L. calostachys* as shown in Table 7. Among the tested plants extracts, methanol extract of *C. decapetala* and water extract of *L. calostachys* were the only inactive extracts against MRSA.

It was noted that methanol extract of *M. kummel*, *A. lahai*, acetone and water extracts of *C. decapetala* had only antibacterial effect against the gram positive bacteria used in the test. Moreover, it was found that all the extracts of *L. calostachys*, acetone and water extracts of *M. kummel*, *A. lahai* and methanol extract of *C. decapetala* exhibited only antibacterial activity against gram negative *P. aeruginosa* but no activity in *E. coli*. Generally, all the extracts of the selected ethnomedicinal plants were not active against *E. coli*. Likewise, acetone, methanol and water extracts of *L. calostachys* showed no inhibition on both *B. cereus* and *C. albicans*.

All the extracts of *M. kummel* and *A. lahai*, including methanol and water extracts of *C. decapetala* exhibited antifungal activity against *C. albicans* (Table11). However, acetone extract of *acacia lahai* revealed the highest antifungal activity with inhibition zone value equal to 13.17 mm.

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

The antimicrobial activity of the four selected plants against *Bacillus cereus* is as shown in Table 7. It was observed that *B. cereus* was inhibited by all the extracts of *M. kummel*, *A. lahai* and *C. decapetala* except that of *L. calostachys* as shown in Table 7. Highest antibacterial activity was shown by methanol extract of *A. lahai* with inhibition zone diameter of 15 mm at a concentration of 200 mg/ml (Table 7) but at a concentration of 25 mg/ml, none of the extracts inhibited the growth of *B. cereus*. Among the plants used, organic extracts of A. *lahai* portrayed a higher inhibition zone as compared to that of *M. kummel* and *C. decapetala*. *M. kummel* aqueous extracts was more active than the aqueous extracts of the rest of the plants (Table 7). The growth inhibition of *B. cereus* by the various extracts from various plants was significantly ($P \leq 0.05$) different. Their significance levels of comparison of all the plants with the positive control at concentrations; 200, 100, 50 and 25 mg/ml 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 $P \leq 0.05$.

Table 7: Growth inhibition of the crude extracts on *Bacillus cereus*

		Mean, zone of inhibition (Mm) ± S.D of three replicates		
plant	concentration (mg/ml)	methanol	acetone	water
L. calostachys	200	-	-	-
	100	-	-	-
	50	-	-	-
	25	-	-	-
M .kummel	200	11.00 ± 0.50	12.28 ± 0.29	12.5 ± 0.05
	100	9.33 ± 1.29	12.17 ± 0.29	11.00 ± 0.00
	50	7.50 ± 0.05	8.50 ± 0.50	8.00 ± 0.00
	25	-	-	-
A. lahai	200	15.00 ± 0.00	13.00 ± 0.50	12.17 ± 0.29
	100	14.00 ± 0.50	11.50 ± 0.00	9.83 ± 1.04
	50	11.00 ± 0.00	8.5 ± 1.32	7.83 ± 0.58
	25	-	-	-
C. decapetala	200	9.17 ± 0.29	10.83 ± 0.29	9.33 ± 0.58
	100	8.33 ± 0.29	9.17± 0.29	8.33 ± 0.53
	50	-	7.50 ± 0.50	-
	25	-	-	-
Gentamicin (40 µg/ml)	-	15.83 ±0.76	15.83 ± 0.76	15.83 ± 0.76
DMSO & Water	-	-	-	-

4.2.2. Antimicrobial activity of the crude extracts on MRSA

Among the tested plants extracts, methanol extract of *C. decapetala* and water extract of *L. calostachys* was not active against MRSA. Effective growth inhibition was noted at higher concentrations of the extracts, whereas at 25 mg/ml it is only acetone extracts of *L. calostachys* that was found active against MRSA (Table 8). However, aqueous extracts of *L. calostachys* were not active in any of the four concentrations. Growth inhibition of MRSA by the various extracts of the four selected plants was found to be significantly different from each other at 95% confidence intervals ($P \leq 0.05$). Their significance levels of all the plants compared with the positive control at 200, 100, 50 and 25 ug/ml were all less than 0.05 showing that all the extracts

had growth inhibitions of MRSA which were significantly different from that of Gentamicin which was used as a positive control.

Table 8: Growth inhibition of the crude extracts on *MRSA*

		Mean, zone of inhibition (mm) ± S.D of three replicates		
plant	concentration (mg/ml)	methanol	acetone	water
L. calostachys	200	11.00 ± 1.00	11.67 ± 0.58	-
	100	9.5 ± 0.00	9.33 ± 0.58	-
	50	-	8.67 ± 0.58)	-
	25	-	7.00 ± 0.00	-
M .kummel	200	10.33 ± 0.58	9.00 ± 0.00	11.12 ± 1.04
	100	9.5 ± 0.50	8.00 ± 0.00	9.5 ± 0.50
	50	-	-	7.83 ± 0.29
	25	-	-	-
A. lahai	200	11.33 ± 0.29	11.00 ± 1.00	11.00 ± 0.00
	100	9.12 ± 1.04	9.83 ± 0.29	8.83 ± 0.29
	50	-	-	-
	25	-	-	-
C. decapetala	200	-	9.83 ± 0.77	9.00 ± 0.00
	100	-	8.00 ± 0.00	7.00 ± 0.00
	50	-	-	-
	25	-	-	-
Gentamicin(40 µg /ml)	-	15.67 ± 1.04	15.67 ± 1.04	15.67 ± 1.04
DMSO & Water	-	-	-	-

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

Antibacterial activity of aqueous, methanol and acetone extracts of the four plants against *P. aeruginosa* were as shown in Table 9. Among the extracts of *C. decapetala*, only methanol extract showed antibacterial activity against this bacterial strain. *L. calostachys* exhibit more

activity among the sampled plants since all its extracts inhibit the growth of *P. aeruginosa.* Methanol extracts of both *M. kummel* and *A. lahai* did not inhibit the growth of *P. aeruginosa* while *M. kummel* acetone extracts had the highest growth inhibition (12.86 mm). *P. aeruginosa* was more sensitive to acetone extracts as compared to methanol and aqueous extracts of the selected medicinal plants. At $P \leq 0.05$, the various extracts of the four plants had growth inhibition of *P. aeruginosa* which was significantly different from each other. Moreover, all the extracts had growth inhibitions of *P .aeruginosa* which were significantly different from that positive control (Gentamicin).

Table 9: Growth inhibition of the crude extracts on *P. aeruginosa*

		Mean, zone of inhibition (mm) ± S.D of three replicates		
plant	concentration(mg/ml)	methanol	acetone	water
L. calostachys	200	11.00 ± 0.00	12.83 ± 0.29	9.00 ± 0.00
	100	10.00 ± 0.00	11.67 ± 0.58	7.67 ± 0.58
	50	9.33 ± 0.58	9.30 ± 0.58	-
	25	8.50 ± 0.50	8.12 ± 0.29	-
M .kummel	200	-	12.86 ± 0.29	12.18 ± 0.29
	100	-	12.00 ± 0.00	11.00 ± 1.00
	50	-	8.67 ± 0.58	8.00 ± 0.00
	25	-	7.67 ± 0.58	-
A. lahai	200	-	10.33 ± 0.58	9.67 ± 0.58
	100	-	9.17 ± 0.29	8.33 ± 0.58
	50	-	8.12 ± 0.29	7.00 ± 0.00
	25	-	7.33 ± 0.58	-
C. decapetala	200	9.83 ± 0.29	-	-
	100	9.00 ± 0.00	-	-
	50	8.12 ± 0.29	-	-
	25	7.33 ± 0.58	-	-
Gentamicin (40 µg/ml)	-	15.12 ± 0.63	15.12 ± 0.63	15.12 ± 0.63
DMSO & Water	-	-	-	-

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

All the extracts of the selected ethno medicinal plants were not active against *E. coli* .The Significance level corresponding to plants and solvent was found to be 0.000 and 1 respectively at $P<0.05$. This showed that growth inhibition of *E. coli* by the various extracts of the four plants was significantly different but there was no significant difference in growth inhibition by the various extracts of the same plant. Significance levels of the comparison of all the plants with the positive control at 200, 100, 50 and 25 ug/ml proved that all the extracts had growth inhibitions of *E. coli* which were significantly different from that of positive control.

Table 10: Growth inhibition of the crude extracts on *E. coli*

		Mean, zone of inhibition (mm) ± S.D of three replicates		
plant	concentration(mg/ml)	methanol	acetone	water
L. calostachy	200	-	-	-
	100	-	-	-
	50	-	-	-
	25	-	-	-
M .kummel	200	-	-	-
	100	-	-	-
	50	-	-	-
	25	-	-	-
A. lahai	200	-	-	-
	100	-	-	-
	50	-	-	-
	25	-	-	-
C. decapetala	200	-	-	-
	100	-	-	-
	50	-	-	-
	25	-	-	-
Gentamicin (40 µ g/ml)	-	14.67 ± 0.29	14.67 ± 0.29	14.67 ± 0.29
DMSO & Water	-	-	-	-

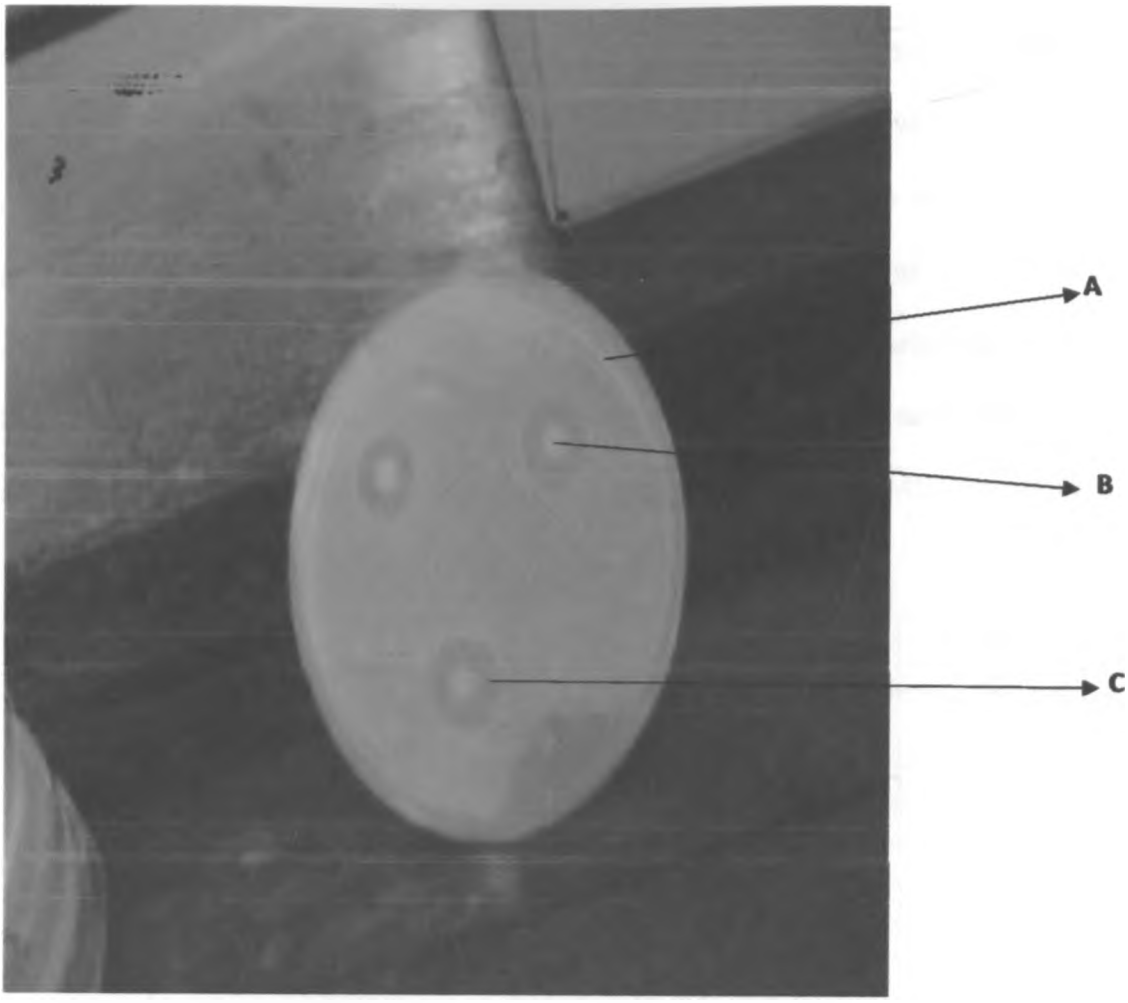
4.2.5. Antimicrobial activity of the crude extracts on *Candida albcans*

Table 11 illustrated that all the extracts of *M. kummel* and *A. lahai*, methanol and methanol extracts of *C. decapetala* exhibited antifungal activity against *C. albicans*. However, acetone extract of *A. lahai* reveal the highest antifungal activity with inhibition zone value equal to 13.17 mm. Acetone, methanol and aqueous extracts of *L. calostachys* showed no inhibition on *C. albicans*. It was evident that growth inhibition of C. *albicans* by the various extracts from the four plants was significantly different from each other ($P \leq 0.05$), while the comparison of growth inhibition by all the plants with the positive control at 200, 100, 50 and 25 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.

Table 11: Growth inhibition of the crude extracts on *candida albicans*

		Mean, zone of inhibition (mm) ± S.D of three replicates		
plant	concentration(mg/ml)	methanol	acetone	water
L. calostachys	200	-	-	-
	100	-	-	-
	50	-	-	-
	25	-	-	-
M .kummel	200	11.00 ± 0.00	11.00 ± 0.00	11.67 ± 0.58
	100	8.67 ± 1.15	9.00 ± 0.00	9.33 ± 1.15
	50	-	7.33 ± 0.58	7.83 ± 0.29
	25	-	-	-
A. lahai	200	12.670 ±.58	13.17 ± 0.29	10.00 ± 0.00
	100	10.00 ± 0.00	12.00 ± 0.00	8.33 ± 0.58
	50	-	7.17 ± 0.29	-
	25	-	-	-
C. decapetala	200	8.83 ± 0.29	-	8.83 ± 0.29
	100	8.00 ± 0.00	-	7.67 ± 0.57
	50	-	-	-
	25	-	-	-
mpotericin B(30µ g/ml)	-	15.83 ± 0.76	15.83 ± 0.76	15.83 ± 0.76
DMSO & Water	-	-	-	-

Figure 6: A representative Photo of antimicrobial assay showing inhibition zone diameter



KEY:

A- Petri dish with growth media

B- Filter paper disk

C- Inhibition zone

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 12. MIC values significally varied with plant samples from 90 mg/ml to 20 mg/ml. *Leucas calostachys* plant recorded more number (3) of least MIC values of 20 mg/ml as compared to the rest of the sampled plants as seen in MRSA and *P. aeruginosa* (Table 12). The MIC values of the test extracts also varied against different test pathogens. The results obtained from these assay revealed that *P. aeruginosa* was the most sensitive bacteria at lower concentrations with highest number of MIC values of 20 mg/ml.

Table 12: Minimum inhibitory concentration (MIC) in mg/ml

Plant	Solvent	*B .cereus*	MRSA	*P. aeruginosa*	*E. coli*	*C. albicans*
Leucas calostachys	Methanol	-	60	20	-	-
	Acetone	-	20	20	-	-
	water	-	-	90	-	-
Mimusops kummel	Methanol	40	70	-	-	70
	Acetone	40	70	20	-	40
	Water	40	40	40	-	30
Acacia lahai	Methanol	30	60	-	-	70
	Acetone	40	60	20	-	40
	water	40	60	40	-	70
Caesalpinia decapetala	Methanol	80	-	20	-	80
	Acetone	50	80	-	-	-
	water	70	90	-	-	80

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

Lethal concentration (LD_{50}) of the crude plant extracts were estimated using finney computer program. The results are summarised in the Table 13. Based on Nguta *et al.*, 2011 evaluation of toxicity, where LD_{50} ranging between 0-500 µg/ml implied high toxicity, LD_{50} between 500-1000 µg/ml implied moderate toxicity and LD_{50} over 1000 µg/ml implied non toxic nature of the extract, all the crude plant extracts were found to be non-toxic. Methanol extract of *Leucas calostachys* had the least toxicity while its acetone extract had the most toxicity with LC_{50} of 9941 µg/ml and 1100µg/ml respectively among the studied extracts (Table 13).

Table 13: Brine-shrimp lethality

Plant species	Solvent	Average mortality				LD_{50}
		1,000 µg/ml	100 µg/ml	10 µg/ml	0 µg/ml	
Leucas calostachys	Water	3.33	1.67	0.00	0.00	0
	Methanol	2.67	1.67	0.33	0.00	9941
	Acetone	2.33	1.33	0.00	0.00	1100
Mimusops kummel	Water	5.67	3.65	2.00	0.00	0
	Methanol	3.33	1.67	0.67	0.00	7130
	Acetone	3.67	3.0	1.33	0.00	5154
Acacia lahai	Water	3.67	1.33	0.67	0.00	0
	Methanol	1.67	1.33	0.00	0.00	4741
	Acetone	1.33	0.33	0.00	0.00	2005
Caesalpinia decapetala	Water	3.33	2.33	0.33	0.00	0
	Methanol	2.67	1.67	0.67	0.00	2559
	Acetone	3.33	1.67	1.33	0.00	2343

4.5. Phytochemical constituents of the crude plant extracts.

The plants extracts showed a positive test for the presence of alkaloids. Saponins were present in all the extracts screened except aqueous extracts of *L. calostachys*, acetone and aqueous extracts *M. kummel*. Flavonoids were present in all organic extracts screened and aqueous extracts of *C. decapetala* only. Sesquiterpenes lactones were found to show a positive test in organic extracts of *L. calostachys* and *M. kummel* (Table 14).

Table 14: Phytochemical constituents of the crude plant extracts.

Plant species	Solvent	Flavonoids	Alkaloids	Saponins	Sesquiterpene lactones
Leucas calostachys	Methanol	+	+	+	+
	Acetone	+	+	+	+
	water	–	+	–	–
caesalpinia decapetala	Methanol	+	+	+	–
	Acetone	+	+	+	–
	water	+	+	+	–
Mimusops kummel	Methanol	+	+	+	+
	Acetone	+	+	–	+
	water	–	+	–	–
Acacia lahai	Methanol	+	+	+	–
	Acetone	+	+	+	–
	water	–	+	+	–

Key: + = present - = absent

CHAPTER FIVE

5.0. DISCUSSION, CONLUSION AND RECOMMENTATION

5.1. Discussion

In the present study, *in vitro* antimicrobial activity of the four selected medicinal plants were evaluated against two gram positive bacteria *(*MRSA, *B. cereus)*, two gram negative bacteria *(E. coli, P. aeruginosas)* and a fungus (*C. albicans*). Results of the study revealed that the tested plant extracts possess potential antimicrobial activity against most tested microorganisms. The tested plant extracts were more active against gram positive bacteria compared to gram negative. The higher sensitivity of Gram-positive bacteria (*B. cereus* and MRSA*)* could be due to possession of an outer peptidoglycan layer which is an ineffective permeability barrier while the Gram-negative bacteria have an outer phospholipid membrane carrying the structural lipopolysaccharide component (Korir *et al*., 2012). The most sensitive bacterium was *Bacillus cereus* which was inhibited by the crude extracts of all the plants except those of *Leucas calostachys*. In contrast, *E. coli* was not sensitive to any of the tested plant extracts.

Traditional practitioners mostly utilized water as the main solvent for active metabolites from medicinal plants, but based on the results of this study, acetone extracts of almost all sampled plants showed the highest degree of antimicrobial activity followed by methanol then water extracts. Similar findings of water extracts having the least antimicrobial activity as compared to organic extracts had also been reported earlier (Allero and Afolayan, 2006). This may be due to better solubility of the active components in an organic solvent (de Boer *et al*., 2005) hence acetone stands as the most effective solvent for extraction of antimicrobial metabolites from the four selected medicinal plants. The better efficacy of acetone was further clearly supported by

MIC results in the study. Nevertheless, water acts as a better solvent for extraction of antimicrobial agents from *M. kummel*.

All the plant extracts tested showed significant antibacterial activity against most bacteria employed on this study, whereas *Candida albicans* was not sensitive to extracts of *L. calostachys* but high and broad spectrum antifungal activity was revealed by extracts of *M. kummel*, *A. lahai* and *C. decapetala*. Investigation of this study revealed that the sampled plants differ in their activities against the tested human pathogens. Results obtained clearly indicated that antibacterial and antifungal 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 (200 mg/ml).

Acacia lahai extracts showed significant antimicrobial activity against the tested pathogenic organisms except *E. coli*. Methanol extract of *A. lahai* exhibited the strongest inhibitory activity against *B. cereus* (15 mm) than any other extract tested on this study. This is in agreement with previous findings on methanol extracts of *Acacia nilotica* (Mahesh and Satish, 2008). Methanol extracts of *A. lahai* had a MIC value of 30 mg/ml on *B. cereus* which was almost similar to findings on *A. nilotica* (40 mg/ml) as documented by Banso, 2009. At lower concentrations, *A. lahai* extracts was more active against *P. aeruginosa* as shown by the least MIC value of 20 mg/ml when compared to other test microbes. Moreover, the extracts of *A. lahai* demonstrated the most potent antifungal activity against *C. albicans* in this study with acetone extract recording the highest zone of inhibition (13.17 mm). Antibacterial and antifungal results of *A. lahai* demonstrated a broad spectrum of activity as compared to other sampled plants in the study. Therefore, these strongly support the traditional utilization of the plant in Sotik Sub-

county in treatment of gastrointestinal problems and pneumonia among many other microbial diseases.

Traditional uses of *M. kummel* as a laxative and galactagogue (Lemmens, 2005), anthelmintic (Kone *et al.*, 2011) and other beneficial pharmacological properties of the genus Mimusops such as anti-microbial properties (Ali, 2008) was previously reported. In the present study, the crude extracts of *M. kummel* showed inhibitory activity against the entire tested microorganisms except *E. coli*. A higher zone of inhibition against *P. aeruginosa* by acetone extracts was recorded (12.86 mm) as demonstrated by Table 9 with a MIC value of 20 mg/ml. Aqueous extracts from *M. kummel* showed a better degree of zone of inhibition against the tested microbial strains as compared to aqueous extracts of other sampled plants. These showed that the plant could possess more polar secondary metabolites compared to other test plants. *M. kummel* demonstrated equal potential in antibacterial and antifungal properties as supported by the close range of inhibitory activity values.

Among all the tested plants, *L. calostachys* extracts were found to have moderate antibacterial activity and was only active against MRSA and *P. aeruginosa* only. Aqueous extracts of *L. calostachys* had no inhibitory effect on MRSA. The highest inhibitory activity was noted on its acetone extracts against *P. aeruginosa*. No inhibition was exhibited by all the extracts of *L. calostachys* on *B. cereus*, *E. coli* as well as *C. albicans*. Sensitivity of *L. calostachys* to MRSA was in line with activity of some other species of the same genus such as *L. zeylanica* which was found to exhibit potent inhibitory activity against *Staphylococcus aureus (*Valsara *et al.,* 1997*).* Antibacterial and antifungal activity of *L. calostachys* was reported for the first time in this study but the plant has been documented for treatment of antimicrobial diseases such as Wounds, dry cough, heartburns, cough, kidney problems, pneumonia, stomach-ache *(*Jeruto *et al.,* 2008).

Fresh leafs had been used to treat chest pain and stomach spasms (Iwu, 1993) and its juice and aqueous extracts in treatment of malaria (Muregi *et al.*, 2004).

Caelsalpinia decapetala extracts showed the least antibacterial and antifungal inhibitory activity among the plants used on this study with inhibition zone ranging from 8.8 mm-10.8 mm. Active secondary metabolites on this plant may be present in insufficient quantities in the crude extracts to show a remarkable activity with the dose levels employed. Acetone extracts of *C. decapetala* possessed better antimicrobial activity in contrast to aqueous and methanol extracts. Fruit extracts from *C. decapetala* have shown inhibitory effect against *C. albicans* (Kumar *et al.*, 2006) which was further supported by its methanol root extracts on the present study. Only methanol extracts of *C. decapetala* were active against *P. aeruginosa* and *C. albicans* while acetone and aqueous extracts showed no inhibition. *B. cereus* was the most susceptible organism to the extracts of *C. decapetala* as higher zones of inhibition was exhibited than on the rest of the microbes employed in the study. Another study has showed that flavonoids isolated from this plant had antioxidant activity (Xiao *et al.*, 2013).

When the selected crude plant extracts were tested for brine shrimp toxicity they were virtually non-toxic on Artemia larvae. Moreover, no mortality was found in the control groups used in the study. From the results, methanol extracts of *L. calostachys* was found to be the least toxic with lc_{50} of 9941 µg/ml while its acetone extracts exhibited the highest LC_{50} value of 1100 µg/ml. Similarly, all the other extracts exhibited very low toxicity, giving LC_{50} values greater than 1000 µg/ml. Although all the extracts were non-toxic to Artemia larvae, differences in LC_{50} values were observed in brine shrimp bioassay. These could be attributed to phylogenetic origins of the tested species which possess varying phytoconstituents. The non toxic effect of *L. calostachys* extracts compares to that of other Leucas species as a study using this model showed that, the

hydroalcoholic extract of *leucas aspera* whole plant exhibited cytotoxicity with LC_{50} of 1,900 µg/ml (krishnaraju *et al.*, 2005). Nguta *et al* 2011 report on toxicity of *acacia seyal* (LC_{50} =5915 µg/ml) was in a close range with that of *A. lahai* (4741 µg/ml) (Table 8). The brine shrimps toxicity of these selected plants was reported for the first time in this study.

It is worth noting that the impressive activity of most extracts against bacteria and *Candida albicans* and the exhibited non-toxicity effect on brine shrimps on this study, support inherent selectivity of the plant extracts for treatment of bacterial and fungal infections. Notable, the aqueous extracts, which in most cases are the ones used by traditional practitioners were also non-toxic on brine shrimps. The non-toxic effect of the screened plant extracts on *Artemia salina* indicates that the concentration of phytochemicals at the employed doses were safe hence justifying continued use of these plants on traditional medication. McLaughlin *et al.*, 1998 outlined that a plant with less toxicity and equally good concentration of these active phytochemicals makes it a good plant for use in traditional medicine.

Phytochemical screening of bioactive constituents showed that methanol extracts had more secondary metabolites, followed by acetone and water lastly. Variably distribution of phytochemicals resulted due to difference in volatility of the solvent used during extraction and plant species major constituents present. Flavonoids and alkaloids were present in all the tested organic crude extracts but flavonoids were absent in aqueous extracts. The higher number of phytochemicals in organic extracts on this study probably explained their comparatively better antimicrobial potential in comparison to aqueous extracts.

Phytochemical Screening of extracts of *A. lahai* revealed the presences of flavonoids in methanol and acetone extracts, alkaloids and saponins in methanol, acetone and aqueous extracts. Alkaloids and saponins phytoconstituents had earlier been documented in stem bark of Acacia

plants (Banso, 2009). Negative results were recorded for sesquertepene lactones in aqueous extracts of all plants and organic extracts of *A. lahai* and *C. decapetala* confirming the absence of this secondary metabolite. *A. lahai* aqueous extracts showed a negative test for flavonoids. Similar results for the absence of flavonoids in aqueous extracts of *A .lahai* had been recorded by other plants of genus Acacia for example *A .nilotica* (Banso, 2009).

Although *Caelsalpinia decapetala* had low antimicrobial activity, it was found to be richer in secondary metabolites such as flavonoids, alkaloids and saponins in all the extracts. Contrarily sesquiterpene lactones were not detected in any of the three extracts of *C. decapetala*. Presence of flavonoids in all the tested extracts agrees with previous chemical investigations on *C. decapetala* that had revealed that the main chemical components are flavonoids (Li *et al.*, 2004, Li *et al.*, 2002, Xiao *et al.*, 2013, Zhang *et al.*, 2008,). Zhang *et al.*, 2008 had reported presence of flavonoids on stem extracts of *C. decapetala* and alkaloids in leaves (Jiang *et al.*, 2001) which are in line with the results of the present study.

Phytochemical analyses of *M. kummel* revealed the presence of saponins, flavonoids, alkaloids and sesquiterpenes lactones in methanol extracts while saponins was absent in acetone extracts. Positive test of Saponins is consistent with the existing phytochemical knowledge ((Nigam *et al.*, 1992; Sahu *et al.*, 1995, Lavaud *et al.*, 1996; Sahu, 1996). Aqueous extracts revealed presence of alkaloids only and this might be due to less solubility of other active constituents in aqueous solutions. Nevertheless, aqueous extracts of *M. kummel* exhibited the highest antimicrobial activity as compared to aqueous extracts of other plants on this study. Misra *et al*, 1968 reported the presence of alkaloids and flavonoids on Mimusops which is in agreement with findings of the current study.

Leucas calostachys leaf extracts demonstrated availability of saponins, alkaloids, flavonoids and sequiterpene lactones in the organic extracts. However aqueous extracts shows the presences of alkaloids only. Jeruto *et al.,* 2008 had also reported similar phytochemical metabolites in *Leucas calostachys.* The presence of more secondary metabolism in *L. calostachys* strengthens its traditional usage to cure an array of antimicrobial infections like pneumonia and stomach-ache among others.

Sodipo *et al.*, 1991 have reported antifungal activity of saponins, but although saponins were present in methanol and acetone extracts of *L. calostachys* and acetone extract of *C. decapetala* they did not elicit antifungal activity against *C. albicans*. The contrary results could be attributed to the tested concentration of the extracts which may have been very little to exert a measurable inhibitory activity against *C. albicans.*

5.2. Conclusion

Bioactivity of methanol, acetone and aqueous extracts of *Leucas calostachys, Mimusops kummel, Acacia lahai* and *Caesalpinia decapetala* on bacterial and fungal strains was remarkable but none of them exhibited antimicrobial activity similar to positive controls. Lower activity of crude extracts as compared to positive controls could be due to presences of antimicrobial metabolites in lower concentration. Generally, alkaloids, saponins and flavonoids tested were present in all the four plant species while sesquiterpene lactones tested positive in *L. calostachys* and *M. kummel.* It is also evident from the study that none of the evaluated crude extracts was toxic to *Artemia Salina*, ascertaining value to the selected medicinal plants for continued use in ethno medicine. Among the studied plant extracts, methanol extracts of *A. lahai* had an overriding efficacy over other plant extracts. From this study *A. lahai* 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 *Leucas calostachys, Mimusops kummel, Acacia lahai* and *Caesalpinia decapetala.*

5.3. Recommendation

This study evaluated some antimicrobial activity, phytochemical composition and acute toxicity of crude plant extracts hence it is worth recommending isolation, purification and structure elucidations. Antimicrobial investigations of isolated metabolites and further evaluation for *in vivo* toxicity can be carried out from the most active crude extract. Nature has demonstrated to be a good source of lead compounds for treatment of microbial infections but only a small proportion of lead compounds have been extracted from plants and evaluated against pathogens of medicinal importance. The current study recommends further evaluation of antimicrobial activity and safety of isolated compounds from those crude extracts found to be active in an attempt to search for optimized lead compounds with antimicrobial activity.

REFERENCES

Adebolu, T.T. and S.A. Oladimeji, 2005. Antimicrobial activity of leaf extracts of *Ocimum gratissimum* on selected diarrhoea causing bacteria in Southwestern Nigeria. Afr. J. Biotechnol., 4: 682-684.

Adenisa SK, Idowu O, Ogundaini AO, Oladimeji H, Olugbade TA, Onawunmi GO, Pais M, 2000. Antimicrobial constituents of the leaves of *Acalypha wilkesiana* and *Acalypha hispida*. *Phytother* Res 14: 371-374.

Ali MA, Abdul MM, Yeasmin SM, Khan AM, Sayeed MA,2008. An evaluation of antimicrobial activities of *Mimusops elengi* Linn.Res. J. Agric. Biol. Sci 4: 871-874.

Allero AA, Afolayan AJ, 2006. Antimicrobial activity of *Solanum tomentosum*. Afr. J. Biotechnol. 5: 369-372.

Al-Momani, W., E. Abu-Basha, S. Janakat, R.A. Nicholas and R.D. Ayling, 2007. *In vitro* antimycoplasmal activity of six Jordanian medicinal plants against three Mycoplasma species Trop. Anim. Health Prod., 39: 515-519.

Aoki, S., Hirakata, Y., Kondoh, A., Gotoh, N., Yanagihara, K., Miyazaki, Y.,Tomono, K., Yamada, Y., Kohno, S., Kamihira, S., 2004. Virulence of metallo-β-lactamase-producing Pseudomonas aeruginosa in vitro and in vivo. Antimicrobial Agents and Chemotherapy 48, 1876–1878.

Ashafa, A. O. T., Orekoya,L.O., Yakubu, M.T., 2012. Toxicity profile of ethanolic extract of *Azadirachta indica* stem bark in male Wistar rats. Asian Pacific Journal of Tropical Biomedicine, 813-817.

Awodele, O., Agbaje, E.O., Ogunkeye, F.A., Kolapo, A.G. and Awodele, D.F., 2011. Towards integrating traditional medicine (TM) into National Health care Scheme (NHCS): Assessment of TM practitioners' disposition in Lagos, Nigeria. Journal of Herbal Medicine, Vol. 1, No. (3-4), pp. 90-94.

Bandow JE, Brotz H, Leichert LIO, Labischinski H, Hecker M, 2003. Proteomic approach to understanding antibiotic action. Antimicro. Agents. Chemotherap. 47: 948-955.

Banso A, 2009. Phytochemical and antibacterial investigation of bark extracts of *Acacia nilotica*. J. Med. Plants Res., 3: 082-085.

Betoni JEC, Mantovani RP, Barbosa LN, Di-Stasi LC, Fernandes A, 2006. Synergism between plant extract and antimicrobial drugs used on *Staphylococcus aureus* diseases. Mem. Inst.

Biesen. C. M. and Dilger. H. (2012). Bridging gaps in health care and healing: Traditional medicine and biomedicine health care sector in Zanzibar. Project report. Freire Universitat B elrin.

Bisignano, G., R. Sanogo, A. Marino, R. Aquino and V. D'Angelo, 2000. Antimicrobial activity of *Mitracarpus scaber* extract and isolated constituents. Lett. Applied Microbiol., 30: 105-108.

Boer. H.J., Koll. A. and Broberg, A. (2005). Anti-fungal and anti-bacterial activity of some herbal remedies from Tanzania. Journal of Ethnopharmacology, Vol. 96. Pp. 461-469.

Bouzada, M.L.M., R.L. Fabri, M. Nogueira, T.U.P. Konno, G.G. Duarte and E. Scio, 2009. Antibacterial, cytotoxic and phytochemical screening of some traditional medicinal plants in Brazil. Pharm. Biol., 47: 44-52.

Chakraborty, A. and A.H. Brantner, 1999. Antibacterial steroid alkaloids from the stem bark of *Holarrhena pubescens*. J. Ethnopharmacol., 68: 339-344.

Ceuppens, S., Rajkovic, A., Heyndrickx, M., Tsilia, V., Van de Wiele, T., Boon, N., Uyttendaele, M., 2011. Regulation of toxin production by Bacillus cereus and its food safety implications. Critical Reviews in Microbiology 37, 188–213.

Chirchir, J., Mungai, G. and Kariuki, P., 2006. Indigenous knowledge and conservation of natural resources: Resource medicinal plants utilisation in Eastern Africa. Proceedings of national museums of Kenya first scientific conference. pp. 106-111.

Clinical and Laboratory Standards Institute (CLSI), 2008. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts: Approved Standard M27-A3, NCCLS, Wayne, PA.

Clinical and Laboratory Standards Institute (CLSI), 2009. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically: Approved Standard, eighth ed. M07-A8. CLSI, Wayne, PA.

Colombo ML, Bosisio E, 1996. Pharmacological activities of *Chelidonium majus* L (Papaveraceae). Pharmacol. Res. 33: 127-137.

Cowan MM, 1999. Plant Products as Antimicrobial Agents. Clin. Microbiol Rev 12(4): 564-582.

Croxen, M.A., Finlay, B.B., 2010. Molecular mechanisms of Escherichia coli pathogenicity. Nature Reviews. Microbiology 8, 26–38.epidemiological cohorts. AIDS 1991;5:519—25.

De Boer, H.J.,Kool,A.,Broberg,A.,Mziray,W.R.,Hedberg,I.,Levenfors,J.J., 2005. Anti-fungal and anti-bacterial activity of some herbal remedies from Tanzania. Journal of Ethnopharmacology96, 461–469.

Dixon R. A., Dey P. M., Lamb C. J., *1983* Phytoalexins: enzymology and molecular biology. Adv. Enzymol. 55:1–69.

Edeoga H.O., D.E. Okwu and B.O Mbaebie, 2005. Phytochemical constituents of some Nigerian medicinal plants. African Journal of Biotechnology vol.4 (7), pp 685-688.

El-Astal, Z.Y., A.E.R.A. Ashour and A.A.M. Kerrit, 2005. Antimicrobial activity of some medicinal plant extracts in Palestine. Pak. J. Med. Sci., 21: 187-193.

Eli-Seedi HR, Ohara T, Sata N, Nishiyama S, 2002. Antimicrobial terpenoids from *Eupatorium glutinosum* (Asteraceae). J. Ethnopharmacol . 81: 293-296.

Feigal DW, Katz MH, Greenspan D, Westenhouse J, Winkelstein W, Lang W, *et al.*, 1991. The prevalence of oral lesions in HIVinfected homosexuals and bisexual men.

Fennell, C.W., Lindsey, K.L., McGaw, L.J., Sparg, S.G., Stafford, G.I., Elgorashi, E.E., Grace, O.M., Van Staden, J., 2004. Assessing African medicinal plants for efficacy and safety: pharmacological screening and toxicology. Journal of Ethnopharmacolog94,205-217

Ferraro, M.J., 2003. Performance Standards for Antimicrobial Disk Susceptibility Tests: Approved Standard. 8th Edn., National Committee for Clinical Laboratory Standards (NCCLS), Wayne, PA., USA., ISBN-13: 9781562384852, Pages: 34

Finney D.J., 1971. Probit analysis, 3rd Ed, Cambridge University press.

Fujioka T., Kashiwada Y., 1994. Anti-AIDS agents. 11. Betulinic acid and platanic acid as anti-HIV principles from *Syzigium claviflorum*, and the anti-HIV activity of structurally related triterpenoids. *J. Nat. Prod.* 57:243–247.

Ganu GP, Jadhav SS, Deshpande AD, 2010. Antioxidant and Antihyperglycemic potential of methanolic extract of bark of *Mimusops elengi* in mice. Res. J. Pharm. Biol. Chem. Sci 1:67-77.

Ghoshal S., Krishna Prasad B. N., Lakshmi V., 1996. *Antiamoebic activity of Piper longum* fruits against *Entamoeba histolytica in vitro and in vivo. J. Ethnopharmacol. 50:167–170.*

Goutam KJ, Dhanamjayarao M, Vani M, 2010. Evaluation of Anthelmintic Potential of *Mimusops elengi* Linn. (sapotaceae) leaf. J. Pharm. Res 3: 2514-2515.

Guha Bakshi D.N., Sensarma P., Pal D.C.,1999. A Lexicon of Medicinal Plants in India, Vol.I, Naya Prokash Publisher, Calcutta.

Gupta MP, Solis PN, Calderon AI, Guionneau-Sinclair F, Correa M, Galdames C, Guerra C, Espinosa A, Alvenda GI, Robles G, Ocampo R, 2005. Medical ethnobotany of the Teribes of Bocas del Toro, Panama. *Journal of Ethnopharmacology* 96:389-401.

Harborne, J.B., 2002. *Phytochemical Methods, A Guide to modern techniques of plant analysi* (3rd ed.) Chapman & Hall London.

Haslam E., 1996. Natural polyphenols *(vegetable tannins)* as drugs: possible modes of action. *J. Nat.* Prod. *59:205–21.*

Hazen KC, 1995. New and emerging yeast pathogens. Clin Microbiol Rev 8:462—78.

Hossain MS, Hossain MA, Islam R, 2004. Antimicrobial and cytotoxic activities of 2-aminobenzoic acid and 2-aminophenol and their coordination complexes with magnesium (Mg-II). Pak J Biol Sci 71: 25-27.

Hubbard CE and Milne-Redhead MA., 1959. Flora of Tropical East Africa, Leguminosae subfamily Mimosoideae. Crown Agents, London.

Islam MA, Sayeed MA, Islam MA, 2002. Terpenes from bark of *Zanthoxylum budrunga* and their cytotoxic activities. Rev Latinoamer Quím 30: 24-28.

Iwu MM, 1993. Handbook of African Medicinal Plants. CRC Press, Florida, USA, p. 39.

J igna, P., and Chanda, S.V., 2007. In vitro Antimicrobial Activity and Phytochemical Analysis of Some Indian Medicinal Plants. Turkey Journal of Biology, 3: 53-58. 8.

Janovska, D., Kubikova, K. and Kokoska, L., 2003. Screening for antimicrobial activity of some medicinal plant species of traditional Chinese medicine. Czech. J. Food Sci. 21: 107, 111.

Jeruto, P., Lukhoba, C., Ouma, G., Mutai, C. and Otieno, D., 2008. Herbal treatments in Aldai and Kaptumo Divisions in Nandi District, Rift Valley Province, Kenya. African Journal of traditional, Complimentary and Alternative medicine. African Ethnomedicine

Jiang, R. –W. S.-C. Ma, P. P.-H. But, T. C. W. Mak, J., 2001. Nat. Prod. 64, 1266.

Johnson J, Kuskowski M, Menard M, Gajewski A, Xercavins M, Garau J, 2006. "Similarity between human and chicken Escherichia coli isolates in relation to ciprofloxacin resistance status". *J Infect Dis* 194 (1): 71–8.

Kanafani, Z. A., and Fowler Jr.V.G., 2006. *Staphylococcus aureus* Infections: New Challenges from an Old Pathogen. Infecc Microbiol Clin 24,182-193.

Kareru G, Kenji M, Gachanja N, Keriko M, Mungai G, 2007. Traditional medicines among the Embu and Mbeere peoples of Kenya. *Afr J Trad CAM 4*: 75-86.

Karem, Z., German-Shashoua, H. and Yarden, O., 2005. Microwave-assisted extraction of bioactive saponins from chickpea (*Cicer arietinum* L.). *Journal of the Science of Food and Agriculture,* Vol. 85, pp. 406–412

Karlowsky, J.A., Draghi, D.C., Jones, M.E., Thornsberry, C., Friedland, I.R., Sahm, D.F., 2003. Surveillance for antimicrobial susceptibility among clinical isolates of *Pseudomonas aeruginosa* and Acinetobacter baumannii from hospitalized patients in the United States, 1998 to 2001. Antimicrobial Agents and Chemotherapy 47, 1681–1688 .

Kiem P.V., Minh C.V., Huong H.T., Lee J.L., Kim Y.H., Asaldecan; Kirtikar K.R., Basu B.D.,1984. Ind Med Plants. p.850.

Kirtikar KR, Basu BD, 1990. Indian medicinal plants. Bidtter, E., Caius J.F., and Mhaskar K.S. (Ed). Periodical Experts Book Company, p.2019.

Kokwaro, J.O., 1993. *Medicinal Plants of East Africa* (2nd ed.) Kenya Literature Bureau,Nairobi.

Kokwaro, J.O., 2009. *Medicinal plants of East Africa* (3rd ed.) University of Nairobi Press, Nairobi.

Kone, W.M., Kamanzi Atindehou, K., Terreaux, C., Hostettmann, K., Traore, D., Dosso, M., 2004. Traditional medicine in North Cote-d'Ivoire screening of 50 medicinal plants for antibacterial activity. J.Ethnopharmacol. 93: 43-49.

Kone´ WM, Vargas M, Keiser J., 2011. Anthelmintic activity of medicinal plants used in Coˆ te d'Ivoire for treating parasitic diseases. Parasitol Res 110(6):2351-62.

Korir R, K, C. Mutai, C. Kiiyukia and C. Bii, 2012. Antimicrobial Activity and Safety of two Medicinal Plants traditionally used in Bomet District of Kenya. *Research Journal of Medicinal Plant, 6: 370-382.*

Kortiranta, A., Lounatmaa, K., and Haapasalo,M.: Epidemiology and pathogenesis of

Krishnaraju AV, Raos TVN, Sundararaju D, 2005. Assessment of bioactivity of Indian medicinal plants using Brine shrimp (Artemia salina) lethality assay. Int J Appl Sci Eng 2: 125-134.

Krishnaraju AV, Rao TVN, Sundararaju D, Vanisree M, Tsay HS, Subbaraju GV, 2005. Assessment of bioactivity of Indian medicinal plants using brine shrimp *(Artemia salina)* lethality assay. Int. J. Appl. Sci. Eng., 3: 125-134.

Kumar VP, Chauban NS, Padu H, Rajani M, 2006 search for antibacterial and antifungal agents from selected Indian medicinal plants.Journal of ethnophaymacological 107,182-188.

Lavaud, C., Massiot, G., Becchi, M., Misra, G., Nigam, S.K., 1996. Saponins from three species of Mimusops. Phytochemistry 41, 887–893.

Lemmens, R.H.M.J., 2005. *Mimusops kummel* Bruce ex A.DC. In: Louppe, D., Oteng-Amoako, A.A. & Brink, M. (Editors). Prota 7(1): Timbers/Bois d'œuvre 1. [CD-Rom].

Lewis K, Ausubel FM, 2006. Prospects for plant-derived antibacterials. Nat. Biotechnol. 24(12): 1504-1507.

Li, X.Z., Zhang, L., Poole, K., 2000. Interplay between the MexA–MexB–OprM multidrug efflux system and the outer membrane barrier in the multiple antibiotic resistance of *Pseudomonas aeruginosa*. Journal of Antimicrobial Chemotherapy 45, 433–436.

Li, M.X.; Zhang, C.Z.; Li, C., 2002. Studies on chemical constituents of Caesalpinia decapetala (Roth) Alston. Zhong Yao Cai 25, 794–795. 4.

Li, M.X.; Zhang, C.Z.; Li, C., 2004. Studies on chemical constituents of Caesalpinia decapetala(Roth) Alston (II). Zhong Yao Cai 35, 741–742. 5.

Mahesh B. and Satish S., *2008:* Antimicrobial Activity of Some Important Medicinal Plant Against Plant and Human Pathogens. World Journal of Agricultural Sciences 4 (S):839 -843,

Mangathayaru K, Lakshmikant J, Sundar NS, Swapna R, Grace XF, Vasantha J., 2005. Antimicrobial activity of *Leucas aspera* flowers. Fitoterapia. 76:752–4.

Manna, A., Abalaka, M.E., 2000. Preliminary screening of the various extracts of *Physalis angulata* (L.) for antimicrobial activities. Spectrum J. 7: 19-125.

Marchese A., Gualco L., Debbia E.A., Bandettini R., Pescetto L., Cavallero A., Ossi M.C., Schito A.M., 2007. Antifungal resistance in Candida spp. isolated in Italy between 2002 and 2005 from children and adults. International Journal of Antimicrobial Agents 29, 179–184.

McLaughlin, J.L., Lingling, L.R., and Anderson, J. E., 1998. The use of biological assays to evaluate botanicals. *Drug Information Journal,* Vol. 32, pp. 513–524. New York, USA.

Miaron OJ, Kassim O, Ekaya, 2004. Indigenous knowledge: the basis of the Maasai Ethnoveterinary Diagnostic Skills. *J Hum Ecol 16*: 43-48.

Michael, J.P., E.C. Chan, R.K. Noel and F.P. Merna, 2003. Microbiology. 5th Edn., Tata McGraw-Hill, New Delhi, India, pp: 627-748.Micro. 38: 204-207.

Miller, J. M., Hair, J. G., Hebert, M., Heber, L., Robert, F. J., and Weyant Jr., R. S., Jr.: Miller, J. M., Hair, J. G., Hebert, M., Heber, L., Robert, F. J., and Weyant Jr., R. S., Jr., 1997. Fulminating bacteremia and pneumonia due to *Bacillus cereus*, J. Clin. Microbiol.,35, 504–507.

Misra G, Mitra CR, 1968. Constituents of leaves, hardwood and root of *Mimusops elengi*. Phytochemsitry 7: 501-502.

Mubarak, S., Robert, A.A., Baskaradoss, J.K., Al-Zoman, K., Al Sohail, A., Alsuwyed, A., Ciancio., 2013. The prevalence of oral *Candida* infections in periodontitis patients with type 2 diabetes mellitus. Journal of Infection and Public Health,article in press.

Muregi FW, Chhabra SC, Njagi ENM, Lang'at-Thoruwa CC, Njue WM, Orago ASS, Omar SA, Ndiege IO, 2004. Anti-plasmodial activity of some Kenyan medicinal plant extracts singly and in combination with chloroquine. Phytother. Res., 18: 379-384.

Musila, M.F., Dossaji, S.F., Nguta, J.M., Lukhoba, C.W. and Munyao, J.M., 2013. *In vivo* antimalarial activity, toxicity and phytochemical screening of selected antimalarial plants. *Journal of Ethnopharmacology,* Vol. 146, pp. 557-561.

Nair, R., Kalariya, T. and Sumitra, C., 2005. Antibacterial Activity of Some Selected Indian Medicinal Flora. Turk J Biol, pp. 29, 41, 47.

Nguta, J.M. and Mbaria, J.M., 2013. Brine shrimp toxicity and antimalarial activity of some plants traditionally used in treatment of malaria in Msambweni district of Kenya. Journal of Ethnopharmacology 148 (2013) 988–992.

Nguta, J.M., Mbaria, J.M., Gakuya, D.W.,Gathumbi, P.K., Kabasa, J.D. and Kiama, S.G., 2011. Biological Screening of Kenya Medical plants using Artemia salina L. (Artemiidae). Pharmacology online, Vol.2, pp. 458-478.

Nguta, J.M, Mbaria, J.M, Mvula, D.W., 2013. Brine shrimp toxicity and in *vitro* antimicrobial activity of *Piliostigma thonningii* (Schum.) Milne-Redh. [Leguminosae-Caesalpinioideae] from

Kenya and Malawi against some pathogens of human and veterinary importance vol.5 (9), pp. 251-256.

Nigam, S.K., Li, X., Wang, D., Misra, G., Yang, C., 1992. Triterpenoidal saponins from Madhuca butyracea. Phytochemistry 31, 3169–3172.

Njoroge GN, Bussmann RW, 2007. Ethnotherapeautic management of skin diseases among the Kikuyus of Central Kenya. *J Ethnopharmacol 111*: 303-307.

Nunes, B.S., Carvalho, F. D., Guilhermino, L.M. and Stappen, G.V., 2006. Use of the genus *Artemia* in ecotoxicity testing. *Environmental Pollution*. Vol. 144, pp. 453-462.

Oreagba, I.A., Oshikoya, K.A. and Amachree, M., 2011) Herbal medicine use among urban residents in Lagos Nigeria, *BMC Complementary and Alternative medicine*, Vol. 11, No. 117.

Orwa C, A Mutua, Kindt R , Jamnadass R, S Anthony. 2009. Agroforestree Database:a tree reference and selection guide version4.0 (http://www.worldagroforestry.org/sites/treedbs/treedatabases.asp) p.336.

Payal JS, Mitesh SG, Mamta BS, Sunita SG, Devdas S., 2003. Study of *Mimusops elengi* bark in experimental gastric ulcers. J. Ethnopharmacol 89: 305–311.

Poole, K., 2004. "Efflux-mediated multiresistance in Gram-negative bacteria". *Clinical Microbiology and Infection* 10 (1): 12–26

Rahman, S., R.B. Blok, H.H. Dahl, D.M. Danks and D.M. Kirby *et al.*, 1996. Leigh syndrome: Clinical features and biochemical and DNA abnormalities. Ann. Neurol., 39: 343-351.arm. Sci 2010; 72: 480-485.

Rana B. K., Singh U. P., Taneja V., 1997. Antifungal activity and kinetics of inhibition by essential oil isolated from leaves of *Aegle marmelos.* J. Ethnopharmacol. 57:29–34.

Romero, C.D., Chopin, S.F., Buck, G., Martinez, E., Garcia, M., Bixby, L., 2005. Antibacterial properties of common herbal remedies of the southwest. J.

Saha K, Mukherjee PK, Mandal SC, Saha BP, Pal M., 1997b. Antiinflammatory evaluation of *Leucas lavandulaefolia* Rees. Extract. Nat. Prod. Sci., 2: 119-122.

Sahu, N.P., 1996. Triterpenoid saponins of Mimusops elengi. Phytochemistry 41, 883–886.

Sahu, N.P., Koike, K., Jia, Z., Nikaido, T., 1995. Novel triterpenoid saponins from Mimusops elengi. Tetrahedron 51, 13435–13446.

Salyers AA, Gupta A, Wang Y., 2004. "Human intestinal bacteria as reservoirs for antibiotic resistance genes". *Trends Microbiol.* 12 (9): 412–6.

Sandhu DS, Heinrich M., 2005. The use of health foods, spices and other botanicals in the Sikh community in London. *Phytotherapy Research* 19:633-42.

Sasikumar, J.M., Thayumanavan, T., Subashkumar, R., Janardhanan, K., & Lakshmanaperumalsamy, P., 2007. Antibacterial activity of some ethnomedicinal plants from the Nilgiris, Tamil Nadu, India. Natural Product Radiance, 6 (1): 34-39. 26.

Scazzocchio F, Comets MF, Tomassini L, Palmery M., 2001. Antibacterial activity of *Hydrastis canadensis* extract and it's major isolated alkaloids. Planta Med. 67: 561-563.

Setzer, W.N., M.C. Setzer, R.B. Bates and B.R. Jakes, 2000. Biologically active triterpenoid of *Syncarpia glomulifera* bark extract from Paluma, North Queensland, Australia. Planta Med , 66: 176-177.

Sohail, M.N., A. Karim, M. Sarwar and A.M. Alhasin, 2011. Onion (*Allium cepa* L.): An alternate medicine for Pakistani population. Int. J. Pharmacol., 7: 736-744.

Sodipo OA, Akanji MA, Kolawole FB, Odutuga AA., 1991. Saponin is the active antifungal principle in Garcinia kola, heckle seed, Biosci. Res. Commun., 3: 171.

Sorgeloos, P., Wielen, C.R and Persoone, G., 1978. The use of Artemia *Nauplii* for Toxicity tests- A critical analysis. *Ecotoxicology and Environmental safety*, Vol. 2, pp. 249-255.

Stapleton PD, Shah S, Anderson JC Hara Y, Hamilton-Miller JMT, Taylor PW., 2004. Modulation of _-lactam resistance in *Staphylococcus aureus* by catechins and gallates. Int. J. Antimicrob. Agents. 23(5): 462-467.

Subbaraju, G.V., Krishnarajua, A.V., Tayi, V.N.R., Sundararajua, D., Hsin-Sheng, T. and Vanisreeb, M., 2005. Assessment of Bioactivity of Indian Medicinal Plants Using Brine Shrimp (Artemia salina) Lethality Assay. *International Journal of Engineering and Applied Sciences*, Vol. 3, No. 2, pp. 125-134. India.

Sultana B, Farooq A and Ashraf M., 2009. Effect of ExtractionSolvent/Technique on the Antioxidant Activity of Selected Medicinal Plant Extracts. Molecules 14:2167-2180.

Valsaraj R, Pushpangadan P, Smitt UW, Adsersen A, Nyman U., 1997. Antimicrobial screening of selected medicinal plants from India. J Ethnopharmacol. 58:75–83.

Wagner, W.L.; Herbst, D.R.; Sohmer, S.H., 1999. Manual of the Flowering Plants of Hawaii; Bishop Museum Special Publication: New York, NY, USA, Volume 2, p. 647. 2.

Waksmundzka, H.M., SheFrma, J. and Kowalska, T., 2008. Thin Layer Chromatography in Phytochemistry. *Chromatography Science Series,* Vol. 99. CRC Press

Wanyoike, G.N., Chabra, S.C., Lang'at, C.C., Omar, S.A., 2004. Brine shrimp toxicity and antiplasmodial activity of five Kenyan medicinal plants, Journal of Ethnopharmacology 90, 129–133.

WHO, 2002. WHO Monographs on Selected Medicinal Plants. Vol. 2, World Health Organization, Geneva, Switzerland, Pages: 246.

WHO., 2002b. Traditional medicine; growing needs and potential. WHO Policy perspectives on medicines. Geneva 1-6.

Xiao-Hua Wei, Sheng-Jie Yang, Na Liang, De-Yu Hu, Lin-Hong Jin, Wei Xue, Song Yang., 2013. Chemical Constituents of Caesalpinia decapetala (Roth) Alston 18, 1325-1336.

Zhang, Q.; Liu, X.T.; Liang, J.Y.; Min, Z.D., 2008. Chemical constituents from the stems of Caesalpinia decapetala. Chin. J. Nat. Med. 6, 168–172. 3.

APPENDICE

Toxicity of the test extracts on Brine shrimp

Leucas calostachys					
solvent	concentration	mortality			
		Trial 1	Trial 2	Trial 3	Average
Water	1000	3	4	3	3.33
	100	3	2	0	1.67
	10	0	0	0	0.00
	0	0	0	0	0.00
	1000	2	3	3	2.67
Methanol	100	2	0	3	1.67
	10	0	0	1	0.33
	0	0	0	0	0.00
Acetone	1000	3	2	2	2.33
	100	0	1	3	1.33
	10	0	0	0	0.00
	0	0	0	0	0.00
Mimusops kummel					
Water	1000	7	4	6	5.67
	100	4	3	4	3.67
	10	3	1	2	2.00
	0	0	0	0	0.00
Methanol	1000	4	2	4	3.33
	100	2	2	1	1.67
	10	0	1	1	0.67
	0	0	0	0	0.00
Acetone	1000	3	4	4	3.67
	100	2	4	3	3.00
	10	2	1	1	1.33
	0	0	0	0	0.00
Acacia lahai					
Water	1000	5	3	3	3.67
	100	1	2	1	1.33
	10	0	2	0	0.67
	0	0	0	0	0.00
Methanol	1000	2	2	1	1.67
	100	1	2	1	1.33

	10	0	0	0	0.00
	0	0	0	0	0.00
Acetone	1000	1	1	2	1.33
	100	0	1	0	0.33
	10	0	0	0	0.00
	0	0	0	0	0.00
Caesalpinia decapetala					
Water	1000	4	2	4	3.33
	100	2	2	3	2.33
	10	0	1	0	0.33
	0	0	0	0	0.00
Methanol	1000	3	3	2	2.67
	100	2	1	2	1.67
	10	2	0	0	0.67
	0	0	0	0	0.00
Acetone	1000	3	5	2	3.33
	100	2	2	1	1.67
	10	2	0	2	1.33
	0	0	0	0	0.00