**KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY,**

**KUMASI, GHANA**


# Antibacterial Activities of some Ghanaian Medicinal Plants against Methicillin Resistant *Staphylococcus aureus* (MRSA) and Methicillin Sensitive *Staphylococcus aureus* (MSSA)

**By**

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**A thesis submitted to the Department of Biochemistry and Biotechnology,**

**College of Science**

**in partial fulfillment of the requirements for the award of**

**MASTER OF SCIENCE DEGREE IN BIOTECHNOLOGY**

JUNE, 2016

## DECLARATION

It is hereby declared that the report of this study is original and was carried out by me and supervised by Dr. F. C. Mills-Robertson. Studies from other authors cited have been duly acknowledged. This work has not been concurrently submitted in candidature for any degree.



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**ABSTRACT**

Plants are noted to have nutritional properties as well as a reservoir for potential drug production and have been part of phytomedicine for many years. Several reports on the antimicrobial activity of different herbs have been documented. Synthetic antibiotics have been the main therapy for resistant bacterial infections; however, the misuse and overuse of antibiotics have become the main factor for the emergence of drug resistant strains of several groups of microorganisms, hence the need to investigate the antimicrobial properties of medicinal plants. The antibacterial activities of the selected plant leaf extracts of various concentrations (100, 150, 200 mg/ml) were tested against clinical isolates of Methicillin Resistant *Staphylococcus aureus* (MRSA) and Methicillin Sensitive *Staphylococcus aureus* (MSSA) as well as seven standard strains of bacteria (*K. pneumoniae* ATCC 33495**,** *Proteus mirabilis* ATCC 49565**,** *S. saprophyticus* ATCC 15305**,** *Salmonella typhi* ATCC 19430**,** *S. aureus* ATCC 25923**,** *E. coli* ATTC 25922 and *P. aeruginosa* ATCC 27853) using modified agar diffusion and minimum inhibitory concentration (MIC) methods. Phytochemical analyses identified compounds such as alkaloids, flavonoids, saponins, tannins, phenols, cardiac glycosides, and terpenoids. *Alchornea cordifolia* gave the highest inhibitory effect ($P < 0.0011$) followed by *Psidium guajava* ($P < 0.0142$) against MRSA whilst the rest of the plants showed no inhibitory effect. *Psidium guajava*, *Alchornea cordifolia, Phyllanthus fraternus, Cnestis ferruginea* and *Hoslundia opposita* had significant effect on the MSSA and the standard bacterial strains ($P < 0.05$). Both leaf extracts of *Alchornea cordifolia* and *Psidium guajava* were highly effective against the MRSA strains with MIC value of 0.195 mg/ml. Generally, the ethanol extracts of the plants had higher antibacterial activity than aqueous extracts. All the standard bacteria strains were susceptible to the selected medicinal plants with the exception of *K. pneumoniae* which was not susceptible even to *Alchornea cordifolia* and *Psidium guajava*. Amongst the seven different plants, *Alchornea cordifolia* and *Psidium guajava* inhibited the growth of resistant isolates of MRSA, and therefore, can be good sources of antimicrobial production which could significantly inhibit the growth of resistant bacteria such as the MRSA.

## DEDICATION

This work is dedicated to the Almighty Allah and my inspiring late Mum, Hajia Afiriwa Alhassan Dantankwa, who gave me life and was my first teacher.

## ACKNOWLEDGEMENT

All praises and thanks go to Almighty Allah, Lord of all, who strengthened and endowed me with knowledge and understanding throughout my course of study. I am with total conviction that His love and blessings enabled me to complete this work. I would like to express my profound gratitude to my supervisor Dr. F. C. Mills-Robertson for his advice, guidance and motivation towards my research and thesis writing. I am thankful to my uncle Ishaq Muhammad of Sweden for his financial assistance.

I am grateful to the staff of the Clinical Analysis laboratory (CAn Lab) of the Department of Biochemistry and Biotechnology, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi as well as staff of the Microbiology Department, Centre for Plant Medicine Research (CPMR), Akuapem-Mampong , for their contributions towards the research work. I am thankful to the staff of Microbiology Unit, Central Laboratory, Korle-Bu Teaching Hospital for their technical and material support during this study.

Special thanks go to the staff of the Department of Biochemistry and Biotechnology, (KNUST) for their contributions and advice towards the research work. I also acknowledge the technical and practical assistance rendered towards this study by Research Assistants; Redeemer Agbolegbe, Bernard Totime, and Chris Yaw Asare at the Microbiology Research Laboratory of the Department of Biochemistry and Biotechnology (KNUST). My sincere gratitude goes to Dr. G. H. Sam, of the Department of Herbal Medicine (KNUST) for his contribution towards the research work. I am also thankful to Dr. A.Y. Tetteh of Department of Biochemistry and Biotechnology for her advice and support offered to me before and during the course of my study. Finally, I am indebted to my friends Richard Gyamfi, Jerry Ampofo Asiama, and Kwasi Ofori Effah for their encouragement towards my master‟s programme.

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| AMP | Ampicillin (10 µg/disc) |
| ATCC | American Type Culture Collection |
| BP | British Pharmacopoeia |
| CHL | Chloramphenicol (30 µg/disc) |
| CLED | Cystine Lactose Electrolyte Deficient |
| CLSI | Clinical and Laboratory Standards Institution |
| COT | Cotrimoxazole (25 µg/disc) |
| CPMR | Centre for Plant Medicine Research |
| CTX | Cefotaxime (30 µg/disc) |
| CRX | Cefuroxime(30 µg/disc) |
| CXC | Cloxacillin (5 µg/disc) |
| CXM | Cefixime (30 µg/disc) |
| DCA | Deoxycholate Citrate Agar |
| DMSO | Dimethyl sulfoxide |
| ERY | Erythromycin(15 µg/disc) |
| ESBL | Extended Spectrum Beta-Lactamases |
| GEN | Gentamicin (10 µg/disc) |
| INT | Iodonitrotetrazolium Chloride |
| KBTH | Korle Bu Teaching Hospital |
| LF | Lactose Fermenters |
| MDR | Multidrug Resistance |
| MHA | Mueller-Hinton Agar |
| MHB | Mueller-Hinton Broth |
| MIC | Minimum Inhibitory Concentration |
| MRSA | Methicillin Resistant *Staphylococcus aureus* |
| MSSA | Methicillin Susceptible *Staphylococcus aureus* |
| NCCLS | National Committee for Clinical Laboratory Standards |
| NLF | Non-Lactose Fermenters |
| PEN | Penicillin (10 µg/disc) |
| PBP2' | Penicillin-Binding Protein 2' |
| TET | Tetracycline (30 µg/disc) |
| WHO | World Health Organization |

# CHAPTER ONE

## 1.0. INTRODUCTION

## 1.1. BACKGROUND OF THE STUDY

Infectious diseases have been troubling mankind even before civilization and they are believed to be present from the very existence of man. The control of infectious diseases has encountered many problems, typical amongst them being the rise in the number of microorganisms that are resistant to antimicrobial agents. Thus, resistances are observed in cases of infections caused by some microorganisms; hence, the failure to respond to conventional treatment. The primary cause of antibiotic resistance is mainly genetic mutation in bacteria with others including abuse of antimicrobial drugs. These problems provide conducive atmosphere for the growth, spread, emergence and persistence of resistant microorganisms. Typical illustration of such resistant microorganism is Methicillin Resistant *Staphylococcus aureus* (MRSA), a very infectious bacterium ordinarily not easy to treat or manage. Over the years, it has developed resistance to beta-lactam antibiotics. MRSA causes nosocomial infection usually associated with overcrowding community or environment such as schools and hospitals. It can flourish in the nearness penicilin-like anti-infection agents, which regularly avoid bacterial development by restraining cell wall material synthesis. The resistance might be due to a resistant gene, *MecA*, which prevents β-lactam anti-infection agents from inactivating the compound transpeptidases that are basic for the production of cell wall (Lowy, 2003). Additionally, there is reoccurrence of deadly diseases like SARS, HIV/AIDS, tuberculosis, malaria and importantly, bioterrorism and many others caused by new microbes (Fauci, 2005). Modern scientific knowledge, discoveries and interventions have not been able to eliminate infectious diseases. There are reports of extremely or multi-

drug resistant organisms that are emerging in the health community; however, available orthodox drugs are very expensive and most of them have some side effects. There is therefore, the need for an alternative medicine which is readily available, efficacious, easier to prepare, cost effective and with little or no toxic side effect for clinical purposes. As per the World Health Organization (WHO, 2008), home grown prescription (herbal medicine) is the well-known form of traditional medicine that uses plants or other plant materials as active ingredients. It is referred to as indigenous or folk medicine (long standing remedies passed on and practiced by lay people) and constitutes knowledge that developed several years ago in various settlements and societies before modern medicine. Ancient people used herbal medicine for treating many infectious diseases using various parts of plants (explants), and the treatment had been very effective long before scientific methods of diagnosis and prognosis of diseases. Thus herbal can serve as an alternative medicine that is easier to prepare. It is estimated that around 80% of the world's populace depends straightforwardly or mostly on home grown prescription for essential health care needs (WHO, 2008). Therefore, plants provide an unlimited range of compounds to humankind, which can produce meaningful results that are cost effective.

## 1.2. PROBLEM STATEMENT

Antibiotics are very important in modern medicine and have been used to fight bacterial infections; however, antibiotics do not work against all infections. Additionally, when

they are utilized improperly and unreasonably, they develop resistance regardless of the adequacy of the expansive range of antimicrobials.

The antibiotic abuse has become the primary factor for the occurrence of drug resistant strains of a variety of microorganisms, typical example being Methicillin Resistant *Staphylococcus aureus* (MRSA). MRSA is an exceptionally infectious bacterium, which is difficult to treat and it is part of the strains of *Staphylococcus aureus*, resistant to betalactam anti-infection agents. Vancomycin is the principal antibiotic accessible to treat genuine contaminations with MRSA, yet lamentably, diminish in vancomycin susceptibility of *S. aureus* and isolation of vancomycin intermediate have as of late been accounted for from numerous nations (Benjamin *et al*., 2010).

The emergence of other resistant strains of bacteria against some broad spectrum antibiotics has now been reported, most especially in the developing countries where most antibiotics are purchased as over-counter-drugs. According to Slack *et al*. (2007), many microbes have developed resistance to aminoglycoside antibiotics, tetracycline, chloramphenicol, erythromycin, clindamycin among others. Available medicines for managing resistant bacteria infections are extremely expensive and researchers are therefore turning their attention on herbal products against resistant strains since plantbased therapeutics are known to be biodegradable, cost effective with no or minimal adverse side effects.

This study focused on the exploration of nature‟s plants to produce a very potent antimicrobial.

## 1.3. JUSTIFICATION

Medicinal plants are utilized for the screening of phytochemical constituents which are required for the production of new drugs (Wadood *et al*., 2013). The systematic search for bioactivities in medicinal plants is now considered to be a rational approach in the discovery of new antimicrobials. The purpose of the present review is to provide an overview of the diverse phytochemicals present in medicinal plants for novel drug development. Additionally, the selected medicinal plant extracts are expected to provide useful information on the antimicrobial activities against resistant strains of clinically isolated bacteria at cost effective manner.

This study will provide data that can be used as informational document on the use of the plant extracts which could provide lead compounds in managing bacterial infections, and such data could be made available to herbal and pharmaceutical industries in Ghana.

Thus, the search for new lead compounds for the development of novel drugs.

## 1.4. HYPOTHESIS

The study hypothesizes that the seven selected medicinal plants have antimicrobial activities that are effective against clinical isolates of MRSA, MSSA and standard bacteria strains.

## 1.5. AIM

To evaluate, *in vitro,* the antimicrobial activities of selected medicinal plants against clinical isolates of MRSA, MSSA and standard bacterial strains.

## 1.6. SPECIFIC OBJECTIVES

- To determine the phytoconstituents of the selected medicinal plants.

- To determine the antimicrobial activities of both ethanol and aqueous extracts of the selected medicinal plants against clinical isolates of MRSA, MSSA and standard bacterial strains.
- To determine the minimum inhibitory concentrations (MICs) of the most active extracts of the selected medicinal plants.

# CHAPTER TWO

## 2.0. LITERATURE REVIEW

## 2.1. TRADITIONAL MEDICINE

Traditional medicine (TM) consists of knowledge systems and experience that evolved over generations in several societies and communities prior to the discovery of modern drugs. This medicine incorporates knowledge, information, methodologies and mineral based pharmaceuticals, plant, spiritual healing, manual procedures and activities. The medicine is administered independently to treat diseases for well-being of the people in the community.

Traditional medicine is also termed as indigenous or folk medicine. It involves treatment of ailments based on accumulated knowledge, experience and idea of body physiology and health preservation passed on from generation to generation. In addition, it is usually done, informally, outside clinical medicinal treatment. Traditional medical practices that are known include traditional Asian (Chinese) medicine and African medicine. These are usually practiced by a segment of people in a community and later become common knowledge to anyone within the community. In most third world nations in Asia, Africa, Latin America and the Middle East, around 70%-95% of the populaces depend on primary health care (WHO, 2011). The value of traditional medicine globally was evaluated at US$ 83 billion every year in 2008, with a rate of expansion that has been exponential (WHO, 2011). Knowledge in traditional medicine is, however, very important for accessing traditional forms of health care which are more affordable than western medicine and therefore has to be protected and preserved.

Maximum recognition and respect should be accorded to those who hold TM knowledge in order to learn from them since scientific based literature on TM gives insufficient proof on efficacy

and safety. In addition, available individual case reports on patients treated with traditional medical care have no control or comparison group to determine the progress of traditional medicine.

## 2.2. HERBAL MEDICINE

Herb is any plant with roots, stems, leaves, seeds or flowers used for flavouring, perfume, food and have therapeutic properties (Lai and Roy, 2004). Herbal medicine is termed as botanical medicine or phytomedicine which represents a type of traditional medicine by which medicinal plant products (seeds, berries, roots, leaves, bark, or flowers) are prepared and usually used to promote health (Mueller and Mechler, 2003). Plants have been utilized for therapeutic purposes much sooner than written history and the medicinal uses were described by ancient Chinese and Egyptian papyrus writings as early as 3,000 BC. Traditionally, herbal medicine is used alongside conventional medicine and it is gradually becoming one of the major ways in advance clinical researches in preventing and treating diseases (Bordeker and Kronenberg, 2002). Pharmaceutical and pharmacological studies have been carried out on many herbal plants but there still exist the problem of insufficient data regarding their efficacies and side-effects (Mueller and Mechler, 2003). Numerous herbs have been utilized for quite a while for asserted medical advantages while natural pharmaceutical products are utilized as dietary supplement to enhance the strength of individuals (Gbenga *et al*., 2013). They are sold as tablets, powder, capsule, tea extracts and fresh or dried plants; in any case, some herbal medicine can bring about health issues due to their ineffectiveness and interaction with conventional drugs. To use herbal products as safely as possible, one needs to consult a physician first and should take a recommended dose under the guidance of well-trained medical

professionals. Regular preferences of herbal medicines are due to viability, safety, cost effectiveness and acceptability (Momin, 1987).

## 2.3. MEDICINAL PLANT AS A SOURCE OF ANTIMICROBIAL AGENTS

Plant antimicrobials have numerous curative potentials and may provide relief from any of the side effects usually associated with synthetic antimicrobials. Many plants with antimicrobial properties are mainly produced during plant secondary metabolism. All plants produce two types of products as part of their normal metabolism. The first product known as the primary metabolites existing in all plants include proteins, amino acids, chlorophyll and sugars, while the second type of products known as the secondary metabolites mainly have a distinctive metabolic products from specific plant species that have almost no role during the developmental stage of plants but synthesized in specialized cells in their life cycle (Kumar, 2004). They include steroids, phenolic compounds, gums, flavonoids, alkaloids, resins, fatty acids and tannins. Plants have the ability to synthesize aromatic substances such as phenolic compounds including phenolic acids, and flavonoids among others. These compounds protect the plant against predation by microbes, herbivores and insects (Bharathi *et al*., 2011). For instance, plants naturally produce a substance known as phytoanticipins which are constantly being synthesized by plants to act as chemical barriers to microorganisms while phytoalexin is produced in response to many stimuli and can impede microbial attack (VanEtten *et al*., 1994).

The secondary metabolites also have impact against major microbial groups including bacteria, fungi, viruses, parasites and these secondary metabolites are capable of triggering definite physiological responses on the body (Hosseinzadeh *et al*., 2015). Additionally, the secondary metabolites aside acting as defense against infection also

prevent the plant from being eaten up by other living organisms. For example, secondary metabolites such as caffeine in coffee, atropine in nightshade and vinca alkaloids in Madagascan periwinkle are some of the poisonous compounds produced to ward off attack (Moudi *et al*., 2013). Secondary metabolites are usually not produced efficiently in isolated cultured cells as a result their production can sometimes be stimulated by elicitor compounds or preparations which are often fungal or plant extracts. Therapeutic treatments are very significant if plant extracts and phytochemicals with beneficial antimicrobial properties are used. For instance, *Xanthoxylum armatum* (Rutaceae) decoction or powder in warm water can be administered orally to treat toothache, constipation, stomach ache, cold and many others (Manadhar, 1987; Joshi and Edington, 1990).

The World Health Organization (WHO) reported that the medicinal plants are the best source of current natural medications got from therapeutic plants and around 80% of people from third world nations use herbal medicine containing compounds got from them (Arunkumar *et al*., 2009).

Currently, crude forms of plant materials used in traditional healing practices are being modified as established drugs and it is estimated that 50% of western drugs are derived from plant materials (Robbers *et al.,* 1996). There is, therefore, the need to investigate medicinal plants by determining their properties and understanding their safety and efficacy. The study of medicinal plants provide basic understanding about plant toxicity and how human and animal may be protected from natural toxins

## 2.4. PLANTS WITH PROMISING ANTIMICROBIAL ACTIVITY

A number of plants are believed to possess biological activities against a wide variety of microorganisms and many of such plants are found in Africa. They include Garcinia kola (Guttiferae), *Acanthospermum hispidum, Nauticlea latifolia, Bridelia atroviridis* and *Zanthoxylum gilletis, Phyllanthus niruri, Cassia alata, Ageratum conyzoides* and *Sida acuta*. These medicinal plants have been studied and are known to have antimicrobial activity against Gram positive and Gram negative organisms (Hoffman *et al.,* 2004; Agyare *et al.,* 2006). In spite of these achievements, many medicinal plants of Africa are still under investigation for their chemical components and bioactivity against pathogenic microorganisms.

This present research is focused on plants such as *Psidium guajava*, *Alchornea cordifolia*, *Hoslundia opposita, Phyllanthus fraternus, Chromolaena odorata*, *Morinda lucida*, and *Cnestis ferruginea* as antimicrobial agents against pathogenic microorganisms such as MRSA and MSSA.

### 2.4.1. *Hoslundia opposita*

*Hoslundia opposita* is a soft shrub which grows up to about 1.2 m high and belongs to the family *Lamiaceae*. The term *Hoslundia* was named after a Guinea naturalist O. Hoslund-Smith while *opposite*, a Latin word, refers to leaves and fruits which are in opposite pairs. The plants are herbaceous perennials which are either erect or spreading and are abundantly found in the gardens of southern part of Africa such as Namibia,

Botswana and Swaziland as well as tropical Africa which include Senegal and Sudan. Leaves on the plants are in opposite directions and are sometimes arranged in threes. The leaves have a strong unpleasant smell alleged to repel bees and used for the collection of

honey. The plant has little white or creamy green blooms and the fruits are berry-like and appealingly orange-red in colour (Akah, 2010). It survives well in both wet and dry areas of Ghana and it is broadly developed in Nigeria where it is ordinarily known as "Oke ota" by the Ibos and "Efirin odan" by the Yorubas whilst in Ghana, it is known as „nunum nini‟ in the Asante twi dialect (Muhammad *et al*., 2012). It has been shown to have several pharmacological benefits, both traditionally and scientifically. Infusion of its leaves is commonly used in African TM for managing and healing many diseases including diabetes (Abbiw *et al*., 2002). Other ailments include sore throat, cold, sore, venereal diseases, herpes, skin diseases, malaria, microbial infections, epilepsy, fever and inflammation (Annan and Dickson, 2008). According to Muhammad *et al*. (2012), intraperitoneal application of the essential oil of *H. opposita* leaf has huge ameliorative impact on alloxan-induced anaemia,

**Plate 1: *Hoslundia opposita***
**(Courtesy; www.google/image.com)**

### 2.4.2. *Alchornea cordifolia*

*Alchornea cordifolia* is a straight perennial bushy shrub or little tree which is around 4 m high and replicated from seeds. It belongs to the sub family *Acalypholdeae* and family *Euphorbiaceae* or spurge family and commonly known as Christmas bush. The leaves are

alternated, not complex and very simple. They are heart shaped at the base with long petioles. The flowers are greenish white with the male blooms being long spike (836cm) while females are straightforward with short stalks. The fruits are three (3) chambered cases containing red seeds (Olaleye *et al.,* 2006). In Africa the plant is well dispersed and mostly used extensively in traditional medicine (Nisar *et al*., 2008). For instance, in tropical Africa, it is used as topical anti-inflammatory substance. The plant contains phytochemicals such as anthranilic acid, gentisinic acid, iso alchorneine, yohimbine, alkaloids and alchorneine (Eliakim-Ikechukwu and Riman, 2010., Duke and Vanquez 1994). The roots, stems, barks and leaves contain terpenoids, steroids, glycosides, flavonoids (2–3%), tannins (about 10%), saponins, carbohydrates and the imidazopyrimidine alkaloids alchorneine, alchornidine and numerous guanidine alkaloids. The leaves also contain a range of hydroxybenzoic acids like gallic acid and its ethyl ester, gentisic acid, anthranilic acid (vitamin L1) and protocatechuic acid, and also ellagic acid (alizarine yellow). A C20 homologue of vernolic acid, alchornoic acid, is found in the seed oil (Mavar- Manga, *et al*., 2007).

In West Africa, the leaves can be chewed fresh or the leafy stems may be prepared as an infusion which can be administered orally for sedative and antispasmodic actions to treat many kinds of diseases such as respiratory problems including sore throat, cough, bronchitis and genital-urinary problems such as female sterility and venereal diseases (Mavar- Manga, *et al*., 2007). Others include intestinal problems such as diarrhoea, gastric ulcers, amoebic dysentery and worms. (Lifongo, *et al*., 2014). The leaves and root bark are usually used as topical treatment for leprosy and as an antidote to snake venom (Marva-Manga *et al*., 2007). It is used as a purgative, by administering as an enema;

however, high doses taken orally are emetic. They are also taken orally to treat anaemia and epilepsy as well as a blood purifier (Mavar- Manga *et al*., 2007).

In West Africa and Congo, the leaves are eaten as an emmenagogue and to facilitate delivery, whilst in Gabon it is used as an abortifacient. (Mavar- Manga *et al*., 2007). In Ghana and Côte d‟Ivoire, the leaves are applied as a haemostatic to stop prolonged menstruation whilst a decoction of roots or leaves is applied to the vagina to stop postpartum haemorrhage and also to treat vaginitis (Kone, 2004).

**Plate 2:** ***Alchornea cordifolia***
**(Courtesy; www.google/image.com)**

### 2.4.3. *Psidium guajava*

*Psidium guajava* is a tropical medicinal plant which belongs to the family *Myrtaceae*. It is commonly termed as guava and a genus of around 100 species of tropical shrub and small trees of about 10 m high which produce fruits within 4 years. The plant is native to Central America, South America and Mexico but currently widely distributed and

cultivated throughout the tropics because they are able to grow easily and bear fruits quickly in variety of soils. Guava is also referred to as the tropical apple of a poor man (Burkil, 1994). Some of the ethnomedicinal uses include the grinding of the guava leaves and the liquid extract applied on cuts, wounds, boils, ulcers, skin and infectious site of the soft tissue as well as area affected by rheumatism (Bala, 2006). The leaves are chewed to treat mouth ulcers, toothache, chest pains, inflamed gums, throat, treatment of leucorrhea, diarrhoea, dysentery, epilepsy and convulsions (Burkil, 1994). Additionally, infusions and decoctions are used as a douche for vaginal discharges and to tighten vaginal walls after childbirth (Burkil, 1994). In some cultures, a decoction of (Lozoya *et al.,* 2002). Its anti-amoebic and antimalarial effects have also been documented (Morton, 1987; Tona *et al.,* 1998).

Commercially, the fruit is consumed raw and also used in making jam, jellies, juice and paste. Guava is both fat and cholesterol free and has an excellent source of fibre, potassium and vitamin A. In addition, the nutrients present in guava including vitamin C, potassium and carotenoids which strengthen and tone up digestive system, these nutrients are significant in gastroenteritis, to disinfect the system. The fruit is pear-like shape, green rind and pinkish or white flesh with small seeds enriched in pectin and iron which contains enzyme pectinase used in jam production and promoting digestion (Barbalho *et al*., 2012). The guava leaves contain useful substances including antioxidants like vitamin C and flavonoids such as quercetin (an antioxidant) that inactivates sorbitol producing enzymes in causing cataracts. The antioxidant can prevent and repair the cells that are damaged by oxidant. Thus combating free radicals produced during metabolism aid in slowing down aging processes and also avoid diseases that are age related including

Rheumatoid arthritis, Alzheimer‟s cancer, heart diseases and cataracts (Barbalho *et al*., 2012)

Guava leaf extracts improve heart health by controlling blood pressure due to the presence of potassium, a mineral element, which acts as electrolyte and function in electrical reaction in the body including the heart (Sao Paulo, 2008). According to Sao Paulo (2008), guava leaf extract inhibited the growth of *Staphylococcus aureus* which are common cause of diarrhoea. The plant has astringent property (fresh and tighter mouth feel after chewing a raw guava fruit or leaves) which build up loose bowls in diarrhoea. The astringents are compounds that are naturally alkaline and have both disinfectant and antibacterial activity. Thus, they help to eliminate extra mucus from the intestine and block microbial growth. Additionally, guava contains large amount of folate which prevents halitosis (bad breath) and a gum disease known as gingivitis. The guava leaves are very effective in weight loss by preventing the conversion of starch to sugar and have anti-allergic properties by preventing histamine release (Guttierrez *et al*., 2008).

**Plate 3:** ***Psidium guajava***

**(Courtesy; www.google/image.com)**

### 2.4.4. *Phyllanthus fraternus*

*Phyllanthus fraternus* belongs to the family *Euphorbiaceae* and has numerous growth diversity including annual and perennial shrubs, climbers, herbs, floating aquatics and pachycaulous succulent. Floral modification and chromosome numbers have a wide variety. It is common in gardens, waste places and along road sides (Koffour and Amoateng, 2011). All *Phyllanthus* species are commonly called leaf flower and exhibit a definite type of growth known as phyllanthoid branching. In Ghana, traditional medical practitioners use the leaves of the plant for managing and curing diseases like diabetes, malaria, jaundice, kidney stones, hypertension, stroke, alcohol-induced liver damage, genital-urinary tract and intestinal infections, anaemia, pain, liver cancer, abdominal pains and diarrhea which are broad spectrum diseases (STEPRI and CSIR,

2007). *Phyllanthus fraternus* was reported to possess antidiabetic (Okoli *et al*., 2010; Kushwah *et al*., 2010), antiviral (Ogata *et al*., 1992), anti-inflammatory activities and analgesic properties (Calixto *et al*., 1998; Santos *et al*., 1994). *Phyllanthus fraternus* also increases oxidative stress which generates various degenerative diseases such as hypertension and cardiovascular problems ((Khairunnuur *et al*., 2010).


**Plate 4:** ***Phyllanthus fraternus***

**(Courtesy; www.google/image.com)**

### 2.4.5. *Chromolaena odorata*

*Chromolaena odorata* is a rapid growing perennial herb which grows up to 3 m in height and fits into the sunflower family known as *Asteraceae*. The plant is native to North America and also found in tropical Asia, part of Australia and West Africa. The common names associated with *Chromolaena odorata* comprise Siam weed, Devil weed, common floss flower and Christmas bush (Chakraborty, *et al*., 2011). In Ghana, the weed may be referred to as Busia, Acheampong or "Abaafo" and they are believed to have been discovered in February 1969, infesting abandoned plots in the Legon Botanical Gardens, and by 1972 it had been observed in the Greater Accra, Central and Western Regions (Timbilla and Braimah, 1996). The plant is glandular and hairy and the leaves have a choking, aromatic smell when crumpled. The leaves are simple, alternate and bright green coloured with long petiole. The seeds are small crowned with silky hairs for wind dispersal. The plant is mostly grown for ornamental and medicinal purposes. Phytochemical screening of the plant identified the cyanogenic glycosides, flavonoids, phytates, saponins, alkaloids and tannins (Igboh *et al.,* 2009). *Chromolaena odorata* in a natural environment is considered as an invasive weed of field crops because it prevents other plant species from growing in area of shifting cultivation especially in West Africa. *Chromolaena odorata* contains a carcinogen known as pyrrolizidine, an alkaloid which cause allergic reactions and poisonous to cattle. In traditional medicine, a decoction of the leaf is used to manage cough and for only malaria when lemon grass and guava leaves are added as part of the ingredient. Other medicinal uses include anti-diarrhoeal, astringent, antispasmodic, antihypertensive, antiinflammatory and diuretic (Iowa *et al.*, 1999). A

decoction of flowers is used as tonic, antipyretic and heart tonic (Bunyapraphatsara and Chokechaijaroenporn, 2000).

**Plate 5: Leaves of *Chromolaena odorata***

**(Courtesy; www.google/image.com)**

### 2.4.6. *Cnestis ferruginea*

*Cnestis ferruginea* is a perennial shrub widely distributed in the savannah region of tropical West Africa. The plant grows around 3.0-3.6 m tall, and densely grown with pinnate leaves and brown fruits, which produces flowers within the months of January to March (Irvine, 1961). In Ghana, it is known as „apose" by the Asantes, „akitase" by the Fantes, „ahuidade" by the Gas, the Guan-Anums call it „adonwaebe" and amongst the Nzema as „pudaegye. *C. ferruginea* posseses medicinal, ornamental and nutritional values and it is used as therapy for many diseases across tropical Africa (Ishola, 2011). The different parts of the plant and preparations have been reported to be used in traditional African medicine to remedy various conditions notably amongst them are painful and inflammatory conditions like periodontitis, headache, bronchitis, eye troubles,

dysmenorrhoea, all manner of pains, migraine, sinusitis, toothache and conjunctivitis ( Ishola, 2011 and Burkil, 1985). In Eastern Nigeria, the plant decoction is used by herbalists to treat rheumatism, gonorrhoea, stroke joint and waist pains, arthritis and syphilis (Okwu and Iroabuchi, 2004). They exhibit antioxidant, anti-inflammatory and membrane stability properties. The aqueous root extract of *C. ferruginea* has also been reported to have anti-stress potential (Ishola and Ashorobi, 2007).

**Plate 6: Leaves of *Cnestis ferruginea* (Courtesy: www.google/image.com)**

### 2.4.7. *Morinda lucida*

*Morinda lucida* Benth. is a tropical West African rainforest tree of about 15 m tall which belongs to the family *Rubiaceae* and it is widely used in West Africa for medicinal purpose (Iwu, 2014). The common names associated with *M. lucida* include “Brimstone tree” (in

English), Sangogo or Bondoukou alongua (in Cote d‟ivoire), Oruwo or Ruwo amongst Yoruba tribes and Huka and Eze-ogu amongst Ibo tribes (both in Nigeria), and atakake in Togo. In Ghana it is termed as “konkroma” in Twi and “amake” in Ewe. The different parts of *Morinda lucida* have been reported to possess medicinal properties. For instance, Oliver-Bever (1986) stated that a decoction of the stem bark is used to treat severe jaundice. *Morinda lucida* leaf extract is also reported to possess hypoglycemic and trypanocidal activities (Taofeeq *et al*., 2010).

**Plate 7: *Morinda lucida***

**(Courtesy: www.google/image.com)**

## 2.5. PHYTOCHEMICALS

There are several organic compounds present in medicinal plants including tannins, alkaloids, glycosides, saponins, phenols, terpenoids, flavonoids, steroids and many others.

These compounds are mostly bioactive and provide physiological actions that are specific on the human body (Edoga *et al*., 2005).

Tannin compounds inhibit microbial growth by causing the bacterial colonies to disintegrate, which results from their interference with the bacterial cell. Tannins and flavonoids possess the ability to increase colonic water and electrolyte re-absorption, therefore, plants containing tannins and flavonoids are used in the treatment of diarrhoea and dysentery (Marvar-Manga *et al*., 2007). It is also reported that glycosides and flavonoids are known to protect against gastrointestinal infections (Marvar-Manga *et al*., 2007). Additionally, saponins are agents that are very active at the surface; they alter the cell wall permeability to allow the exit of essential components from the cell or the entry of poisonous substances at the cell surface (Marvar-Manga *et al*., 2007).

Phenol is generally a protoplasmic poison and toxic to all types of cells. Precipitation of protein occurs with high concentration of phenol, while at low concentrations it denatures protein without coagulating them. It is free to penetrate the tissues because of its denaturing activity (Marvar-Manga *et al*., (2007). Most alkaloids are readily soluble in alcohol and fairly soluble in water, however, their salts are usually soluble (Doughari, 2012). The antibacterial properties of alkaloids have been reported as possessing the capability to intercalate with DNA of microorganisms to prevent cell division and growth (Abioye *et al*., 2003).

Cardiac glycosides are identified as compound used to lower blood pressure as reported by Yadav and Agarwala (2012). They are mainly steroids with an innate capacity to perform powerful and specific action on the heart (cardiac) muscle when given to human or animal through an injection (Hamuel, 2012., Yadav and Agarwala, 2011). Terpenoids are generally non-polar molecules and will dissolve well in non-polar solvents and semi-

polar solvents. Again, as a general rule, terpenoids tend to be oily and high percentage ethanol soluble (Rispail *et al*., 2005). Terpenoids demonstrate some significant pharmacological activities such as anti-inflammatory, anti-viral, antimalarial, anti-bacterial activities, anticancer and inhibition of cholesterol synthesis (Mahato and Sen 1997). Terpenoids play a very important role by killing the

herbivorous insects and attracting mites that are useful (Kappers *et al*., 2005).

## 2.6. INFECTIOUS MICROBES

Microorganisms are group of organisms that can only be visualized with a microscope, yet abundant on Earth (NAIAID, 2009). The group includes bacteria, fungi (moulds and yeast), parasites (protozoans and metazoans), microscopic algae and viruses. Some are pathogenic while others are non-pathogenic or opportunistic pathogens (Tortora *et al*., 2001).

Infectious microbes are therefore, pathogenic organisms that cause infectious diseases. The disease these microbes cause could spread directly or indirectly from one person to another or they could be zoonotic (Pinner *et al*., 1996). These microbes are the principal cause of death in the world and the consistent increase in infectious diseases has been attributed to increase in antibiotic resistance (Pinner *et al*, 1996). In view of this, scientists are working assiduously towards the discovery of new antimicrobials with minimum toxicity but maximum potency against resistant infectious microbes (Roger and Clire, 1999).

### 2.6.1. *Staphylococcus aureus*

*Staphylococcus aureus* is an opportunistic pathogen that can bring about various ailments as a result of infections to different body tissues. The organism is usually carried on the

human body asymptomatically (without showing any symptoms). It is a Gram positive, coagulase positive coccus which is part of the family *Staphylococcaceae.* The term *Staphylococcus* was obtained from the Greek word *staphyle*, which means a cluster of grapes, and kokkos, which means berry. When viewed under the microscope, it looks like a bunch of grapes or little round berries and it is commonly known as Staph.

*Staphylococcus aureus* is an effective pathogen on account of blend of bacterial immune-attack strategies which incorporate production of carotenoid pigment and staphyloxanthin, responsible for the golden colour properties of *S. aureus* colonies. The pigment serves as a virulent factor, basically as bacterial antioxidant which helps the organism to attack the reactive or responsive oxygen species utilized by the host immune system to destroy pathogen. *Staphylococcus aureus* is likewise catalase-positive and in this manner ready to change hydrogen peroxide ($H_2O_2$) to water ($H_2O$) and oxygen ($O_2$). In addition, *S. aureus* is coagulase positive and this property can be used to differentiate a small percentage of *S. aureus* from most staphylococci.

It is also a normal flora in the nostril of 30% of healthy persons but may be found transiently on the skin (Slack *et al.*, 2007). In most instances, the bacteria do not cause infection, since human skin serves as a natural protective mechanism to bacteria and other harmful organisms. Therefore, the damage to the skin enables bacteria entry to cause infections. The infections are usually by direct contact with infected lesions such as pus and dried exudates discharged from wounds. Chronic shedders can infect themselves and others. The infections may also be acquired nosocomially (Jawetz and Levinson, 1996).

### 2.6.2. Methicillin-resistant *Staphylococcus aureus* (MRSA)

MRSA is a bacterium which is extremely tougher to cure or manage and belongs to one of the most dangerous strains of *Staphylococcus aureus* that has acquired a gene called *mecA* which makes it resistant to all beta-lactam antibiotics including cephalosporins and penicillins. These antimicrobials are no more very potent when it comes to treating an MRSA infection. MRSA is also referred to as oxacillin-resistant *Staphylococcus aureus* (ORSA) as well as multidrug-resistant *Staphylococcus aureus* (Karygianni and Manuel, 2014). MRSA is in some cases called a "super bug", showing a strain of microbes that is impervious to one or more antibiotic(s) that would typically treat the microscopic organisms.

Nucleic acid enhancement tests, for example, the polymerase chain reaction (PCR), can be utilized to identify the *mecA* gene, which is the most well-known gene that links oxacillin resistance in staphylococci. *MecA* PCR tests cannot recognize novel resistance mechanism, for example, mecC or extraordinary phenotypes, for example, borderline oxacillin resistance. Acquisition of *mecA* gene, however, renders treatment of MRSA more challenging especially to the physicians. This is because the standard antimicrobials used to cure the pathogens are no longer potent and available alternative therapies are also less effective. As a result, MRSA that cause healthcare-associated infections become multiple resistant to other ordinary antimicrobial agents, such as tetracycline, erythromycin, clindamycin and fluoroquinolones that are readily available. Thus, if the necessary measures are not taken, MRSA infections can cause overstay at the hospital leading to patient death. There is, therefore, the need to search for an alternative but readily available and affordable antimicrobials to treat MRSA infection and the best solution is using medicinal or herbal products.

### 2.6.2.1. Mechanism of resistance of MRSA

Methylcytosine DNA also known as mec DNA contains *mecA* gene. This gene is mostly found among staphylococcal species or bacterial cells. The area assigned as mec DNA is accepted to have begun from the genome of another bacterial species and coordinated into the chromosome of *S. aureus* cell in the past or generations ago. The presence of the *mecA* gene may permit a bacterium to be resistant to anti-infection agents (antibiotics), for example, methicillin, penicillin and other penicillin-like anti-toxins (CFSPH, 2012).

The most usually known transporter of the *mecA* gene is the bacterium known as MRSA. It is additionally found in Staphylococcus aureus and *Streptococcus*

*pneumoniae* strains resistant to penicillin-like anti-infection agents (CFSPH, 2012) Staphylococcal resistance to oxacillin/methicillin happens when an isolate yields an adjusted penicillin-binding protein, PBP2a, encoded by the *mecA* gene. The penicillinbinding protein variant binds beta-lactams with lower affinity, rendering it resistant to this class of antimicrobial agents. In *Staphylococcus* species, *mecA* is spread on the SCC*mec* genetic component (CFSPH, 2012).

The *mecA* gene does not permit the ring structure of penicillin-like antibiotics to invade the enzymes (transpeptidases) that helps bacterial cell wall formation, hence the bacteria is made to undergo typical replication. The *mecA* gene encodes the protein PBP2A (Penicillin binding protein) involved in bringing together the bacterial cell wall and it is poorly inactivated by beta lactam antibiotics (Ito *et al*., 2003). Thus, the enzyme transpeptidase enhances bacterial cell wall synthesis in the presence of beta-lactams. The MRSA gene, *mecA*, is carried on the staphylococcal chromosomal cassette mec

(SCCmec), and it is a large genetic component which is also mobile. Strains that are susceptible to these beta lactam antibiotics are referred to as methicillin-sensitive *Staphylococcus aureus* (MSSA).

### 2.6.2.2. Classification and the site of transmission of MRSA

MRSA is categorized by virtue of where the disease was initially obtained. For example, MRSA that is transmitted overwhelmingly in clinics or hospitals, is known as hospital-acquired MRSA (haMRSA) while those transmitted outside clinics/hospitals from individual to individual is known as community-acquired MRSA (caMRSA). On the other hand, MRSA that is common among production animals and mostly found in people who are in customary contact with animals because of their profession is termed as livestock-associated MRSA (laMRSA).

### 2.6.2.3. Mode of transmission of MRSA

*S. aureus* is an infectious pathogen on human and as a result its strains such as methicillin-sensitive and methicillin-resistant strains well noted as typical pathogens on the skin, nose and the nasopharynx of some people (Slack *et al*., 2007) Transmission of MRSA happens through direct contact with somebody who has an active infection or who is a carrier of the contamination (colonized).Poor hygienic practices constitute the major MRSA transmission paths and also through a contaminated objects (CFSPH, 2012).

According to the Center for Food Security and Public Health (CFSPH, 2012), when an individual comes into contact with a contaminated surface or object, that person may become a carrier of the bacteria (colonized). An active infection with MRSA/MSSA can develop when a person is colonized. MRSA can also be spread from mother to new born child during delivery, on fomites and in aerosol. Colonization with *S. aureus*

(MRSA/MSSA) can happen any time after birth, and transmission might be transient or persistent.

#### 2.6.2.4. Diagnostic test and incubation period of MRSA

MRSA infection, including colonization, can be diagnosed by culturing to identify the organism. MRSA can colonize more than one site in human, however, isolates of *S. aureus* (MRSA/MSSA) from an infection, can be identified by genetic testing or antibiotic susceptibility testing on methicillin resistant strains. The appearance of the mecA gene indicates MRSA usually carried out by amplification test based on polymerase chain reaction (PCR) to recognize this gene, as the gold standard for identification. A latex agglutination method or test can also detect the penicillin-binding protein, 2PBP2a, the product of *mecA* gene. This is because the binding protein is part of the aggregation of the cell wall in bacteria but inactivated poorly by beta-lactam antibiotics. *S. aureus* infections in humans have a high variable incubation period. For instance, clinically in susceptible patients it becomes obvious between 4 to 10 days after
exposure; however, after an unknown period of carriage of the infection asymptomatically, leads to opportunistic infections.

#### 2.6.2.5. Clinical signs and symptoms of MRSA

Ordinarily, MRSA causes gentle infections on the skin, such as sores or boils; however, it can also cause severe skin infections when chance on surgical wounds, the bloodstream, the lungs and the urinary tract. MRSA bacteria cause skin infections with the signs and symptoms, such as cellulitis (infection of the skin or the fat and tissue underneath the skin), abscesses (collection of pus or discharge in or under the skin), carbuncles (infections bigger than an abscess usually with several openings to the skin), impetigo

(skin infection with blisters filled with pus), styes ( infection of an oil gland of the eyelid), and rashes (reddish skin or have red colour shaded area ) and boils (pusfilled infections of hair follicles).



#### 2.6.2.6. Risk factors for MRSA infection

MRSA infections are readily available in facilities like hospitals, nursing homes and other health centers, and usually common among people who have weak immune systems in such facilities. Infections can appear around surgical wounds or invasive devices, like catheters or implanted feeding tubes; however, typical skin tissue resist the development of MRSA infection, rather persons with depressed immune systems (HIV) and individuals with cuts, abrasions, chronic diseases (diabetes or cancer) or chronic skin disease, pneumonia (lung infection) are more susceptible to MRSA infection. Additionally, most infections caused by MRSA are limited to the skin and can be life threatening when they get access into the bloodstream and spread to internal organs. They can develop into more severe symptoms to indicate internal infections which include: chills and fever, weakness or fatigue, shortness of breath, severe headache, muscle aches, nausea, acute pain and rashes all over the body which need immediate medical attention.

#### 2.6.2.7. Disinfection of MRSA

MRSA which is a strain of *S. aureus* is vulnerable to some disinfectants like sodium hypochlorite, alcohols (effectively 70% ethanol), quaternary ammonium compounds, iodophors, phenolics, glutaraldehyde, formaldehyde, and iodine and alcohol combination. The MRSA bacteria can also be killed by moist heat (121°C for a minimum of 15 minutes) or dry heat (160-170°C for at least 1 hour).

#### 2.6.2.8. Prevention of MRSA infection

This could be made possible by washing the hands and avoiding direct contact with nasal secretions and wound. Hand washing plays an important role in preventing MRSA transmission from humans and also serves as one of an infection control measures. Patients with MRSA skin lesions should keep the lesions covered with clean, dry bandages. Avoid direct exposure to uncovered MRSA-infected wound and people who are susceptible. High risk patients are quarantined until the screening test proof negative for MRSA (CFSPH, 2012).

#### 2.6.2.9. Treatment of MRSA Infection

Antibiotics are used to manage clinical cases and in many instances, surgical implants or detached are also done. Currently, antibiotic therapy is based on susceptibility testing; however, all MRSA strains are considered to be resistant to ß-lactam antibiotics, irrespective of the test results. In the light of the above reason, there is a need to search for effective but readily available antimicrobials to treat infections caused by MRSA.

### 2.6.3. Methicillin-sensitive *Staphylococcus aureus* (MSSA)

Methicillin-sensitive *Staphylococcus aureus* is a type of bacteria which exists harmlessly on the skin and in the noses, in about 30% of people. Additionally, individuals are said to be colonized with MSSA infection when present on their bodies or in their noses; however, MSSA colonisation incidentally causes no harm to individuals, but may cause an infection when it enters the body most especially amongst sick persons. MSSA can infect any wound on the skin and cause local infections like abscesses or boils. MSSA

may cause severe infections referred to as septicaemia (blood poisoning) when it gets access to the bloodstream; however, unlike MRSA, MSSA is more sensitive to antibiotics and therefore easier to treat.

## 2.7. STANDARD BACTERIAL STRAINS

### 2.7.1. *Staphylococcus aureus*

*Staphylococcus aureus* belongs to the *Micrococcaceae* family (Konemam *et al*., 1992). All staphylococci are Gram positive with an average diameter of 1 µm. The cocci are usually arranged in grape-like clusters, but they may also appear singly or in pairs. They are non-motile, non-sporing and usually non-capsulated (Slack *at al*., 2007). They are non-fastidious with a large temperature range of 10–42 $^0$C, with optimum growth of 3537 $^0$C. Individual bacteria colonies are round and about 2-3 mm diameter, with surface that is shiny and smooth. The colonies are opaque and normally exhibit golden-yellow pigment on blood agar. *S. aureus* ferments lactose and coagulate plasma (Cheesbrough, 2007). The infections may also be acquired nosocomially (Jawetz and Levinson, 1996). Diseases triggered by *S. aureus* are grouped into pyogenic and toxin-mediated infections. The pyogenic infections include boils, carbuncles, wound infection, abscesses, impetigo, mastitis, bacteraemia, osteomyelitis, pneumonia and endocarditis while toxic-mediated infections encompass scalded skin syndrome, pemphigus neonatorum, toxic shock syndrome and food poisoning (Slack *et al*., 2007).

### 2.7.2. *Staphylococcus saprophyticus*

*S. saprophyticus* is a Gram-positive bacteria and a coagulase negative *Staphylococcus* (CoNS), (Cheesbrough, 2007). It is the second most important CoNS that have a special

medical niche. Coagulase-negative staphylococcal species are important agents of hospital-acquired infections associated with the utilisation of implanted prosthetic devices and catheters (Harvey *et al*., 2007). *S. saprophyticus* causes acute urethritis, especially in sexually active women, and catheter-associated urinary tract infections in elderly men. In women who are young, *S. saprophyticus* is, after *Escherichia coli*, the most frequent causative agent of acute urinary tract infections (UTI) (Koneman *et al*., 1992**)**. There are cases of *S. saprophyticus* associated endocarditis according to a study reported by Laskova *et al.* (2008), and it is believed to be resulting from prosthesis. They also presumed that the causative agent might have entered the body through a tunneled catheter for haemodialysis, even though the catheter site of insertion demonstrated no signs of infection. The achievement of this organism as a urinary tract pathogen is ascribed to the properties like possession of a specific cell wall-anchored adhesin, the urease production and a proliferation of transport systems which aid adaptation to the human urinogenital environment (Kuroda *et al*., 2005).

### 2.7.3. *Salmonella typhi*

Salmonellae are members of the *Enterobacteriaceae*, Gram-negative bacilli and facultatively anaerobic. They are able to thrive on a wide array of simple media and differentiated from other members of the family by their biochemical features and structure of antigen (Slack *et al*., 2007). With the exception of *S. Pullorum-gallinarum*, all salmonellae are actively motile. They are non-sporing, and with the exception of *S. typhi* which is capsulated, the rest are non-capsulated (Cheesbrough, 2007). *Salmonella typhi* causes enterocolitis, enteric fever such as typhoid fever, and septicaemia with metastatic abscesses (Jawetz and Levinson, 1996).

Typhoid and paratyphoid bacilli are fundamentally human parasites. Humans are the principal host with most infections traceable to sewage or human. All other salmonellae have animal hosts (Slack *et al*., 2007). Even though data is incomplete, an estimated 10 to 500 incidence of typhoid per 100 000 population occur throughout the developing nations every year (Slack *et al*., 2007). *Salmonella* infections are mainly associated with hygiene and sanitation; hence commensurate with the high prevalence in developing countries (Cheesbrough, 2007). Ingested *Salmonella* attack epithelial cells of the small intestine (Jawetz and Levinson, 1996) and then penetrate the endothelial cells to the submucosa to be engulfed by the macrophages. The macrophages carry the *Salmonella* to the reticuloendothelial system where the bacteria multiply intracellularly, causing lymphoid hyperplasia and hypertrophy. Some of the organisms reenter the bowels via the liver and gallbladder (Harvey *et al*., 2007).

### 2.7.4. *Proteus mirabilis*

They are morphologically pleomorphic, and actively motile especially at 20-28 $^{0}$C. On solid media, especially blood agar, they exhibit a discontinuous swamming characteristic (Slack *et al*., 2007). They are non-capsulated and have the ability to break down urea. When cultured aerobically, they produce a characteristic fishy smell (Cheesbrough, 2007). Members of the genus *Proteus* are found in the human colon, soil and water, as well as faecally contaminated materials (Koneman *et al*., 1992). They cause disease only when they are outside the enteric tract. *Proteus* infections are believed to result from ascending spread of the organism from the anal region (Jawetz and Levinson, 1996).

Common cause of urinary tract infection (UTI) is *Proteus mirabilis* usually present in elderly and young males and often following catheterization or cystoscopy (Slack *et al*, 2007). Infections are also associated with calculi (Slack *et al*, 2007). It is frequently a secondary attack to damaged tissues, pressure sores, ulcers and burns (Cheesbrough, 2007). Septicaemia generally occurs only in patients with serious underlying disease conditions or complications of UTI. Meningitis due to *Proteus* has also been reported (Slack *et al*, 2007). The vigorous motility of *Proteus* species may contribute to their ability to invade the urinary tract (Slack *et al*, 2007). They produce urease which hydrolyses urea in the urine to form ammonia, making the urine pH alkaline (Koneman *et al*., 1992).

### 2.7.5. *Pseudomonas aeruginosa*

*Pseudomonas aeruginosa* belongs to the family *Pseudomonadaceae,* genus *Pseudomonas* and species *aeruginosa. P. aeruginosa* is a non-sporing, non-capsulated, Gram-negative bacillus (Cheesbrough, 2007). It has one or two polar flagella which makes the microbe motile. It is an obligate aerobe but can grow anaerobically if nitrate is available as a terminal electron acceptor (Slack *et al*., 2007). Most strains produce diffusible pigments; greenish-blue pigment (pyocinin), yellow-green fluorescent pigment (fluorescein), pyorubrin (red pigment), melanin (brown pigment) and others (Slack *et al*., 2007). It has a distinctive grape-like smell due to the production of 2aminoacetophenone (Cheesbrough, 2007). They are non-fermentors and derive energy from carbohydrates strictly by oxidation. The oxidation involves electron transport by cytochrome c, hence making them oxidase-positive (Jawetz and Levinson, 1996).

*P. aeruginosa* can be obtained from a wide range of environmental sources. It has the ability to persist and multiply in moist and humidified environs and on equipment in hospital wards, bathrooms and kitchens (Slack *et al*., 2007). The microbe may even thrive in some disinfectants (Jawetz and Levinson, 1996). The major source of acquisition of *P. aeruginosa* in cystic fibrosis (CF) appears to be from the environment (Speert *et al*., 2002). *P. aeruginosa* infection used to be common among immunocompromised HIV patients before the introduction of HAART (Highly Active Antiretroviral Therapy) (Marjorie *et al.*, 2000). *P*. *aeruginisa* causes numerous diseases in the world including skin infections, especially burn site, wounds, pressure sores, and ulcers (Cheesbrough, 2007). Otitis externa, varicose ulcers, corneal infection, acute selflimiting folliculitis, cystic fibrosis and other infections among immune compromised patients are also common (Slack *et al*., 2007).

### 2.7.6. *Klebsiella pneumoniae*

*Klebsiella pneumoniae* belong to family *Enterobacteriaceae*, genus *Klebsiella,* and species *pneumoniae.* Members of the genus *Klebsiella* are Gram-negative rods, aerobes and facultative anaerobes, 1-2 µm long, non-motile and usually capsulated, lactose, glucose and sucrose fermentors (Cheesbrough, 2007). On a solid non-differential media, *Klebsiella* colonies appear grayish white and extremely mucoid. It grows within temperature range of 12-43 $^{0}$C with optimum growth at 37 $^{0}$C (Slack *et al*., 2007).

*Klebsiella pneumoniae* causes diseases outside the enteric tract (Jawetz and Levinson, 1996). The diseases include respiratory tract infections like lung abscesses and bronchopneumonia. Additionally, it infect the urinary tract and surgical wounds

(Cheesbrough, 2007) while other strains are incorporated in pyogenic liver abscesses (Alvarez *et al*., 2001).

### 2.7.7. *Escherichia coli*

*Escherichia coli* belong to family *Enterobacteriaceae*, genus *Escherichia,* and species *coli*. They are Gram-negative rods and motile with peritrichous flagella. They are generally found in the smaller intestine of warm blooded animals; however, majority of the strains are harmless yet in humans, other serotypes cause food poisoning. The harmless strains are portion of normal micro flora of an alimentary canal which help in the production of vitamin K12 (Brooks *et al*., 2007). *E. coli* is the most abundant facultative anaerobe in the enteric tract aside the obligate anaerobe, *Bacteroides* (Jawetz and Levinson, 1996). It ferments lactose, a property used to distinguish it from *Salmonella* and *Shigella* (Jawetz and Levinson, 1996). Most strains are motile and even though optimum growth is obtained at 36-37 $^0$C, most strains can grow within the range 18-44 $^0$C (Cheesbrough, 2007).

*E. coli* is the leading cause of Urinary Tract Infection (UTI) in both males and females (Jawetz and Levinson, 1996). UTI is common in females compared to male because of the difference in the anatomical architecture of the human body (Slack *et al*., 2007). *E. coli* associated UTI, like many other *Enterobacteriaceae* is usually as a result of ascending spread of the organism from the anal region (Jawetz and Levinson, 1996). It is believed that strains that cause UTI are geographically specific (Slack *et al*., 2007). Pathogenic strains consumed via contaminated food or water may colonize the GIT and cause diarrhoea especially in infants; however, if systemic infections occur it can lead to neonatal meningitis (Slack *et al*., 2007). In newborns, *E. coli* infection normally emanate

from colonization of the parental vagina (Jawetz and Levinson, 1996). Thedifferent strains of *E. coli* cause different diseases. For instance, Enteropathogenic *E. coli* (EPEC) causes infantile enteritis especially in developing countries (Slack *et al*., 2007). Enterotoxigenic *E. coli* (ETEC) causes community acquired and travellers‟ diarrhoea (Slack *et al*., 2007). Entero-invasive *E. coli* (EIEC) causes shigellosis-like disease while Entero-aggregative *E. coli* (EAggEC) causes chronic diarrhoea and vomiting, especially in children (Cheesbrough, 2007). Verocytotoxin-producing *E. coli* (VTEC) causes watery diarrhoea, haemorrhagic colitis and haemolytic uraemic syndrome (Slack *et al*., 2007). *E. coli* is also the predominant cause of bacterial pyogenic liver abscesses (PLA) (Alvarez *et al*., 2001).

*E. coli* possesses or has the ability to produce pili, capsule, endotoxin and enterotoxins, responsible for damaging host cells (Slack *et al*., 2007). EIEC and EPEC invade intestinal epithelia cells, especially those of the jejunum and ileum, dysfunctioning the functional morphology and physiology of these cells (Slack *et al*., 2007). The pathological difference between EIEC and EPEC is that the former is similar to shigellosis and not age bound while the latter is more limited to infants (Jawetz and Levinson, 1996). VTEC produces toxins (VT1 and VT2) similar to *Shigella dysenteriae* type 1, and function as such (Slack *et al*., 2007). ETEC produces heat labile toxins (LT-I and LT-II) and heat-stable toxins (ST-I and ST-II) which function to distort the normal purpose of cAMP and cGMP (Slack *et al*., 2007). The pathogenic mechanism of EAggEC is not well established (Slack *et al*., 2007).

## 2.8. ANTIBIOTICS

Antibiotics are one class of antimicrobials that are relatively harmless to the host. It may also be defined as microbial products or their derivatives which at appropriate dose selectively kill or inactivate the growth of other microorganisms.

They are manufactured as secondary metabolites predominantly by organisms that utilized soil as habitat. Majority of these organisms form some type of a spore or dominant cell, subsequently there is a belief that they have a link between antibiotic production and processes of sporulation. Amongst the moulds, the prominent antibiotic producers are *Penicillium* and *Cephalosporium* which are the chief source of the beta- lactam antibiotics (penicillin and its relatives) whilst in bacteria, the *actinomycetes*, fundamentally *streptomycetes* species produce different kinds of antibiotics like aminoglycosides (Streptomycin), macrolides (erythromycin), and tetracycline. Endospore-forming *Bacillus* species produce polypeptides antibiotics such as polymyxin and bacitracin. The primary cause of antibiotic resistance may be genetic mutation in bacteria and other cause may include incorrect and unreasonable use of antimicrobial drug which invariably provides conducive atmosphere for the growth, appearance, spread and persistence of resistant microorganisms. As a result, the longer the duration of exposure of the antibiotics to the host, the higher the risk of developing resistance, irrespective of the efficacy of the need for the antibiotic. The selection of antibiotics to treat an infection depends on factors such as site of the infection, the causative organism and the state of the patient (Tortora *et al*., 2001).

## 2.9. BRIEF HISTORY OF ANTIMICROBIAL AGENT

The chemotherapy of modern antimicrobial started in1929 with Fleming‟s discovery of the penicillin as a very potent bactericidal whilst in 1935 Domagk discovered synthetic chemicals (sulfonamides) with broad antimicrobial activity. During World War II in the mid 1940‟s there was the requirement for antibacterial agent and penicillin was isolated, purified and injected into experimental animals where it was found to cure infections as well as inconceivably low toxicity to the animals. This prompted antibiotic chemotherapy and a serious search for antimicrobial agents of comparative properties and low toxicity to organisms that might be effective to the treatment of infectious diseases. Later, quick isolation of streptomycin, chloramphenicol and tetracycline were then used as chemotherapy. During 1950‟s these antibiotics and a few different antimicrobial agents were in clinical use (Saga and Yamaguchi, 2009).

## 2.10. IMPORTANT PROPERTIES OF ANTIMICROBIAL AGENTS

The use of plants in medicine is gaining popularity for some time now; for example, in cancer treatment, using anti leukaemic alkaloids and vincristine plants such as *Catharanthus roseussyn,* and *Vinca roseus* (Nelson, 1982). A huge quantity of medicinal plants have been documented as appreciable natural resources containing antimicrobial compounds (Mahady, 2005).The extracts of medicinal plants also provide significant potential for the new antimicrobial agent‟s development which is potent against infections presently very hard to cure with conventional drugs (Iwu *et al.,* 1999). Clinically, antimicrobial agent is very significant because of its selective toxicity. Thus, in bacteria, the biochemical processes somehow vary from those in animal cells and the difference may serve as advantage in chemotherapy.

An antimicrobial is an agent that destroys microorganisms or inactivates their growth. Antimicrobial drugs may be grouped primarily based on the microorganisms they act against. For instance, antibacterial and antifungal used against bacterial and fungi respectively. Antimicrobial may also be classified by virtue of their functions. Agents that damage microbes and inactivate their growth are respectively microbicidal and microbiostatic. Additionally, antimicrobial agents are grouped into main classes, namely; disinfectants (such as bleach) which are nonselective antimicrobials that destroy a vast range of microbes on non-living surfaces to avoid the spread of infection. Another main group is antiseptics which are usually used as topical treatment to living tissue to reduce infection especially, during surgery. Lastly, there are antibiotics which are administered orally to kill or inactivate microorganisms within the body. Some adverse effects exhibited by antibiotics on the host are mainly immune-suppression, hypersensitivity and allergic reactions. This condition has challenged scientists to look for new antimicrobial agent mainly from medicinal plants which are cheap and readily available to treat infectious diseases.

## 2.11. MECHANISMS OF ANTIMICROBIAL RESISTANCE

Frequently found resistance mechanisms are as follows:

### 2.11.1. Enzymatic Inactivation of drugs

It is one of the most commonest resistance mechanism for antibiotics of natural origin such as aminoglycosides (enzymatic phosphorylation, acetylation or adenylation and βlactams, enzymatic hydrolysis by β-lactamases). Genes coding for such enzymes will make the bacterial cell resistant when they are available as extra hereditary (genetic)

component on plasmids. This may be one of the reasons why such genes have become so prevalent on resistance plasmids (R-plasmids) as reported by Brown and Wright, (2005).

### 2.11.2. Mutational Alteration of Target Proteins

Completely artificial compounds like fluoroquinolones are doubtful to end up inactivated by the enzymatic mechanisms as portrayed above; in any case, bacteria can still become resistant by virtue of mutations that make the target protein less vulnerable to the agent. For instance, fluoroquinolone resistance is primarily as a result of mutation in the target protein, and DNA topoisomerases. This type of resistance is caused by alteration of chromosomal gene(s) and this is not easily transferred to other cells because, the recipient will still retain the drug-susceptible and will enable unaltered target enzyme eventually remain susceptible. Nevertheless, such mutants will become more dominance in the presence of selective pressure. Fluoroquinolones resistance is rapidly increasing in almost all groups of pathogens (Brown and Wright, 2005).

### 2.11.3. Acquisition of genes for less susceptible target proteins from other species

Scientists discovered that when MRSA is produced it contains methicillin-resistant penicillin-binding protein called PBP-2A or 2‟, whose expression is predominantly induced by methicillin and other β-lactams. The gene is sited in a large segment of DNA (30–60 kb) which seemingly obtained from an organism rather than *S. aureus* and contains other genes coding for resistance to macrolides and aminoglycosides. The presence of a plasmid with

genes coding for the conventional β-lactamase and for tetracycline resistance, make these strains resistant to all available agents except vancomycin. (Brown and Wright, 2005).

### 2.11.4. Target bypass

A product of fermentation vancomycin, from *streptomycetes* has a strange mode of activity. The reason is that instead of inactivating an enzyme, vancomycin rather binds to the substrate (the lipid-linked disaccharide pentapeptide) which is a precursor of cell wall peptidoglycan. As a result of this mechanism, some scientists presumed that it would be difficult to produce resistance against vancomycin. Resistant strains of vancomycin are, however, currently dominance among enterococci because enterococci being the ordinary occupant of human intestinal tract are naturally resistant against βlactams, aminoglycosides, macrolides and tetracycline. This vancomycin-resistant strain of enterococci has now become prevalence in the surroundings of hospitals and other health facilities to colonize the patients which cause infections that are tougher to cure. Investigation of resistance mechanism indicated that the end of pentapeptide D-Ala-DAla where vancomycin binds was replaced by an ester structure D-Ala-D-lactic acid (Lac) in the resistant strain. This structure still allows the cross links development in peptidoglycan but it is not bound by vancomycin (Clardy *et al*., 2006).

### 2.11.5. Preventing Drug Access To Target

An active efflux process can reduce access to drug discovered first with tetracycline or at least in Gram-negative bacteria to reduce the influx across the barrier of the outer membrane. The latter mechanism may be harmful to the bacterial growth since the influx of nutrients is also minimized. Nevertheless, it is also found in some species of enteric

bacteria as a way of „"last- resort"" resistance to current version of β-lactams that withstand inactivation by most common β-lactamases. The efflux process is now recognized for an important role it demonstrates in resistance to many drugs as a result of the discovery of multi-drug efflux pump (Brown and Wright, 2005).

# CHAPTER THREE

## 3.0. MATERIALS AND METHODS

## 3.1. MATERIALS

### 3.1.1. Study site and clinical specimens

The test organisms, clinical isolates of Methicillin Resistant *Staphylococcus aureus* (MRSA) and Methicillin Sensitive *Staphylococcus aureus* (MSSA) were obtained from the Central laboratory of Microbiology Department, Korle Bu Teaching Hospital (KBTH), Accra. The confirmation of the test organisms and evaluation of the antimicrobial activities of the fractions from *Psidium guajava*, *Alchornea cordifolia*, *Hoslundia opposita, Phyllanthus fraternus, Chromolaena odorata*, *Morinda lucida* and *Cnestis ferruginea* were done at the Microbiology Laboratory of the Department of Biochemistry and Biotechnology, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi. The extracts were also evaluated using seven standard bacterial strains of *K. pneumoniae* (ATCC 33495), *Proteus mirabilis* (ATCC 49565), *S. saprophyticus* (ATCC 15305), *Salmonella typhi* (ATCC 19430), *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922), and *P. aeruginosa* (ATCC 27853) obtained from the Microbiology Department, Centre for Plant Medicine Research (CPMR), Akuapem-Mampong.

### 3.1.2. Chemicals, media, and reagents

Iodonitrotetrazolium chloride (INT) was purchased from Fluka Biochemika (SigmaAldrich), Austria. Nutrient agar was obtained from Merck, Germany. Bacteriological Peptone, Dimethylsulphoxide (DMSO) and Sabouraud (4% Glucose) agar were obtained from Sigma-Aldrich, Germany. Mueller-Hinton agar and 30ug/disc chloramphenicol were purchased

from Oxoid Ltd, England. Ampicillin 30 µg/disc, Cefotaxime 30 µg/disc, Ceftriaxone 30 µg/disc, Cefuroxime 3 µg/disc, Cotrimoxazole 25 µg/disc, Gentamicin 10 µg/disc, and Tetracycline 30 µg/disc (Ernest Chemist, Accra) were purchased from Lynch Medical Services, Accra-Ghana.

### 3.1.3. Study design

This study is a quantitative experimental research conducted to address the problems of resistance shown by bacteria against some available broad spectrum synthetic antibiotics in managing or treating resistant bacterial infections. Two (2) treatment groups, comprising aqueous and ethanol extracts of seven (7) medicinal plants were evaluated against eleven (11) MRSA strains, twenty-four (24) MSSA strains and some seven (7) standard bacteria strains to explore nature"s plants for antibacterial agents against the strains. The samples were triplicated in a factorial design of 2x3x7x11, 2x3x7x24 and 2x3x7x7 respectively for MRSA, MSSA and standard bacterial strains. The quantitative data collected from the various diameter zones of inhibitions displayed on a glass plates were measured in millimeters using a ruler. The data collected were subjected to statistical analysis using two-way ANOVA under Bonferroni for which values compared at a significant level of 0.05 using GraphPad Prism version 5.0.

## 3.2. METHODS

### 3.2.1. Collection and identification of the plant materials

The plant materials (leaves) were harvested from the wild (Adawso in the Eastern Region, Ghana) early in the morning using cleaned machetes and knife. The collected plant materials were placed in separate clean baskets or mesh bags and transported immediately to the CPMR laboratories. The plant parts were authenticated by a

(Mention the name of the person) Taxonomist at the Plant Development Department, Centre for Plant Medicine Research (CPMR) at Akuapem-Mampong, Ghana, with sample voucher/specimen at the herbarium of the Department.

### 3.2.2. Preparation of crude extract from the plant materials

The fresh plant materials were exposed to the suitable preliminary processing like removal of unwanted materials and contaminants, washing, sorting and cutting. The fresh plant materials were air-dried in a shade at room temperature, pulverized and stored in air-tight bags

### 3.2.3. Aqueous extract preparation

Aqueous fractions (decoctions) of the plant materials were prepared as stated by the method of Mills-Robertson *et al*., (2014). Briefly, 500 g of the dried powdered plant sample was saturated in 5000 ml of water for 30 minutes and then boiled for another thirty minutes. The extracts were then concentrated by simmering at reduced temperature for 30 minutes. The resultant extracts were cooled, lyophilized and stored in a refrigerator at 4ºC until needed.

### 3.2.4. Ethanol extract preparation

Five hundred grams (500 g) of each pulverized plant materials was cold macerated with 70% ethanol for 3 days (Quasie, *et al*., 2010). The ethanol extracts were concentrated using Heidolph rotary evaporator (LABOROTIA 4000, Germany) at a temperature of 35 °C. The concentrates obtained were reconstituted in water (500) and the process repeated to ensure complete removal of residual ethanol.. Twenty five millilitres (25 ml) of the concentrated ethanol

extracts were poured into various flasks and lyophilized using a Heto Power Dry LL3000 freeze-dryer (Jouan Nordic, R507/200 gr., Germany) for 24 hours. The dried powders were stored in air-tight containers and refrigerated until needed (Quasie, *et al*., 2010).

**3.2.5. Preparation of ethanol and aqueous stock solutions for the bioassays** In the preparation of stock solutions for the bioassays, 100 mg/ml, 150 mg/ml, and 200 mg/ ml of the lyophilized ethanol products were prepared using 20% DMSO (Sigma, D5879, Germany) for the determination of the antimicrobial activity assay. In the case of the aqueous extracts concentrations of 100 mg/ml, 150 mg/ml and 200 mg/ml of lyophilized aqueous products were prepared using sterile distilled water. The stock solutions were stored in a refrigerator until needed.

## 3.3. PHYTOCHEMICAL SCREENING OF THE EXTRACTS

The methods defined by Odebiyi and Sofowora (1978), and Fennell *et al*. (2004), were used to screen for the presence of the phytoconstituents in both the ethanol and aqueous extracts. The phytoconstituents assayed included saponins, cardiac glycosides, phenols, tannins, alkaloids, flavonoids and terpenoids.

### 3.3.1. Test for Alkaloids (Wagner's reagent)

A fraction of the extract (2 ml) was treated with 3-5 drops of Wagner‟s reagent (2 g of potassium iodide and1.27 g of iodine in 100 ml of water) and the formation of reddish brown colouration (or precipitate) was observed indicating presence of alkaloids.

### 3.3.2. Test for flavonoids (Alkaloid reagent test)

Two millilitres (2 ml) of the extract was mixed with few drops of 20% sodium hydroxide solution. Formation of deep yellow colour which became colourless on addition of dilute hydrochloric acid, indicated the presence of flavonoids.



### 3.3.3. Test for phenols (Ferric chloride test)

A fraction (2 ml) of each extract was treated with aqueous 5% ferric chloride ($FeCl_3$) and observed for the formation of deep blue or black colour indicating presence of phenol..

### 3.3.4. Saponins (Foam test)

Two millilitres (2 ml) of each extract was added to 6 ml of water in a test tube. The mixture was shaken strongly and observed for formation of stable foam that confirmed the presence of saponins.

### 3.3.5. Tannins (Braymer's test)

Two millilitres (2 ml) of each extract was treated with 10% alcoholic ferric chloride solution and observed for the formation of blue or greenish colour solution.

### 3.3.6. Terpenoids (Salkowki's test)

One millilitre (1ml) of chloroform was added to 2ml of each extract followed by a few drops of concentrated sulphuric acid ($H_2SO_4$). A reddish brown precipitate produced instantly indicated the presence of terpenoids.

### 3.3.7. Cardiac glycosides (Keller Kelliani's test)

Five millilitres (5 ml) of each extract was treated with 2 ml of glacial acetic acid in a test tube and a drop of ferric chloride solution ($FeCl_3$) added (Sayed, 2016). The test tube was slightly tilted and 1ml concentrated sulphuric acid gently poured into the test tube. A brown ring at the interface indicated the presence of deoxysugar characteristic of cardenolides. A violet ring may appear below the ring while in the acetic acid layer, a greenish ring may form.

## 3.4. LABORATORY ANALYSIS

### 3.4.1. Culture interpretation and biochemical identification test

The cultured bacteria were morphologically identified on the agar plates. Differential media of CLED, DCA and MacConkey agars aided in distinguishing Gram-negative bacteria that were lactose fermenters (LF) and non-lactose fermenters (NLF). Gram staining, biochemical, and other microbiological procedures for *Echerichia coli, Proteus mirabilis, Salmonella typhi, Klebsiella* spp. and *Pseudomonas aeruginosa* were also performed as defined by Cowan and steel, (1993). A portion of the bacteria colony was transferred from the media to a drop of physiological saline on a slide with a sterile inoculating loop and it was spread evenly on the slide. The smear was fixed on the slide by flaming using Bunsen burner and allowed to air dry. The bacteria smear was stained with crystal violet for one (1) minute. Acetone-alcohol (1:1) was carefully dropped onto the smear, allowed to run off and then washed with water. Safranin was used as a counter stain for one (1) minute and finally washed off with water. The slides were allowed to air dry and examined under low power 100X objective oil immersion. The gram-positive bacteria stained blue or purple whilst gram-negative, light pink.

Additionally, oxidase tests were performed on all the NLFs to aid in the identification of *Pseudomonas* species, which are oxidase positive. In this procedure, a small size filter paper was saturated with a few drops of oxidase reagent (tetra-methyl-*p*phenylenediamine dihydrochloride) and a colony of bacteria was picked with an inoculating loop and the microbe smeared on the filter paper. *Pseudomonas* bacteria oxidize the oxidase reagent to a deep purple colour (Gordon and Mcleod, 1928). Isolated colonies from pure Gram-negative rod (GNR) bacteria were picked using sterile bacteriological wire loops and inoculated into tubes of peptone water (Liofilchem, Roseto Degli Abruzzi, Italy), urea agar slants ( Liofilchem, Roseto Degli Abruzzi, Italy), citrate slants ( Liofilchem, Roseto Degli Abruzzi, Italy), triple sugar iron agar slants (Liofilchem, Roseto Degli Abruzzi, Italy), and motility test media. The inoculated biochemical media were incubated aerobically at 35-37$^{o}$C for 18 to 24 hours. The biochemical reactions were examined after the incubation. Drops of Kovac"s reagent (Oxoid, Hamphire, UK) were added to peptone water cultures for reactions suggestive of indole production. For each isolate, reactions of biochemical tests were compared to that of reference strains for species identification.

### 3.4.2. Disk diffusion susceptibility of MRSA to the test extracts

The National Committee for Clinical Laboratory Standard method was used (NCCLS, 2000). Direct colony inoculum preparation was performed using clinical isolates of the bacteria swabbed on Mueller Hinton agar. One microgram (1 ug) oxacillin disk was then placed on the Mueller Hinton agar and incubated for 24 hrs at 35° C. The results were interpreted as follows; Resistant (MRSA) : ≤10 mm zone size of inhibition; confirmed with Oxacillin Screening Agar: 11-12 mm zone size of inhibition, Susceptible ≥13 mm zone size of inhibition indicated MSSA.

### 3.4.3. Oxacillin agar screening test for MRSA

The oxacillin screening plate was used in addition to or as a backup method to detect MRSA. This method was adopted from the National Committee for Clinical Laboratory Standards (NCCLS, 2000). Mueller Hinton agar with 4% NaCl and 6 ug/ml of Oxacillin was prepared. A direct colony inoculum preparation of the bacteria was also made. An inoculating loop was dipped into test tube containing inoculum suspension and streaked to a quadrant of the plate. It was then incubated for 24 hours at 35 °C. The result was interpreted as greater than one (> 1) colony or light film of growth implying oxacillin/methicillin-resistant (Wei Wang, 2009).

### 3.4.4. MRSA-screen latex agglutination test

#### 3.4.4.1. Reagent preparation

The kits were first brought to room temperature and the reagents used as supplied by the manufacturer. The reagents were gently shaken to ensure latex formed homogenous suspensions before use.

#### 3.4.4.2. Latex agglutination procedure

Each specimen was alloted and labelled two circles on the test card, one as test and the other as control. Fifty micro liters (50 µL) of the specimen was placed onto each of the test and the control circles. To the test circle, 1 drop (25 µL) of Sensitized Latex was added and to the control circle,1 drop (25 µL) of Control Latex was also added. With separate mixing sticks, each specimen was thoroughly mixed over the area of the circle. The test card was rotated by hand or mechanical rotary platform for 3 minutes. The test card was

placed on the bench and the agglutination patterns read. Thus clear agglutination within three minutes indicated the presumptive presence of PBP2'.

### 3.5. Inoculum preparation

The stock cultures of MRSA, MSSA and selected standard bacteria strains were subcultured onto fresh nutrient agar (Marck 1.05450, Germany) plates and incubated for 24 hrs. at 37ºC to obtain pure working cultures and stored in a refrigerator. Well-isolated colonies of three to five of the same morphological type of each organism were suspended in test tubes containing 5 ml of sterilized Mueller-Hinton broth (Fluka 70192, Spain) and incubated overnight at 37 °C for between 14 and 16 hours. The overnight culture was then sub-cultured onto fresh sterilized Mueller-Hinton broth and incubated for between 2 to 4 hours to attain the turbidity of 0.5 McFarland standards (Mills-Robertson *et al*., 2009).

### 3.6. ANTIMICROBIAL ACTIVITY ASSAY (MODIFIED AGAR DIFFUSION METHOD)

A modification of the agar diffusion method was used to investigate the antimicrobial activities of the crude extracts as designated by the National Committee for Clinical Laboratory Standards (2002) and Mills-Robertson *et al.*, (2014; 2015). The process involves time limit of 15 minutes. After the turbidity of the inoculum suspension was adjusted, a sterilized swab was aseptically immersed into the suspension, rotated numerous times and firmly pressed on the inner wall of the tube above the fluid to eliminate excess inoculum from the swab. The dried surface of a Mueller-Hinton agar (Oxoid, CM0337, Oxoid Ltd, England) plates were inoculated by swabbing the bacteria on the entire surface of the sterile agar. This method was repeated two more times, turning

the plate approximately 60° each time to ensure a uniform distribution of inocula. A sterilized cock borer of an internal diameter of about 4 mm was used to punch five holes in the medium. A sample each of 100, 150 and 200 mg/ml of the plant extracts were dispensed into the respective labeled holes. Antibiotic disc of 30 µg chloramphenicol (Oxoid, CT00143 Oxoid Ltd, England) and 20% v/v DMSO (Sigma, D5879, Germany) were used as positive and negative controls respectively. Triplicates of each plate were made and the procedure repeated. The plates were kept in the refrigerator for about 4 hours for complete diffusion of the extract and incubated at 37°C for 24 hours (Mills-Robertson *et al*., 2015). After the incubation period, a sterilized ruler was used to measure the diameter of each zone of inhibition in millimeters (mm).

### 3.6.1. Susceptibility of the Isolates to Antibiotics

All the isolates were subjected to antimicrobial susceptibility test using the Kirby-Bauer disc diffusion method as described by NCCLS (2002). Peptone water subcultures from 16-18 hours cultures which had achieved the 0.5 McFarland turbidity standards were applied to seed uniformly the entire surface of the Mueller-Hinton agar plates. After allowing the plates to dry, antibiotics disks [ampicillin (10 µg/disc), penicillin (10·ug/disc), cloxacillin (5 µg/disc), erythromycin(15 µg/disc), flucoxacillin (30 µg/disc), tetracycline (30 µg/disc), gentamicin (10 µg/disc), cotrimoxazole (25 µg/disc), chloramphenicol (30 µg/disc)], and some newer generation antibiotics including cefuroxime (30µg/disc)] were carefully placed on the surface of the plates and incubated at 37 ºC for 16-18 hours. The zones of inhibition of the various antibiotics were measured with a meter rule by taking the diameter of the zones. The results were compared with the

standard antimicrobial sensitivity chart and recorded as sensitive, intermediate or resistant to the antibiotics.

### 3.6.2. Micro–dilution method

The Minimum Inhibitory Concentration (MIC) was determined for the most active extracts. Tetracycline (MAST Diagnostics, Bootle, United Kingdom) was used to perform micro-dilution test. Twenty five milliliter (25 ml) stock solution of tetracycline (30 µg/ml) was prepared by following manufacturer‟s instruction. The stock solutions were stored at 2-8$^0$C for maximum of 7 days. Mueller-Hinton broth (Liofilchem, Roseto Degli Abruzzi Italy) was used as the diluents for the Minimum Inhibitory Concentration (MIC) test because it acted as a medium for the growth of the bacteria. Each test isolate was emulsified in the Mueller-Hinton broth (MHB) and incubated aerobically for 8-12 hours at 35-37$^o$C. The inoculum was then adjusted to 0.5 McFarland standard and the suspension further diluted to provide an inoculum of $10^5$cfu/ml.

**3.6.2.1. Determination of minimum inhibitory concentration (MIC)** The MIC values of the crude ethanol extract and that of aqueous extract and their fractions were determined using the microplate dilution as described by Eloff (1998). One hundred microlitres (100 µl) each of 100, 150 and 200 mg/ml of the ethanol extract were added to 100 µl of sterile bacteriological peptone in the first well in the 96-well microplate separately and mixed well with a micropipette. One hundred microlitres (100 µl) of this dilution was transferred to the bacteriological peptone in subsequent wells yielding two-fold serial dilution in the original extract. The process was repeated for the other fractions in other columns of the microplate. A reference antibiotic, tetracycline (30 µg/ml) was

also serially diluted in another column of the microplate as a positive control test. Hundred microlitres (100 µl) of actively growing test organisms (0.5 McFarland) was added to each of the dilutions except the negative control, and the microplates covered and incubated at 37 $^{0}$C for 24 hours. After the incubation period, 40 µl of 0.2 mg/ml Indonitrotetrazolium chloride (INT) was added to each of the wells. The microplates were then examined after additional 30 to 120 minutes incubation. Bacterial growth was indicated by a red colour (conversion of the INT to formazan), and the lowest concentration at which the red colour is apparently invisible compared to the next dilution was taken as the MIC value.

#### 3.6.2.2. Determination of the minimum bactericidal concentration (MBC)

The MBC values were deduced from those wells with lowest concentrations at which no growth (colour development) was detected after culture for 24 hours of incubation as described by Nester *et al.* (2004). Briefly, a drop of sample from each of those wells were transferred to fresh nutrient agar plates and incubated for 24 hours at 37 °C. The plates were subsequently examined for the presence or absence of living organisms. The minimum concentration at which plates showed no microbial growth was regarded as the MBC

### 3.7. STATISTICAL ANALYSIS OF DATA

The results were analyzed using GraphPad Prism version 5. The mean zones of inhibition for each isolate were analyzed and the mean difference between the extract types were compared using two-way ANOVA under Bonferroni at a P-value < 0.05 considered statistically significant.

## 3.8. ETHICS

This study received ethical clearance from the Research and Protocol Review Committee on Human Research Publications and Ethics of School of Medical Sciences, Kwame Nkrumah University of Science and Technology (KNUST) and Komfo Anokye Teaching Hospital (KATH), Kumasi.

# CHAPTER FOUR

## 4.0. RESULTS

## 4.1. PHYTOCONSTITUENTS OF THE TEST PLANTS

The study discovered the presence of phytoconstituents like saponins, flavonoids, phenols, tannins and alkaloids in the aqueous fractions of all the seven different plants. Terpenoids were absent in *Alchornea cordifolia* and *Phyllanthus fraternus* whilst glycosides were absent in *Cnestis ferruginea*, *Hoslundia opposita* and *Chromolaena odorata* (Table1).

For the ethanol extract, the active compounds present were saponins, alkaloids, flavonoids, phenols, tannins, and terpenoids in all the seven extracts. Alkaloids and glycosides were absent in *Chromolaena odorata* while glycosides were absent in *Alchornea cordifolia*, *Cnestis ferruginea* and *Phyllanthus fraternus* (Table 1).

**Table 1: Phytoconstituents identified in the aqueous and ethanol extracts**

| Parameters | *Psidium guajava* | | *Morinda lucida* | | *Alchorneacor difolia* | | *Cnestis Ferruginea* | | *Hoslundia opposite* | | *Chromolaena odorata* | | *Phyllunthus fraternus* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Aq | Eth | Aq | Eth | Aq | Eth | Aq | Eth | Aq | Eth | Aq | Eth | Aq | Eth |
| Saponins | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Alkaloids | + | + | + | + | + | + | + | + | + | + | + | - | + | + |
| Flavonoids | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Phenols | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Tannins | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Terpenoids | + | + | + | + | - | + | + | + | + | + | + | + | - | + |
| Cardiac Glycosides | + | + | + | + | + | - | - | - | - | + | - | - | + | - |

+ = POSITIVE - = NEGATIVE Aq = AQUEOUS Eth = ETHANOL

## 4.2. BIOCHEMICAL IDENTIFICATION OF TEST MICROBES

Latex agglutination test was used and clear agglutination within three minutes (3 mins) indicated the presumptive presence of Penicillin-Binding Protein 2' (PBP2') which confirmed the presence of MRSA.

## 4.3. ANTIBIOTIC SUSCEPTIBILITY TESTS

The antibiotic susceptibility test was performed on all the eleven (11) MRSA and twenty-four (24) MSSA strains based on the Kirby-Bauer disk diffusion technique as described by NCCLS (2002). The zones of inhibitions exhibited by standard antibiotics against MRSA were 15 mm for Erythromycin, 14 mm for Tetracycline, 19 mm for Cotrimoxazole,

22 mm for Gentamycin. The rest of the standard antibiotics could not inhibit the growth of the microbes (Fig. 4.1).

The zones of inhibition exhibited by the standard antibiotics against MSSA were 16 mm for ERY, 19 mm for TET, 23 mm for COT, and 24 mm for GEN. The rest of the standard antibiotics (AMP, CHL, FLU, CRX, PEN, and CLX) could not inhibit growth of the microbes (Fig. 4.2). Zones of inhibition for MSSA were greater than that of MRSA (Fig. 4.1 and Fig 4. 2).

It was observed that MRSA and MSSA strains were resistant to AMP, CLX, CHL, FLU, CRX and PEN, whilst the remaining antibiotics demonstrated varying degrees of resistance and susceptibility as indicated in Figures 4.1 and 4.2.

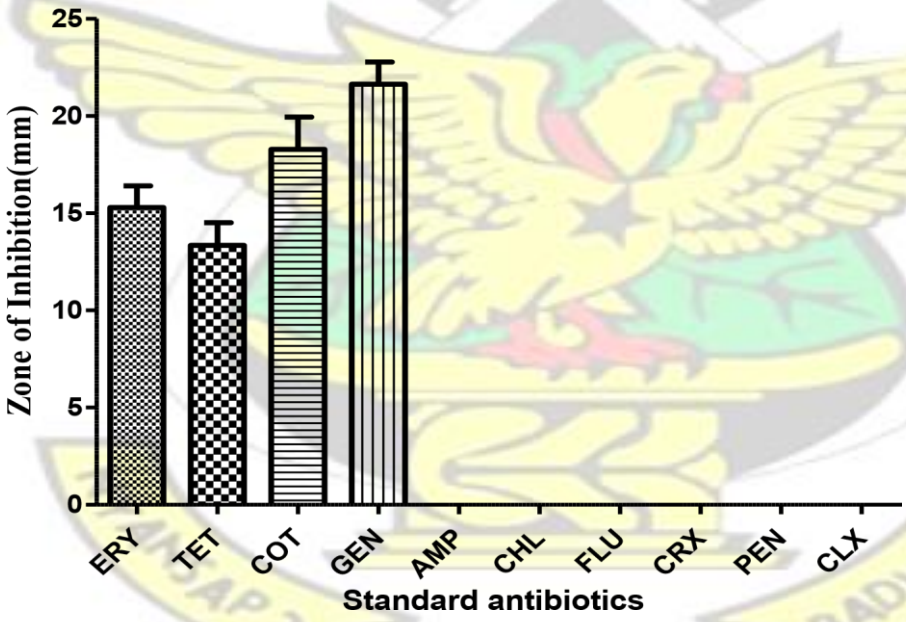



**Figure 4. 1: Susceptibility of the MRSA to the standard Antibiotics**

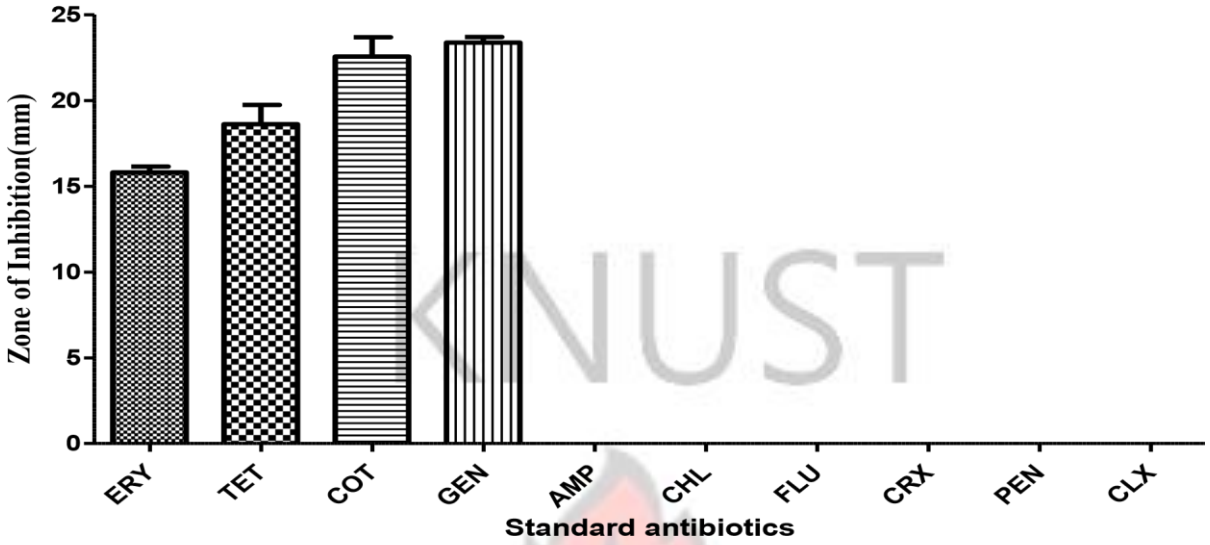



**Figure 4. 2: Susceptibility of the MSSA to the standard antibiotics**

## 4.4. ANTIBACTERIAL ACTIVITIES OF THE SEVEN MEDICINAL PLANTS

Amongst the seven different plants evaluated against the various bacterial strains, only *Psidium guajava* and *Alchornea cordifolia* had activity against the MRSA strains. A total of five plant extracts were effective against MSSA strains while four inhibited the growth of the standard bacteria strains.

### 4.4.1. Susceptibility of the MRSA strains to the *P. guajava*

All the eleven test strains of MRSA were susceptible to both ethanol and aqueous extracts of *P. guajava* with varying degree of susceptibility. The average zones of inhibition ranged from 10.00 to 23.70 mm for the ethanol extract and 15.30 to 21.00 mm for the aqueous extracts (Appendix 5). The average zone of inhibition of MRSA by the ethanol and the aqueous extracts of *P. guajava* were respectively 18.02 mm and 16.60 mm (Fig. 4.3). On the average, the ethanol extracts had higher zones of inhibition than the aqueous extracts.

### 4.4.2. Susceptibility of the MRSA strains to *A. cordifolia*

The *A. cordifolia* extracts inhibited the growth of all the isolated microbes used with zones of inhibition ranging from 10.70 to 28.30 mm and 15.00 to 27.30 mm for the ethanol and the aqueous extracts respectively (Appendix 6). The average zone of inhibition was 18.63 mm for ethanol and 16.30mm for the aqueous extract. On the average, the ethanol extracts had higher zones of inhibition than the aqueous extracts (Fig. 4.4).

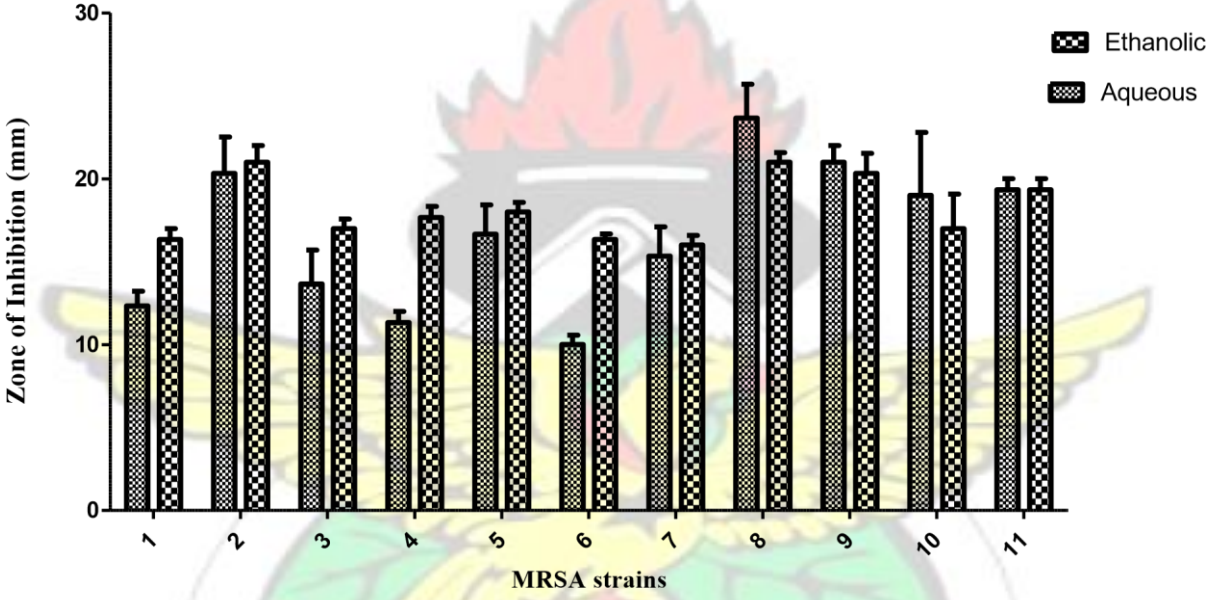


**Figure 4.3: Susceptibility of the MRSA to the extracts of *P. guajava***

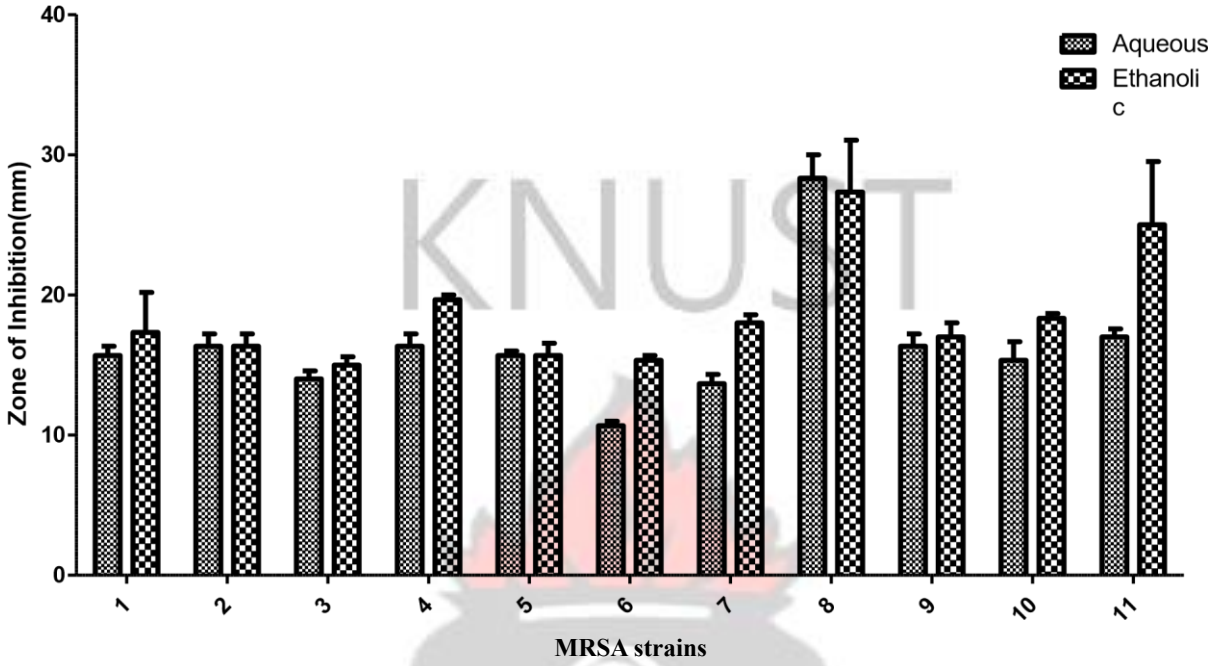



**Figure 4.4: Susceptibility of the MRSA to the extracts of *A. cordifolia***

### 4.4.3. Susceptibility of the MSSA strains to the seven medicinal plants

The results from the study indicated that, all the twenty four test microbes were susceptible to the ethanol and aqueous extracts of *Psidium guajava, Alchornea cordifolia, Hoslundia opposita*, *Phyllanthus fraternus*, and *Cnestis ferruginea* (Appendices 7-11). Five of the seven medicinal plants were effective against the MSSA and their average zones of inhibition were respectively 35.40 mm and 33.23 mm for the ethanol and aqueous extracts of *P. guajava,* 41.79 mm for ethanol and 30.78 mm for the aqueous extracts of *A. cordifolia,* 32.90 mm for ethanol and 29.98 mm for the aqueous extracts of *P. fraternus*, and 38.00 mm for ethanol and 37.54 mm for the aqueous extracts of *C. ferruginea*, In the case of the *H. opposita*, only the ethanol extract was reactive with an average diameter zone of inhibition of 16.08 mm (Figures 4.5 to 4.9.).

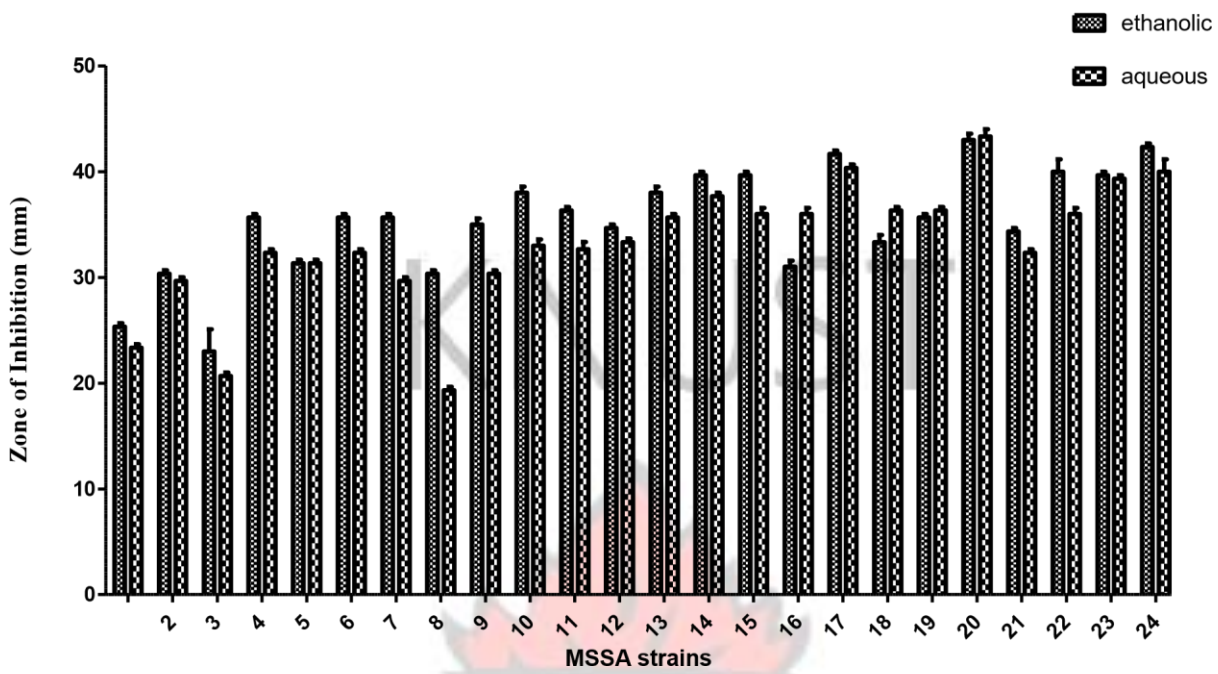



**Figure 4. 5: Susceptibility of the MSSA to the extracts of *P. guajava***

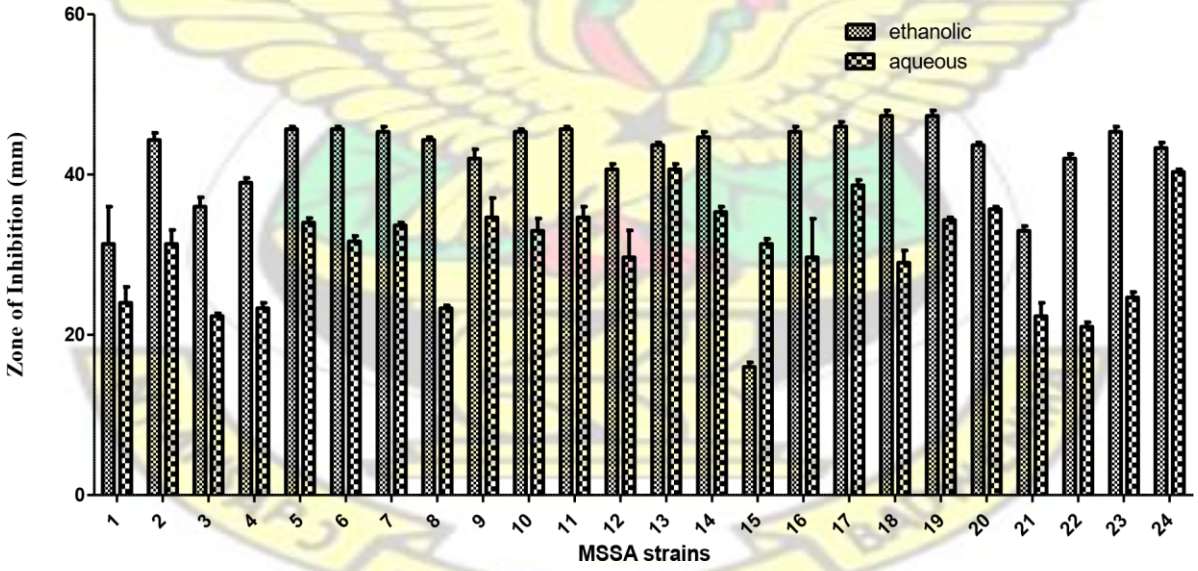



**Figure 4.6: Susceptibility of MSSA to the extracts of *A. cordifolia***

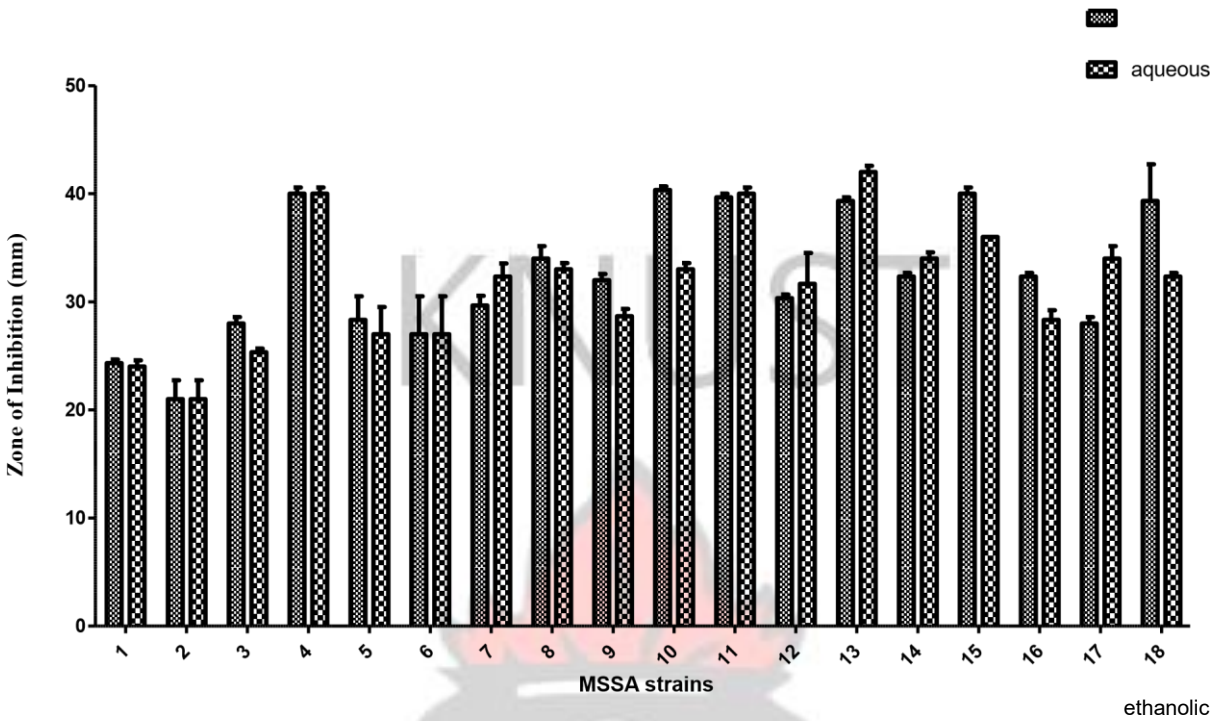



**Figure 4.7: Susceptibilityof the MSSA to the extracts of *P. fraternus***

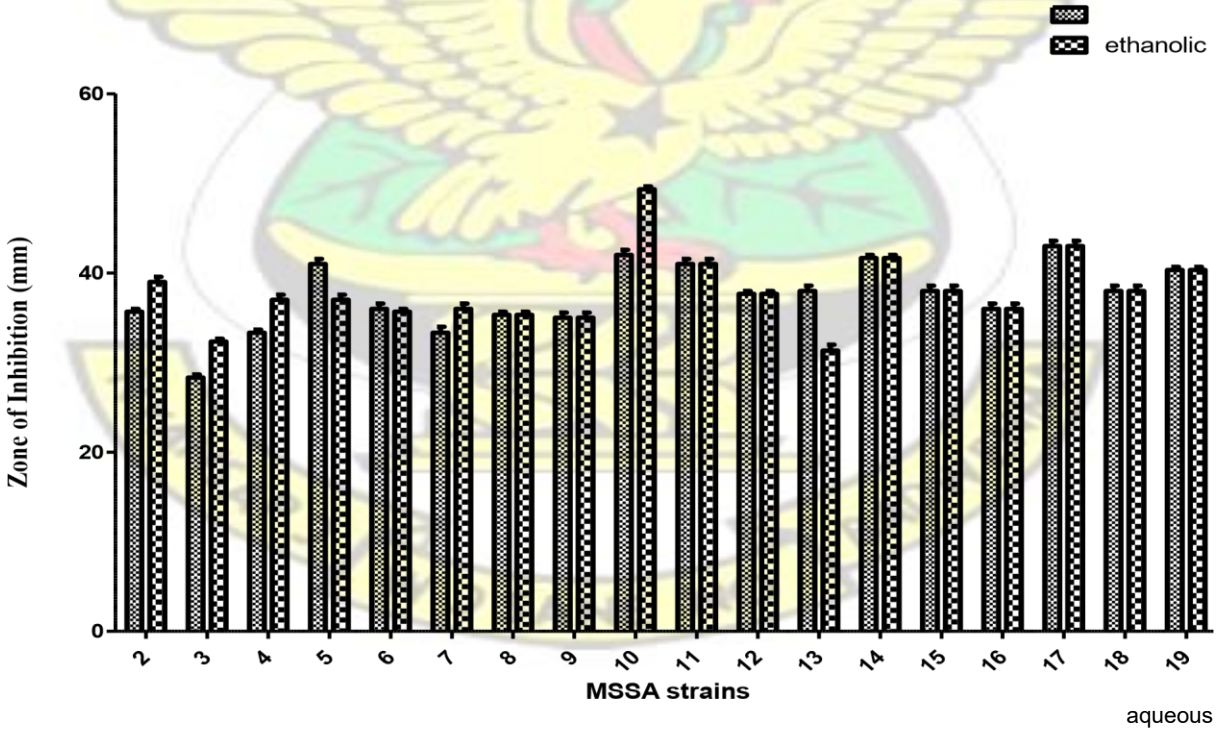



**Figure 4.8: Susceptibility of the MSSA to the extracts of *C. ferruginea***

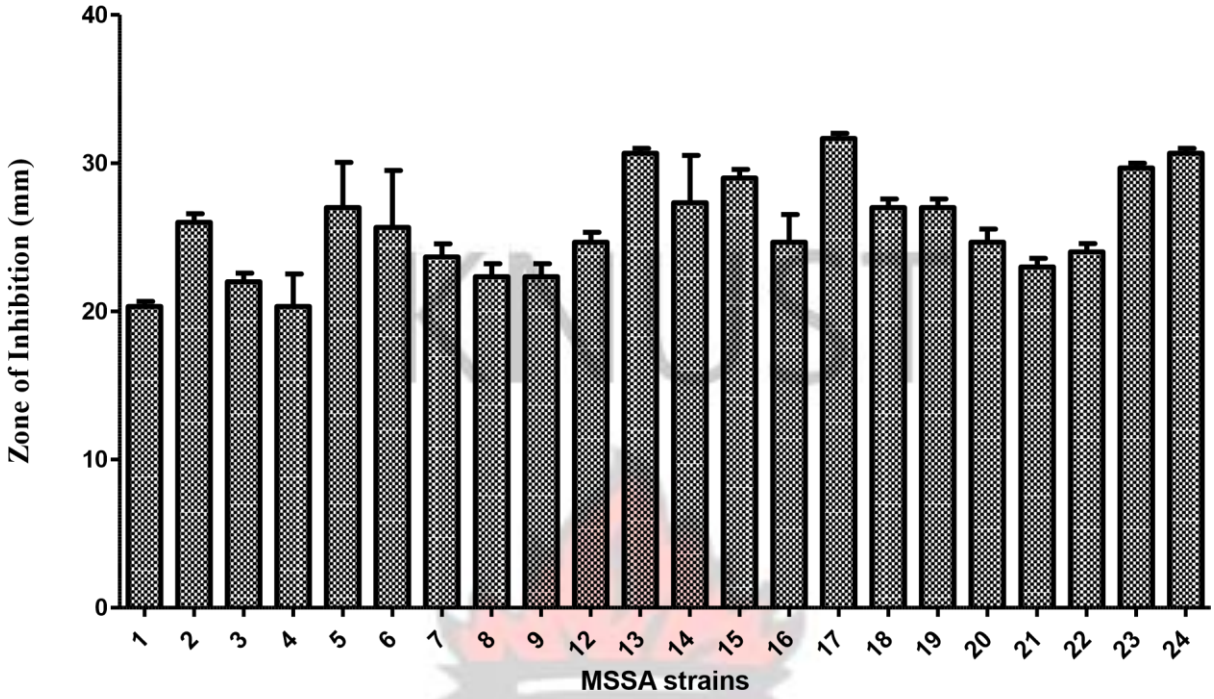



**Figure 4.9: Susceptibility of the MSSA to the ethanolic extract of *H. opposita***

**4.4.4. Susceptibility of the standard bacteria to the seven medicinal plants** The antimicrobial susceptibilities exhibited by the standard bacterial strains are presented in Appendices 3-4 and 14-21. Four of the seven plants were active against the standard bacteria strains. The zones of inhibition averaged 14.81 mm for the ethanol and 13.42 mm for the aqueous extracts of the *P. guajava*, 13.71 mm for ethanol and 13.00 mm for the aqueous extracts of *A. cordifolia,* 16.90 mm for the ethanol and 7.38 mm for the aqueous extracts of *P. fraternus*, 18.86 mm for the ethanol and 17.81mm for the aqueous extracts of *C. ferruginea* (Figures 4.10 to 4.13).

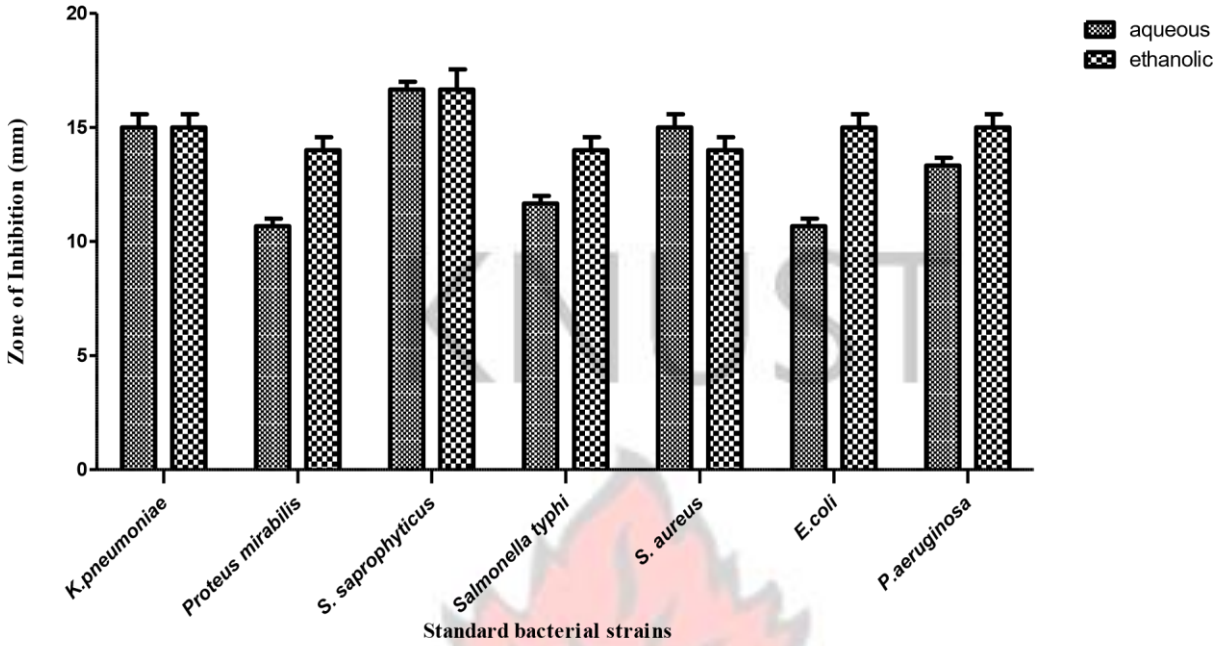



**Figure 4. 10: Susceptibility of the Standard bacteria to the extracts of *P. guajava***

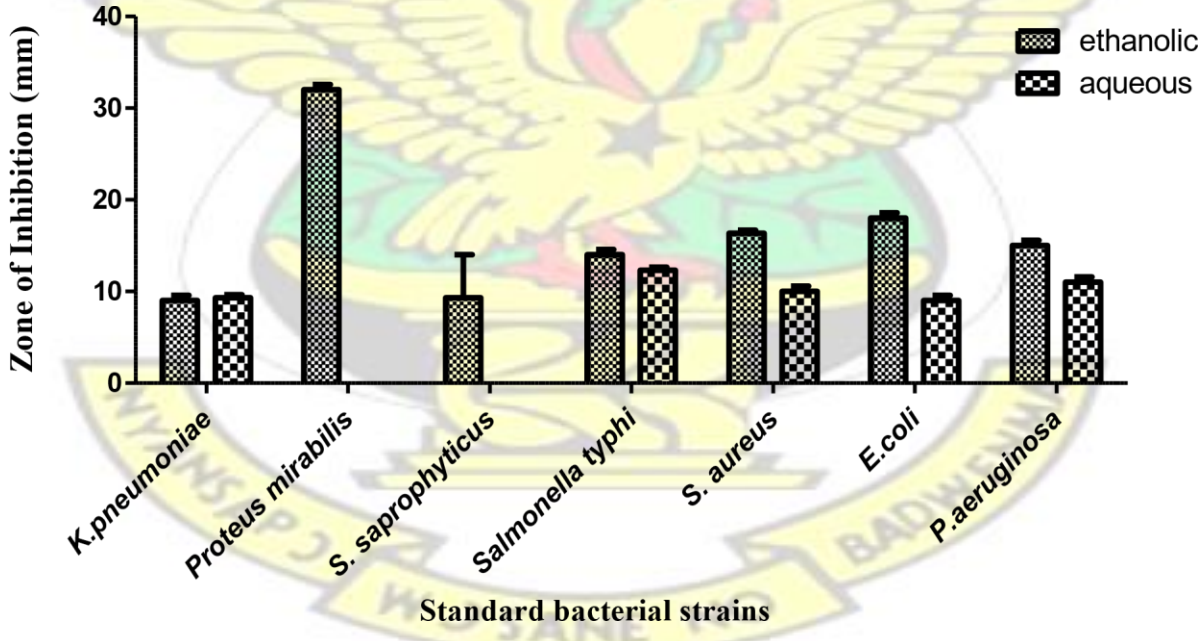



**Figure 4. 11: Susceptibility of the Standard bacteria strains to the extracts of *P. fraternus***

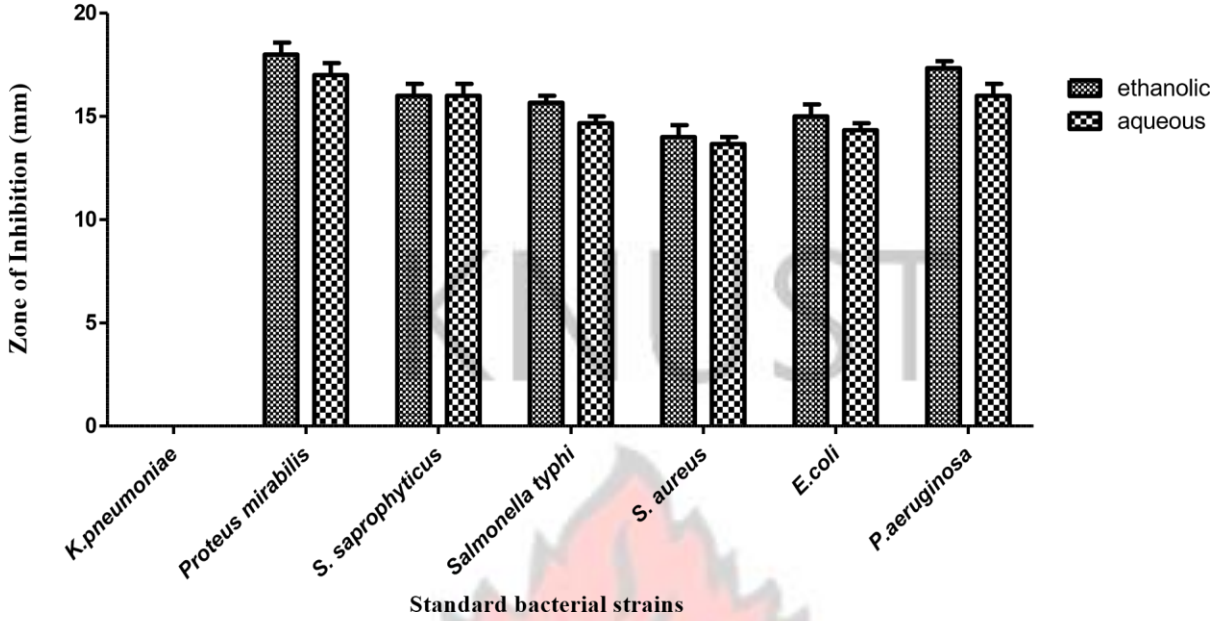



**Figure 4. 12: Susceptibility of the Standard bacteria to the extracts of *A. cordifolia.***

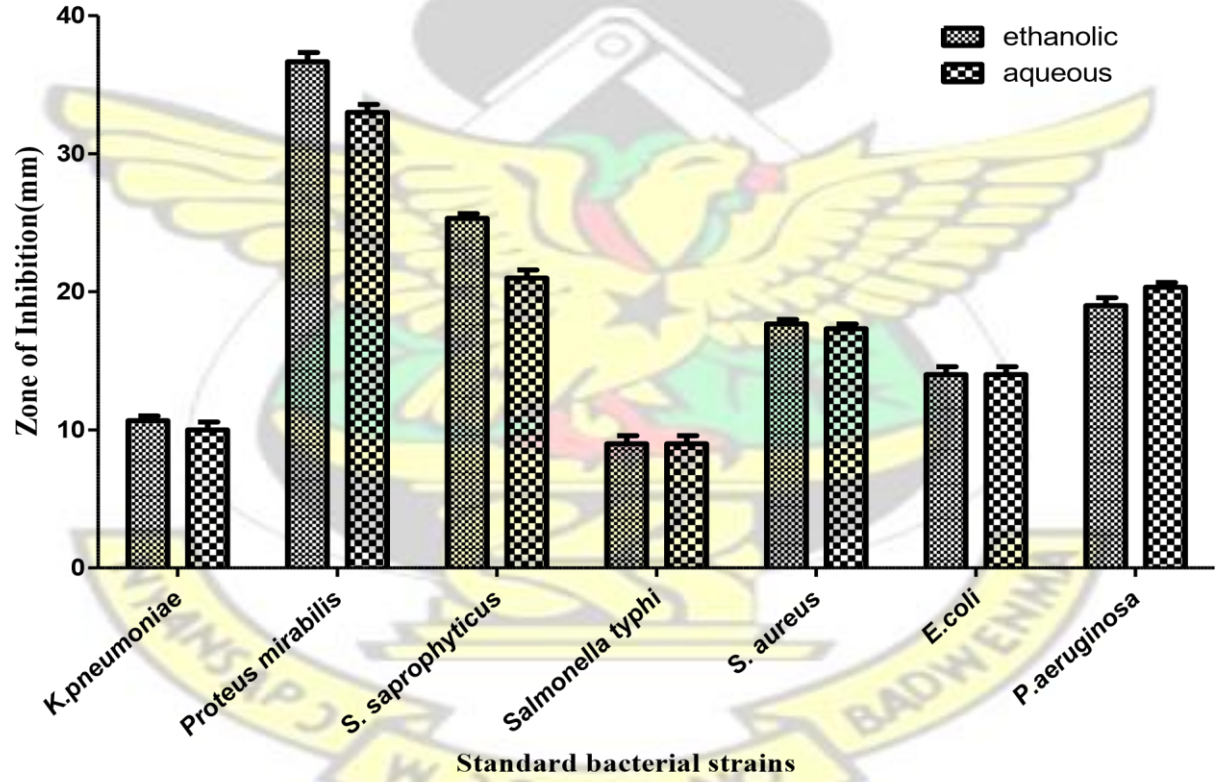



**Figure 4. 13: Susceptibility of the standard bacteria o the extracts of *C. ferruginea***

## 4.5. MICs of the extracts

The MIC values of the medicinal plant extracts determined against susceptible bacteria are shown in Appendices 12 and 13.

The MICs of *Alchornea cordifolia* on MRSA ranged from 0.195 to 0.390 mg/ml and 0.195 to 1.563 mg/ml respectively for the ethanol and the aqueous extracts (Figure 4.14). The average MICs was 0.28 mg/ml for ethanol and 0.59 mg/ml for the aqueous extract. The MIC and average MIC for tetracycline on MRSA were 3.75 µg/ml and 10.57 µg/ml respectively.

The MICs of the *Psidium guajava* on MRSA ranged between 0.195 and 0.390 mg/ml for the ethanol extract while that of the aqueous extract ranged from 0.195 to 0.781 mg/ml (Figure 4.15). The average MICs were 0.27 mg/ml for the ethanol and 0.44 mg/ml for the aqueous extract.

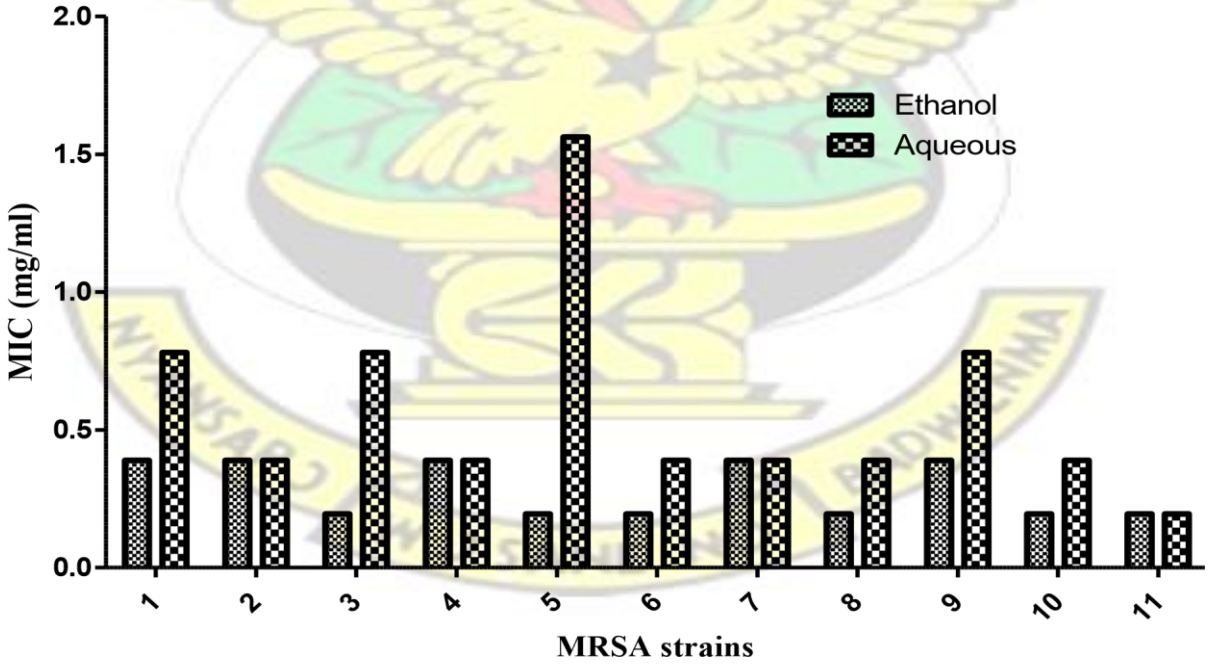


**Figure 4. 14: MICs of the extracts of *A. cordifolia* on the MRSA**

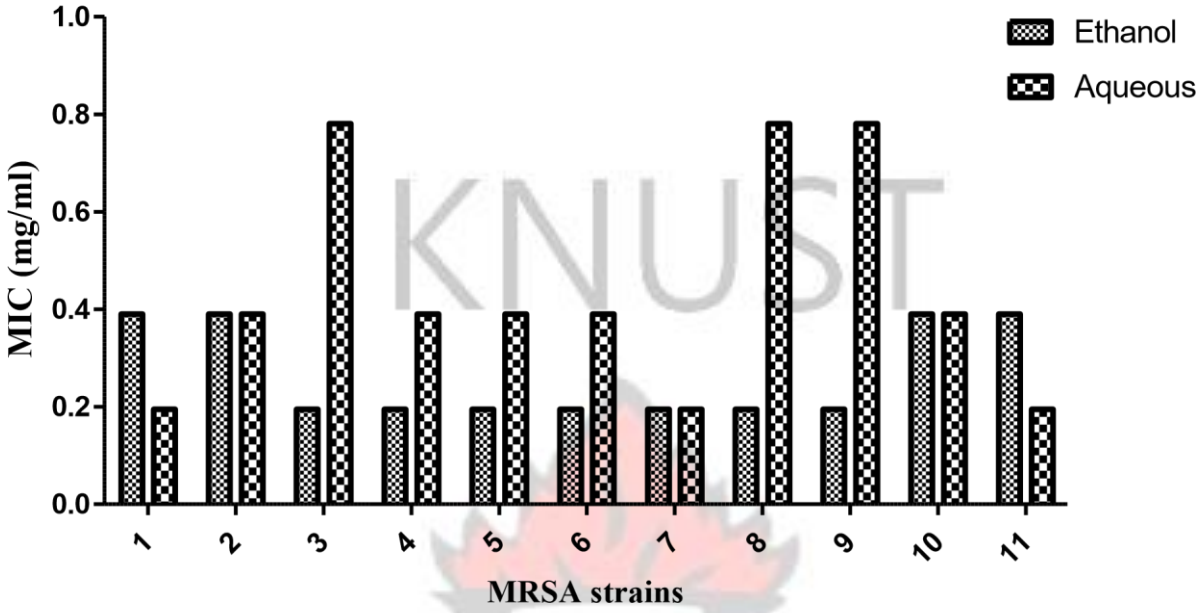



**Figure 4.15: MICs of the extracts of *P. guajava* on the MRSA.**

The MICs of the *Alchornea cordifolia* on MSSA ranged between 0.195 and 1.563 mg/ml for the ethanol extract while that of the aqueous ranged from 0.195 to 1.563 mg/ml (Figure 4.16). The average MICs were 0.45 mg/ml for ethanol and 1.28 mg/ml for the aqueous extracts. The MIC and the average MIC for tetracycline on MSSA were 3.75 µg/ml and 10.31µg/ml respectively.

In the case of the *Psidium guajava,* the ethanol extract had MICs ranged between 0.195 and 0.781 mg/ml while that of the aqueous ranged from 0.390 to 1.563 mg/ml (Figure 4.17). The average MICs were 0.36 mg/ml for ethanol and 1.33 mg/ml for the aqueous extract.

For *Cnestis ferruginea,* the ethanol extract had MICs ranged between 0.718 mg/ml and 1.563 mg/ml whereas that aqueous extract had 1.563 mg/ml for all test microbes. The average MICs was 0.68 mg/ml for ethanol and 1.56 mg/ml for the aqueous extract.

For the *Phyllanthus fraternus*, the ethanol extract had MICs that ranged between 0.195 and 1.563 mg/ml while that of the aqueous extract ranged from 0.390 to 1.563 mg/ml (Figure 4.18). The average MICs were 0.83 mg/ml for ethanol and 1.02mg/ml for the aqueous extract.

The *Hoslundia opposita*, on the other hand, had MICs ranged between 0.390 and 1.563 mg/ml for the ethanol. The aqueous extract was not active on MSSA strains. The average MIC was 0.28 mg/ml for the ethanol extract.

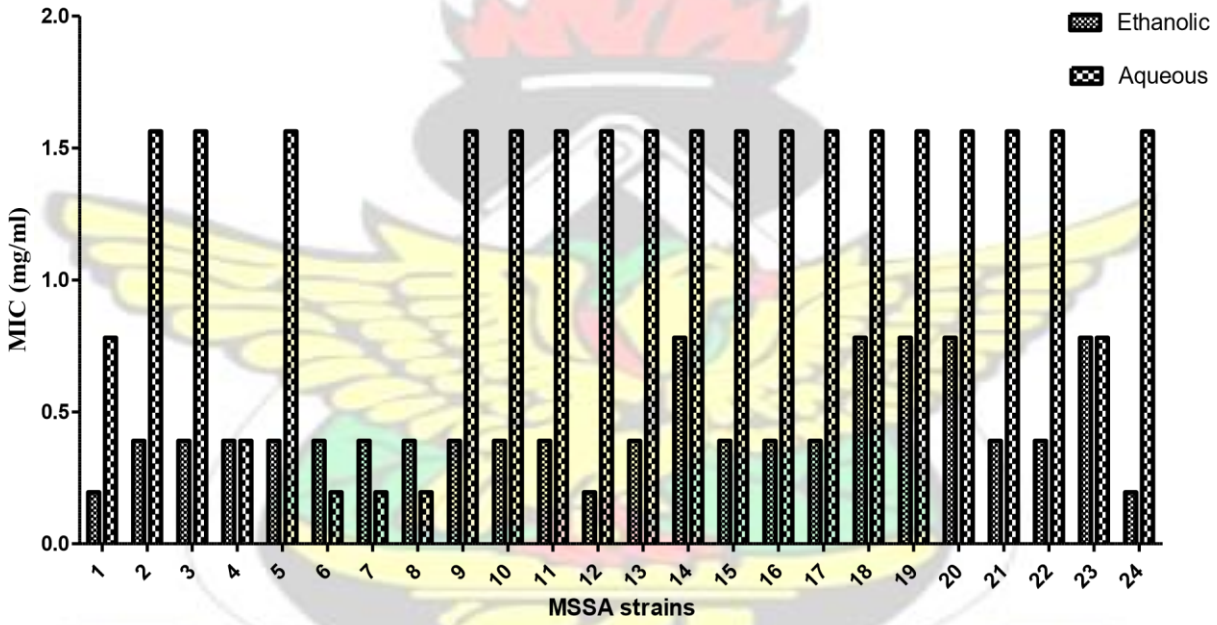



**Figure 4. 16: MICs of the extracts of *A. cordifolia* on MSSA**

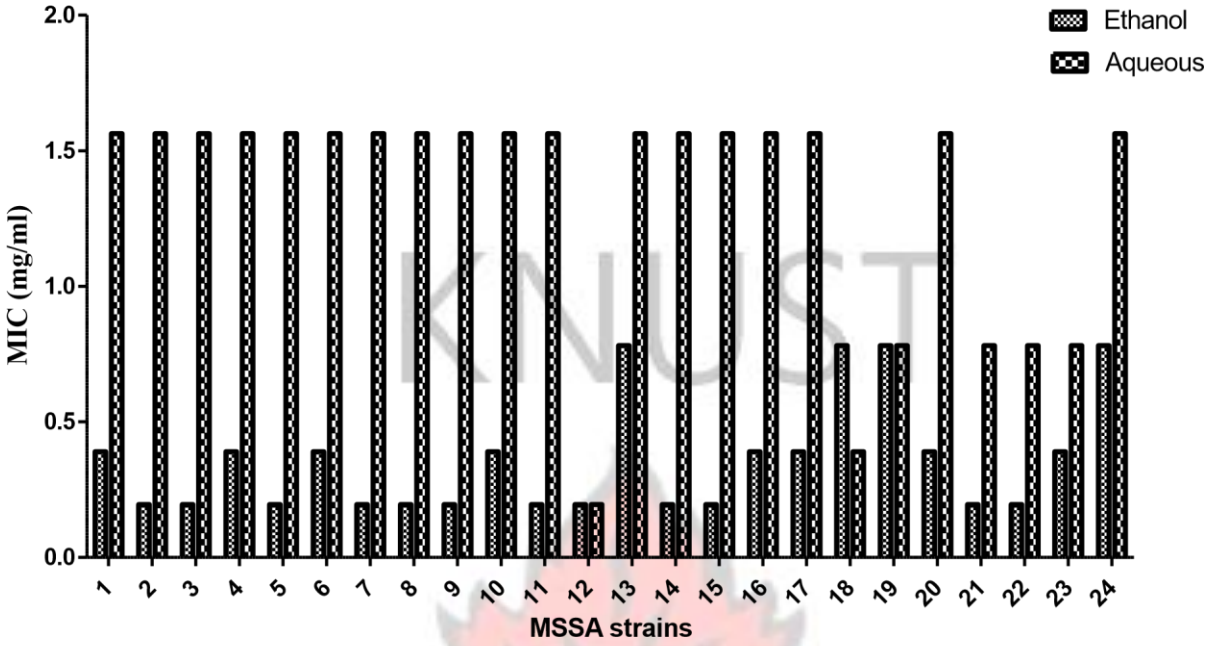



**Figure 4. 17: MICs of the extracts of *Psidium guajava* on the MSSA**

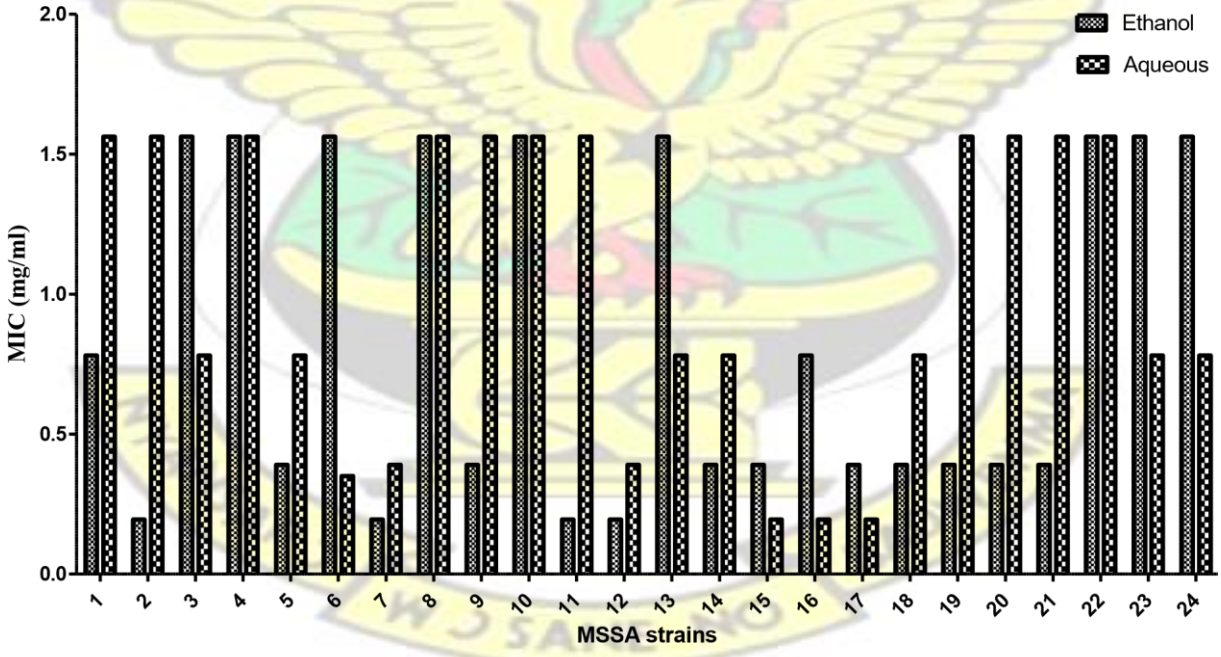


**Figure 4. 18: MICs of the extracts of *P. fraternus* on the MSSA**

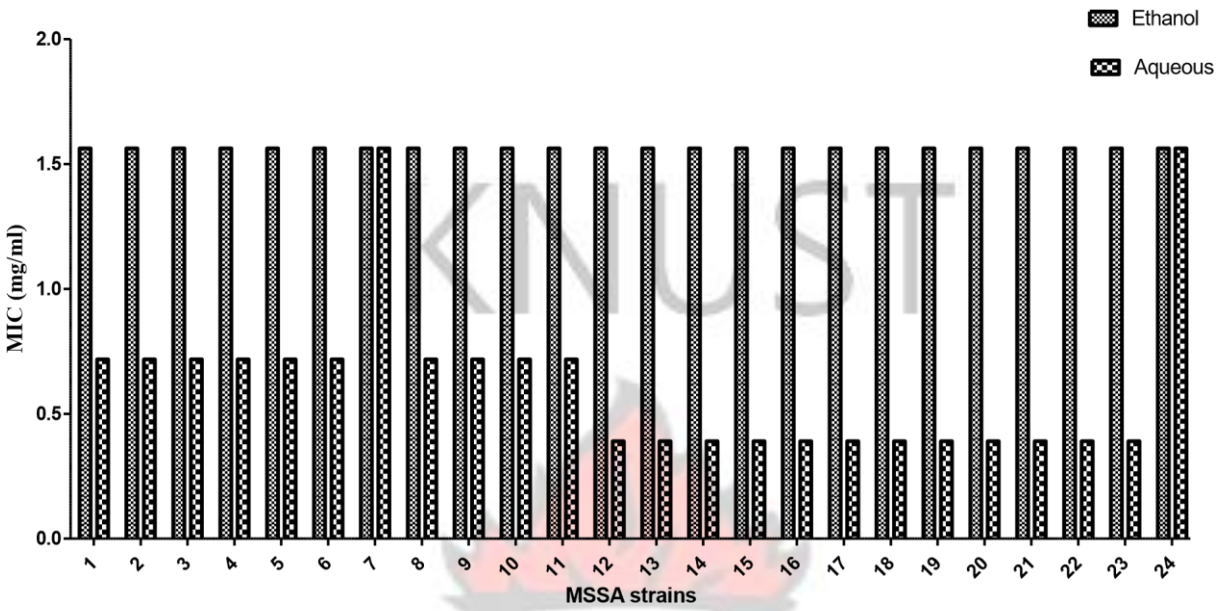



**Figure 4. 19: MICs of the extracts of *C. ferruginea* on MSSA**

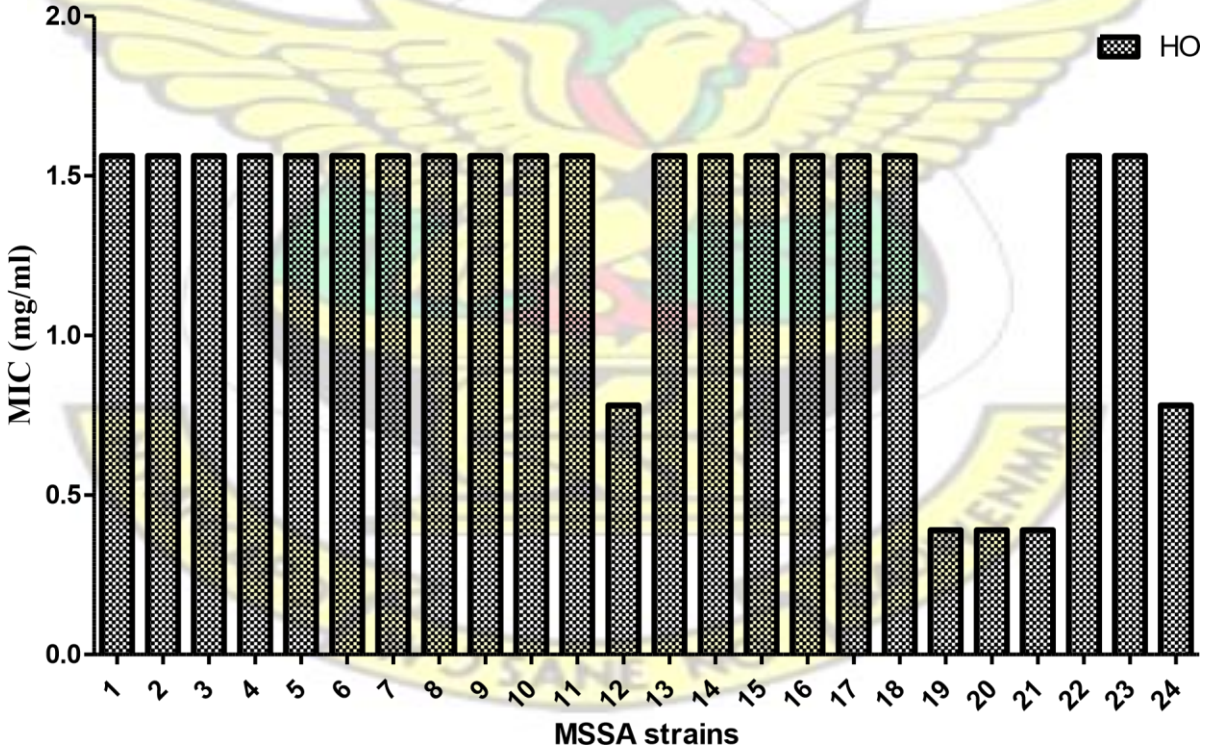



**Figure 4. 20: MICs of the ethanol extract of *H. opposita* on the MSSA**

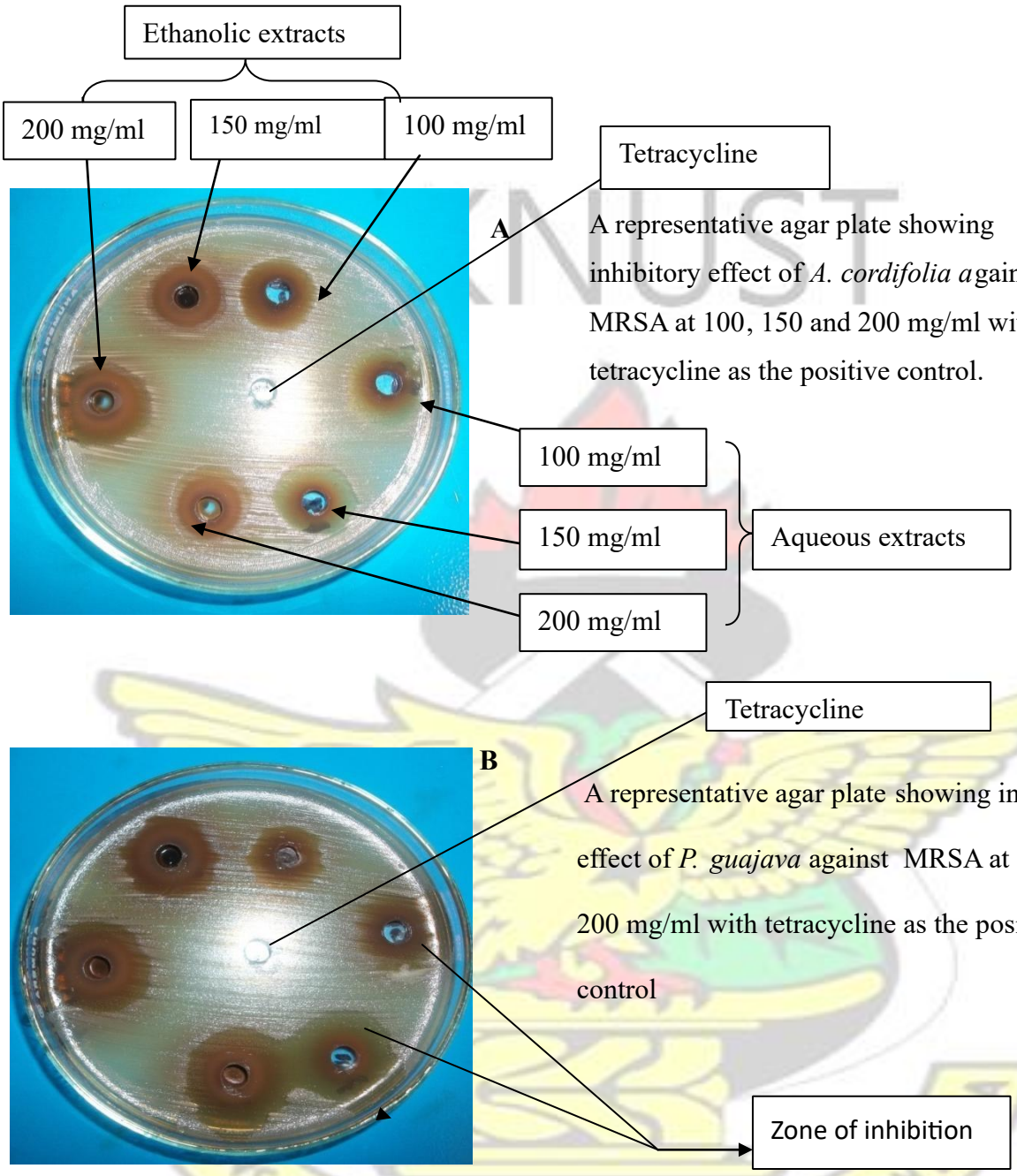
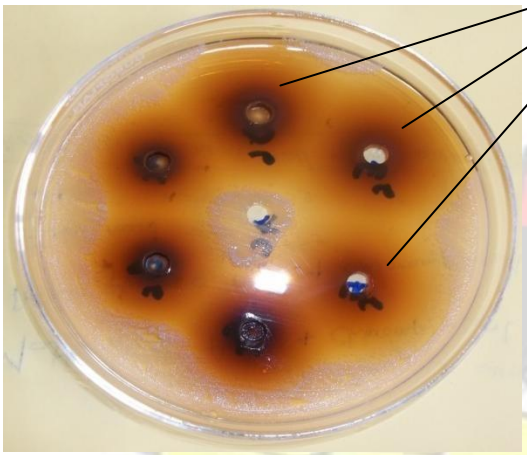
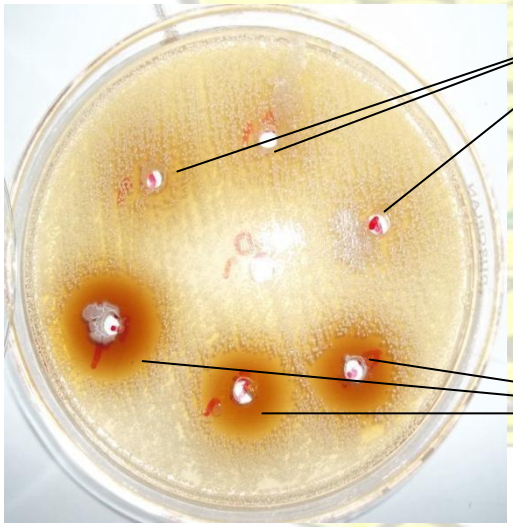



**Plate 8: A and B showing antimicrobial susceptibility test of MRSA against the selected medicinal plant extracts**



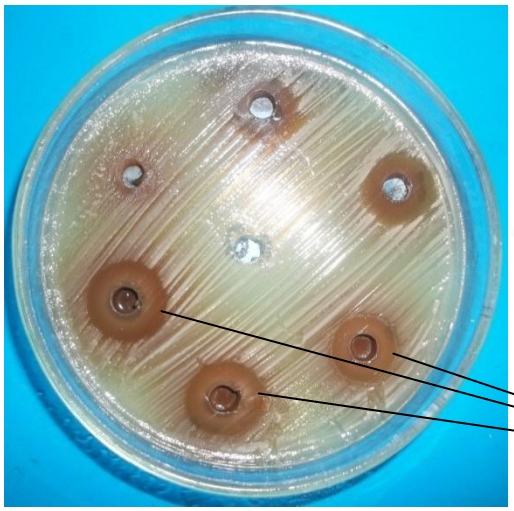

C. A representative agar plate of *Alchornea cordifolia* leaves extract showing inhibitory effect against clinical isolates of bacteria MSSA at 100, 150, 200 mg/ml .



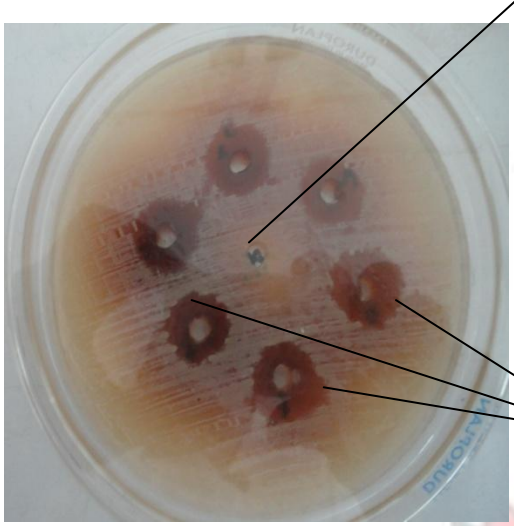

D. A representative agar plate of

inhibitory effect against clinical isolates of bacteria, MSSA at 100, 150, 200 mg/ml

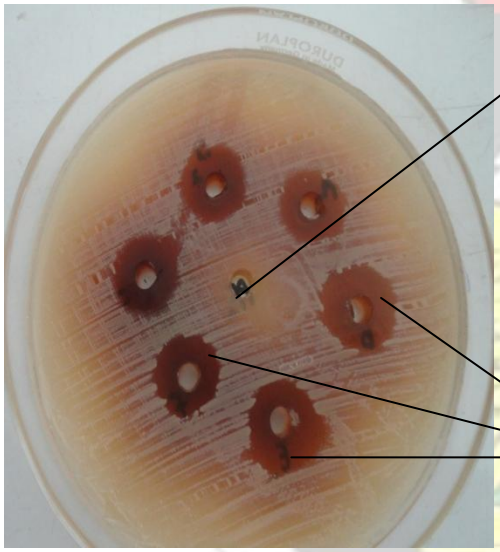
*Hoslundia opposita l*eaves extract showing




**E.** A representative agar plate showing inhibitory effect of *Phyllanthus fraternus* leaves extract showing inhibitory effect against clinical isolates of bacteria, MSSA at 100, 150, 200mg/ml




**F.** A representative agar plate of *Psidium guajava* leaves showing inhibitory effect against clinical isolates of bacteria MSSA.




**G**. A representative agar plate of *Cnestis ferruginea* leaves showing inhibitory effect against clinical isolates of bacteria MSSA at 100, 150, 200 mg/ml.

**Plate 9: C, D E, F, G showing antimicrobial susceptibility test of some selected medicinal plants extract against the MSSA in both aqueous and ethanolic**

# CHAPTER FIVE

## 5.0. DISCUSSION

The results of the phytochemical analysis on the crude extracts revealed the presence of medically active compounds in the seven different plants studied. Ethanol and water were able to extract the active compounds present in the leaves of the plants. There were, however, other plants whose active compounds were present in the aqueous extract but absent in that of ethanol extract. For example, cardiac glycosides were present in the aqueous extract of *Alchornea cordifolia* and *Phyllanthus fraternus* but not detected in the ethanolic extract while terpenoids were also not detected in aqueous extract but present in ethanol extract.

Effective determination of any biologically active compounds from plant material is mostly dependent on type of solvent used in the extraction process. Organic solvents usually used to extract bioactive compounds include acetone, ethanol and methanol (Eloff, 1998). Ethanol is the most usually utilized organic solvent by the manufacturers of herbal drug in the light of the fact that the final products can be securely utilized internally by purchasers of herbal concentrates (Low Dog, 2009). Also, the bioactivity of plant concentrates (extracts) relies on the water and ethanol concentration utilized as a part of the extraction procedure (Ganora, 2008). In spite of the fact that an awesome research has been performed to evaluate the antibacterial activity of medicinal plants, ideal extraction of bioactive compounds has not been well settled for several plants. Phytochemicals are noted to contribute medicinal in addition to physiological properties to plants in the curative treatment of numerous disorders. The presence of phytochemicals in plants, therefore, signifies as an important source for production of useful drugs (Radha

and Vijayakumari, 2013). Phytochemicals are commonly present in a wide range of plants and they are well-established to prevent the growth of bacterial pathogens (Cowan, 1999; Medina *et al*., 2005; Romero *et al*., 2005). The present study revealed the existence of saponins, flavonoids, alkaloids, phenols and tannins present in all the seven different plant extracts (Table1).

Saponin is a form of a glycosidic bond made up of a sugar molecule and the aglycone part normally called sapogenin. They are generally soluble in water which is polar hence its presence in the aqueous extract. The non-polar portion called aglycone dissolves in ethanol. Saponins have been shown to have antibacterial property with the method of activity ascribed to their capacity to bring about spillage of proteins and certain enzymes from bacterial cells. They are, however, harmless to human when taken orally and have beneficial property of lowering cholesterol level in the body (Amos-Tautua *et al*., 2011). Saponins are also important for the activity of cardiac glycosides according to Doughari (2012). Yadav and Agarwala (2011), reported that saponins have antimicrobial activity as well as inhibitory effect on inflammation. According to Doughari and Hamuel (2012), tannins are high molecular weight compounds and hydroxylated phenolic substance believed to be cytosolic poison which leak cellular content of microorganism (Marva-Manger *et al*., 2007).

Additionally, both aqueous and ethanol extracts of the seven different plants tested positive for phenols. Phenols are soluble in both polar and semi-polar solvents (Sharad and Swati, 2012). This confirms the results obtained for flavonoids and tannins in this study. Both tannins and flavonoids are phenolic compounds and thus possess basic

„acidic‟ hydroxyl group attached to an aromatic phenyl ring. Flavonoids are phenolic compounds (aromatic ring linked to C6 –C3 units) identified to be produced by plants in reaction to infections caused by microbe, hence possess antimicrobial agents against a varied range of microorganisms. Cowan (1999), reported that any phenol group attached by many hydroxyl groups are believed to be related to their relative toxicity to microorganisms, with confirmation that increased in hydroxylation is toxicity dependent. Flavonoid action is presumed to complex with bacterial cell wall aside complex with the extracellular and soluble proteins (Majorie, 1996). Additionally, they exhibit strong anticancer activities and are effective antioxidant as well (Okwu, 2004).

Alkaloids have been related to herbal medicine for many years with some of their common biological properties being cytotoxicity and antibacterial activity. Most alkaloids are readily soluble in alcohol and though they are sparingly soluble in water, their salts are usually soluble (Doughari and Hamuel, 2012). Thus, their presence in both the aqueous and ethanol extracts in this study. The antibacterial properties of alkaloids have been accounted for as a consequence of their capacity to intercalate with DNA of microorganisms to prevent cell division and growth (Abioye *et al*., 2003).

Cardiac glycosides are known to reduce pressure of the blood according to Radha and Vijayakumari, (2013) and Yadav and Agarwala, (2012). They are fundamentally steroids with an innate ability to perform definite and intense activity for the most part on the heart (cardiovascular) muscle when applied on man or animal through injection (Yadav and Agarwala, 2011).The aqueous extracts of *A. cordifolia* and *P. fraternus* in this study, tested positive for the presence of cardiac glycosides but their ethanol extracts tested negative. Cardiac glycosides are made up of glycosides and aglycone portions. According to Sharad

and Swati, (2012), the glycoside portion dissolves in water whilst the aglycone portion dissolves in organic solvents but a greater number of glycoside in a cardiac glycoside confers polarity, thereby increasing solubility, hence the presence of cardiac glycosides in the aqueous extracts.

Terpenoids are generally non-polar molecules and will dissolve well in non-polar solvents and semi-polar solvents. As a general rule, terpenoids tend to be oily and high percentage ethanol soluble (Rispail *et al*., 2005). This explains why the ethanol extracts (semi-polar medium) of *A. cordifolia* and *P. fraternus* had terpenoids but the aqueous extracts (polar) medium tested negative to terpenoids. Terpenoids have demonstrated some significant pharmacological activities such as anticancer, anti-inflammatory, antiviral, anti-malarial, anti-bacterial activities and inhibition of cholesterol synthesis (Mahato and Sen 1997). Terpenoids play a very important role by killing the

herbivorous insects and attracting mites that are useful (Kappers *et al*., 2005).

Amongst the seven plants evaluated, the extract of *Alchornea cordifolia* and *Psidium guajava* were found to be more active against all the MRSA and MSSA strains as well as the standard bacterial strains. These two plants may be useful in managing and curing resistant infections caused by these microbes compared to conventional drugs that are more expensive. The leaves and root bark of *Alchornea cordifolia* are exceptionally used to cure leprosy and as an antitoxin to snake venom (Marva-Manga *et al*., 2007) and decoction of roots or leaves is applied to the vagina to stop partum haemorrhage and to treat vaginitis ( Kone, 2004). Phenols, terpenoids, saponins and flavonoids found in the leaves of guava have been seen to exert anti-bacterial properties (Ogbonnia *et al*., 2008) and together with the alkaloids and tannins in a synergistic way, might be liable for the

inhibition of growth observed in both the *Alchornea cordifolia* and *Psidium guajava* used in the study. Plants like *Phyllanthus fraternus, Hoslundia opposita* and *Cnestis ferruginea* were also effective against the MSSA strains and the standard bacterial strains. Currently, there is sparse information on the antimicrobial properties of most plants, though some scientists have attributed the antimicrobial activities to the presence of secondary metabolites such as flavonoids, phenolics, alkaloids and biterpenoids (VanEtten *et al*., 1994; Badreldin *et al*., 2005; Rios and Recio, 2005; Olaleye, 2007).

This study also revealed that antibacterial activity increased significantly with increase in extract concentration and as a result, the highest concentration (200 mg/ml) gave the maximum inhibition. The interpretation of average diameter zone of inhibition of test organisms were adopted from Veeramuthu *et al.,*(2006), who considered diameter zone of inhibition greater than or equal to14 mm as being high antimicrobial activity. The zone of inhibition is an area around the antibiotic/antimicrobial agent where no organism grows. In this study, there was high antimicrobial activity for both *Alchornea cordifolia* and *Psidium guajava* on the MRSA strains with the ethanol extracts having higher zones of inhibition than that of the aqueous extracts ( $P = 0.0011$, $P = 0.0142$ respectively) which may be due to the higher solubility of active compounds in the ethanol than in water (Njoroje *et al*., 2012).

The MSSA strains were also more susceptible to the antimicrobial activity of the aqueous and the ethanol extracts of the seven different plants than the MRSA strains.

Both *A. cordifolia* and *P. guajava* had P value $< 0.0001$ while that of *C. ferruginea* was $P = 0.0014$, however, there was no significant difference between the aqueous and ethanol extracts of *P. fraternus* ($P = 0.0589$).The variation in the degrees of antimicrobial activities

of the extracts on the clinical isolates is presumed to be due to differences in responses by the isolates to different active compounds present in the plant (Anyanwu and Nwosu, 2014). The effectiveness of the plants against these bacteria gives a basis for the potency against the resistant strains such as MRSA in this present study.

The inhibitory activity of each extract of *A. cordifolia* and *P. guajava* at 200 mg/ml against MRSA was averagely very good according to Veeramuthu *et al.,* (2006) as indicated in Figures 4.3 and 4.4.

For the standard bacteria strains, a common observation between the effectiveness of the aqueous and ethanol extracts was that they inhibited almost all the Gram-positive test organisms (*S. saprophyticus* and *S. aureus*) and the Gram negative test organisms (*K. pneumoniae*, *Proteus mirabilis*, *S. typhi*, *E. coli,* and *P. aeruginosa*). There was a significant difference between the aqueous and ethanol extracts against standard bacteria strains (*P. fraternus* and *P. guajava* had P value < 0.0001while that *of A. cordifolia* and *C. ferruginea* were P = 0.0185, P = 0.0004 respectively). In the case of the Gram positive bacteria, about 90% of the cell wall of Gram-positive bacteria is made of peptidoglycan, which is a non-regulatory structure compared to the cell membrane. The cell wall is, therefore, unable to perform the function of selective permeability; consequently it allows substances to filter through it. The inhibition of Gram-negative capsule-forming organisms, notably*, S. typhi* and *E. coli* by both aqueous and ethanol extracts could be due to the presence of phytochemical constituents capable of paralyzing the defensive potential of the capsule. With the exception of the *A. cordifolia* extract, the Gram-negative organisms showed varying resistant patterns to both the aqueous and ethanol extracts of the medicinal plants used. This may be partly or wholly due to the fact that Gram-negative

bacteria have cell wall made of only 20% peptidoglycan surrounded by the periplasmic space that contains many hydrolytic enzymes, including β-lactamase, which destroy potentially dangerous foreign substances present in this space

The antibiotic susceptibility test performed on all the 11 MRSA strains based on the Kirby-Bauer disk diffusion technique as described by NCCLS (2002) demonstrated a high resistance pattern to almost all the antibiotics used in the study. There was, however, a significant difference between the standard antibiotics against MRSA/MSSA strains ($P < 0.0001$).It was detected that, all the microbes were resistant to AMP, CLX, CHL, CRX, FLU and PEN, while the rest of the antibiotics showed variable degrees of resistance and susceptibility. The high resistant pattern observed in the antibiogram of the test microbes probably confirmed the reality of antimicrobial resistance worldwide, with higher rates occurring in developing countries (Okeke *et al*., 1999). More affordable antibiotics like ampicillin, cloxacillin, flucoxacillin, chloramphenicol and penicillin, however, recorded no zone of inhibition. There are reports of high rates of resistance to chloramphenicol, streptomycin, ampicillin, sulphonamides and tetracycline occurring in animals and humans in the developed countries such as the European countries and in the United States (Poppe *et al*. 1998). The simultaneous utilization of antimicrobial agents in both human and veterinary medicines has enlarged the range of resistance to cover trimethoprim, fluoroquinolones and third-generation cephalosporins (Rabsch *et al*. 2001). In this regard, there should be strict monitoring of the usage of antibiotics not only in humans but also in animals. There are also natural ways that microbes develop resistance and a good pharmaco-vigilance policies well adhered to will help curb the prevalence rate of antibiotic resistance (Jawetz and Levinson, 1996).

The MIC is an assessment tool for bacteriostatic activity of antibacterial agent and low MIC indicates the potency of the plant constituents or compounds against pathogens (ElMahmood, 2009).

The agar diffusion and MIC using the micro-dilution method were used in the study to generate quantitative results that provided a clearer interpretation. According to Mani, *et al*. (2014) and Allen *et al*. (1991), low levels of antimicrobial activity of plant extracts are not detectable in agar diffusion method. MIC is one of a known reliable and appropriate assessment tool or method that has been identified as more accurate than agar disc diffusion (Eloff, 1998 and Mani, *et al*., 2014). This could explain the variation in the MIC and agar diffusion test in which low concentrations of both *A*. *cordifolia* and *P*. *guajava* (0.195 mg/ml) inhibited the growth of the highly resistant MRSA compared to the agar diffusion test which gave an inhibitory effect at 100 mg/ml. The most active extracts were *A*. *cordifolia* and *P*. *guajava*. Analysis on MIC data for MRSA strains, revealed that both ethanol and aqueous extracts of *P. guajava* were virtually the same ($P < 0.0743$) suggesting that both extracts have the same potency with few non-polar compounds from the ethanol and polar molecules from water while there was a significant difference between aqueous and ethanol extracts of *A. cordifolia* ($P = 0.0320$). Additionally, MIC data for MSSA showed a significant difference ($P < 0.0001$) between aqueous and ethanol extracts of *A. cordifolia*, *P. guajava* and *C. ferruginea* whereas *P. fraternus* had no significant difference ( $P = 0.2540$ ). In this regard, aqueous extraction may be preferred by our local folks and herbalists due to the availability and cost effectiveness of using water instead of ethanol. The low MICs observed are of crucial importance which could be employed in the standardization of the crude plant extract; however, without

standardization the crude extract could be contaminated and will have less patronage and this confirms the reason why orthodox medical practitioners find it difficult to accept herbal medicine (WHO, 2000). The study also revealed how different solvent systems can be used to extract active compounds in medicinal plants.

The modified agar diffusion assay revealed that ethanol extracts had slightly higher potency over the aqueous extracts. This may be attributed to the fact that ethanol was able to extract phytoconstituents from the different medicinal plants with more potent antimicrobial activity than water. The detected difference may be due to solubility of the active compounds in ethanol than in water. Additionally, Alo *et al*. (2012) has reported that the inactivity of plant extracts may be due to oldness (or age) of plant, harvesting time of plant materials, extracting solvent and method of extraction.

The present study support the medicinal uses of plants like *Psidium guajava* and *Alchornea cordifolia* in the management of resistant microbes. Thus, medicinal plants should be encouraged to be used worldwide for therapeutic purposes since the prolonged utilization of antibiotics has led to drug resistant of clinical isolates. Medicinal plants possess potent anti-microbial activity which justifies its continuous use in traditional medicine as an alternative antimicrobial agent since very few potent antimicrobials are produced annually. Therefore, the discovery of antimicrobial plant extracts could be an effective way to control clinical infections, especially resistant bacteria such as MRSA.

# CHAPTER SIX

## 6.0. CONCLUSIONS AND RECOMMENDATIONS

## 6.1. CONCLUSIONS

Phytochemical analysis of the crude extracts identified the presence of medically active compounds such as saponins, alkaloids, flavonoids, phenols, tannins, terpenoids and cardiac glycosides in the seven medicinal plants. These phytoconstituents known to confer medicinal properties to plants (Bashir *et al*., 2012) were found in both *Alchornea cordifolia* and *Psidium quajava*, the most active of the selected seven medicinal plants. *Alchornea cordifolia* and *Psidium guajava* had very high antibacterial activities and were able to inhibit the growth of the highly resistant MRSA and also MSSA strains. The very wide zones of inhibition exhibited by these plants on the MRSA show that they have great potential as a remedy for resistant bacteria infections. The aqueous and ethanol extracts generally had almost the same potency but with ethanol extracts having a slightly better antimicrobial activity than that of water using the modified agar diffusion test. Therefore, aqueous extraction would be more preferred by herbalists and the rural folks due to its availability and cost effectiveness.

Finally, medicinal plants as a primary source of drugs used in the traditional and herbal medicine as alternative medicines for the curative treatment of various diseases is acceptable after necessary standardization is carefully observed.

## 6.2. RECOMMENDATIONS

It is recommended that more studies should be done using different solvent systems for extracting the important compounds in medicinal plants such as *Alchornea cordifolia* and *Psidium guajava*. Additionally, the antimicrobial assay of *A. cordifolia* and *P. guajava* should be done in synergy to determine the extent of the activity. Finally antifungal and antiviral properties of the extracts could also be determined.



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# APPENDICES

## Appendix 1: Preparation of reagents and chemicals

### A: Preparation of 20% DMSO Stock solution

A 20ml of DMSO (Sigma, D5879, Germany) was measured into a 100ml volumetric flask and diluted with sterile water up to the 100ml mark. The solution was stored at 2-8$^0$C.

### B: Preparation of INT stock solution

INT (Iodonitrotetrazolium chloride, Fluka Biocheika, 58030, Sigma-Aldrich, Austria) stock was prepared by dissolving 2mg of INT crystals in 10ml of sterile distilled water.

The stock solution was kept in a refrigerator (2-8$^0$C) in a brown (amber) sterile bottle.

## Appendix 2: Preparation of culture media

A: Bacteriological Peptone (Sigma- Aldrich, P0556, Germany)

i. Formula (g/litre) Peptone 10.0g Sodium chloride

5.0 pH 7.2+0.2

ii. Preparation

This media was prepared according to the manufacturer‟s standard. The medium was heated until completely dissolved and then distributed into capped test tubes before autoclaving at $121^0$C for 15 minutes.



**B: Mueller-Hinton Agar (Oxoid, CM0337, Oxoid Ltd, England)**

i. Composition (g/litre)

Beef, dehydrated infusion 300

Casein hydrolysate 17.5

Starch 1.5

Agar 17.0 pH

7.4+0.2 ii.

Preparation

This medium was prepared by following the producer‟sinstruction. It was boiled to dissolve completely after which it was sterilized by autoclaving at $121^0$C for 15 minutes.

After sterilization, the medium was allowed to cool to $50^0$C and poured aseptically. Approximately 25ml volumes were dispensed into an 85mm sterile Petri Dishes and allowed to set. The agar plates were stored at 2-$8^0$C. Sterility check was done on each batch of plates by incubating a plate at $37^0$C aerobically for 18-24 hours. The media quality was checked by inoculating randomly selected media with control bacteria. **C: Mueller Hinton broth**

i. Composition (g/litre)

Beef infusion solids 2.0

Acid hydrolyzed casein 17.5

Starch 1.5

Calcium ions 0.05

Magnesium ions 0.02 pH

7.3+0.1

ii. Preparation



Broths were prepared according to the manufacturer‟s instruction (Liofilchem, Italy). The mixture was warmed until completely dissolved and then dispensed into appropriate bottles before autoclaving at $121^0$C for 15 minutes.

**D: MacConkey Agar**

i. Composition (g/litre)

Peptone 20.0

Lactose 10.0

Bile salts 5.0

Neutral red 0.075

Agar 12.0 pH

7.4+0.2 ii.

Preparation

Media was prepared according to the manufacturer‟s standards (Liofilchem, Italy). After sterilization, the media was cooled to about $55^0$C, and then approximately 25ml volumes were dispensed into 85mm sterile Petri dishes and allowed to set.

**E: Cystine Lactose Electrolyte Deficient (CLED) Agar**

i. Composition (g/litre)

Peptone 4.0

Lab-Lemco 3.0

Tryptone 4.0

Lactose 10.0
L-cystine 0.128

Bromothymol blue 0.02

Agar 15.0 pH 7.3+0.2

ii. Preparation

Manufacturer‟s standard (Liofilchem, Italy) was followed. The medium was boiled to dissolve completely. Sterilization was done at $121^0$C for 15 minutes. After the medium had cooled, it was mixed and dispensed aseptically into sterile Petri dishes and allowed to set. Sterility and media quality checks were done on them.

**F: Blood and Chocolate Agar** i.

Composition (g/litre)

„Lab-Lemco‟ 10.0

Peptone neutralized 10.0

Sodium chloride 5.0

Agar 15.0 pH

7.3+0.2 ii.

Preparation

Blood agar (Liofilchem, Italy) was prepared according to the manufacturer‟s instruction. The dehydrated blood agar base was heated to dissolve entirely. After sterilization at $121^0$C for 15 minutes, the medium was cooled to $50^0$C and 7% sterile sheep blood was added to the agar based, mixed well and poured aseptically into Petri dishes.

Chocolate agar: A double strength blood agar base was prepared as was done for blood agar except that sterile 2% w/v haemaglobin powder added to the double strength agar base, mixed and poured aseptically into 90mm Petri dishes.

**G: Preparation of culture media**

Nutrient Agar (Marck, 1.05450, Germany), Bacteriological Peptone (Sigma-Aldrich, P0556, Germany), Sabouraud 4% glucose agar (Fluka Biocheika, 84088, Sigma- Aldrich, India), and Mueller-Hinton agar (Oxoid, CM0337, Oxoid Ltd, England) were used. The media were prepared according to manufacturer‟s instruction or protocol.

**Appendix**

**3: Susceptibility of MRSA to Standard Antibiotics**

| Test organisms (MRSA strains) | Gen | Ery | on m) Tet | Cot |
|---|---|---|---|---|
| **1** | 18 | 17 | 23 | 21 |
| **2** | - | - | 16 | - |
| **3** | - | 11 | 10 | 24 |
| **4** | 15 | 15 | 9 | 14 |
| **5** | - | 14 | 19 | 22 |
| **6** | 19 | 14 | 14 | 23 |
| **7** | 16 | 9 | 24 | 24 |
| **8** | 11 | - | 17 | - |
| **9** | - | - | 24 | 23 |
| **10** | 12 | - | 23 | 22 |
| **11** | **16** | **-** | **22** | **-** |

**Appendix**

**4: Susceptibility of MSSA to Standard Antibiotics**

| Test organisms (MSSA trains) | Zone of Inhibition (mm) | | | |
|---|---|---|---|---|
| | | | Ery | |
| **1** | 11 | 15 | - | 21 |
| **2** | 17 | 8 | 14 | 24 |
| **3** | 14 | 19 | 22 | 23 |
| **4** | - | - | 14 | 18 |
| **5** | 15 | 20 | 25 | 24 |
| **6** | 16 | 21 | 25 | 24 |
| **7** | 14 | 20 | 25 | 25 |
| **8** | - | - | 24 | 23 |
| **9** | 15 | 20 | 14 | 23 |
| **10** | 16 | 20 | 23 | 24 |
| **11** | 16 | 21 | 26 | 23 |

**5: Susceptibility of the MRSA to the aqueous and ethanol extracts of *Psidium guajava.***

| Test | Zone of Inhibition (mm) | | | | Test Organisms | Zone of Inhibition (mm) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | Mean | | 1 | 2 | 3 | Mean |
| | | | | | 1 | 17 | 15 | 17 | 15.3 |

**Appendix**

**Organisms**

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Aqueous Extract** | 1 | 11 | 14 | 12 | 12.3 | **Ethanolic Extract** | 2 | 22 | 19 | 22 | 21.0 |
| | 2 | 23 | 16 | 22 | 20.3 | | 3 | 17 | 16 | 18 | 17.0 |
| | 14 | 17 | 13.7 | | | | 4 | 19 | 17 | 27 | 17.6 |
| | 4 | 12 | 10 | 12 | 11.3 | | 3 10 | | | | |
| | | | | | | | 5 | 18 | 17 | 19 | 18.0 |
| | 6 | 9 | 1 | 10 | 10.0 | | 5 16 20 14 16.7 | | | | |
| | | | | | | | 6 | 17 | 16 | 16 | 16.3 |
| | 8 | 24 | 20 | 27 | 23.7 | | 7 | 16 | 15 | 17 | 16.0 |
| | 12 | 16 | 18 | 15.3 | | | 8 | 21 | 22 | 20 | 21.0 |
| | 10 | 12 | 20 | 25 | 19.0 | | 9 | 18 | 22 | 21 | 20.3 |
| | | | | | | | 10 | 13 | 18 | 20 | 17.0 |
| | 11 | 18 | 20 | 20 | 19.3 | | 7 9 | 20 | 23 | 20 | 21.0 |
| | | | | | | | 11 | 18 | 20 | 20 | 19.3 |

Total mean: 182.60 mm

Average: 16.60 mm

Total mean: 198.20 mm

Average: 18.02 mm

**Appendix**

**6: Susceptibility of the MRSA to the aqueous and ethanol extracts of *Alchornea cordifolia***

| | Test Organisms | Zone of Inhibition (mm) | | | | | Test Organisms | Zone of Inhibition (mm) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | Mean | | | 1 | 2 | 3 | Mean |
| Aqueous Extract | 1 | 17 | 15 | 15.7 | | Ethanolic Extract | 1 | 14 | 15 | 23 | 17.3 |
| | 2 | 15 | 16 | 18 | 16.3 | | 2 | 15 | 16 | 18 | 16.3 |
| | 3 | 14 | 13 | 15 | 14.0 | | 3 | 14 | 25 | 26 | 15.0 |
| | 4 | 15 | 18 | 16 | 16.3 | | 4 | 20 | 19 | 20 | 19.7 |
| | 5 | 15 | 16 | 16 | 15.7 | | 5 | 14 | 16 | 17 | 15.7 |
| | 6 | 11 | 11 | 10 | 10.7 | | 6 | 16 | 15 | 15 | 15.3 |
| | 7 | 19 | 17 | 18 | 18.0 | | 7 | 15 | 13 | 13 | 13.7 |
| | 8 | 30 | 30 | 25 | 28.3 | | 8 | 18 | 18 | 15 | 17.0 |
| | 9 | 18 | 18 | 19 | 18.3 | | 9 | 18 | 16 | 15 | 16.3 |
| | 10 | 14 | 18 | 14 | 15.3 | | 10 | 20 | 30 | 32 | 27.3 |
| | 11 | 16 | 18 | 17 | 17.0 | | 11 | 16 | 30 | 29 | 25.0 |

**Total mean: 179.30 mm** **Total mean: 204.90 mm**

**Average: 16.30 mm** **Average: 18.63 mm**

**7: Susceptibility of MSSA to the *Alchornea cordifolia* (Agar Diffusion Test).**

| Test | Zone of Inhibition (mm) | Test | Zone of Inhibition (mm) |
|---|---|---|---|

**Appendix**

**Ethanolic Extract**

| Organisms | 1 | 2 | 3 | Mean |
|---|---|---|---|---|
| | 24 | 40 | 30 | |
| | 46 | | | |
| | | 44 | 43 | |
| | 38 | | | |
| | | 34 | 36 | 31.3 |
| | 40 | | | |
| | 46 | 38 | 39 | 44.3 |
| | 46 | 46 | 45 | 36.0 |
| | 46 | | | |
| **1** | 44 | 46 | 45 | 39.0 |
| **2** | 42 | 46 | 44 | 45.7 |
| **3** | 46 | | | |
| | | 44 | 45 | 45.7 |
| **4** | 46 | | | |
| **5** | 40 | 40 | 44 | 45.3 |
| **6** | 43 | 45 | 45 | 44.3 |
| **7** | 44 | 46 | 45 | 42.0 |
| **8** | 16 | | | |
| | 46 | 42 | 40 | 45.3 |
| **9** | 46 | 44 | 44 | 45.7 |
| **10** | 48 | | | |
| | | 44 | 46 | 40.7 |
| **11** | 48 | 15 | 17 | 43.7 |
| **12** | 44 | | | |
| **13** | 34 | 46 | 44 | 44.7 |
| **14** | 42 | 47 | 45 | 16.0 |
| **15** | 46 | 46 | 48 | |
| **16** | | 46 | 48 | 45.3 |
| **17** | | | | 46.0 |
| **18** | | 43 | 44 | 47.3 |
| **19** | | 32 | 33 | 47.3 |
| **20** | | 43 | 41 | 43.7 33.0 |
| **21** | | 46 | 44 | |
| **22** | | | | 42.0 |
| **23** | | | | 45.3 |
| **24** | 44 | 42 | 44 | 43.3 |

**TM: 1002.90 mm A: 41.79 mm**

**Aqueous Extract**

| Organisms | 1 | 2 | 3 | Mean |
|---|---|---|---|---|
| **1** | 20 | 26 | 26 | 24.0 |
| **2** | 34 | 28 | 32 | 31.3 |
| **3** | 22 | 22 | 23 | 22.3 |
| **4** | 22 | 24 | 24 | 23.3 |
| **5** | 34 | 33 | 35 | 34.0 |
| **6** | 31 | 33 | 31 | 31.7 |
| **7** | 34 | 34 | 33 | 33.7 |
| **8** | 23 | 24 | 23 | 23.3 |
| **9** | 38 | 30 | 36 | 34.7 |
| **10** | 30 | 34 | 35 | 33.0 |
| **11** | 32 | 36 | 36 | 34.7 |
| **12** | 34 | 23 | 32 | 29.7 |
| **13** | 40 | 42 | 40 | 40.7 |
| **14** | 34 | 36 | 36 | 35.3 |
| **15** | 30 | 32 | 32 | 31.3 |
| **16** | 34 | 20 | 35 | 29.7 |
| **17** | 38 | 38 | 40 | 38.7 |
| **18** | 26 | 30 | 31 | 29.0 |
| **19** | 34 | 35 | 34 | 34.3 |
| **20** | 36 | 35 | 36 | 35.7 |
| **21** | 19 | 24 | 24 | 22.3 |
| **22** | 20 | 21 | 22 | 21.0 |
| **23** | 24 | 26 | 24 | 24.7 |
| **24** | 40 | 41 | 40 | 40.3 |

**TM: 738.7 mm A: 30.78 mm**

**8: Susceptibility of MSSA to *Psidium guajava* (Agar Diffusion Test)**

| | 1 | 2 | 3 | Mean |
|---|---|---|---|---|
| **1** | | | | |

| | 1 | 2 | 3 | Mean |
|---|---|---|---|---|
| **1** | | | | |

## Appendix

**Ethanolic Extract**

| | | | | | |
|---|---|---|---|---|---|
| **2** | 25 | 25 | 26 | | |
| **3** | 30 | 31 | 30 | | |
| **4** | 26 | 19 | 24 | | |
| **5** | 36 | 36 | 35 | | |
| **6** | 31 | 31 | 32 | 25.3 | |
| **7** | 35 | 36 | 36 | 30.3 | |
| **8** | 35 | 36 | 36 | 23.0 | |
| **9** | 31 | 30 | 30 | 35.7 | |
| **10** | 37 | 34 | 36 | 31.3 | |
| **11** | 36 | 38 | 39 | 35.7 | |
| **12** | 34 | 37 | 36 | 35.7 | |
| **13** | 38 | 35 | 35 | 30.3 | |
| **14** | 40 | 37 | 39 | 35.0 | |
| **15** | 40 | 39 | 40 | 38.0 | |
| **16** | 32 | 39 | 40 | 36.3 | |
| **17** | 42 | 31 | 30 | 34.7 | |
| **18** | 32 | 42 | 41 | 38.0 | |
| **19** | 36 | 34 | 34 | 39.7 | |
| **20** | 42 | 35 | 36 | 39.7 | |
| **21** | 34 | 44 | 43 | 31.0 | |
| **22** | 40 | 34 | 35 | 41.7 | |
| **23** | 40 | 42 | 38 | 33.3 | |
| | | 40 | 39 | 35.7 | |
| | | | | 43.0 34.3 | |
| | | | | 40.0 | |
| | | | | 39.7 | |
| **24** | 43 | 42 | 42 | 42.3 | |

**Aqueous Extract**

| | | | | |
|---|---|---|---|---|
| | 23 | 23 | 24 | 23.3 |
| **2** | 29 | 30 | 30 | 29.7 |
| **3** | 20 | 21 | 21 | 20.7 |
| **4** | 32 | 33 | 32 | 32.3 |
| **5** | 31 | 31 | 32 | 31.3 |
| **6** | 32 | 32 | 33 | 32.7 |
| **7** | 29 | 30 | 30 | 29.7 |
| **8** | 19 | 20 | 19 | 19.3 |
| **9** | 31 | 30 | 30 | 30.3 |
| **10** | 34 | 32 | 33 | 33.0 |
| **11** | 34 | 32 | 32 | 32.7 |
| **12** | 34 | 33 | 33 | 33.3 |
| **13** | 36 | 36 | 35 | 35.7 |
| **14** | 38 | 37 | 38 | 37.7 |
| **15** | 36 | 37 | 36 | 35.0 |
| **16** | 35 | 36 | 37 | 36.0 |
| **17** | 40 | 41 | 40 | 40.3 |
| **18** | 36 | 37 | 36 | 36.3 |
| **19** | 36 | 36 | 37 | 36.3 |
| **20** | 44 | 44 | 42 | 43.3 |
| **21** | 32 | 32 | 33 | 32.3 |
| **22** | 36 | 35 | 37 | 36.0 |
| **23** | 40 | 39 | 39 | 39.3 |
| **24** | 40 | 38 | 42 | 40.0 |

**Appendix**

| Test Organisms | Zone of Inhibition (mm) | Test Organisms | Zone of Inhibition (mm) |
|---|---|---|---|






**TM: 849.70 mm A: 35.40 mm** **TM: 797.50 mm A: 33.23 mm**

**Appendix 9: Antimicrobial Activity Screening for *Phyllanthus fraternus* against strains of MSSA (Agar Diffusion Test).**

| | Test Organisms | Zone of Inhibition (mm) | | | |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | Mean |
| Ethanolic Extract | **1** | 24 | 24 | 25 | 24.3 |
| | **2** | 24 | 18 | 21 | 21.0 |
| | **3** | 28 | 29 | 27 | 28.0 |
| | **4** | 40 | 39 | 41 | 40.0 |
| | **5** | 24 | 30 | 31 | 28.3 |
| | **6** | 20 | 30 | 31 | 27.0 |
| | **7** | 28 | 30 | 31 | 29.7 |
| | **8** | 32 | 34 | 36 | 34.0 |
| | **9** | 32 | 33 | 31 | 32.0 |
| | **10** | 40 | 41 | 40 | 40.3 |
| | **11** | 40 | 40 | 39 | 39.7 |
| | **12** | 30 | 31 | 30 | 30.3 |
| | **13** | 39 | 40 | 39 | 39.3 |
| | **14** | 32 | 33 | 32 | 32.3 |
| | **15** | 40 | 39 | 41 | 40.0 |
| | **16** | 32 | 33 | 32 | 32.3 |
| | **17** | 28 | 27 | 29 | 28.0 |
| | **18** | 36 | 35 | 37 | 46.0 |

**Total mean: 592.50 mm**

**Average: 32.90 mm**

| | Test Organisms | Zone of Inhibition (mm) | | | |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | Mean |
| Aqueous Extract | **1** | 24 | 25 | 23 | 24.0 |
| | **2** | 24 | 18 | 21 | 21.0 |
| | **3** | 26 | 25 | 25 | 25.3 |
| | **4** | 39 | 40 | 41 | 40.0 |
| | **5** | 22 | 30 | 29 | 27.0 |
| | **6** | 20 | 30 | 31 | 27.0 |
| | **7** | 34 | 30 | 33 | 32.3 |
| | **8** | 34 | 33 | 32 | 33.0 |
| | **9** | 28 | 30 | 28 | 28.7 |
| | **10** | 34 | 33 | 32 | 33.0 |
| | **11** | 40 | 39 | 41 | 40.0 |
| | **12** | 26 | 35 | 34 | 31.7 |
| | **13** | 42 | 41 | 43 | 42.0 |
| | **14** | 34 | 35 | 33 | 34.0 |
| | **15** | 36 | 35 | 37 | 36.0 |
| | **16** | 28 | 27 | 30 | 28.3 |
| | **17** | 32 | 36 | 34 | 34.0 |
| | **18** | 32 | 32 | 33 | 32.3 |

**Total mean: 539.60 mm**

**Average: 29.98 mm**

**Appendix :**

**10 Susceptibility of MSSA to the *Cnestis ferruginea* (Agar Diffusion Test).**

**Aqueous Extract**

| Test Organisms | Zone of Inhibition (mm) | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | Mean |
| **1** | 40 | 39 | 40 | 39.7 |
| **2** | 36 | 35 | 36 | 35.7 |
| **3** | 28 | 29 | 28 | 28.3 |
| **4** | 34 | 33 | 33 | 33.3 |
| **5** | 42 | 41 | 40 | 41.0 |
| **6** | 36 | 35 | 37 | 36.0 |
| **7** | 34 | 32 | 34 | 33.3 |
| **8** | 36 | 35 | 35 | 35.3 |
| **9** | 35 | 34 | 36 | 35.0 |
| **10** | 42 | 43 | 41 | 42.0 |
| **11** | 40 | 41 | 42 | 41.0 |
| **12** | 38 | 37 | 38 | 37.7 |
| **13** | 38 | 37 | 39 | 38.0 |
| **14** | 42 | 41 | 42 | 41.7 |
| **15** | 38 | 37 | 39 | 38.0 |
| **16** | 36 | 35 | 37 | 36.0 |
| **17** | 44 | 43 | 42 | 43.0 |
| **18** | 38 | 37 | 39 | 38.0 |
| **19** | 40 | 41 | 40 | 40.3 |

**Total mean: 713.30 mm**

**Ethanolic Extract**

| Test Organisms | Zone of Inhibition (mm) | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | Mean |
| **1** | 37 | 39 | 40 | 38.7 |
| **2** | 38 | 39 | 40 | 39.0 |
| **3** | 32 | 33 | 32 | 32.3 |
| **4** | 38 | 37 | 36 | 37.0 |
| **5** | 38 | 37 | 36 | 37.0 |
| **6** | 36 | 35 | 36 | 35.7 |
| **7** | 36 | 35 | 37 | 36.0 |
| **8** | 36 | 35 | 35 | 35.3 |
| **9** | 35 | 34 | 36 | 35.0 |
| **10** | 50 | 49 | 49 | 49.3 |
| **11** | 40 | 41 | 42 | 41.0 |
| **12** | 38 | 37 | 38 | 37.7 |
| **13** | 32 | 32 | 30 | 31.3 |
| **14** | 42 | 41 | 42 | 41.7 |
| **15** | 38 | 37 | 39 | 38.0 |
| **16** | 36 | 35 | 37 | 36.0 |
| **17** | 44 | 43 | 42 | 43.0 |
| **18** | 38 | 37 | 39 | 38.0 |
| **19** | 40 | 41 | 40 | 40.3 |

**Total mean: 722.30 mm**

**Appendix :**

**Average: 37.54 mm** **Average: 38.00 mm**

**11 Susceptibility of MSSA to *Hoslundia opposita* (Agar Diffusion Test)**

| Test Organisms | Zone of Inhibition (mm) | | | | Test Organisms | Zone of Inhibition (mm) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | Mean | | 1 | 2 | 3 | Mean |
| **1** | 20 | 21 | 20.3 | **1** | 20 | | | | |
| **2** | 26 | 27 | 25 | 26.0 | **2** | | | | |
| **3** | 21 | 22 | 23 | 22.0 | **3** | | | | |
| **4** | 16 | 23 | 22 | 20.3 | **4** | | | | |
| **5** | 23 | 25 | 33 | 27.0 | **5** | | | | |
| **6** | 18 | 30 | 29 | 25.7 | **6** | | | | |
| **7** | 25 | 22 | 24 | 23.7 | **7** | | | | |
| **8** | 22 | 21 | 24 | 22.3 | **8** | | | | |
| **9** | 22 | 21 | 24 | 22.3 | **9** | | | | |
| **10** | 24 | 24 | 26 | 24.7 | **10** | **11** | **12** | **13** | **14** |
| **11** | 30 | 31 | 31 | 30.7 | **15** | | | No Inhibition | |
| **12** | 30 | 21 | 31 | 27.3 | **16** | **17** | **18** | **19** | **20** |
| **13** | 28 | 29 | 30 | 29.0 | **21** | 30 | 30 | 29 | 29.7 | **21** |
| **14** | 27 | 21 | 26 | 24.7 | **22** | 30 | 31 | 31 | 30.7 | **22** |
| **15** | 31 | 32 | 32 | 31.7 | | | | | |
| **16** | 26 | 27 | 28 | 27.0 | | | | | |
| **17** | 26 | 27 | 28 | 27.0 | | | | | |
| **18** | 26 | 23 | 25 | 24.7 | | | | | |
| **19** | 22 | 24 | 23 | 23.0 | | | | | |
| **20** | 23 | 24 | 25 | 24.0 | | | | | |

**Ethanoloic Extract**

**Aqueous Extract**

**Total mean: 353.80 mm**

**Average: 16.08 mm**

**12 Minimum Inhibitory**

**Concentration of extracts**

**Appendix :**

| Test organisms (MRSA) | *Alchornea cordifolia* | | *Psidium guajava* | | Tetracycline (µg/ml) |
|---|---|---|---|---|---|
| | Ethanol (mg/ml) | Aqueous (mg/ml) | Ethanol (mg/ml) | Aqueous (mg/ml) | |
| **1** | 0.390 | 0.781 | 0.390 | 0.195 | 7.5 |
| **2** | 0.390 | 0.390 | 0.390 | 0.390 | 3.75 |
| **3** | 0.195 | 0.781 | 0.195 | 0.781 | 7.5 |
| **4** | 0.390 | 0.390 | 0.195 | 0.390 | 7.5 |
| **5** | 0.195 | 1.563 | 0.195 | 0.390 | 3.75 |
| **6** | 0.195 | 0.390 | 0.195 | 0.390 | 3.75 |
| **7** | 0.390 | 0.390 | 0.195 | 0.195 | 7.5 |
| **8** | 0.195 | 0.390 | 0.195 | 0.781 | 15 |
| **9** | 0.390 | 0.781 | 0.195 | 0.781 | 30 |
| **10** | 0.195 | 0.390 | 0.390 | 0.390 | 15 |
| **11** | 0.195 | 0.195 | 0.390 | 0,195 | 15 |

**Appendix :**

| | | | | | |
|---|---|---|---|---|---|
| **Average** | 0.28 mm | 0.59 mm | 0.27 mm | 0.44 mm | 10.57 mm |

**Appendix 13: Minimum Inhibitory Concentration of test microbes (MSSA)**

| Test | AC | | AC | PG | | PG | PF | | PF | CF | CF | HO | Tert |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | 0.195 | 0.781 | 0.390 | 1.563 | 0.781 | 1.563 | 1.563 | 0.718 | 1.563 | 7.5 | | | |
| **2** | 0.390 | 1.563 | 0.195 | 1.563 | 0.195 | 1.563 | 1.563 | 0.718 | 1.563 | 3.75 | | | |
| **3** | 0.390 | 1.563 | 0.195 | 1.563 | 1.563 | 0.781 | 1.563 | 0.718 | 1.563 | 7.5 | | | |
| **4** | 0.390 | 0.390 | 0.390 | 1.563 | 1.563 | 1.563 | 1.563 | 0.718 | 1.563 | 7.5 | | | |
| **5** | 0.390 | 1.563 | 0.195 | 1.563 | 0.390 | 0.781 | 1.563 | 0.718 | 1.563 | 3.75 | | | |
| **6** | 0.390 | 0.195 | 0.390 | 1.563 | 1.563 | 0.390 | 1.563 | 0.718 | 1.563 | 3.75 | | | |
| **7** | 0.390 | 0.195 | 0.195 | 1.563 | 0.195 | 0.390 | 1.563 | 1.563 | 1.563 | 7.5 | | | |
| **8** | 0.390 | 0.195 | 0.195 | 1.563 | 1.563 | 1.563 | 1.563 | 0.718 | 1.563 | 15 | | | |
| **9** | 0.390 | 1.563 | 0.195 | 1.563 | 0.390 | 1.563 | 1.563 | 0.718 | 1.563 | 30 | | | |
| **10** | 0.390 | 1.563 | 0.390 | 1.563 | 1.563 | 1.563 | 1.563 | 0.718 | 1.563 | 15 | | | |
| **11** | 0.390 | 1.563 | 0.195 | 1.563 | 0.195 | 1.563 | 1.563 | 0.718 | 1.563 | 15 | | | |
| **12** | 0.195 | 1.563 | 0.195 | 0.195 | 0.195 | 0.390 | 1.563 | 0.390 | 0.781 | 7.5 | | | |
| **13** | 0.390 | 1.563 | 0.781 | 1.563 | 1.563 | 0.781 | 1.563 | 0.390 | 1.563 | 7.5 | | | |
| **14** | 0.781 | 1.563 | 0.195 | 1.563 | 0.390 | 0.781 | 1.563 | 0.390 | 1.563 | 7.5 | | | |
| **15** | 0.390 | 1.563 | 0.195 | 1.563 | 0.390 | 0.195 | 1.563 | 0.390 | 1.563 | 15 | | | |
| **16** | 0.390 | 1.563 | 0.390 | 1.563 | 0.781 | 0.195 | 1.563 | 0.390 | 1.563 | 3.75 | | | |
| **17** | 0.390 | 1.563 | 0.390 | 1.563 | 0.390 | 0.195 | 1.563 | 0.390 | 1.563 | 7.5 | | | |
| **18** | 0.781 | 1.563 | 0.781 | 0.390 | 0.390 | 0.781 | 1.563 | 0.390 | 1.563 | 30.0 | | | |
| **19** | 0.781 | 1.563 | 0.781 | 0.781 | 0.390 | 1.563 | 1.563 | 0.390 | 0.390 | 15.0 | | | |
| **20** | 0.781 | 1.563 | 0.390 | 1.563 | 0.390 | 1.563 | 1.563 | 0.390 | 0.390 | 15.0 | | | |
| **21** | 0.390 | 1.563 | 0.195 | 0.781 | 0.390 | 1.563 | 1.563 | 0.390 | 0.390 | 7.5 | | | |
| **22** | 0.390 | 1,563 | 0.195 | 0.781 | 1.563 | 1.563 | 1.563 | 0.390 | 1.563 | 3.75 | | | |
| **23** | 0.781 | 0.781 | 0.390 | 0.781 | 1.563 | 0.781 | 1.563 | 0.390 | 1.563 | 7.5 | | | |
| **24** | 0.195 | 1.563 | 0.781 | 1.563 | 1.563 | 0.781 | 1.563 | 1.563 | 0.781 | 3.75 | | | |
| Average | 0.45 | | 1.28 | 0.36 | | 1.33 | 0.83 | | 1.02 | 1.56 | 0.65 | 1.35 | 10.31 |

Tetracycline – Initial concentration of 30 µg/ml

AC- *Alchornea cordifolia* PG- *Psidium guajava* PF-*Phyllanthus fraternus* CF-*Cnestisferruginea* HO-*Hoslundia opposite* Tert-Tetracycline

**Appendix**

**14: Susceptibility of microbes to the aqueous extract of *P. guajava***

| Test Organisms | Zones of inhibition (mm) | | | | Standard Deviation | Standard Error of Mean | Variance |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | Mean | | | |
| *K. pneumonia* ATCC 33495 | 15 | 16 | 14 | 15.00 | 0.00 | 0.00 | 0.00 |
| *Proteus mirabilis* ATCC 49565 | 11 | 12 | 10 | 11.00 | 0.00 | 0.00 | 0.00 |
| *S. saprophyticus* ATCC 15305 | 16 | 17 | 17 | 16.67 | 0.58 | 0.33 | 0.33 |
| *Salmonella typhi* ATCC 19430 | 12 | 13 | 11 | 12.00 | 0.00 | 0.00 | 0.00 |
| *S. aureus* ATCC 25923 | 14 | 15 | 16 | 15.00 | 1.00 | 0.58 | 1.00 |
| *E. coli* ATTC 25922 | 11 | 12 | 10 | 11.00 | 0.00 | 0.00 | 0.00 |
| *P. aeruginosa* ATCC 27853 | 13 | 14 | 13 | 13.33 | 0.58 | 0.33 | 0.33 |

**Total mean: 94.00 mm** **Average: 13.42 mm**

**Appendix 15: Susceptibility of microbes to the ethanolic extract of *P. guajava***

| Test Organisms | Zones of inhibition (mm) | | | | Standard Deviation | Standard Error of Mean | Variance |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | Mean | | | |
| *K. pneumonia* ATCC 33495 | 15 | 15 | 15 | 15.00 | 0.00 | 0.00 | 0.00 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *Proteus mirabilis* ATCC 49565 | 14 | 14 | 14 | 14.00 | 0.00 | 0.00 | 0.00 |
| *S. saprophyticus* ATCC 15305 | 15 | 17 | 18 | 16.67 | 1.53 | 0.88 | 2.33 |
| *Salmonella typhi* ATCC 19430 | 13 | 14 | 15 | 14.00 | 1.00 | 0.58 | 1.00 |
| *S. aureus* ATCC 25923 | 13 | 15 | 14 | 14.00 | 1.00 | 0.58 | 1.00 |
| *E. coli* ATTC 25922 | 15 | 15 | 15 | 15.00 | 0.00 | 0.00 | 0.00 |
| *P. aeruginosa* ATCC 27853 | 14 | 15 | 16 | 15.00 | 1.00 | 0.58 | 1.00 |

**Total mean: 103.67 mm Average: 14.81 mm**

**Appendix 16: Susceptibility of microbes to the ethanolic extract of *P. fraternus***

| Test Organisms | Zones of inhibition (mm) | | | | Standard Deviation | Standard Error of Mean | Variance |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | Mean | | | |
| *K. pneumonia* ATCC 33495 | 8 | 10 | 9 | 9.00 | 0.00 | 0.00 | 0.00 |
| *Proteus mirabilis* ATCC 49565 | 31 | 32 | 33 | 32.00 | 1.00 | 0.58 | 1.00 |
| *S. saprophyticus*ATCC 15305 | 14 | 15 | 13 | 14.00 | 0.00 | 0.00 | 0.00 |
| *Salmonella typhi*ATCC 19430 | 15 | 13 | 14 | 14.00 | 0.00 | 0.00 | 0.00 |
| *S. aureus*ATCC 25923 | 16 | 16 | 17 | 16.33 | 0.58 | 0.33 | 0.33 |
| *E. coli* ATTC 25922 | 17 | 18 | 19 | 18.00 | 1.00 | 0.58 | 1.00 |
| *P. aeruginosa*ATCC 27853 | 14 | 15 | 16 | 15.00 | 1.00 | 0.58 | 1.00 |

**Appendix**

**Total mean: 118.33 mm** **Mean average: 16.90 mm**

**Appendix 17: Susceptibility of microbes to the aqueous extract of *P. fraternus***

| Test Organisms | Zones of inhibition (mm) | | | | Standard Deviation | Standard Error of Mean | Variance |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | Mean | | | |
| *K. pneumonia* ATCC 33495 | 10 | 9 | 9 | 9.33 | 0.58 | 0.33 | 0.33 |
| *Proteus mirabilis* ATCC 49565 | 0 | 0 | 0 | 0.00 | 0.00 | 0.00 | 0.00 |
| *S. saprophyticus* ATCC 15305 | 0 | 0 | 0 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Salmonella typhi* ATCC 19430 | 12 | 12 | 13 | 12.33 | 0.58 | 0.33 | 0.33 |
| *S. aureus* ATCC 25923 | 10 | 11 | 9 | 10.00 | 0.00 | 0.00 | 0.00 |
| *E. coli* ATTC 25922 | 8 | 9 | 10 | 9.00 | 1.00 | 0.58 | 1.00 |
| *P. aeruginosa* ATCC 27853 | 11 | 10 | 12 | 11.00 | 0.00 | 0.00 | 0.00 |

**Total mean: 51.66 mm** **Average: 7.38 mm**

**18: Susceptibility of microbes to the ethanolic extract of *A. cordifolia*.**

| Test Organisms | Zones of inhibition (mm) | | | | Standard Deviation | Standard Error of Mean | Variance |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | Mean | | | |
| *K. pneumonia* ATCC 33495 | 0 | 0 | 0 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Proteus mirabilis* ATCC 49565 | 18 | 19 | 17 | 18.00 | 0.00 | 0.00 | 0.00 |
| *S. saprophyticus*ATCC 15305 | 16 | 17 | 15 | 16.00 | 0.00 | 0.00 | 0.00 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *Salmonella typhi*ATCC 19430 | 15 | 16 | 16 | 15.67 | 0.58 | 0.33 | 0.33 |
| *S. aureus*ATCC 25923 | 14 | 15 | 13 | 14.00 | 0.00 | 0.00 | 0.00 |
| *E. coli* ATTC 25922 | 14 | 14 | 17 | 15.00 | 0.00 | 0.00 | 0.00 |
| *P. aeruginosa* ATCC 27853 | 17 | 17 | 18 | 17.33 | 0.58 | 0.33 | 0.33 |

**Total mean: 96.00 mm** **Average: 13.71 mm**

**Appendix 19: Susceptibility of microbes to the aqueous extract of *A. cordiforlia*.**

| Test Organisms | Zones of inhibition (mm) | | | | Standard Deviation | Standard Error of Mean | Variance |
|---|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **Mean** | | | |
| *K. pneumonia* ATCC 33495 | 0 | 0 | 0 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Proteus mirabilis* ATCC 49565 | 17 | 16 | 18 | 17.00 | 0.00 | 0.00 | 0.00 |
| *S. saprophyticus* ATCC 15305 | 16 | 15 | 17 | 16.00 | 0.00 | 0.00 | 0.00 |
| *Salmonella typhi* ATCC 19430 | 14 | 15 | 15 | 14.50 | 0.58 | 0.33 | 0.33 |
| *S. aureus* ATCC 25923 | 13 | 14 | 14 | 13.50 | 0.58 | 0.33 | 0.33 |
| *E. coli* ATTC 25922 | 14 | 14 | 15 | 14.00 | 0.58 | 0.33 | 0.33 |
| *P. aeruginosa* ATCC 27853 | 16 | 15 | 17 | 16.00 | 0.00 | 0.00 | 0.00 |

**Total mean: 91.00 mm** **Average: 13.00 mm**

**Appendix :**

**20 Susceptibility of microbes to the ethanolic extract of *C. ferruginea*.**

| Test Organisms | Zones of inhibition (mm) | | | | Standard Deviation | Standard of Error Mean | Variance |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | Mean | | | |
| *K. pneumonia* ATCC 33495 | 11 | 10 | 11 | 10.67 | 0.58 | 0.33 | 0.33 |
| *Proteus mirabilis* ATCC 49565 | 36 | 36 | 38 | 36.67 | 1.15 | 0.67 | 1.33 |
| *S. saprophyticus*ATCC 15305 | 25 | 24 | 26 | 25.00 | 0.00 | 0.00 | 0.00 |
| *Salmonella typhi*ATCC 19430 | 9 | 8 | 10 | 9.00 | 0.00 | 0.00 | 0.00 |
| *S. aureus*ATCC 25923 | 18 | 18 | 17 | 17.67 | 0.58 | 0.33 | 0.33 |
| *E. coli* ATTC 25922 | 15 | 14 | 13 | 14.00 | 0.00 | 0.00 | 0.00 |
| *P. aeruginosa*ATCC 27853 | 19 | 18 | 20 | 19.00 | 0.00 | 0.00 | 0.00 |

**Total mean: 132.01 mm** **Average: 18.86 mm**

**Appendix 21: Susceptibility of microbes to the aqueous extract of *C. ferruginea***

| Test Organisms | Zones of inhibition (mm) | | | | Standard Deviation | Standard Error of Mean | Variance |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | Mean | | | |
| *K. pneumoniae*ATCC 33495 | 10 | 9 | 11 | 10.00 | 1.00 | 0.58 | 1.00 |
| *Proteus mirabilis* ATCC 49565 | 32 | 33 | 34 | 33.00 | 1.00 | 0.58 | 1.00 |
| *S. saprophyticus*ATCC 15305 | 21 | 20 | 22 | 21.00 | 1.00 | 0.58 | 1.00 |

**Appendix :**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *Salmonella typhi*ATCC 19430 | 9 | 10 | 8 | 9.00 | 0.00 | 0.00 | 0.00 |
| *S. aureus*ATCC 25923 | 17 | 17 | 18 | 17.33 | 0.58 | 0.33 | 0.33 |
| *E. coli* ATTC 25922 | 13 | 14 | 15 | 14.00 | 1.00 | 0.58 | 1.00 |
| *P. aeruginosa*ATCC 27853 | 20 | 20 | 21 | 20.33 | 0.58 | 0.33 | 0.33 |

**Total mean: 124.66 mm** **Average: 17.81 mm**

**22 Susceptibility of microbes to the tetracycline**

| Test Organisms | Zones of inhibition (mm) | | | | Standard Deviation | Standard Error of Mean | Variance |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | Mean | | | |
| *K. pneumonia* ATCC 33495 | 18 | 19 | 17 | 18.00 | 0.00 | 0.00 | 0.00 |
| *Proteus mirabilis* ATCC 49565 | 12 | 11 | 13 | 12.00 | 0.00 | 0.00 | 0.00 |
| *S. saprophyticus* ATCC 15305 | 16 | 18 | 19 | 17.67 | 1.53 | 0.88 | 2.33 |
| *Salmonella typhi* ATCC 19430 | 12 | 13 | 11 | 12.00 | 0.00 | 0.00 | 0.00 |
| *S. aureus* ATCC 25923 | 13 | 12 | 12 | 12.33 | 0.58 | 0.33 | 0.33 |
| *E. coli* ATTC 25922 | 15 | 14 | 16 | 15.00 | 1.00 | 0.58 | 1.00 |
| *P. aeruginosa* ATCC 27853 | 17 | 16 | 18 | 17.00 | 0.00 | 0.00 | 0.00 |

**Total mean: 104.03 mm** **Average: 14.86 mm**

**Appendix 23: Susceptibility of microbes to the ethanolic extract of *C. odorata***

**Appendix :**

| Test Organisms | Zones of inhibition (mm) | | | | Standard Deviation | Standard Error of Mean | Variance |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | Mean | | | |
| *K. pneumonia* ATCC 33495 | 0 | 0 | 0 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Proteus mirabilis* ATCC 49565 | 18 | 19 | 17 | 18.00 | 0.00 | 0.00 | 0.00 |
| *S. saprophyticus*ATCC 15305 | 0 | 0 | 0 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Salmonella typhi*ATCC 19430 | 0 | 0 | 0 | 0.00 | 0.00 | 0.00 | 0.00 |
| *S. aureus*ATCC 25923 | 0 | 0 | 0 | 0.00 | 0.00 | 0.00 | 0.00 |
| *E. coli* ATTC 25922 | 0 | 0 | 0 | 0.00 | 0.00 | 0.00 | 0.00 |
| *P. aeruginosa*ATCC 27853 | 14 | 13 | 15 | 14.00 | 0.00 | 0.00 | 0.00 |

**24 Susceptibility of microbes to the aqueous extract of *C. odorata***

| Test Organisms | Zones of inhibition (mm) | | | | Standard Deviation | Standard Error of Mean | Variance |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | Mean | | | |
| *K. pneumonia* ATCC 33495 | 0 | 0 | 0 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Proteus mirabilis* ATCC 49565 | 17 | 19 | 15 | 17.00 | 0.00 | 0.00 | 0.00 |
| *S. saprophyticus*ATCC 15305 | 0 | 0 | 0 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Salmonella typhi*ATCC 19430 | 0 | 0 | 0 | 0.00 | 0.00 | 0.00 | 0.00 |

**Appendix :**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *S. aureus*ATCC 25923 | 0 | 0 | 0 | 0.00 | 0.00 | 0.00 | 0.00 |
| *E. coli* ATTC 25922 | 0 | 0 | 0 | 0.00 | 0.00 | 0.00 | 0.00 |
| *P. aeruginosa*ATCC 27853 | 12 | 11 | 12 | 11.67 | 0.58 | 0.33 | 0.33 |



**Appendix 25: Susceptibility of microbes to the aqueous extract of *H. opposite***

| Test Organisms | Zones of inhibition (mm) | | | | Standard Deviation | Standard Error of Mean | Variance |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | Mean | | | |
| *K. pneumonia* ATCC 33495 | 0 | 0 | 0 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Proteus mirabilis* ATCC 49565 | 0 | 0 | 0 | 0.00 | 0.00 | 0.00 | 0.00 |
| *S. saprophyticus*ATCC 15305 | 0 | 0 | 0 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Salmonella typhi*ATCC 19430 | 0 | 0 | 0 | 0.00 | 0.00 | 0.00 | 0.00 |
| *S. aureus*ATCC 25923 | 0 | 0 | 0 | 0.00 | 0.00 | 0.00 | 0.00 |
| *E. coli* ATTC 25922 | 0 | 0 | 0 | 0.00 | 0.00 | 0.00 | 0.00 |
| *P. aeruginosa*ATCC 27853 | 0 | 0 | 0 | 0.00 | 0.00 | 0.00 | 0.00 |

**Appendix**

**26 : Susceptibility of microbes to the ethanolic extract of *H. opposita***

| Test Organisms | Zones of inhibition (mm) | | | | Standard Deviation | Standard of Error Mean | Variance |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | Mean | | | |
| *K. pneumonia* ATCC 33495 | 0 | 0 | 0 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Proteus mirabilis* ATCC 49565 | 0 | 0 | 0 | 0.00 | 0.00 | 0.00 | 0.00 |
| *S. saprophyticus*ATCC 15305 | 0 | 0 | 0 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Salmonella typhi*ATCC 19430 | 0 | 0 | 0 | 0.00 | 0.00 | 0.00 | 0.00 |
| *S. aureus*ATCC 25923 | 0 | 0 | 0 | 0.00 | 0.00 | 0.00 | 0.00 |
| *E. coli* ATTC 25922 | 0 | 0 | 0 | 0.00 | 0.00 | 0.00 | 0.00 |
| *P. aeruginosa*ATCC 27853 | 0 | 0 | 0 | 0.00 | 0.00 | 0.00 | 0.00 |

**Appendix 27 Susceptibility of microbes to the aqueous extract of *M. lucida***

| Test Organisms | Zones of inhibition (mm) | | | | Standard Deviation | Standard Error of Mean | Variance |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | Mean | | | |
| *K. pneumonia* ATCC 33495 | 0 | 0 | 0 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Proteus mirabilis* ATCC 49565 | 0 | 0 | 0 | 0.00 | 0.00 | 0.00 | 0.00 |
| *S. saprophyticus*ATCC 15305 | 15 | 15 | 15 | 15.00 | 0.00 | 0.00 | 0.00 |
| *Salmonella typhi*ATCC 19430 | 0 | 0 | 0 | 0.00 | 0.00 | 0.00 | 0.00 |
| *S. aureus*ATCC 25923 | 0 | 0 | 0 | 0.00 | 0.00 | 0.00 | 0.00 |
| *E. coli* ATTC 25922 | 0 | 0 | 0 | 0.00 | 0.00 | 0.00 | 0.00 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *P. aeruginosa*ATCC 27853 | 7 | 7 | 7 | 7.00 | 0.00 | 0.00 | 0.00 |

**Appendix 28: Susceptibility of microbes to the ethanolic extract of *M. lucida***

| Test Organisms | Zones of inhibition (mm) | | | | Standard Deviation | Standard Error of Mean | Variance |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | Mean | | | |
| *K. pneumonia* ATCC 33495 | 10 | 10 | 10 | 10.00 | 0.00 | 0.00 | 0.00 |
| *Proteus mirabilis* ATCC 49565 | 0 | 0 | 0 | 0.00 | 0.00 | 0.00 | 0.00 |
| *S. saprophyticus*ATCC 15305 | 13 | 13 | 12 | 12.67 | 0.58 | 0.33 | 0.33 |
| *Salmonella typhi*ATCC 19430 | 11 | 11 | 11 | 11.00 | 0.00 | 0.00 | 0.00 |
| *S. aureus* ATCC 25923 | 0 | 0 | 0 | 0.00 | 0.00 | 0.00 | 0.00 |
| *E. coli* ATTC 25922 | 10 | 11 | 11 | 10.67 | 0.58 | 0.33 | 0.33 |
| *P. aeruginosa*ATCC 27853 | 11 | 11 | 11 | 11.00 | 0.00 | 0.00 | 0.00 |

**Appendix**



**29: Ethical Clearance**

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY
**COLLEGE OF HEALTH SCIENCES**
**SCHOOL OF MEDICAL SCIENCES / KOMFO ANOKYE TEACHING HOSPITAL**
***COMMITTEE ON HUMAN RESEARCH PUBLICATION AND ETHICS***

Our Ref: CHRPE/AP/007/14

20th January, 2014.

Mr. Ali Alhassan Dantankwa
Department of Biochemistry
and Biotechnology
School of Medical Sciences
KNUST-KUMASI.

Dear Sir,

LETTER OF APPROVAL

*Protocol Title* *"In Vitro Evaluation of Selected Medicinal Plants Against Methicillin Resistant Staphylococcus Aureus (MRSA)."*

*Proposed Site:* Biotechnology Laboratory, Department of Biochemistry and Biotechnology, KNUST, Kumasi.

Sponsor: *KNUST/KATH, Bench Fees.*

Your submission to the Committee on Human Research, Publications and Ethics on the above named protocol refers.

The Committee reviewed the following documents:

- A completed CHRPE Application Form.
- Research Proposal.

The Committee has considered the ethical merit of your submission and approved the protocol. The approval is for a fixed period of one year, renewable annually thereafter. The Committee may however, suspend or withdraw ethical approval at anytime if your study is found to contravene the approved protocol.

Data gathered for the study should be used for the approved purposes only. Permission should be sought from the Committee if any amendment to the protocol or use, other than submitted, is made of your research data.

The Committee should be notified of the actual start date of the project and would expect a report on your study, annually or at close of the project, whichever one comes first. It should also be informed of any publication arising from the study.

Thank you Sir, for your application.

Yours faithfully,

Rev. Prof. John Appiah-Poku.
**Honorary Secretary**
**For: CHAIRMAN**

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