COMPARATIVE STUDY OF THE ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL PROPERTIES OF *Allium sativum* (GARLIC) AND *Zingiber officinale* (GINGER) EXTRACTS ON SOME CLINICAL ISOLATES

BY

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CERTIFICATION

This is to certify that this research work was carried out by Eccepacem Chukwudebe with matriculation number 16/27/MMI006, under my supervision and it is a reflection of the student's input.

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DEDICATION

I dedicate this work to God Almighty who has guided me through the tough times.

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ABSTRACT

A comparative analysis of the antimicrobial activity and phytochemical properties of *Allium sativum* (garlic) and *Zingiber officinale* (ginger) extract was carried out on some clinical isolates. Garlic and ginger samples were extracted using ethyl acetate, acetone and methanol as extracting solvents using standard methods. Phytochemical screening was done using standard procedures. *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and *Candida albicans* were collected and were further identified using standard biochemical tests. Antimicrobial studies were carried out using Agar disc diffusion method and zones of inhibitions of garlic and ginger extracts were measured in millimetres. Minimum inhibitory, bactericidal and fungicidal concentrations of the garlic and ginger extracts were determined using broth dilution technique. Tetracycline, Ampicillin and Chloramphenicol antibiotics were used as positive control. Extracting solvents served as negative control. The result of the study showed that the phytochemicals in both garlic and ginger samples included alkaloids, flavonoids, tannins, saponins, glycosides and cardiac glycosides. The results of this study also revealed that methanolic extract of ginger gave the highest zones of inhibition of 26.3±1.15 mm at 200 mg/ml concentration against *S. aureus* while methanolic extract of garlic gave the highest zones of inhibition of 25.3±0.58 mm against *P. aeruginosa*. Also, ethyl acetate extract of garlic and acetone extract of ginger were observed to show the lowest zones of inhibition (9.66±8.39 mm and 9.66±8.50 mm) at same concentrations against *E. coli* and *P. aeruginosa* respectively. Tetracycline (TET 10g) and Gentamycin (GEN 10g) gave highest zone of inhibition of 23.0 ±2.00 mm and 22.0 ± 2.00 mm respectively against *S. aureus* and *C. albicans*. Ethyl acetate extract of garlic and ginger gave Minimum inhibitory concentration value of 100 mg/ml against *S. aureus, E. coli* and *P.*

aeruginosa respectively. Acetone extract of garlic showed Minimum inhibitory concentration against *S. aureus, P. aeruginosa* and *C. albicans* at 100 mg/ml respectively while Acetone extract of ginger gave Minimum inhibitory concentration at 200 mg/ml against *E.coli* and 100 mg/ml against *P. aeruginosa*. Methanolic extract of garlic gave Minimum inhibitory concentration at 200 mg/ml against *E.coli*, 100 mg/ml against *S. aureus* and *C. albicans* and 50 mg/ml against *P. aeruginosa* while the methanolic extract of ginger gave Minimum inhibitory concentration at 100 mg/ml against *E. coli, P. aeruginosa* and *C. albicans* and 50 mg/ml against *S. aureus*. The Minimum bactericidal concentration for acetone extract of garlic was 100 mg/ml against *S. aureus*. Also, methanolic extract of garlic and ginger gave Minimum bactericidal concentration at 200 mg/ml and 100 mg/ml against *E.coli* and *P. aeruginosa* respectively. The Minimum fungicidal concentration for all solvent extracts of garlic and ginger against *C. albicans* gave no result.

It was thus concluded that at 100 mg/ml and 200 mg/ml concentrations, garlic and ginger extracts could be effective against the clinical isolates and could serve as an alternative source of drugs. In recommendation therefore, plant sample extracts should be standardized by the appropriate bodies so as to confirm its safe use. Proper preservations and sustainable use of such plant resources especially considering the growth rate of multi- resistant drug strain of bacteria worldwide should be promoted.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of Study

Medicinal plants, also known as 'Chemical Goldmines', contain natural chemicals which are relevant and acceptable in human and animal systems, all of which are not being synthesized in laboratories. Many secondary bioactive metabolites in plant are important and used in a number of pharmaceutical companies. Man, over the years, has been dependent on plants for the treatment of diseases. Of the 250,000 higher plant species on earth, more than 80,000 are medicinal plants (Sacchetti *et al.,* 2005). The use of plant extracts as alternative therapy is a worldwide practice but this is mostly carried out in tropical countries (Naqvi *et al.,* 1991; Elvin-Lewis, 2001).

A number of medicinal plants (herbs) have been found and are in use in Ethno-medicine by traditional healers in the eradication of many diseases. According to Sofowora (1993), traditional medicinal plants as alternative therapy ranks highest among plants used in the investigations of antimicrobial properties. This could be due to their high traditional medicinal use, their capacity to produce a large number of organic chemicals of high structural diversity and also the ease with which research are carried out on the plants (Naqvi *et al.,* 1991). These discoveries have shown that extracts from plants contain both primary and secondary metabolites like alkaloids, glycosides, terpenoids, saponins, steroids, flavonoids, tannins, quinones and coumarins (Das *et al.,* 2010). These biological molecules are the source of plant-derived antimicrobial substances (Srivastava *et al.,* 2013). Some of these bio-products are highly efficient in their antioxidant, analgesic, antipyretics, cardio-protective, anti-inflammatory, antimicrobial, antispasmodics, and immune-modulatory

potentials (Sofowora, 1993; Okigbo *et al.,* 2009). The parts used for research are leaves, barks, roots, stems, flowers, fruits and even the seeds depending on the analysis (Farombi, 2003; Nguelefack *et al.,* 2005). The plant derived medicines may be used in many different forms including: powder, liquid or mixtures which could be uncooked or cooked such as, liniments, ointments and incisions.

1.2 (*Allium sativum* (Garlic)

Allium sativum (Garlic) belongs to the Liliaceae family. The genus *Alliium* includes garlic, scallions, onions, chives, leeks, shallots and asparagus. It is an herbaceous plant with height of 20-40 cm. It contains essential minerals such as phosphorus, potassium, magnesium, zinc, calcium, and iron, as well as trace minerals like iodine, sulfur, and chlorine. It is a rich source of vitamins like folate, thiamine, niacin, and vitamin C, A, K, and B_6. In terms of organic compounds, it is one of the rare sources of allicin and allisatin (Shobana *et al.,* 2009). Garlic is mainly used as herb to spice up many foods in various different cultures. It also contains substances which together functions to prevent disease and age-related conditions (Anon, 2009). Scientific and clinical studies have proven that garlic can increase immunity, fight against infectious diseases and inflammation and reduce cancer risk, cardio-vascular disease and dementia (Rahman, 2010; Labu *et al.,* 2019). The unique taste and smell are usually attributed to the presence of sulfur compounds, such as alliin, γ-glutamyl and their derivatives (Chan *et al.,* 2012). It has been estimated that the cysteine sulfoxides and γ-glutamylcysteine peptides are non-volatile and that garlic contains over 82% of the total sulfur (WHO, 1999). Organic-soluble allyl sulfur compounds found in garlic are alliin, ajoene, diallyl sulfide (DAS), diallyl disulfide (DADS) and diallyl trisulfide (DATS), while the water-soluble sulfur compounds of garlic may occur especially after alcoholic

fermentation and the parent compounds; alliin and gamma-glutamyl S-allylcysteine; are converted to S-allylcysteine (SAC), S-allylmercaptocysteine (SAMC) and others (Chekki *et al.,* 2014). Allicin is the most predominant thiosulphate in garlic that is responsible for its peculiar smell, antibacterial effect and is also toxic to insects (Cunha, 2012). The organosulfur compounds derived from garlic such as alliin, allicin and diallyl sulfide are known to have antioxidant properties (Tapiero, 2004).

Initial reports on antimicrobial activity of garlic showed that allicin (allyl 2-propene thiosulfinate) a notable flavonoid in garlic is formed when garlic cloves are crushed (Ross *et al.,* 2000). One milligram of alliin is considered equivalent to 0.45 mg of allicin (WHO, 1999). Allicin formation follows the action of an enzyme, allinase of the bundle sheet cells upon the alliin of the mesophyll cells. When crushed, *A. sativum* yields allicin, a powerful antibiotic and antifungal compound (phytoncide). Studies have shown that these essential oil, water and ethanol extracts inhibit the growth of *Bacillus* species, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida* species (Ankri, 1999; WHO, 1999).

1.3 *Zingiber officinale* (Ginger)

Zingiber officinale (Ginger) a member of the Zingiberaceae family, is a perennial herbaceous plant (Gupta and Sharma, 2014). It is commonly used as a spice and a medicinal plant (Ali *et al.,* 2008). It is a semi-woody perennial herb, 3 to 4 feet high from the root stock. It grows rapidly and the leaves and flowers are used medicinally. The plant produces an orchid like flower with petals that are greenish yellow streaked with purple colour. Chemical analysis of ginger shows that it contains over 400 different compounds (Ali *et al.,* 2008). The major constituents in ginger rhizomes are carbohydrates (50–70%), lipids (3–

8%), terpenes, and phenolic compounds (Grzanna *et al.,* 2005). Terpene components of ginger include zingiberene, β-bisabolene, α-farnesene, β-sesquiphellandrene, and α-curcumene, while phenolic compounds include gingerol, paradols, and shogaol. These gingerols (23–25%) and shogaol (18–25%) are found in higher quantity than others. Besides these, amino acids, raw fiber, ash, protein, phytosterols, vitamins (e.g., nicotinic acid and vitamin A), and minerals are also present (Langner *et al.,* 1998; Shukla and Singh, 2007). The aromatic constituents include zingiberene and bisabolene, while the pungent constituents are known as gingerols and shogaols (Tyler, 1994). Other gingerol- or shogaol-related compounds (1–10%), which have been reported in ginger rhizome, include 6-paradol, 1-dehydrogingerdione, 6- gingerdione and 10-gingerdione, 4- gingerdiol, 6-gingerdiol, 8-gingerdiol, and 10-gingerdiol, and diarylheptanoids (Ali *et al.,* 2008). The characteristic odor and flavor of ginger are due to a mixture of volatile oils like shogaols and gingerols (Tyler, 1994). Ginger is thought to act directly on the gastrointestinal system to reduce nausea. It is also used to treat morning sickness, colic, upset stomach, gas, bloating, heartburn, flatulence, diarrhea, loss of appetite, and dyspepsia (discomfort after eating), thus enhancing the digestion of food (Ali *et al.,* 2008). The consumption of ginger rhizome is a typical traditional remedy to relieve common health problems including pains, infections, nausea and vomiting (Li *et al.,* 2019). Furthermore, ginger has been reported to act as pain relief for arthritis, muscle soreness, chest pain, low back pain, skin burns, stomach pain, and menstrual pain. It is also used for treating upper respiratory tract infections, cough and bronchitis, as an anti-inflammatory agent, and to reduce high blood pressure (Qidwai *et al.,* 2003; Shukla and Singh, 2007). Ginger is also used as a flavoring agent in beverages, and also serves as a fragrance in soaps and cosmetics (Alam, 2013).

1.4 Statement of Problem

The increase in multidrug resistance among pathogenic microbes caused by the increased usage of antibiotics as observed in recent times and their ability to transmit and acquire resistance to drugs used in treatment of diseases have become a huge problem (Cohen, 1992; Abimbola *et al.,* 1993). Infectious diseases are the world's major threat to human health and account for almost 50,000 deaths every day (Ahmad and Beg, 2001). Many obligate and opportunist pathogens are becoming increasingly resistant to most available drugs at an alarming rate that is unmatched by the development of new drugs (Newman *et al.,* 2003). Also most antibiotics packaged and consumed have been discovered to have great side effect on the body organs particularly the liver and kidney during metabolism of the consumed drugs since they do not dissolve easily and quickly (Bhanu *et al*., 2013). According to WHO (1999), it was noted that antimicrobial resistance is on the rise with millions of deaths every year and that numerous factors have been identified that affect antimicrobial use and any strategy designed to improve the use of antimicrobial agents must take all of these factors into account. These factors according to them could be misuse of drugs, lack of knowledge, inadequate diagnosis and incorrect drug selection for treatment of infection, financial gain, multi resistant nature of microorganisms to drugs and self-medication. Despite these, among the estimated 400,000 plants species known and used, only 6% have been studied for biological activities and about 15% have been subjected to phytochemical screening (Cohen, 1992). The phytochemical content and pharmacological actions, if any, of many plants having medicinal potential remain unassessed by rigorous scientific research to define efficacy and safety (Ahn, 2017). Also only about 1% of Nigerian medicinal plants had been scientifically evaluated for potential chemotherapeutic/

medicinal value Nwankwo and Osaro (2014) while approximately 20% of the plants in the world have been subjected to pharmacological/ biological screening (Mothana and Lindequist, 2005). To eradicate this menace, further and broader researches should be conducted so as to enhance discovery of new antibiotics (especially from various plant parts) which are easily available and have considerably less side effects Doughari (2006); Khulbe and Sati (2009) and also to understand the genetic mechanisms of resistance of pathogenic organisms to antibiotics. It is therefore necessary to carry out a study of the antimicrobial effect and phytochemical properties of crude extract of some selected plant samples (garlic and ginger) on some selected microbial isolates.

1.5 Justification

The emergence of new and trending infections caused by organisms in recent years has become a threat to the existence of man. Man is forced by the ecosystem to co-exist with these organisms of which majority are pathogenic. The immunity of man to these prevalent organisms is reduced because of exposure to hazardous substances found in the environment. Synthetic antibiotics have for decades helped in combating these pathogenic organisms. Unfortunately most of these organisms are gaining resistance to the discovered antibiotics and pharmacologists are in the battle to provide or discover new antibiotics to fight them (both already invading microorganisms and the newly growing organisms). This study is therefore geared towards comparing the antimicrobial potency of plant samples (garlic and ginger) against regular antibiotics so as to ascertain which is more active against some selected pathogens. The research will serve to further project the relevance of medicinal plants in the struggle to combat microorganisms.

The findings of this research would also serve as the basis for further research on the antimicrobial properties of plant samples.

1.6 Aim of the Study

The aim of the study is to compare antimicrobial activity and phytochemical screening of garlic bulb and ginger rhizome extract on some clinical isolates.

1.7 Objectives of the Study

The objectives of this study are to:

i. obtain the ethyl acetate, acetone and methanolic extracts of garlic bulb and ginger rhizome sample
ii. carryout qualitative and quantitative phytochemical screening on garlic bulb and ginger rhizome crude extracts.
iii. determine the antimicrobial activities of the garlic bulb and ginger rhizome extracts on some bacterial and fungal clinical isolates.
iv. compare the antimicrobial activities of garlic bulb and ginger rhizome extracts against that of commercially purchased antimicrobial drugs.
v. determine the minimum inhibitory concentration, minimum bactericidal and minimum fungicidal concentrations of garlic bulb and ginger rhizome extract on the clinical isolates.

1.8 Significance of the Study

For years, synthetically produced antibiotics have been used in treatment and management of a vast array of diseases and infections. However, there has been an increase in resistance to these synthetically produced antibiotics. The significance of this study therefore is to improve and validate the use of alternative medicinal sources especially ginger and garlic. Humankind serves to gain from an alternative to synthetically produced antibiotics. This research attempts to shed more light on alternative sources of treatments of diseases especially ginger and garlic. The study will serve as a resource base to other scholars and researchers interested in carrying out further research in this field. It will also add to the existing body of literature on the subject.

CHAPTER TWO

2.0 LITERATURE REVIEW

Infectious diseases are the world's major threat to human health and account for almost 50,000 deaths every day (Ahmad and Beg, 2001). According to WHO (1999), it was noted that antimicrobial resistance is on the rise with millions of deaths every year and that numerous factors have been identified that affect antimicrobial use and any strategy designed to improve the use of antimicrobial agents must take all of these factors into account. These factors according to them could be misuse of drugs, lack of knowledge, inadequate diagnosis and incorrect drug selection for treatment of infection, financial gain, and self-medication. According to the Centers for Disease Control and Prevention, 'each year in the United States, at least 2 million people become infected with bacteria that are resistant to antibiotics and at least 23,000 people die each year as a direct result of these infections, this is even worse in the case of developing nations like Nigeria.

The use of medicinal plants is growing worldwide, because of the increasing toxicity and allergic manifestations of the synthetic drugs (Bhanu *et al.,* 2013). Many obligate and opportunist pathogens are becoming increasingly resistant to most available drugs at an alarming rate that is unmatched by the development of new drugs (Newman *et al.,* 2003). Among the estimated 400,000 plants species, only 6% have been studied for biological activities and about 15% have been subjected to phytochemical screening (Cohen, 1992). Traditional knowledge to solve health problems of mankind and animals exist in all countries of the world (Rukangira, 2001). According to Khan (1996), the investigation of certain indigenous plants for their antimicrobial properties is very useful and there is

increasing interest in plants as source of agents to fight microbial and treatment of several infections (Aburjai *et al.,* 2001).

2.1 History of Medicinal Plants

Human use of plants as medicines could be dated back to the Middle Age, which is about 60,000 years ago, according to fossil records (Fabricant and Farnsworth, 2001). The first records that were written on clay tablets in cuneiform were from Mesopotamia and dated from about 2600 BC. Some of the substances that were used include oils of *Cedrus* species (cedar) and *Cupressus sempervirens* (cypress), *Glycyrrhiza glabra* (licorice), *Commiphora* species (myrrh) and *Papaver somniferum* (poppy juice), most of these are still in use today for treating diseases ranging from colds to parasitic infections and inflammation (Gurib-Fakim, 2006). Health care in ancient time used leaves, flowers, stems, berries and roots of herbs for their therapeutic or medicinal value. These medicines initially took the form of crude drugs such as tinctures, teas, poultices, powders, and other herbal formulations (Balick and Cox, 1996; Samuelsson, 2004). Knowledge of the specific plants to be used and the methods of application for particular ailments were passed down through oral history and information regarding medicinal plants was eventually recorded in herbals (Balunasa and Kinghorn, 2005).

2.2 General Uses of Medicinal Plants

Medicinal plants (otherwise referred to as herbs, herbal medicines, or phytomedicinals) remain the known form of medicine in most countries. Over three quater of the earth's population depend primarily on raw plant products to meet their daily health care needs (Barrett and Kieffer, 2001). Most of the collected plant materials are used fresh in order to obtain the extract from the whole plant or parts of it, which could be leaves, roots, flowers or

fruit. In the case of woody plants, the bark, roots and other parts are used. Carminatives such as ginger, cloves and coriander are also usually added as fresh or dried materials (Roa and Arora, 2004). For example, bearberry (*Arctostaphylos uva-ursi*) and cranberry juice (*Vaccinium macrocarpon*) have been reported to treat urinary tract infections while species such as ginger (*Zingiber officinale*), lemon balm (*Melissa officinalis*), garlic (*Allium sativum*) and tea tree (*Melaleuca alternifolia*) are known as broad-spectrum antimicrobial agents (Heinrich *et al.,* 2004). Some plant extracts with great medicinal value are the stem bark decoction of *Albizia gummifera,* which is used in the treatment of venereal diseases (Buwa and Van Staden, 2006), leaves of *Glyphaea brevis*, which are macerated in water and are used to cure intestinal diseases and hepatitis (Noumi and Yomi, 2001) and oil extracts of the roots, seeds and stem barks of *Monodora myristica,* which are used to treat scabies, helminthiasis, malaria and dysenteric syndromes (Okpekon *et al.,* 2004).

Many plants used as traditional medicines are now being validated through scientific research by isolating its bioactive compounds for direct use in medicines. For instance, drug discovery from medicinal plants led to the isolation of early drugs such as morphine from opium, cocaine, codeine, digitoxin and quinine, some of which are still in use (Samuelsson, 2004; Balunasa and Kinghorn, 2005). Recently a drug, β-methoxypsoralen, has been produced from the plant *Ammi majus* (bishop's weeds), which was reported by Egyptian medical practitioners to treat vitiligo, psoriasis and other skin disorders, as well as T-cell lymphoma (Gurib-Fakim, 2006).

2.3 Microbial Infections

Microbial infections are caused by microorganisms. In other to cause diseases, microorganisms must reach the site which they are targeting, attach to that site, multiply and avoid attack by the immune system of the individuals. This site of entry into the body is called the portal of entry. According to Collier (1998), there are different sites for microbes to enter human body and these are: oral route, respiratory system, wounds, urogenital tract. Micro-organisms are ubiquitous in nature and most of them are pathogenic thereby causing diseases while others are non-pathogenic in nature. Some bacteria for example live in symbiotic relationship with plant roots, while other types of bacteria aid food digestion in humans. On the other hand, some bacteria can cause diseases which can even lead to death in some cases (Collier, 1998). Infection by bacteria happens when harmful bacteria start reproducing and multiplying at a fast rate in the body causing mild to severe infections, the latter of which is exemplified by tuberculosis, cholera and plague. Bacterial infections like ear infection occur mostly in children. Some infections are specific to the organ they invade; for example surgical wounds may get infected with *S. aureus*. Treatment with appropriate antibiotics, which act through either by killing the bacteria or prevent their reproduction, can cure diseases caused by bacterial infections. Penicillin which is still in use was the first antibiotics discovered. Other drugs such as tetracycline, erythromycin, bacitracin, fluroquinolones, and cephalosporins, are commercially available and have being of help to humanity in controlling infection. The biggest threat however is the emergence of some bacterial strains that have developed resistance to one or more types of antibiotics (Chopra *et al.,* 2002).

2.4 Types and Sources of Antimicrobials

There exist different types of antimicrobials. These include antibiotics, anti-viral, anti-fungal, anti-protozoan. Antibiotics are used in the treatment of bacterial infections and can be obtained from either natural or synthetic sources. Examples of those with a natural origin are phenyl-propanoids (chloramphenicol), polyketides (tetracycline), aminoglycosides (streptomycin, gentamycin), macrolides (erythromycin), glycopeptides (vancomycin) and second-generation β-lactams (cephalosporins). Those from synthetic sources are sulphonamides, quinolones and oxazolidinones. Most antibiotics exert their action either by inhibition of the bacterial cell wall or protein synthesis. Exceptions are the quinolones that inhibit DNA synthesis, and the sulphonamides that inhibit the synthesis of metabolites used for the synthesis of deoxyribonucleic acid (DNA) (Singh and Barrett, 2006). Most anti-viral, anti-fungal, anti-protozoa and anti-cancer drugs however are obtained from synthetic sources. Because of the re-occurring resistance of pathogenic microorganisms to antibiotics, as well as the side effects presented by these antibiotics, investigation on other sources of antimicrobials, such as medicinal plants, for their antimicrobial properties is in practice. Plants produce secondary metabolites (phytochemicals), which have demonstrated their potential as antibacterial if used alone and as synergists of other antibacterial agents. Phytochemicals frequently act through different mechanisms than conventional antibiotics and could therefore be of use in the treatment of resistant bacteria (Abreu *et al.,* 2012).

2.4.1 Natural Products in Antimicrobial Agents

Antimicrobial agents are chemical or biological substances used to kill or prevent the growth of microorganisms. A great number of these agents already exist and their actions on micro-

organisms are due to the presence of certain substances in plants. Medicinal plants are rich source of wide variety of secondary metabolites belonging to chemical classes such as sterols, alkaloids, glycosides, saponins, flavonoids, tannins, and carbohydrates are generally superior in their anti-microbial activities (Cowan, 1999). The study of natural products involves isolation in a pure form of these compounds and investigation of their structure, formation, use, and purpose in the organism. Secondary metabolites appear to function primarily in defense against predators and pathogens and in providing reproductive advantage as intra-specific and inter specific attractants (Cowan, 1999).

Natural products have been the greatest source of successful antibiotic compounds throughout history. Over the years, 80% of the bioactive, natural products isolated have been antibiotic compounds, and this trend is continuing (Newman *et al.,* 2003). In particular, bacteria and fungi have been a reliable source for the discovery of useful antibiotic compounds. For example, the penicillins and cephalosporins are both clinically used drug classes derived from fungal natural product structures. However, most of these lead have been derived from terrestrial organisms, and in recent times, totally the discovery of new biologically active chemical compounds has diminished. In this respect, the oceans, which cover over 70% of the planet and are home to an enormous biodiversity of species, offer vast opportunities in the discovery of new bio-active natural products (Newman and Cragg, 2004). Natural products have the potential to provide medicine with a source of novel structures that are unobtainable from sources such as combinatorial synthesis. Nature is capable of producing complex molecules with multiple chiral centers that are designed to interact with biological systems (Cordell, 2000). These compounds are often used by the producing organism as a self-defense mechanism (Rocha *et al.,* 2001). Plants also known to

have special ability to synthesize aromatic substances, most of which include alkaloids, quinones, flavones, tannins, phenols or their oxygen-substituted derivatives (Agrawal *et al.,* 2012). Quinones are another class of compounds, which are characteristically highly reactive. They are responsible for the browning reaction of cut or injured fruits and vegetables and are known to complex irreversibly with nucleophilic amino acids in proteins often leading to inaction (Stern *et al.,* 1996). For that reason, the potential range of Quinone anti-bacterial effects is great. Plants are known to synthesize flavonoids in response to microbial infections (Agrawal *et al.,* 2012). Hence it should not be surprising that they have been found to be effective antimicrobial substances against a wide array of microorganisms. Their activity is probably due to their ability to complex with extra cellular and soluble proteins and to complex with bacteria cell walls. Catechins flavonoids have been extensively researched due to their occurrence in oolong green teas. According to Tsuchiya *et al.* (1996), it was noticed some time ago that these teas exerted anti-microbial activity and that they contain a mixture of Catechin compounds. These compounds as reported by Sakanaka *et al.* (1992); Borris (1996); Tsuchiya *et al.* (1996), inhibited *in vitro Vibrio cholerae Streptococcus mutans* and other bacteria and microorganisms. Flavonoid compounds also exhibit inhibitory effects against multiple viruses.

Furthermore, terpenoids are active against bacteria, fungi and viruses (Fujioka and Kashiwada, 1994; Mendoza *et al.,* 1997; Suresh *et al.,* 1997). In 1997, it was reported that 60% of essential oil derivatives examined to date were inhibitory to fungi while 30% inhibited bacteria (Chaurasia and Vyas, 1997). The triterpenoid betullinic acid is just one of the several terpenoids which have been shown to inhibit HIV. The terpenoid, petalostemmumol obtained from the ethanol soluble fraction of the purple prairie clover

showed excellent activity against *Bacillus subtilis* and *Staphylococus aureus* and less activity against gram negative bacteria as well as *Candida albicans* (Hufford *et al.,* 1993). Alkaloids are another group of compounds that show antimicrobial activity. The diterpenoid alkaloids from the plants of *Ranuniculaceae* family are commonly found to have antimicrobial activity (Rahman and Choudhary, 1995; Omulokoli *et al.,* 1997). Solamargine, a glycol alkaloid from the berries of *Solanum khastanum* and other alkaloids may be useful against HIV infection as well as intestinal infections associated with AIDS (Mcmahon *et al.,* 1995; Mcdevitt *et al.,* 1996). Although some secondary metabolites may not be associated with a pronounced activity on any organisms, it may be working synergistically with other chemicals in the plant to provide the much-needed protection for the plant to survive (Abeywardhana *et al.,* 2014).

2.5 Some Phytochemicals in Natural Products

Phytochemical is a natural bioactive compound found in plants, such as vegetables, fruits, medicinal plants, flowers, leaves and roots that work with nutrients and fibres to act as a defense system against diseases or more accurately, to protect against disease. Unlike vitamins and minerals, they have no nutritional value. They can however influence various body processes. They work together with nutrients and dietary fibre to protect the body against diseases, slow the aging process and reduce the risk of many diseases such as cancer, heart disease, stroke, high blood pressure (Igwenyi *et al.,* 2011). Phytochemicals are divided into two groups, which are primary and secondary constituents; according to their functions in plant metabolism. Primary constituents comprises of common sugars, amino acids, proteins and chlorophyll while secondary constituents consists of alkaloids, terpenoids, phenolic compounds, flavonoids, tannins and so on (Krishnaiah *et al.,* 2009). The

phytochemical compounds identified in the plant extract like flavonoids and saponins been generally found to be haemolytic, anticancer and anti-inflammatory compounds and not analgesic (Evans, 2002; Ajali, 2004).

The phytochemical screening of *Zingiber officinale* rhizome also revealed the presence of polyphenols and reducing sugars this results compared favourable well with the one reported by Osabor *et al.* (2015) for cola lepidota seeds. Polyphenols have been implicated in medical circle to protect person against ageing and can inhibit cancer growth (Rahman, 2010). The extract also contains glycosides known to possess anti-neoplastic properties (Kar, 2007). Cyanogenic glycosides are known to be active against slugs and snails (Harbone, 1998; Umerie *et al.,* 2007),

2.5.1 Flavonoids

Flavonoids are water soluble polyphenolic molecules and therefore belong to the polyphenol family. Together with carotenes, flavonoids are also responsible for the coloring of fruits, vegetables and herbs. Flavonoids possess antioxidant properties and ensure healthy circulation of blood. It helps to strengthen capillaries wall. The compound is sometimes referred to as phytoestrogens. Phytoestrogens are associated with relief of menopausal systems, reduction of osteoporosis, improvement of blood cholesterol levels, and lowering the risk of certain hormone-related cancers and coronary heart disease (Rahman, 2010). Flavonoids have antioxidant activities as well as much health promoting effects viz., anti-allergic, anti-cancer, anti-oxidant, anti-inflammatory, anti-thrombotic, vasoprotective, tumour inhibitory and anti-viral effects. These effects have been associated with the influence of flavonoids on arachidonic acid metabolism. Some flavonoid containing plants

are diuretics (e.g. buchu), antispasmodic (e.g. liquorice) and others have antimicrobial properties (Trease and Evans, 2002). Epidemiological studies have shown that heart diseases are inversely related to flavonoid intake and that flavonoids prevent the oxidation of LDL therefore reducing the risk for the development of atherosclerosis (Prohp and Onoagbe, 2012). The presence of flavonoids in the leaves of *Cissampelos mucronata* which have hypoglycaemic and anti-diabetic properties have also been documented (Tanko *et al.,* 2007). Effects of flavonoids, quercetin and ferulic acid on pancreatic β-cells leading to their proliferation and secretion of more insulin have been proposed by Mahesh and Menon (2004) and Sri-Balasubashini *et al.* (2004) as the mechanism of their hypoglycaemic activity in streptozotocin-induced diabetic rats. These are justifications for the use of the extracts of *Allium sativum* in the treatment of *Diabetes mellitus*. Flavonoids exhibit dramatic effects on immune and inflammatory cells; these can be either immunosuppressant or immune-stimulatory (Huang *et al.,* 2001). In some cases, the immunosuppressant effect is not caused by direct cytotoxicity of the flavonoids themselves. Some studies indicate that the effects are possible only when these cells are physiologically-activated. Flavonoids detected in *Allium sativum* bulbs could be used in the treatment of various disease conditions like edema, toothache, fever, common cold, diarrhea and dental caries. These could be possible as the root extracts contains some antibacterial activities. The flavonoids are acting on bacteria by inhibiting its protein synthesis (Hong-Xi and Song, 2001).

2.5.2 Tannins

Tannins may decrease protein quality by decreasing digestibility, and palatability. Other nutritional effects which have been attributed to tannins include damage to the intestinal tract, toxicity of tannins absorbed from the gut, and interference with the absorption of iron,

and a possible carcinogenic effect (Osagie and Eka, 1998). In addition, tannin has astringent properties, hastens the healing of wounds and inflamed mucous membrane. Plants with tannins are used for healing of wounds, varicose ulcers, hemorrhoids, frost- bite and burns (Tanko *et al.,* 2007). Tannins are known to possess immuno stimulating activites. Well known Ayurvedic formulation, *Triphala churna* contains *Terminalia chebula*, *Terminalia belenica* and *Emblica officialis*, which are rich in tannin and has been reported for its immune-stimulating activity (Kumar and Subrahmanyam, 2013). Tannins are also secondary metabolites in plants. They are glycosides of protocatechic acids. Tannins have astringent properties, hasten the healing of wounds and inflamed mucous membrane (Njoku and Akumefula, 2007). Their astringent property makes them useful in preventing diarrhea and controlling hemorrhage due to their ability to precipitate proteins, mucus and constrict blood vessels (Kokwaro, 2009). This is the reason why traditional healers use plants reach in tannins to treat wounds and burns since they are able to cause blood clotting. Some tannins have been reported to inhibit HIV replication selectively besides the use of diuretics (Argal and Pathak, 2006). This shows how traditional medicinal plants rich in tannins can be used to control this dangerous disease. According to Chung *et al.* (1998), many tannin molecules have been shown to reduce the mutagenic activity of a number of mutagens. The growths of many fungi, yeast, bacteria and viruses have been proven to be inhibited by tannins. Tannins have also been reported to exert physiological effects, such as to accelerate blood pressure, decrease the serum lipid level and produce liver necrosis and module immune responses. The dosage and kind of tannins are critical to these effects (Chung *et al.,* 1998).

2.5.3 Alkaloids

Alkaloids have been implicated in its analgesic activities. Morphine and morphine-type alkaloids are classed as narcotic or opiate analgesics and are strong agonists (Way *et al.,* 2001). These substances mediate their action by binding to opiate receptors in the central nervous system, causing inhibition of ascending pain pathways and altering the perception of and response to pain, thus producing generalized central nervous system depression (Takemori *et al.,* 1999; Umerie *et al.,* 2007). They are the main ingredients used in the preparation of analgesic drugs. The presence of alkaloids in *Allium sativum* shows the potential of the extract to have an analgesic, anti-inflammatory and adaptogenic effects, which help the host (man and animal) to develop resistance against disease and endurance against stress (Gupta, 1994).

2.5.4 Saponins

These are heterogeneous groups of natural products with a marked hormonal activity, strong expectorant and aid in the absorption of nutrients (Rahman, 2010). Saponins are steroid or triterpenoid glycosides characterized by their bitter or astringent taste, foaming properties and their haemolytic effect on red blood cells. Saponins possess both beneficial (cholesterol-lowering) and deleterious (cytotoxic permeabilization of the intestine) properties and also exhibit structure dependent biological activities (Osagie and Eka, 1998). Saponins cause a reduction of blood cholesterol by preventing its reabsorption (Prohp and Onoagbe, 2012). Also, it has also been documented that saponins have antitumor and anti-mutagenic activities and can lower the risk of human cancers, by preventing cancer cells from growing. Saponins are believed to react with the cholesterol rich membranes of cancer

cells, thereby limiting their growth and viability (Roa *et al.,* 1995). Plants produce saponins to fight infections by parasites and in humans saponins help the immune system and also protect against viruses and bacteria. The non-sugar part of saponins has a direct antioxidant activity which may result in reduced risk of cancer and heart diseases (Prohp and Onoagbe, 2012). Steroidal saponins have the ability of drastic reduction in cholesterol levels and raises high density lipoprotein (HDL). Saponins are used by the folkloric remedies of Kashmir (India) in treating wounds, help in blood clotting and enteric ulcers problems (Roa *et al.,* 1995). This is because of their ability to cause red blood cells to precipitate and coagulate (Just *et al.,* 1998; Maobe *et al.,* 2013). Saponins have also being associated with inhibitory effect on inflammation and used to prevent hypercholesterolemia, antibiotic activity, anti-inflammatory and anti-diabetic (Maobe *et al.,* 2013). The presence of saponins shows the potential of the plants to be used to produce mild detergents and intracellular histochemistry staining to allow antibody access to intercellular proteins (Just *et al.,* 1998). They have been found to treat hypercholesterolemia, hyperglycemia, antioxidant, anti-inflammatory, central nervous system activities, anticancer and weight loss (Just *et al.,* 1998).

2.5.5 Terpenoids

These have medicinal value such as anti-carcinogenic, antimalarial, antimicrobial and diuretics activity (Pichersky and Gershezon, 2002; Deganhardt, 2003). Terpenoids have also shown a great potential in treatment against disease causing microorganisms. Terpenoids have exhibited antibacterial activity against *E. coli*, *Staphylococcus* species*, P. aeruginosa,* methillin-resistant *S. aureus*, *Proteus mirabilis, K. pneumoniae*, *Listeria monocytogenes, Enterobacter cloacae*, yeast *Candida albicans* and fungi, *Aspergillus flavus* (Santos *et al.,*

2008; Piera *et al.,* 2011; Leandro and Vargas, 2012). Terpenoids have been proved scientifically to kill mosquito larvae (Anthoney *et al.,* 2014).

2.6 Mechanism of Action of Plant Secondary Compounds

Plant secondary compounds are usually classified according to their biosynthetic pathways. A good example of a widespread metabolite family is the phenolics, because these molecules are involved in lignin synthesis, they are common to all higher plants. Phenolic compounds are potent antioxidants and free radical scavengers which can act as hydrogen donors, reducing agents, metal chelators and singlet oxygen quenchers (Chew *et al.,* 2009). Studies have shown that phenolic compounds such as catechin and quercetin are very efficient in stabilizing phospholipid bilayers against peroxidation induced by reactive oxygen species (Gulcin *et al.,* 2010; Gulcin, 2010). Flavonoids, which are a subclass of phenolics, are known to be synthesized by plants in response to microbial infection and they have been found *in vitro* to be effective antimicrobial substances against a wide array of microorganisms. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls (Cowan, 1999). Tannins and flavonoids are thought to be responsible for antidiarrhoeal activity by increasing colonic water and electrolyte reabsorption (Palombo, 2006). Terpenoids are condensation products of C_5 isoprene units which are important constituents of essential oils (Pichersky and Gershenzon, 2002). They have been shown to be active against bacteria, fungi, viruses, and protozoa. The mechanism of action of terpenes is not fully understood but is speculated to involve membrane disruption by the lipophilic compounds (Cowan, 1999). Alkaloids are the best known nitrogen-containing metabolites of plants and are sparsely distributed in the plant kingdom, but much more specific to defined plant genera and species. This is probably

due to the limited supply of nitrogen in plants (Harborne, 1999). Alkaloids have been found to have antimicrobial properties with microbiocide effects against *Giardia* and *Entamoeba* species as well as antidiarrhoeal effects, which are probably due to their effects on transit time in the small intestine (Cowan, 1999).

In a research carried out by Nitta *et al.* (2002), the active extracts obtained from the bark of *Shorea hemsleyana* and roots of *Cyphostemma bainessi* were separated into their components and these exhibited strong inhibitory activity against methicillin-resistant *Staphylococcus aureus*. These active compounds were identified as stilbene derivatives.

2.7 Selected Clinical Isolates

Resistant pathogenic gram positive and gram negative clinical isolates was selected for the course of this study.

2.7.1 *Staphylococcus aureus*

S. aureus is a strain of gram positive bacteria that belongs to the family of Micrococcaceae (Punopas, 2002). *S. aureus* is a spherical cell having a diameter about 1μm. It is a non-motile, non-spore forming and facultative anaerobe, so it grows by anaerobic respiration or fermentation. *S. aureus* are present in clusters (Harris *et al.,* 2002). Microscopically, it looks like a branch of grapes (Ranpal, 2009). *S. aureus* appears as a golden yellow colony on rich medium agar plate and hemolytic on blood agar plate. *S. aureus* can be differentiated from the other species by showing positive results in mannitol fermentation, deoxyribonuclease and coagulase tests (Franklin, 1998). *S. aureus* can cause various types of skin infections such as abscess, impetigo, carbuncles, boils, furuncles and folliculitis. These are

opportunistic infections as it usually occurs due to the present of previous skin injuries like insect bites, ulcers, burns or wounds (Punopas, 2002; Zaidan *et al.,* 2005; Ranpal, 2009). Pyogenic abscess is the basic skin lesion that is caused by *S. aureus*. In addition, *S. aureus* is able to cause a more serious infection such as toxic shock syndrome (TSS) by exhibiting some extracellular toxins. *S. aureus* also can cause food poisoning when a person consumes contaminated food that contained enterotoxins produced by *S. aureus*. These staphylococcal enterotoxins are resistant to high temperature. In addition, *S. aureus* also causes scaled skin syndrome, an exfoliative dermatitis which damages the epidermal layer of skin. This syndrome mostly occurs in the newborn and young children (Punopas, 2002).

2.7.2 *Escherichia coli*

E. coli is a gram negative bacterium, belonging to the family of Enterobacteriaceae. *E. coli* is a straight rod cell with having a size of 2.0 - 6.0μm long and 1.1 - 1.5μm in diameter. It is a mobile, non-spore-forming, facultative anaerobe and chemo-organotroph. It can appear as either single or in pairs. *E. coli* show positive results in methyl red test, catalase test and indole test, but negative results in oxidase and citrate tests (Huang *et al.,* 2001; Brooks *et al.,* 2004). *E. coli* forms milky white colonies on the LB agar and is known to cause watery diarrhea in young children especially in developing countries. They exist in the alimentary tract of humans and animals. There are different strains of *E.coli* of which the majority are harmless commensals in the gut. However some strains like (O157: H7) can cause several severe illness such as diarrhoea; anaemia, kidney failure or urinary tract infections. A person can easily be infected by *E.coli* from contaminated food, water or direct contact (Collier, 1998).

2.7.3 *Pseudomonas aeruginosa*

P. aeruginosa is a common gram negative, rod shaped bacterium that can cause disease in plants and animals, including humans. It belongs to the family Pseudomonadaceae. A species of considerable medical importance, *P. aeruginosa* is a multidrug resistant pathogen recognized for its ubiquity, its intrinsically advanced antibiotics resistance mechanisms, and its association with serious illnesses and hospital acquired infections such as a ventilator-associated pneumonia and various sepsis syndromes. *P. aeruginosa* is citrate, catalase and oxidase positive. It is found in soil, water, skin flora and most man made environments. It thrives not only in normal atmosphere but also in low oxygen atmosphere, moist surfaces and can aggregate into enduring biofilms. Serious infections caused by *P. aeruginosa* can occur in patients under treatment in hospitals or those with compromised or weak immune systems. In some cases pseudomonas infections can lead to death, especially if it co-infects people who have pneumonia, blood infections or in case of infection during or after surgery (Awada *et al.,* 2003).

2.7.4 *Candida albicans*

C. albicans is an opportunistic pathogenic yeast that is a common member of the human gut flora. It belongs to the family Saccharomycetaceae. It is generally referred to as dimorphic fungus since it grows both as yeast and filamentous cells. *C.albicans* is easily cultured in the laboratory and can be studied both in-vivo and in-vitro. *C. albicans* are able to grow in different environments, both as a commensal and as a pathogen. *C. albicans* can cause both superficial and local infections (Awada *et al.,* 2003).

2.8 Antimicrobial Properties of Selected Plant Crude Extracts

2.8.1 Antimicrobial Properties of *Allium sativum* (Garlic)

Numerous researches have demonstrated that allicin, one of the active ingredients of fresh crushed garlic exhibits different antimicrobial activity (Ankri and Mirelman, 1999; Ross *et al.,* 2001; Goncagul, 2010). It has been shown that allicin in its pure form displays antibacterial activity against a broad spectrum of gram positive and gram-negative bacteria, particularly antifungal activity against *Candida albicans*, anti-parasitic activity and antiviral activity (Ankri and Mirelman, 1999). Allicin and its derivatives inhibit the cysteine protease, thereby acting anti-parasitic on the human and animal pathogenic protozoa (Waag *et al.,* 2010). El-Mahmood (2009) reported that *S. aureus* (NSA1) was most susceptible to the active principles present in garlic, which had a zone of growth inhibition diameter of 28 mm in water, 23, 24 and 32 mm in chloroform, ethanol and metronidazolem respectively in his work on Efficacy of crude extracts of garlic (*Allium sativum* Linn.) against nosocomial *Escherichia coli, Staphylococcus aureus, Streptococcus pneumoniae and Pseudomonas aeruginosa.* Vuddhakul *et al.* (2007) observed that garlic extracts inhibited the growth of *S. aureus* in their work on Inhibitory activity of Thai condiments on pandemic strain of *Vibrio parahaemolyticus* which was contrary to the observation of Onyeagba *et al.* (2004) who studied the antimicrobial effects of garlic (*Allium sativum*), ginger (*Zingiber officinale*) and lime (*Citrus aurantifolia*) reported that crude extracts of garlic did not exhibit any in vitro inhibition on the growth of test organisms including *Staphylococcus* sp.

Han *et al.* (1995) reported that the antibiotic activity of 1mg of allicin, is equated to that of 15 IU of penicillin in their studies on a spectrophotometric method for quantitative

determination of allicin and total garlic thiosulfinates. Hughes and Lawson (1991) in their study of Antimicrobial effects of garlic (*Allium sativum L.*), elephant garlic (*Allium ampeloprasum*), and onion (*Allium cepa*), garlic compounds and commercial garlic supplement products, found that the antimicrobial activity of garlic is completely abolished when the thiosulfinates (e.g allicin) are removed from the extract. Tynecka *et al.* (1993) reported in their research on the effect of various environmental conditions on the antimicrobial activity of *Allium ursinum* that the antimicrobial activity of *Allium ursinum* juice decreases on storage above 4 ^{0}C.

Garlic has been shown to inhibit growth of fungal elements equally along with the drug ketoconazole, when tested on the fungi *Malassezia furfur*, *Candida albicans*, other *Candida* sp. as well as 35 strains of various dermatophyte species (Shams-Ghahfarokhi *et al.,* 2006). Mahmoodhi *et al.* (2006) recently studied 30 volunteer individuals with blood cholesterol higher than 245 mg/dl, subjecting them to ingest 5g raw garlic twice a day for 42 days and concluded that consumption alone of garlic can decrease serum lipids and may be effective in mild cases, but should probably not be relied on as the main therapeutic agent for hyperlipidemia. Other recent animal work also corroborates the beneficial effect of using boiled or raw garlic, the forms most often used most commonly (Gorenstein *et al.,* 2006). In one animal study by Yeh *et al.* (2006) the addition of aged garlic extract decreased plasma total homocysteine concentration by 30%. Recent in vitro studies by Benavides *et al.* (2007) have confirmed the vasoactive ability of garlic's sulfur compounds whereby red blood cells convert garlic's organic polysulfides into hydrogen sulfide, a known endogenous cardio-protective vascular cell signaling molecule.

Yin (2003) conducted a study on antioxidant and antimicrobial protection of diallyl sulfide (DAS), diallyl disulfide (DADS), S-ethyl cysteine (SEC), n-acetyl cysteine (NAC) for five inoculated pathogenic bacteria; *Salmonella typhimurium, Escherichia coli, Listeria monocytogenes, Staphyllococcus aureus and Campylobacter jejuni;* and showed that diallyl sulfide and diallyl disulfide exhibited both antioxidant and antimicrobial protection contrarily to both S-ethyl cysteine and n-acetyl cysteine that might directly stabilize the redox status or protein structure.

Many research works have shown that garlic is effective against bacteria such as *Helicobacter pylori*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, *Escherichia coli***,** *Staphylococcus aureus*, *Proteus vulgaris*, *Proteus mirabilis***,** *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Bacillus cereus***,** *Salmonella typhimurium*, *Salmonella paratyphi* B and C, *Vibrio cholerae***,** *Corynebacterium diphtheriae* and *Streptococcus faecalis* (Boboye and Dayo-Owoyemi, 2004; Calsamiglia *et al.,* 2007). Garlic has antifungal activity against *Cryptococcus neoformans*, thus it is used in the treatment of cryptococcal meningitis (Boboye and Dayo-Owoyemi, 2004). Venugopal and Venugopal (1995) showed that garlic could be an effective anti-dermatophytic agent. It is equally antagonistic against other fungi including *Aspergillus flavus***,** *Candida albicans*, *Alternaria* species, *Rhodotorula* and *Torulopsis* (Arora and Kaur, 1999; Lemar *et al.,* 2005). Rajabather (1994) reported immune boosting ability of garlic when administered with viral vaccine in AIDS condition. Garlic has also been shown to possess antiparasitic effect mainly against protozoan parasites including *Trypanosoma* strains, *Entamoeba histolytica*, *Giardia lamblia* and *Hymenolepsis nana* (Nok *et al.,* 1996; Ross, 1999). Garlic can be used on microorganisms that have particularly developed resistance to antibiotics. This can be seen in the study of Tsao and

Yin (2001) who explained that garlic oil and four diallyl sulphides showed *in vitro* activity against antibiotic-resistant *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*.

It has been proved by Sofowora (1993) that garlic prevents malignant tumors, especially digestive cancers. Hughes and Lawson (1991) showed that the antimicrobial activity of garlic is completely abolished when the thiosulfinates (e.g. allicin) are removed from the extract. Also, upon reduction of allicin to diallyl disulfide, the antibacterial activity is greatly reduced (Tsao and Yin, 2001). Nok *et al.* (1996) showed that allicin exhibits its antimicrobial activity mainly by immediate and total inhibition of RNA synthesis, although DNA and protein syntheses are also partially inhibited, suggesting that RNA is the primary target of allicin action. Allicin interferes with RNA production and lipid synthesis. If RNA cannot be produced, or produced in less amount then protein synthesis will be severely affected. It would be stopped at every stage due to the absence of messenger RNA, ribosomal RNA and transfer RNA. If amino acids and proteins cannot be produced then growth and development of the organism will not occur as they are essential for all parts of cell structure. Also, as lipid synthesis is affected, other parts of the cell are interfered with. The main effect being that the phospholipid biolayer of the cell wall cannot form correctly in both Gram positive and Gram negative bacteria. The structural differences of the bacterial strains may also play a role in the bacterial susceptibility to garlic constituents (Srinivasan-Durairaj *et al.,* 2009). Since the cells of Gram-negative bacteria have beside a peptidoglycan layer also an outer lipid membrane, for a substance to exhibit any antibacterial activity on these bacteria, the lipid membrane must at least be partially dissolved or pores created in it to act on the permeability of the membrane, leading to molecules and ions from bacterial cells leaking, and at the end, cracking the cell (Didry *et al.,* 1992). Recently, ajoene has also

been shown to inhibit quorum sensing in *P. aeruginosa*. The combination of garlic extracts with antibiotics leads to partial or total synergism (Didry *et al.,* 1992). Studying the antimicrobial properties of garlic indicated that garlic is full of anion compounds including nitrates, chlorides, sulfides and organosulphur compounds that can be easily resolved in water and are responsible for antibacterial properties (Shobana *et al.,* 2009).

Fujisawa *et al.* (2008) reported chemical and biological stability of garlic aqueous and alcoholic extracts and their effects on both Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria. Researches have indicated *S. aureus* as a very sensitive bacterium to aqueous and alcoholic extracts. However, tests revealed that the antimicrobial activity of garlic is totally dependent on the allicin compound, which is three times more effective on Gram-positive bacteria than Gram-negative ones. Such results will be accounted for the membrane lipid content of these two bacteria because *E. coli* has a membrane lipid content 10 times higher than *S. aureus,* making allicin unable to reach the goal, as they are trapped in this lipid content. Though, according to Onyeagba *et al.* (2004) the crude extracts of garlic and ginger applied singly and in combination did not exhibit any *in vitro* inhibition on the growth of test organisms including *Staphylococcus* spp. In contrast the study has clearly shown that for *S. aureus* with inoculums density of 10^4 CFU/ml, garlic in concentration of (15.00 - 60.00 mg/ml) was capable of causing the inhibition of bacteria growth. Using the same protocol garlic has a bactericidal effect at the lower concentration of 30.00 mg/ml for clinical isolate of *S. aureus*. However, this concentration level may vary as different authors have stated; for instance 160mg/ml was observed by Sivam *et al.* (1997). This might be due to the garlic species variation in different countries, the processing difference of the garlic species and the inoculums' densities. The bactericidal effect of garlic might be due to the

structural characteristics of organisms which play a role in the bacterial susceptibility to garlic constituents Hughes and Lawson (1991) particularly *S. aureus* contains only 2% lipid so that the lipid content of the membranes will have an effect on the permeability of allicin and other garlic constituents. Hence these phenomenons may favor the destruction of the cell wall and genetic materials of *S. aureus*.

2.8.2 Antimicrobial Properties of *Zingiber officinale Roscoe* (Ginger)

Singh *et al.* (2008) in their work on Chemistry, antioxidant and antimicrobial investigations on essential oil and oleoresins of *Zingiber officinale* reported that the antimicrobial activity of Ginger may be due to the considerable amounts of phenolic compounds. Malu *et al.* (2008) reported in their study on antibacterial activity and medicinal properties of ginger (*Z. officinale)* that sesquiterpenoids are the main component of ginger which is the attributes of its antibacterial activity. According to Massar and Jinan (2012) who researched on the inhibitory effect of the ethanolic extract of *Corianrum sativum, Vitis vinifera* and *Zingiber officinale* on the growth of *S. aureus* from milk of cows infected with clinical mastitis, ginger extract used against the growth of pathogenic *S. aureus* isolated from milk of some local cows infected with clinical mastitis showed antibacterial effects. Also, aqueous extract of Ginger roots used for antibacterial activity against various Gram negative and Gram positive bacteria showed clear antibacterial activity against pathogenic bacteria, and when this activity was enhanced with the increasing of concentrations, the extract gave highest activity against *S. aureus* (Suhad *et al.,* 2012; Bandna, 2013).

According to Kapoor (1997), in a study on the antifungal activities of fresh juice and also a study using aqueous extracts of turmeric and ginger (*Zingiber officinale*) against some

selected fungi recorded the inhibitory action of plant samples against Fungi species selected. Guptha and Ravishankar (2005) in their work on the antimicrobial activity of garlic, ginger, carrot and turmeric pastes against *E. coli* in laboratory buffer and ground beef, reported that Ginger extract showed antifungal activity against *Candida albicans*. Atai *et al.* (2009) who researched the Inhibitory effect of ginger extract on *Candida albicans* revealed that extract of rhizomes of ginger had pronounced inhibitory effect against *Candida albicans*. Dugasani *et al.* (2010) found that in the scavenging of DPPH, superoxide and hydroxyl radicals, the pattern of effectiveness is as follows: [6]-shogaol > [10]-gingerol > [8]-gingerol> [6]-gingerol, which implies that increasing the carbon chain length of the extract will increase the efficacy. Ginger extracts and individual constituents have been reported in in-vitro studies to suppress the growth of a variety of common infectious bacteria including *Staphylococcus aureus* and *Listeria monocytogenes*. Norajit *et al.* (2007) noted that Commercial ginger paste demonstrated antimicrobial activity toward *Escherichia coli O157:H7* in laboratory buffer and ground beef. Gupta and Ravishankar (2005) were also able to enhance the antimicrobial efficacy of drugs in the treatment of drug-resistant enterococci. Nagosh *et al.* (2006) reported the considerable capacity of gingerols and phenolic metabolites found in ginger in inhibiting the growth of *Helicobacter pylori* and also enhances the effectiveness of drugs targeting this bacterium, suggesting a new potential use of ginger in combating *H. pylori* related gastrointestinal diseases. According to Mahady *et al.* (2003) in his animal studies, revealed that ginger extracts exhibited the capacity to protect mice against infections caused by several microbes. Again, according to Schnitzler *et al.* (2007) in the susceptibility of drug-resistant clinical herpes simplex virus type 1 strains to essential oils of ginger, thyme, hyssop, and sandalwood, ginger had the capacity to suppress

virus growth process in in-vivo anti-parasitic activity in sheep given at a dose of 1 to 3 g of ginger powder/kg body weight. Boer *et al.* (2005) reported that bioactive compounds show better solubility in water miscible organic solvents. White (2007), in his studies on the antimicrobial activity of ginger against different microorganisms discovered that the essential oil from Ginger, has not just antimicrobial activity, but can also inhibit multiplication of colon bacteria. Adeshina *et al.* (2011) reported antibacterial activity of fresh red and white *A. cepa* (Onion) and *Z. officinale* (Ginger) juice against multidrug resistant bacteria viz *P. aeruginosa, S. aureus, E. coli and S. typhi* isolated from salad was using agar well diffusion and agar dilution methods.

2.9 Significance of Antimicrobial Susceptibility Testing

In testing for new antimicrobials or antibiotics, evaluation of biological activity is essential for the assessment of susceptibility of pathogens to the antimicrobial agent. Antimicrobial susceptibility testing is used in pathology to determine the resistance of certain microbial strains to different antimicrobials and in pharmacology research it is used to determine the efficacy of novel antimicrobials from biological extracts against different microorganisms (Das *et al.,* 2010). Microbial growth or its inhibition can be measured in a number of ways, these are: viable counts, direct microscopic counts, turbidity measurement, bioluminescence and fluorimetry (Grare *et al.,* 2008). Of the various antimicrobial susceptibility methods employed, the disk diffusion method and the broth microdilution method are mostly used to evaluate the effect of the plant extracts or any other antimicrobial on disease-causing pathogens. The disk diffusion method is used in determining the zones of inhibition exhibited by the plant extracts, while the broth microdilution method, which has been recommended by the Clinical and Laboratory Standards Institute (2006), is used in

determining the minimum inhibitory concentration (MIC) of plant extracts. This method is less cumbersome, less expensive and quite reproducible when compared with the disk diffusion method. The use of microplates allows large amounts of data to be generated quickly. Bacterial growth could be assessed either visually by grading turbidity or better spectrophotometrically by measuring optical density (Grare *et al.,* 2008). The disadvantage of visual assessment of bacterial growth is that it lacks objectivity and precision; whereas the accuracy of spectrophotometric readings may be hampered by (i) additives or antibacterial compounds that affect the spectral characteristics of growth media, (ii) the aggregation of bacteria, or (iii) bacterial pigments (Eloff, 1998). Colorimetric methods therefore could represent an alternative approach, using tetrazolium salts as indicators, since bacteria convert them to coloured formazan derivatives that can be quantified (Grare *et al.,* 2008).

2.10 Extraction Techniques of Plant Extracts

In the analysis of medicinal plants, extraction is the most important first step because it is necessary to extract the desired chemical components from the plant materials for further separation and characterization (Sasidharan *et al.,* 2011). Different extraction techniques are available, but the most common ones used in plants extraction are the conventional techniques. In conventional extraction, the release of the desired compounds traditionally requires soaking and maceration in mild solvents (Chan *et al.,* 2012). Decoction in water is broadly employed in traditional Chinese medicinal practices and is an effective method that can be considered in cases where the presence of a chemical solvent is undesirable (Das *et al.,* 2010). Other solvents that can be used in conventional extraction are acetone, petroleum ether and hexane. Liquid nitrogen has also been used as a form of extraction in some

research work (Karuna *et al.,* 2000). Techniques such as lyophilization (Grover *et al.* 2000; Chen *et al.* 2003) and sonification (Chukwujekwu *et al.* 2009; Yang *et al.* 2009) are further methods that can be employed other than solvent extraction.

Non-conventional methods that can be used are the supercritical fluid extraction and microwave-assisted techniques. The advantages presented by these two non-conventional techniques are short extraction time and solvent-free active compounds.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Collection and Identification of Plant Samples

Bulbs and rhizomes of garlic (*Allium sativum)* and ginger (*Zingiber officinale*) plants respectively were purchased from Eleyele market in Ido Local Government Area of Ibadan, Oyo State. The plant samples were identified at the taxonomy unit of the Forestry Research Institute of Nigeria (FRIN) Ibadan, Oyo State. The purchased plant samples were allocated specimen numbers SPR 103 and SPR 104 and identification vouchers were issued to this effect.

3.2 Processing of Plant Samples

Garlic (*Allium sativum)* and ginger (*Zingiber officinale*) plant samples were processed in the Bio-Medicinal Research Centre Laboratory. The Samples were washed, air dried at room temperature (25 ^{0}C) before milling using the electric grinder into powdered form. They were kept in tight dry plastic containers to keep them air tight till they were required for further analysis.

3.3 Collection of Test Clinical Isolates

Clinical isolates were collected from University of Ilorin Teaching Hospital, Oke-Oyi, Kwara State. The four clinical isolates collected for this study were *Staphylococcus aureus, Escherichia coli, Candida albicans* and *Pseudomonas aeruginosa*.

3.4 Sterilization of Glass Ware

All glass ware used were washed, sterilized by oven drying so as to prevent introduction of foreign organisms. Work bench was disinfected. Laminar flow cabinet was used during research so as to prevent contamination and introduction of foreign organisms.

3.5 Preparation of Media

3.5.1 Mueller Hinton Agar Medium (MHA)

The medium was prepared by dissolving 33.9 g of Mueller Hinton Agar Medium (Hi Media) in 1000 ml of distilled water. The dissolved medium was autoclaved at 121 ^{0}C for 15 minutes. The autoclaved medium was cooled, mixed and poured onto 100 mm petri plates (25 ml/plate).

3.5.2 Mueller Hinton Broth Medium

Twenty-one grams of the medium was dissolved in 1000 ml of distilled water. The dissolved medium was autoclaved at 15 lbs. pressure at 121 ^{0}C for 15 minutes.

3.5.3 Sabouraud Dextrose Agar Medium (SDA)

Forty-seven grams of SDA (Hi Media) was suspended in 1000 ml of distilled water. The medium was dissolved and was then autoclaved at 121 ^{0}C for 15 minutes.

3.5.4 Sabouraud Dextrose Broth Medium (SDA)

Thirty grams of SDA (Hi Media) was suspended in 1000 ml of distilled water. The medium was dissolved and was then autoclaved at 121 ^{0}C for 15 minutes.

3.5.5 Nutrient Agar Medium(NA)

Twenty-eight grams of nutrient agar was suspended in 1000 ml of distilled water. The medium was dissolved and was then autoclaved at 121 ^{0}C for 15 minutes.

3.6 Preparation of Inoculum

Clinical isolates were sub cultured on nutrient agar and Potato dextrose agar for 24 hours at 37 ^{0}C and 48 hours at 30 ^{0}C. A loopful of test organism was taken from agar slants into test tubes containing nutrient broth and Potato dextrose broth. The test tubes were then incubated for 24 hours at 37 ^{0}C and 48 hours at 30 ^{0}C.

3.7 Identification of Test Clinical Isolates

Gram stain was carried out according to the method of Fawole and Oso (2004). Also, catalase, citrate and oxidase tests were carried out according to the methods of Cheesebrough (2005). *Candida albicans* was also cultured on Potatoes Dextrose Agar. It was later incubated at 37 ^{0}C for 48 hours. Colonies having cream pasty color with yeast smell indicated the presence of *Candida albicans*

3.7.1 Gram stain

A few drops of distilled water were added onto a sterile glass slide. A loopful of the bacterium was transferred and spread in circular motion over a small area of the slide. The smear was allowed to air dry. The microorganism was heat fixed by placing the bottom of the slide to heat for approximately 30 seconds without exposing. Forceps were used to hold the slide above the sink, after which the slide was flooded with crystal violet for 1 minute. Rinsing of the slide with distilled water was done for 5 seconds. Grams iodine solution was applied and allowed to act for 1 minute as a mordant. The slide was thoroughly rinsed with distilled water. Excess water was drained from the slide and the slide was blotted so that alcohol used for decolourization is not diluted. A few drops of 95 % ethanol were applied onto the slide for 10 seconds and washed off with tap water. The slide was drained to remove excess water. A few drops of safranin solution were used to counter stain the slide for 30 seconds, rinsed off with tap water, drained blotted out to dry with paper. The slide was read with oil immersion lens of the slide of the microscope at high power (x 1000).

3.7.2 Catalase test

A small amount of growth from the culture was smeared onto a sterile microscope slide. A few drops of hydrogen peroxide were added onto the smear. Formation of bubbles confirms the presence of *E. coli* (Cheesebrough, 2005).

3.7.3 Citrate test

A small amount of inoculum was added in a tube containing citrate medium, it was incubated at 37 ^{0}C for 24hours. Growth in the citrate medium indicate the presence of *S. aureus* (Cheesebrough, 2005).

3.7.4 Oxidase test

Commercially prepared oxidase test paper disk was moistened with distilled water. Using a wire loop organism was smeared on the filter paper. It was observed for 30 seconds. The presence of colour change from plain to purple confirmed the presence of *P. aeruginosa* (cheesebrough, 2005).

3.8 Extraction of Garlic and Ginger Samples:

3.8.1 Ethyl acetate Extraction of Garlic and Ginger Samples

Fifty grams of milled garlic (*Allium sativum)* bulb and ginger (*Zingiber officinale*) rhizome plant parts were soaked in 200 ml of ethyl acetate (extracting solvent). This was stirred with a glass rod and left on the shaker for 12 hours. It was then filtered using sterile double Whatsman filter paper. The solution was then passed through the Rotary evaporator. Filtrate was left in the desiccator for future use.

3.8.2 Acetone Extraction of Garlic and Ginger Samples

Fifty grams of milled garlic (*Allium sativum)* bulb and ginger (*Zingiber officinale*) rhizome plant part were soaked in 200 ml of acetone (extracting solvents). This was stirred with a

glass rod and left on the shaker for 12 hours. It was then filtered using sterile double Whatsman filter paper. The solution was then passed through the Rotary evaporator. Filtrate was left in the desiccator for future use.

3.8.3 Methanol extraction of Garlic and Ginger Sample

Fifty grams of milled garlic (*Allium sativum)* bulb and ginger (*Zingiber officinale*) rhizome plant part were soaked in 200 ml of methanol (extracting solvents). This was stirred with a glass rod and left on the shaker for 12 hours. It was then filtered using sterile double Whatsman filter paper. The solution was then passed through the Rotary evaporator. Filtrate was left in the desiccator for future use.

3.8.4 Sterility Test of extracts

After extraction and concentration, extracts were tested for sterility, by streaking it on freshly prepared sterile Nutrient Agar which was incubated for 24 hours at 37 ^{0}C. Growth on the nutrient agar indicate the presence of microorganism on the extracts.

3.9 Qualitative Phytochemical Screening of Various Extracts of Plant Sample:

This was performed using the methods described by Nweze *et al.* (2004) and Senthilkumar and Reetha (2009). The samples were screened for cardiac glycosides, alkaloids, flavonoids, triterpenes and steroids, anthocyanin and betacyanin, phenols. Tannins, saponin, glycosides, carbohydrate, reducing sugar, phlobatannins and anthraquinones.

3.9.1 Test for Cardiac Glycosides

A powdered garlic (*Allium sativum)* bulb and ginger (*Zingiber officinale*) rhizome plant samples (1 g) was extracted with 10 ml of 80 % ethanol for 5 minutes on a water bath at 100 ^{0}C. The extract was filtered and diluted with equal volume of distilled water. A few drops of lead acetate solution were added, shaken and filtered after standing for few minutes. The

filtrate was then extracted with aliquots 10 ml of chloroform; the extract was divided into two portions in evaporating dish and evaporated to dryness on a steam bath. One portion from above was dissolved in 2 ml of glacial acetic concentration, one drop of $Fecl_3$ solution in a clean test tube. Two millilitres of concentrated sulphuric acid was then poured down the side of the tube so as to form a layer below the acetic acid. The formation of a purple or reddish – brown or brown ring at the interface and a green interface and a green colour in the acetic layer was taken for a positive result (Sofowora, 1993). The second portion was mixed with 1ml of 2 % 3,5-dinitrobenzoic acid in ethanol. The solution was made alkaline with 5 % NaOH after mixing. The formation of a transient purple colour, which turned brown on standing, was considered positive (Senthilkumar and Reetha, 2009).

3.9.2 Test for Tannins

One gram of powdered garlic (*Allium sativum)* bulb and ginger (*Zingiber officinale*) rhizome plant samples were boiled in 10 ml distilled water, filtered when hot and cooled. The filtrate was adjusted to 10 ml with distilled water. Then, a few drops of 1 % ferric chloride reagent were added to 1 ml of the filtrate. The mixture was observed for the formation of either blue, dark brown, blue black, green or green- black colouration or precipitate (Senthilkumar and Reetha, 2009).

3.9.3 Test for Saponins

One gram of powdered garlic (*Allium sativum)* bulb and ginger (*Zingiber officinale*) rhizome plant samples were boiled with 10 ml of distilled water for 10 minutes. The samples were filtered while hot, cooled and the following tests were performed:

Frothing Test: Two and half millilitre of the filtrate was diluted to 10 ml with distilled water and shaken vigorously for 20 minutes. The formation of 1 cm layer of persistent foam indicates the presence of saponins (Sofowora, 1993).

Emulsifying Property: Two drops of olive oil were added to 2.5 ml of the filtrate and shaken vigorously for 30 minutes. Observation was made for the formation of stable emulsion (Sofowora, 1993).

3.9.4 Test for Flavonoids

One gram of garlic (*Allium sativum)* bulb and ginger (*Zingiber officinale*) rhizome plant samples material were boiled with 10 ml of ethanol. To 5 ml of the samples garlic (*Allium sativum)* and ginger (*Zingiber officinale*) was added 2 drops of ferric chloride. A dusty green colour was considered positive. To 5 ml of plant sample, garlic (*Allium sativum)* and ginger (*Zingiber officinale*) a small quantity of dilute sodium hydroxide was added and drops of concentrated hydrochloric acid were run down the side of the tube. A reddish colour indicated the presence of flavonoids (Nweze *et al.,* 2004).

3.9.5 Test for Alkaloids

One gram of powdered garlic (*Allium sativum)* bulb and ginger (*Zingiber officinale*) rhizome plant samples was stirred in 10 ml of 10 % (v/v) concentrated hydrochloric acid on a steam bath followed by filtration. One millilitre of the filtrate was mixed with few drops of Meyer's reagent. To another 1 ml of the filtrate was added few drops of Wagner's reagent. Few drops of Dragendroff reagent was added to another 1ml of the filtrate. The mixtures were observed for turbidity or formation of precipitate which indicated the presence of alkaloids (Nweze *et al.,* 2004).

3.9.6 Test for Anthocyanin and Betacyanin

To 1 g of garlic (*Allium sativum)* bulb and ginger (*Zingiber officinale*) rhizome plant samples, 1 ml of 2N sodium hydroxide was added and heated for 5 minutes at 100 ºC. Formation of bluish green colour indicated the presence of anthocyanin and formation of yellow colour indicates the presence of betacyanin (Senthilkumar and Reetha, 2009).

3.9.7 Test for Glycosides

To 2 g of garlic (*Allium sativum)* bulb and ginger (*Zingiber officinale*) rhizome plant samples, 1 ml of glacial acetic acid plus 5 % of ferric chloride were added. To these 3 drops of concentrated sulphuric acid were added. Presence of greenish blue colour indicated the presence of glycosides (Sofowora, 1993).

3.9.8 Test for Steroids and Triterpenes

Exactly 0.2 grams of ethanol extract was dissolved in 1ml acetic anhydride and then 1ml of dichloromethane. The solution was transferred into a dry test tube by means of pipette. Two millilitres of concentrated sulphuric acid were added at the bottom of the test tube. At the contact zone of the liquid, a brownish- red ring is formed; the supernatant layer became greenish denoting the presence of steroids and triterpenes (Senthilkumar and Reetha, 2009).

3.9.9 Test for Phenols

To 1 g of the powdered garlic (*Allium sativum)* bulb and ginger (*Zingiber officinale*) rhizome plant samples, 2 ml of distilled water followed by 5 drops of 10 % ferric chloride was added. Formation of blue or green colour indicates presence of phenols (Senthilkumar and Reetha, 2009).

3.9.10 Test for Phlobatannins

Deposition of a red precipitate when an aqueous extract of each plant sample was boiled with 1 % aqueous hydrochloric acid was taken as evidence for the presence of phlobatinins (Senthilkumar and Reetha, 2009)

3.9.11 Borntrager's Test

Borntrager's Test for *Free Anthraquinones:* Powdered garlic (*Allium sativum)* bulb and ginger (*Zingiber officinale*) rhizome plant samples (0.5 g) were shaken with 5 ml of chloroform for 10 minutes, filtered and 5 ml of 10 % ammonia solution was added to filtrate. The mixture was shaken and the presence of a pink, red or violet colour in the ammonia phase indicated the presence of antraquinones (Nweze *et al.,* 2004).

Borntrager's Test for *Combined Anthraquinones:* One gram of powdered garlic (*Allium sativum)* bulb and ginger (*Zingiber officinale*) rhizome plant samples was boiled with 5 ml of 10 % HCl for 5minutes and filtered while hot. The cooled filtrate was partitioned against equal volumes of chloroform (2 ml) avoiding vigorous shaking. A clean pipette was then used to transfer the chloroform layer to a clean tube taking care not to include the aqueous layer. An equal volume of 10 % ammonia was added to the chloroform extract. A pink, red or violet colour in the aqueous layer was considered positive (Nweze *et al.,* 2004).

3.10.0 Quantitative Phytochemical Screening

3.10.1 Alkaloid Determination

Five grams of the garlic (*Allium sativum)* bulb and ginger (*Zingiber officinale*) rhizome plant samples were weighed into a 250 ml beaker and 200 ml of 10 % acetic acid in ethanol was added and covered and allowed to stand for 4 hours. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated

ammonium hydroxide was added drop-wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected. This was washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed (Obadoni and Ochuko, 2001).

3.10.2 Saponin Determination

The method used was that of Obadoni and Ochuko (2001). The garlic (*Allium sativum)* bulb and ginger (*Zingiber officinale*) rhizome plant samples were ground and 20 grams of each were put into a conical flask and 100 cm^3 of 20 % aqueous ethanol were added. The samples were heated over a hot water bath for 4 hours with continuous stirring at about 55 ^{0}C. The mixture was filtered and the residue re-extracted with another 200 ml of 20 % ethanol. The combined extracts were reduced to 40 ml over water bath at about 90 ^{0}C. The concentrate was transferred into a 250 ml separatory funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. Sixty millilitres of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5 % aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight; the saponin content was calculated as percentage.

3.10.3 Flavonoid Determination

Ten grams of the garlic (*Allium sativum)* bulb and ginger (*Zingiber officinale*) rhizome plant samples was extracted repeatedly with 100 ml of 80 % aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No 42 (125

mm). The filtrate was later transferred into a crucible and evaporated to dryness over a water bath and weighed to a constant weight (Bohm and Kocipai-Abyazan, 1994).

3.10.4 Tannin Determination

Five hundred milligrams of the garlic (*Allium sativum)* bulb and ginger (*Zingiber officinale*) rhizome plant samples was weighed into a 50 ml plastic bottle. Fifty milliliter of distilled water was added and shaken for 1 h in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtrate was pipetted out into a test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.I N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 minutes (Bohm and Kocipai-Abyazan, 1994).

3.10.5 Preparation of 0.5ml McFarland's Standard

This was done by adding 0.5 ml of 1.175 % of anhydrous Barium chloride drop-wise to 99.5 ml of 1 % sulphuric acid in 100 ml volumetric flask and constantly swirling. This was mixed for 3-5 minutes until solution appeared homogeneous and free of clumps. Optical density of solution was read using a spectrophotometer and wavelength of 625 nm was taken which is the accepted range for McFarland 0.5 ml. This was then dispensed into a glass screw cap tube and sealed with paraffin and then stored at room temperature until needed. The approximate cell density corresponding to 0.5 McFarland is 1×10^8 Cfu/ml.

3.11 Antimicrobial Sensitivity Testing of the Extract on the Clinical Isolates

Using agar disc diffusion techniques as described by Kirby-Bauer technique (Bauer *et al.,* 1966; John and James, 1999), various concentrations of plant extract garlic (*Allium sativum)* bulb and ginger (*Zingiber officinale*) rhizome (200 mg/ml, 100 mg/ml 80 mg/ml, 60 mg/ml, 40 mg/ml and 20mg/ml) were used. Paper discs of 0.3 diameter in size was impregnated

with the selected plant extracts. It was left to dry for 10 minutes, before placing unto the surface of the already inoculated Mueller Hinton Agar/Sabouraud Dextrose Agar plate and allowed to diffuse for half an hour on the surface of the medium. The plates were incubated at 37 ^{0}C for 24 hours and the zones of inhibition around each disc were measured for sensitivity, mild sensitivity or resistance. Diameters of the inhibition zones were measured using a metre rule. The antibacterial activity was expressed as the mean zone of inhibition diameters (mm) produced by the plant extract. Each assay was carried out in triplicates under strict aseptic conditions. The absence of zones of inhibition was interpreted as the absence of activity (Kohner *et al.,* 1994; Mathabe *et al.,* 2006). The activities were expressed as resistant, if the zones of inhibition was less than 7 mm, intermediate or mildly sensitive (8-10 mm) and sensitive if more than 11 mm (Assam *et al.,* 2010).

3.11.1 Antibiotic Sensitivity Testing Against Clinical Isolate

Commercially prepared antibiotics discs (Ampicillin (AMP- 10ug), tetracycline (TET-10ug) and chloramphenicol (C-10ug)) were used to determine the drug sensitivity and resistance pattern of bacteria. These discs were placed on the plates inoculated with different strains of approximate cell density corresponding to 0.5 McFarland is 1×10^{8} Cfu/ml. These served as positive control. Disc containing extracting solvents served as negative control.

3.11.2 Minimum Inhibitory Concentrations (MIC)

This was carried out by broth dilution techniques according to European Society of Clinical Microbiology and Infectious Diseases (ESCMID, 2003). Different concentrations of ethyl acetate, acetone and methanol extracts of bulbs and rhizomes of garlic (*Allium sativum)* and ginger (*Zingiber officinale*) from 200 mg/ml to 25 mg/ml, (200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml) were prepared using extracting solvent following a two-fold dilution.

Standardized test organisms in broth culture (Mueller Hinton broth and Sabouraud Dextrose broth) were used. Nine milliliter of broth culture together with 0.5 ml of standardized organism and 0.5 ml of extract was dispensed in test tubes and incubated for 24 hours at 35 ^{0}C. The presence of turbidity indicated growth of organism while tube with less concentration, having no turbidity was recorded as Minimum Inhibitory Concentrations (MIC). This was done in triplicates. Ampicillin served as positive control while the last column of test tubes was left blank (that is without extract or antibiotics). This served as negative control.

3.11.3 Minimum Bactericidal (MBC)

This was done using the method of Andrews (2006). Pour plate method was used to determine MBC from the tubes positive to MIC. Plates were incubated for 24 hours at 35 ^{0}C. Plates showing no further growth of organisms were taken as the Minimum Bactericidal (MBC). Ampicillin served as positive control while the extracting solvents served as negative control.

CHAPTER FOUR

4.0 RESULTS

The results of the phytochemical analysis, antimicrobial analysis, minimum inhibitory, and minimum bactericidal concentration of plant extract garlic (*Allium sativum)* and ginger (*Zingiber officinale*) (garlic bulb and ginger rhizome) are as follows.

4.1 Qualitative Phytochemical Analysis Garlic (*Allium sativum)* Bulb and Ginger (*Zingiber officinale*) Rhizome Plant Sample

The qualitative phytochemical substances present in garlic (*Allium sativum)* bulb and ginger (*Zingiber officinale*) rhizome plant crude extracts includes tannin, alkaloid, saponin, steroid, flavonoid, glycoside, cardiac glycoside (Table 1). Anthraquinone was found present only in garlic bulb. On the other hand, Phenol was found present in ginger rhizome sample and not in garlic bulb. All others were found absent in both garlic bulb and ginger rhizome (Table 1).

4.2 Quantitative Phytochemical Analysis Garlic (*Allium sativum)* Bulb and Ginger (*Zingiber officinale*) Rhizome Plant Sample

The quantitative phytochemical screening of garlic (*Allium sativum)* bulb plant samples gave flavonoids (15.00±0.05mg/g), alkaloids (55.0±2.66 mg/g,) and saponin (11.70 ±2.17 mg/g) and tannin (0.139±0.01ug/g). On the other hand, ginger (*Zingiber officinale*) rhizome, the concentrations were revealed as flavonoids (44.40±0.08mg/g), alkaloids (56.00 ±1.00 mg/g), saponin (0.50±0.32 mg/g), and tannin (15.09 ±0.58 ug/g) as shown in table 2.

Table 1: Qualitative Phytochemical Screening of Garlic (Bulb) and Ginger (Rhizome) Samples.

Phytochemical constituent	Garlic	Ginger
Tannin	+	+
Alkaloid	+	+
Saponin	+	+
Steroid	+	+
Flavonoid	+	+
Phenol	-	+
Glycoside	+	+
Cardiac glycoside	+	+
Phlobatannin	-	-
Anthocyanin	-	-
Anthraquinone	+	-

Key: + = present, - = absent

Table 2: Quantitative Phytochemical Screening of Garlic (Bulb) and Ginger (Rhizome) samples

Plant samples	Flavonoids (mg/g)	Alkaloid (mg/g)	Saponin (mg/g)	Tannin (µg/g)
Garlic	15.00±0.05	55.00±2.66	11.70±2.17	0.139±0.01
Ginger	44.40±0.08	56.00±1.00	0.50±0.32	15.09±0.58

Values presented are means of triplicate readings and standard deviation of quantitative phytochemical screening of garlic bulb and ginger rhizome samples.

4.3 Antimicrobial Activities of Ethyl acetate Extract of Garlic and Ginger against Clinical Isolates

The zones of inhibition of the antimicrobial activities of plant extracts garlic (*Allium sativum)* bulb and ginger (*Zingiber officinale*) rhizome on selected clinical isolates at various concentrations is as shown in table 3. The crude ethyl acetate forms of the extracts of garlic and ginger exhibited varying degree of antimicrobial activities against the test organisms. *S. aureus* was most sensitive to ethyl acetate extract of garlic with a zone of inhibition of 18.00±3.00 mm at a concentration of 200 mg/ml while *E. coli* was the least sensitive with a zone of inhibition of 9.66±8.39 mm at same concentration. On the other hand, *E. coli* was the most sensitive to ethyl acetate extract of ginger with a zone of inhibition of 21.60±1.15 mm at a concentration of 200 mg/ml while the least sensitive to ethyl acetate extract of ginger was *C. albicans* with a zone of inhibition of 11.00±1.00 mm at same concentration (Table 3). All other concentrations gave zones of inhibitions ranging from sensitive to mild to insensitive as shown in table 3.

4.4 Antimicrobial Activities of Acetone Extract of Garlic and Ginger against Clinical Isolates

The zones of inhibition of the antimicrobial activities of plant extracts garlic (*Allium sativum)* bulb and ginger (*Zingiber officinale*) rhizome on selected organisms at various concentrations is as shown in table 4. The crude acetone forms of the extracts of garlic and ginger exhibited varying degree of antimicrobial activities against the test organisms. *P. aeruginosa* was most sensitive to acetone extract of garlic with a zone of inhibition of 21.00±1.00 mm at a concentration of 200 mg/ml while *C. albicans* was the least sensitive with a zone of inhibition of 13.33±2.08 mm at same concentration. On the other hand, *S.*

aureus was the most sensitive to acetone extract of ginger with a zone of inhibition of 13.66±1.53 mm at a concentration of 200 mg/ml while the least sensitive to acetone extract of ginger was *P. aeruginosa* with a zone of inhibition of 9.66±8.50 mm at same concentration. All other concentrations gave zones of inhibitions ranging from sensitive to mild, to insensitive (Table 4).

4.5 Antimicrobial Activities of Methanol Extract of Garlic and Ginger against Clinical Isolate

The zones of inhibition of the antimicrobial activities of plant extracts garlic (*Allium sativum)* bulb and ginger (*Zingiber officinale*) rhizome on selected organisms at various concentrations is as shown in table 5. The crude methanolic forms of the extracts of garlic and ginger exhibited varying degree of antimicrobial activities against the test organisms. *P. aeruginosa* was most sensitive to methanol extract of garlic with a zone of inhibition of 25.23±0.58 mm at a concentration of 200 mg/ml while *C. albicans* was the least sensitive with a zone of inhibition of 15.33±0.58 mm at same concentration. On the other hand, *S. aureus* was the most sensitive to methanol extract of ginger with a zone of inhibition of 26.33±1.16 mm at a concentration of 200 mg/ml while the least sensitive to methanol extract of ginger was *P. aeruginosa* with a zone of inhibition of 21.00±1.00 mm at same concentration. All other concentrations gave zones of inhibitions ranging from sensitive to mild to insensitive (Table 5).

Table 3: Antimicrobial Activities of Ethyl acetate Extracts of Garlic (bulb) and Ginger (rhizome) against the Clinical Isolates

Plant samples	Clinical isolates	Concentration(mg/ml)/Average Zone of Inhibition(mm)					
		200	100	80	60	40	20
Garlic	*E. coli*	9.66 ± 8.39	7.00±6.08	4.66±4.16	3.00±1.00	2.00±0.00	1.00±1.00
	S. aureus	18.00±3.00	10.00±1.00	7.00±1.00	3.33±1.16	1.70±0.58	1.33±1.16
	P. aeruginosa	12.66±0.58	7.33±0.58	6.33±0.58	2.66±0.58	1.00±1.00	1.00±0.00
	C. albicans	14.00±12.17	8.70±1.15	7.66±2.08	3.33±0.58	1.33±0.58	0.66±0.58
Ginger	*E. coli*	21.60±1.15	12.33±2.65	9.70±0.58	6.70±0.58	2.70±1.53	1.33±0.58
	S. aureus	15.33±3.05	10.33±0.58	7.66 ±0.58	2.66±0.58	1.00±0.00	0.66±0.58
	P. aeruginosa	12.66±11.02	12.33±1.53	9.33±1.16	5.33±0.58	2.33±0.58	1.00±0.00
	C. albicans	11.00±1.00	6.33±5.51	6.33±1.53	1.66±1.16	1.00±1.00	0.33±0.58

Values presented are means of triplicate readings and standard deviation of zones of inhibition of garlic and ginger ethyl acetate extracts against the clinical isolate

Table 4: Antimicrobial Activities of Acetone Extracts of Garlic (bulb) and Ginger (rhizome) against the Clinical Isolates

Plant samples	Clinical isolates	Concentration(mg/ml)/Average Zone of Inhibition(mm)					
		200	100	80	60	40	20
Garlic	*E. coli*	15.00±1.00	7.33±6.43	6.00±5.19	5.33±1.53	2.33±1.16	0.33±0.58
	S. aureus	19.00±1.00	13.00±1.00	10.00±1.00	7.00±1.00	3.00±0.00	1.00±0.00
	P. aeruginosa	21.00±1.00	14.33±1.16	10.00±2.00	5.66±0.58	2.00±2.00	1.00±1.00
	C. albicans	13.33±2.08	9.66±2.08	8.33±1.53	4.66±0.58	1.70±1.53	0.66±1.16
Ginger	*E. coli*	11.00±9.64	10.33±1.53	8.00±1.73	3.00±2.66	1.33±0.58	0.66±0.58
	S. aureus	13.66±1.53	12.30±1.16	9.66±0.58	6.00±1.00	2.00±1.00	1.00±0.00
	P. aeruginosa	9.66±8.50	16.33±1.53	12.30±1.16	7.30±0.58	2.66±1.16	0.66±1.15
	C. albicans	12.70±2.52	7.33±6.43	8.00±0.00	4.00±1.00	1.33±1.16	0.00±0.00

Values presented are means of triplicate readings and standard deviation of zones of inhibition of garlic and ginger acetone extracts against the clinical isolates

Table 5: Antimicrobial Activities of Methanolic Extracts of Garlic (bulb) and Ginger (rhizome) against the Clinical Isolates

Plant samples	Clinical isolates	Concentration(mg/ml)/Average Zone of Inhibition(mm)					
		200	100	80	60	40	20
Garlic	*E. coli*	15.33±13.31	11.66±0.58	6.00±5.29	3.30±2.88	2.33±2.08	1.30±0.58
	S. aureus	20.66±4.04	16.66±1.53	12.30±0.58	8.33±1.53	4.66±1.53	1.33±1.16
	P. aeruginosa	25.23±0.58	17.70±1.53	13.33±1.53	9.30±0.58	6.70±0.58	2.70±0.58
	C. albicans	15.33±0.58	11.66±1.53	9.33±1.16	5.66±0.58	3.00±1.00	0.30±0.58
Ginger	*E. coli*	21.23±1.53	12.33±0.58	7.66±1.16	4.33±0.58	2.33±0.58	1.00±1.00
	S. aureus	26.33±1.16	17.33±1.16	13.70±1.16	9.30±1.53	7.00±0.00	3.30±0.58
	P. aeruginosa	21.00±1.00	13.00±1.00	9.33±0.58	4.70±4.04	2.30±2.08	1.336±0.58
	C. albicans	22.33±2.08	16.66±1.53	13.00±1.00	8.66±1.16	5.33±0.58	2.00±1.00

Values presented are means of triplicate readings and standard deviation of zones of inhibition of garlic and ginger methanol extracts against the clinical isolates

4.6 Antibiotics Sensitivity Testing against Clinical Isolates

E. coli was sensitive to ampicillin (AMP-10ug), Tetracycline (TET-10ug) and chloramphenicol (C-10ug) with zones of inhibitions of 15.00±1.00 mm, 21.00±1.00 mm and 12.00±2.00 mm respectively (table 6). Also *S. aureus* was sensitive to ampicillin, Tetracycline and chloramphenicol with zones of inhibition of 21.00±1.00 mm, 23.00±2.00 mm and 18.00±0.64 mm respectively. *P. aeruginosa* also showed sensitivity to ampicillin, Tetracycline and chloramphenicol with zones of inhibition of 17.00±0.29 mm, 20.00±1.00 mm and 14.00±0.46 mm respectively.

Table 6: Antibiotics Sensitivity Testing against Clinical Isolate and Extracting Solvents

Clinical isolates	Antibiotics/ Zone of Inhibition(mm) (Positive control)			Negative Control (Extracting Solvents)
	Ampicillin (AMP-10ug)	**Tetracycline (TET-10ug)**	**Chloramphenicol (C-10ug)**	
E. coli	15.00 ±1.00	21.00 ±1.00	12.00 ± 2.00	0.00
S. aureus	21.00 ±1.00	23.00 ±2.00	18.00 ±0.65	0.00
P. aeruginosa	17.00 ±0.29	20.00 ±1.00	14.00 ±0.46	0.00

*Values presented are means of triplicate readings and standard deviation of zones of inhibition of antibiotics against the clinical isolates.

*Extracting solvents are Ethyl acetate, Acetone and Methanol.

4.7 Minimum Inhibitory Concentration and Bactericidal Concentration of Garlic and Ginger Extract on Clinical Isolates

4.7.1 Minimum Inhibitory Concentration of Garlic and Ginger Extract on Clinical Isolate

The minimum inhibitory concentration (MIC) values of garlic (*Allium sativum)* bulb and ginger (*Zingiber officinale*) rhizome against the clinical isolates is shown in table 7. Ethyl acetate extract of garlic gave MIC value of 100 mg/ml against *S. aureus* while ethyl acetate extract of ginger gave MIC value of 100 mg/ml against *E. coli* and *P. aeruginosa* respectively. Acetone extract of garlic showed MIC against *S. aureus* (100 mg/ml), *P. aeruginosa* (100 mg/ml), *C. albicans* (200 mg/ml) respectively while acetone extract of ginger showed MIC against *E. coli* (200 mg/ml) and *P. aeruginosa* (100 mg/ml) respectively. Methanolic extract of garlic showed MIC against *E. coli* (200 mg/ml), *S. aureus* (100 mg/ml), *P. aeruginosa* (50 mg/ml) and *C. albicans* (100 mg/ml) while the methanolic extract of ginger showed MIC against *E. coli* (100 mg/ml), *S. aureus* (50 mg/ml), *P. aeruginosa* (100 mg/ml) and *C. albicans* (100 mg/ml).

4.7.2 Minimum Bactericidal Concentration of Plant Extracts on Clinical Isolates

The minimum bactericidal concentration of garlic (*Allium sativum)* bulb and ginger (*Zingiber officinale*) rhizome extracts on clinical isolates is as shown in table 8. Acetone extract of garlic gave MBC of 100 mg/ml against *S. aureus.* Also methanolic extract of garlic gave MBC against *E. coli* at 200 mg/ml while Methanolic extract of ginger gave MBC against *P. aeruginosa* at concentration of 200 mg/ml.

Table 7: Minimum Inhibitory Concentration of Garlic (bulb) and Ginger (rhizome) Extracts on Clinical Isolates

Solvents	Plant samples	Clinical isolates/MIC(mg/ml)			
		E. coli	*S. aureus*	*P. aeruginosa*	*C. albicans*
Ethyl acetate	Garlic	-	100	-	-
	Ginger	100	-	100	-
Acetone	Garlic	-	100	100	200
	Ginger	200	-	100	-
Methanol	Garlic	200	100	50	100
	Ginger	100	50	100	100

Note: - = Absence of MIC

Table 8: Minimum Bactericidal of Garlic (bulb) and Ginger (rhizome) Extracts on Clinical Isolates

Solvents	Plant samples	Clinical isolates/MBC (mg/ml)		
		E. coli	*S. aureus*	*P. aeruginosa*
Ethyl acetate	Garlic	-	-	-
	Ginger	-	-	-
Acetone	Garlic	-	100	-
	Ginger	-	-	-
Methanol	Garlic	200	-	-
	Ginger	-	-	100

Note: - = Absence of MIC

CHAPTER FIVE

5.0 DISCUSSION

The medicinal potency of plants lies in the bioactive phytochemical constituents that definitely produces biological and physiological actions in the human system (Akinmoladun *et al.,* 2007). The presence of the phytochemical properties in the garlic and ginger crude extract of garlic with concentrations values as shown in table 2, is in agreement with the work of Idowu *et al.* (2008), Aravind *et al.* (2013), Osabor *et al.* (2015) and Vijayakumar *et al.* (2015). These bioactive compounds such as alkaloids, saponins, tannins and flavonoids as seen in table 2, are known to have curative activity against varieties of pathogens (Usman *et al.,* 2009). The presence of bioactive compounds such as flavonoids, saponins, alkaoids, tannins, steroids, and anthraquinones in the plant sample gives these plant extracts their antimicrobial properties. For example, flavonoids possess antioxidant properties and ensure healthy circulation of blood and also strengthen capillary wall (Rahman, 2010). Plants that have flavonoids are known to have antimicrobial properties (Trease and Evans, 2002). The presence of flavonoids in garlic increases its usefulness in the treatment of diarrhoea, toothache (Huzaifa *et al.,* 2014). Flavonoids acts on bacteria by inhibiting its protein synthesis (Hong-Xi and Song, 2001). They possess anti-cancerous, anti-inflammatory antimicrobial and anti-allergic activity (Balch and Balch, 2000) and may therefore be useful in therapeutic roles (Jisika *et al.,* 1992). Tannins are known to have astringent properties, promote wound healing and inflamed mucous membrane (Njoku and Akumefula, 2007; Huzaifa *et al.,* 2014). Alkaloids content of garlic and ginger are high (Table 2). This is in agreement with the work of Gupta (1994). Alkaloids are said to have analgesic, anti-

inflammatory effect in man and animal thereby causing host to form resistance against diseases and stress (Gupta, 1994). Saponins are known to possess useful cholesterol lowering, harmful cytotoxic permeabilization of the intestine properties, and structure dependent biological activities (Osagie and Eka, 1998). Saponins also causes blood cholesterol decrease, have antitumor and anti-mutagenic activities which helps to reduce cancer risk in humans (Roa *et al.,* 1995; Prohp and Onoagbe, 2012). The presence of saponin helps plants to defend itself against humans and parasites. It also helps plants to fight against various microorganisms most especially fungi (Ogu *et al.,* 2012; Huzaifa *et al.,* 2014). Plants containing saponins have ability to precipitate and coagulate red blood cells (Sood *et al.,* 2012).

Malu *et al.* (2008) reported in his study of antibacterial activity and medicinal properties of ginger (Zingiber officinale) that sesquiterpenoids are the main component of ginger which confer its antibacterial activity. The essential oil from Ginger, according to Guptha and Ravishankar, (2005) also give ginger its antimicrobial properties.

Generally, all extracts (methanol, acetone and ethyl acetate) of both garlic and ginger were observed to be active against all the test organisms. Garlic and ginger crude plant extract showed antimicrobial properties against all selected test organisms. Zone of inhibition was observed to range from sensitive to intermediate and then to resistant. Sensitivity implied that the plant extract could inhibit the growth of that particular organism at the given level of concentration. For example, the methanolic extract of garlic and ginger showed antimicrobial activities against the clinical isolates (Table 5). Ethyl acetate and acetone extract of garlic and ginger gave high zones of inhibitions against clinical isolates (Tables 3 and 4). This is therefore in line with the work of Onyeagba *et al.* (2004) and Abeywardhana

et al. (2014) who stated that the phytochemicals present in the plant sample improves organism's sensitivity to plant and also the presence of secondary metabolites in plant sample is associated with a pronounced activity on any organism. Also it is in line with the findings of Cowan (1999) who stated that the medicinal plants are rich sources of wide variety of secondary metabolites belonging to chemical classes such as sterols, alkaloids, glycosides, saponins, flavonoids, tannins, and carbohydrates which are generally superior in their anti-microbial activities.

It can be observed from the results that higher concentrations of extract gave higher zones of inhibitions than the lower concentrations of extract. In comparison, it is observed that there is a difference between zones of inhibition for each concentration of extract as shown in tables 3, 4, and 5. Bacon *et al.* (2017) stated that the higher the concentration of extract to solvent the more the zones of inhibition. This is in line with the findings of Cheremisinoff (2003); Bacon *et al.* (2017). According to Cheremisinoff (2003), this could be as a result of increasing concentration of extract and also because of the differences in interaction between phytochemicals and solvent that may account for differences in microbial activity of extracts of different solvents.

Furthermore, it was also observed that the solvent of extraction, its polarity and method of extraction affected the degree of antimicrobial activity. This is based on the observation from this study which revealed that the solvent methanol (Table 5) with a higher polarity had much higher antimicrobial activity than those with lower polarity (Table 3 and 4) in all concentrations. This agrees with the findings of Ekwenye and Elegalam (2005); Singh *et al.* (2008), who stated that the solvent used for preparation of the spice extract plays a major role in the inhibitory effect of the spice. A concentration of two hundred milligram/milliliter

methanolic extract of ginger gave the overall highest zone of inhibition against *S. aureus* (Table 5). This is in line with the findings of Onyeagba *et al.* (2004). Other than solvent polarity mentioned above, the reason for disparity again maybe as a result of organisms' cell wall which according to Strika *et al.* (2016) suggests that the nature of the cell wall determines penetration of plant extracts. The outer membrane of gram negative bacteria is said to endow the bacteria surface with strong hydrophobicity and acts as a strong permeability barrier. Despite this aforementioned reason, garlic methanol extracts gave the second maximum zone of inhibition against *P. aeruginosa* (Table 5) which makes this findings contrary to the findings of Chandarana *et al.*, 2005; Hassan *et al.*, 2012; Strika *et al.* 2016) who proved that maximum zone of inhibition was against *E. coli* . Ginger is seen to have more effect on microorganisms (Tables 3 and 5) probably because of higher concentration of bioactive components. This is contrary to the work of Gull *et al.* (2012) who proved that garlic had higher inhibitory properties against microorganisms than ginger.

In comparing results with the highest value obtained using plant extract against clinical isolates to the highest value obtained in positive control, antibiotics was seen to have lesser zone of inhibition. This suggests that plant extract used in this study has more inhibitory property than Tetracycline. This could be as a result of plants extract concentrations and solvent used for extraction. This is contrary to the findings of Nascimento *et al.* (2000) and Peggy (2006) who in their study found out that antibiotics gave more inhibitory property than plant extracts. The MIC was observed in garlic and ginger for all extracting solvents at various concentrations against clinical isolates as shown in Table 7. The variation in MIC of plant crude extract may probably be as a result of the constituents as well as nature of the clinical isolate used. This result is in line with the findings of Nok *et al.* (1996). Minimum

bactericidal concentration was observed on acetone extract of garlic and methanolic extract of garlic used against clinical isolates (Table 8). This implies that the concentration of the extracts used have better inhibitory properties and can be used for the treatment of *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. This is in line with the findings of Kumar *et al.* (2007).

5.1 CONCLUSION

The antimicrobial activities of plant crude extract of garlic and ginger against clinical isolates emphasizes the usefulness of both plant samples in combating pathogenic microbes. They can be used as a potential inhibitor of food pathogens. The findings of this research also supports the traditional knowledge of local users, it confirms and validates scientific findings for the use of these plants against microbial activities.

5.2 RECOMMENDATIONS

It is recommended that further scientific research should be done on garlic and ginger plant samples so as to standardize its use. The use of plants for medicinal purposes should be encouraged. Also, more research on herbal medicine should be encouraged. Furthermore, plant extracts should be standardized by the appropriate bodies so as to confirm its safe use. Again proper preservations and sustainable use of such plant resources especially considering the growth rate of multi- resistant drug strain of bacteria worldwide should be promoted.

REFERENCES

Abeywardhana, K.W., Abeysinghe, D.C., Dharmadasa, R.M. and Aththanayake, A.M.L. (2014). Determination of Optimum Maturity Stage for *Ocimum lanctum L*. Grown Under Different Growing Systems in Terms of Therapeutically Active Secondary Metabolites.*World Journal of Agricultural Research*. 2(4): 159-162.

Abimbola, K.A., Obi, C.L., Alabi, S.A, Olukoya, D.K. and Ndip, R.N. (1993). Current Status on Biotyping Antibiogram and Plasmid Profiles of *E.coli* Isolates. *East Afr. Med. J*. 70: 207- 210.

Aboutaleb, N., Kuijper, E.J. and Van- dissel, J.T. (2014). Emerging Infectious Colitis. *Current Opinion in Gastroenterology*. 30(1): 106–115.

Abreu, A.C., Mcbain, A.J. and Simões, M. (2012). Plants as Sources of New Antimicrobials and Resistance Modifying Agents. *Natural Product Report*. 29: 1007-1021.

Aburjai, T.M., Darwish, S., Al- Khahl, A., Mahaj, Z.N.H. and Al- Abbadi, A. (2001). Screening of Antibiotic Resistance Inhibitors from Local Plant Materials against Two Different Strains of *Pseudomonas aeruginosa. Journal of Ethnopharmacol*. 76: 39-44.

Adamu, M., Naidoo, V. and Eloff, J.N. (2014). The Antibacterial Activity, Antioxidant Activity and Selectivity Index of Leaf Extracts of Thirteen South African Tree Species Used in Ethno-Veterinary Medicine to Treat Helminth Infections. *BMC Veterinary Research*. 10(1): 1-15

Adeshina, G.O., Jibo, S., Agu, V.E. and Ehinmidu, J.O. (2011). Antibacterial Activity of Fresh Juices of *Allium cepa* and *Zingiber officinale* against Multidrug Resistant Bacteria. *Int J Pharma Biosci*. 2: 289–295.

Agrawal, S., Kulkarni, G.T. and Sharma, V.N. (2012). Antimicrobial and Anti-Inflammatory Activities of Bark of Four Plant Species from Indian Origin. *Webmed Central Pharmaceutical Sciences*. 3(10): 2010-2012.

Ahmed, I. and Beg, A. Z. (2001). Antimicrobial and Phytochemical Studies of 45 Indian Medicinal Plants Against Multi Drug Resistant Human Pathogens. *Journal of Ethnopharmcol*. 74(2): 113- 114

Ahn, K. (2017). The Worldwide Trend of Using Botanical Drugs and Strategies for Developing Global Drugs. *BMB Reports*. 50(3): 111-116.

Aida-Gabara, D., Mamaa, S., Manuela, D., Madya, C. and Maxa. R. (2006). The African Baobab Tree (*Adansonia digitata L.*): Principal Characteristics and Uses.61: 55-69.

Ajali, U. (2004). Saponins: In: Chemistry of Biocompounds.1st Ed Rhyce Karex Publishers, Enugu,Nigeria. 167-175

Akinmoladun, A.C., Ibukun, E.O., Afor, E., Obuotor, E.M. and Farombi, E.O. (2007). Phytochemical Constituent and Antioxidant Activity of Extract from the Leaves of *Ocimum gratissimum*. *Sci. Res. Essay*. 2: 163-166.

Alam, P. (2013). Densitometric HPTLC Analysis of 8-gingerol in *Zingiber officinale* Extract and Ginger-Containing Dietary Supplements, Teas and Commercial Creams. *Asian Pacific Journal of Tropical Biomedicine*. 3(8): 634–638.

Ali, B. H., Blunden, G., Tanira, M. O. and Nemmar, A. (2008). Some Phytochemical, Pharmacological and Toxicological Properties of Ginger (*Zingiber officinale Roscoe*): A review of Recent Research. *Food and Chemical Toxicology*. 46 (2): 409–420.

Anan, I. K., Hudson, J.B., De souza, C., Akpagana, K., Tower, G.H.N., Arnason, J.T. and Gbeassor, M. (2000). Investigation of Medicinal Plants of Togo for Antiviral and Antimicrobial Activities. *Pharmaceutical Biology*. 39(1): 40–45.

Andrade R.J. and Tulkens. P.M. (2011). Hepatic Safety of Antibiotics Used in Primary Care. *Journal of Antimicrobial Chemotherapy*. 66(7): 1431-1446.

Andrews, J.M. (2005): BSAC Standardized Disc Susceptibility Testing Method (Version4). *J. Antimicrob. Chemother*. 56: 60-76.

Ankri, S. and Mirelman, D. (1999). Antimicrobial Properties of Allicin from Garlic. *Microbes and Infection*. 1:125-129.

Ankri. (1999). Antimicrobial Properties of Allicin from Garlic. *Microbes and Infection*. 125-135.

Anon. (2009). www.growingtaste.com/vegetables/garlic.shtml

Anthoney S.T., Mutuku, C.N. and Obey J.K. (2014). Phytopharmacological Analysis of Chemical Constituents of Infused *Vernonia adoensis* Roots. *International Journal of Pharmacology and Toxicology*. 4(1): 28-33.

Aravind, G., Debjit, B., Duraivel, S. and Harish, G. (2013). Traditional and Medicinal Uses of Carica Papaya. *J.Med Plants Stud*. 1(1): 7-15.

Argal, A. and Pathak, A.K. (2006). CNS Activity of *Calotropis gigantean* Roots. *Journal of Ethnopharmacology*. 19: 425-428.

Arora, D.S. and Kaur, J. (1999). Antimicrobial Activity of Spices. *Int. J. Antimicrob. Agents*. 12: 257-262.

Assam, J.P., Dzoyem, J.P., Pieme, C.A. and Penlap, V.B. (2010). In vitro Antibacterial Activity and Acute Toxicity Studies of Aqueous-Methanol Extract of *Sida rhombifolia Linn*. (*Malvaceae*). *Journal of the International Society for Complementary Medicine Research* (ISCMR). 10:40

Atai, Z., Atapour, M. and Mohseni, M. (2009). Inhibitory Effect of Ginger Extract *on Candida albicans. Am. J. Appl. Sci*. 6: 1067- 1069.

Atawodi, S.E., Bulus, T., Ibrahim, S., Ameh, D.A., Nok, A.J., Mamman, M. And Galadima. M. (2003). In Vitro Trypanocidal Effect of Methanolic Extracts of Some Nigerian Savannah Plants. *African Journal of Biotechnology*. 2 (9): 317-321.

Awada, A., Bleiberg, H., De- Valeriola. (2003) Phase I Clinical and Pharmacology of the Epothiloneanalog. *Proc Am Soc Clin Oncol*.

Ayele, Y., Kim, J., Park, E., Kim, Y., Retta, N., Dessie, G., Rhee, K. Koh, K. Nam and Kim. H.S. (2013). A Methanol Extract of *Adansonia digitata L*. Leaves Inhibits Pro-Inflammatory INOS Possibly via the Inhibition of NF-Kb Activation. *Biomolecules and Therapeutics*. 21(2): 146–152.

Bacon, K., Boyer, R., Denbow, C., O'keefe, S., Neilson, A. and Williams, R. (2017). Evaluation of Different Solvents to Extract Antibacterial Compounds. *Food Sci Nutr*. 5: 497-530.

Balch, J.F. and Balch, P.A. (2000). Prescription for Nutritional Healing. New York: A very, Penguin Putnam Inc. 267-270.

Balick, M.J. and Cox. P.A. (1996). Plants, People, and Culture: The Science of Ethnobotany. New York: *Scientific American Library*.

Balunasa, M.J and Kinghorn. A.D. (2005). Drug Discovery from Medicinal Plants. *Life Sciences*. 78(5): 431–441.

Bandna, C. (2013). Antibacterial Effect of Garlic (*Allium sativum*) and Ginger (*Zingiber officinale*) against *Staph.aureus*, *Salmonella typhi*, *E. coli* and *Bacillus cereus*. *J. Micro. Biotech. and Food Sci*. 2: 2481-29491.

Barrett, B. And Kieffer. D. (2001). Medicinal Plants, Science, and Health Care. *Journal of Herbs, Spices and Medicinal Plants*. 8(2-3): 1-36.

Baum, D.A. (1995b). A systematic revision of *Adansonia* (*Bombacacea*).*Annals of the Missouri Botanical Garden.* 82 (3): 440-470.

Benavides, G.A., Squadrito, G.L., Mills, R.W., Patel, H.D., Isbell, T.S., Patel, R.P., Darley-Usmar, V.M., Doeller, J.E. and Kraus, D.W. (2007). Hydrogen Sulfide Mediates the Vaso-activity of Garlic. *PNAS*. 104: 17977-17982

Bhanu, P., Santos, K.V., Gondi, S., Manjunath, K.G., Rajendaran, S.C. and Raj, N. (2013). Neutrophilic eccrine hidradenitis: A newculprit- carbamazepine. *Indian Journal of Pharmacology*.45: 91-92.

Bhatnagar, S., Bhandari, N. Mouli, U.C. and Bhan, M.K.. (2004). Consensus Statement of IAP National Task Force: Status Report on Management of Acute Diarrhoea. *Indian Pediatrics*. 41(4): 335–348.

Bielaszewska, M., Mellmann. A., Zhang, W., Kock, R., Fruth, A., Bauwens, A., Peters, G. and Karch. H. (2011). Characterisation of The *Escherichia Coli* Strain Associated With an Outbreak of Haemolytic Uraemic Syndrome in Germany*: A Microbiological Study. The Lancet Infectious Diseases*. 11(9): 671–676.

Boboye, B. and Owoyemi, I.D. (2004). Antibacterial Effect and Minimum Inhibitory Concentrations of Garlic (*Allium sativum*) Extract on Some Human Pathogenic Bacteria. *Biosci. Biotechnol. Res. Asia*. 1: 37-40.

Boer, H.J., Kool, A., Mizirary, W.R., Hedberg, I. and Levenfors, J.J. (2005). Antifungal and Antibacterial Activity of Some Herbal Remedies from Tanzania. *J Ethanopharmcol*. 96: 461–469.

Bohm, B.A. and Kocipai-Abyazani M.R. (1994). Flavonoids and Condensed Tannins from Leaves of Hawaiian Vaccinium and Vcalycinium. *Pacific Science*.48: 456-463

Borris, R.P. (1996). Natural Products Research: Perspectives from a Major Pharmaceutical Com- Pany. *Journal of Ethnopharmacol.* 51: 367-375.

Bourgaud, F., Gravot, A., Milesi, S. and Gontier, E. (2001). Production of Plant Secondary Metabolites: A Historical Perspective. *Plant Science*.161: 839–851.

Brooks, D. M., Pando,V. L., Ocmin, P.A. and Tejada, R.J. (2004). Resource Separation in a Napo-Amazonian Tinamou Community. Ornitol. Neotrop. 15: 323–328.

Buwa, L.V. and Van Staden. J. (2006). Antibacterial and Antifungal Activity of Traditional Medicinal Plants Used against Venereal Diseases in South Africa. *Journal of Ethnopharmacology*. 103 (1): 139–142.

Calsamiglia, S., Busquet, M., Cardozo, P.W., Castillejos, L. and Ferret, A. (2007). Essential Oils as Modifiers of Rumen Microbial Fermentation. *J. Dairy Sci*. 90: 2580-2595

Chan, C., Ngoh. G. and Yusoff. R. (2012). A Brief Review on Anti Diabetic Plants: Global Distribution, Active Ingredients, Extraction Techniques and Acting Mechanisms. *Pharmacognosy Review*. 6 (11): 22–28.

Chandarana, H., Baluja, S. and Chanda, S.V. (2005). Comparison of Antibacterial Activities of Selected Species of Zingiberaceae Family and Some Synthetic Compounds. *Turk J Biol* . 29: 83–97.

Chaurasia, S.C. and Vyas, K.K. (1997). *In Vitro* Effect of Some Volatile Oil against *Phytophthora Parasitica* Var. Piperina. *Journal of Indian Medical Research*, 1977: 24-26.

Cheesbrough, M. (2005). District Laboratory Practice in Tropical Countries part 2. Cambridge University Press, UK. 105 – 194.

Chekki , R.Z., Snoussi , A., Hamrouni, I. and Bouzouita, N. (2014) . Chemical Composition, Antibacterial and Antioxidant Activities of Tunisian Garlic (*Allium sativum*)

Essential oil and Ethanol extract. *Mediterranean Journal of Chemistry*. 3(4): 947-956

Chen, H.L., Wang, C.H., Chang, C.T. and Wang. T.C. (2003). Effects of Taiwanese Yam (Dioscorea Japonica Thunb Var.Pseudojaponica Yamamoto) on Upper Gut Function and Lipid Metabolism in Balb/C Mice.Nutrition. 19: 646–651.

Chen, J., Huang, L., Wu, S., Kuo, S. H. T. and Hsiang, C. (2007).Ginger and Its Bioactive Components Inhibit Enterotoxigenic *Escherichia coli* Heat-Labile Enterotoxin-Induced Diarrhea in Mice. *J Agric Food Chem*.55: 8390-8397.

Cheremisinoff, N.P. (2003). Industrial Solvents Handbook, 2nd Rev. Edn., Marcel Dekker Inc., New York, Usa. 580

Chew, Y.L., Goh, J.K. and Lim. Y.Y. (2009). Assessment of In-Vitro Antioxidant Capacity and Polyphenolic Composition of Selected Medicinal Herbs from Leguminosae Family in Peninsular Malaysia. *Food Chemistry*.119: 373–378.

Chopra, I., Hesse, L. and O'Neill, A.J. (2002). Exploiting Current Understanding of Antibiotic Action for Discovery of New Drugs. *Journal of Applied Microbiology*. 92(1): 47

Chukwujekwu, J.C., Lategan, C.A., Smith, P.J., Van Heerden, F.R. and Van Staden, V.J. (2009). Antiplasmodial and Cytotoxic Activity of Isolated Sesquiterpene Lactones from the Acetone Leaf Extract of *Vernonia colorata*. *South African Journal of Botany*.75: 176–179.

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

Clinical and Laboratory Standards Institute (CLSI, 2006): *Methods for the Dilution, Antimicrobial Susceptibility Test for Bacteria that Grow Aerobically*. Approved Standards, 7th Edition, (MO7A7) Vollanova. 112-115.

Cohen, M.L. (1992). Epidemiology of Drug Resistance. In: Implications for a Post-Antimicrobial Era. *Science.* 257: 1050-1055.

Collier, L., Balows, A. and Sussman, M. (1998). *Topley and Wilson's Microbiology and Microbial Infections*, *9th Edition*, *Volume 2*: Systematic Bacteriology, Eds., Arnold, London. 731–782.

Cordell, G.A. (2000). Biodiversity and Drug Discovery: A Symbiotic Relationship. *Phytochemistry*. 55: 463-480.

Cowan, M.M. (1999). Plant Products as Antimicrobial Agents. *Clinical Microbiology Reviews*. 12(4): 564–582.

Craig, W. J. (1999). Health-Promoting Properties of Common Herbs. *American Journal of Clinical Nutrition.* 70(3): 491–499.

Cunha. (2012). *American journal of Botany*. 17-19.

Das, K., Tiwari, R.K.S. and Shrivastava, D.K. (2010).Techniques for Evaluation of Medicinal Plant Products as Antimicrobial Agents: Current Methods and Future Trends. *J Med Plants Res*. 4: 104–111.

Deganhardt, J. (2003). Attracting Friends to Feast on Foes: Engineering Terpene Emission to Make Crop Plant More Attractive to Herbivore Enemies. *Current Opinion in Biotechnology*. 14: 169-176.

Demin, G. and Yingying, Z. (2010). Comparative Antibacterial Activities of Crude Polysaccharides and Flavonoids from *Zingiber officinale* and their Extraction. *American Journal of Tropical Medicine.* 5: 235–238.

Deresse, D. (2010). Antibacterial Effect of Garlic (*Allium sativum*) On *Staphylococcus aureus*: An In-Vitro Study. *Asian J Med Sci.* 2(2): 62– 65.

Didry, N., Dubreuil, L. And Pinkas, M. (1992). Antimicrobial Activity of Naphthaquinones and *Allium* Extracts Combined With Antibiotics. *Pharm. Acta Helv.* 67: 148-151.

Doughari, J.H. (2006). Antimicrobial Activity of *T. indica Linn. Tropical Journal Pharm. Res*. 5(2): 597 – 603.

Dugasani, S., Pichika, M R., Nadarajah, V. D., Balijepalli, M. K., Tandra, S. and Korlakunta, J. N. (2010). Comparative Antioxidant and Anti-inflammatory Effects of [6]-gingerol, [8]-gingerol, [10]-gingerol and [6]-shogaol. *J. Ethnopharm*. 127: 515-520.

Ekwenye, U.N. and Elegalam, N.N. (2005). Antibacterial Activity of Ginger (*Zingiber Officinale Roscoe* and Garlic (*Allium sativium L*.) Extracts on *Escherichia coli* and *Salmonella typhi*. *International Journal of Molecular Medicine and Advance Science*. 4: 411-416

EL-mahmood, M.A. (2009). Efficacy of Crude Extracts of Garlic (*Allium sativum Linn*.) against Nosocomial *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pneumoniea* and *Pseudomonas aeruginosa*. *Journal of Medicinal Plants Research*. 3(4): 179-185.

Eloff, J.N. (1998). A Sensitive and Quick Microplate Method to Determine the Minimal Inhibitory Concentration of Plant Extracts for Bacteria. *Planta Medica*. 64: 711–713.

Elvin-Lewis, M. (2001). Should We Be Concerned About Herbal Remedies. *J. Ethnopharmacol*. 75: 141-164

European Committee for Antimicrobial Susceptibility Testing of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID). (2003). Determination of Minimum Inhibitory Concentrations (MICS) of Antibacterial Agents by Broth Dilution. *Clinical Microbiology and Infection.* 9(8)

Evans, W.C. (2002). Trease and Evans Pharmacognosy. 15thed Saunders Company Ltd, Edinburgh. 56.

Fabricant, D.S. and Farnsworth, N.R. (2001). The Value of Plants Used in Traditional Medicine for Drug Discovery. *Environmental Health Perspectives*. 109(1): 69-75.

Farombi, E.O. (2003). African Indigenous Plants with Chemotherapeutic Potentials and Biotechnological Approach to the Production of Bioactive Prophylactic Agents. *African Journal of Biotechnology*. 2(12): 662-671.

Fawole, M.O. and Oso, B.A. (2004). Characterisation of Bacteria: Laboratory Manual of Microbiology 4th edition. Spectrum Books Limited, Ibadan, Nigeria. 24 - 33

Ficker, C., Smith, M. and Akpagana, K. (2003). Bioassay-Guided Isolation and Identification of Antifungal Compounds from Ginger. *Phytother Res*.17: 897-902.

Foster, S and Duke, J. (1990). *A field guide to medicinal plants*. Houghton miffilin Co, Boston.

Franklin, D.L. (1998). *Staphylococcus aureus* Infections. *N. Engl.J. Med*. 339(8): 520-532

Fujioka, T. and Kashiwanda, Y. (1994).Anti- AIDS Agents. Betullinic Acid and Platonic Acid as Anti-HIV Principles from Syzigumclaviflorum, and the Anti-HIV Activity of Structurally Related Triterpenoids. *Journal of Natural Product*. 57: 243-247.

Fujisawa, H., Suma, K., Origuchi, K., Kumagai, H., Seki, T. and Ariga, T. (2008).Biological and Chemical Stability of Garlic-derived Allicin. *J Agric Food Chem*. 56(11): 4229–4235.

Galvez, J., Crespo, M.E., Zarzuelo, A., De witte, P. and Spiessens. C. (1993). Pharmacological Activity of a Procyanidin Isolated from Sclerocarya Birrea Bark: Antidiarrhoeal Activity on Isolated Guinea-Pig Ileum. *Phytotherapy Research*. 7: 25–28.

Goncagul, G. (2010). Antimicrobial Effect of Garlic (*Allium sativum*). *Recent Pat Antiinfect Drug Discov*. 5(1): 91-93.

Gorinstein, S., Leontowicz, H., Leontowicz, M., Drzewiecki, J., Najman, K., Katrich, E., Barasch, D., Yamamoto, K. and Trakhtenberg, S. (2006). Raw and Boiled Garlic Enhances Plasma Antioxidant Activity and Improves Plasma Lipid Metabolism in Cholesterol- Fed rats. *Life Sci*. 78(6): 655-663.

Grare, M., Fontanay, S., Cornil, C., Finance, C. and Duval, R.E. (2008). Tetrazolium Salts for MIC Determination in Microplates: Why? Which Salt to Select? How?. *Journal of Microbiological Methods*. 75(1): 156–159.

Grover, J.K., Vats. V. and Rathi, S.S. (2000). Medicinal Plants of India with Anti-Diabetic Potential. *Journal of Ethnopharmacology*. 73: 461–470.

Grzanna, R., Lindmark, L. and Frondoza, C.G. (2005). Ginger—an Herbal Medicinal Product with Broad Anti-inflammatory Actions. *Journal of Medicinal Food*. 8(2): 125–132.

Gulcin, I. (2010). Antioxidant Properties of Resveratrol: A Structure-Activity Insight. Innovative Food Science Emerging Techniques. 11: 210–218.

Gulcin, I., Bursal, E., Sehitoglu, M.H., Bilsel, M. and Goren, A.C. (2010). Polyphenol Contents and Antioxidant Activity of Lyophilized Aqueous Extract of Propolis from Erzurum, Turkey. *Food and Chemical Toxicology*. 48: 2227–2238.

Gull, I., Saeed, M., Shaukat, H., Aslam, S.M., Samra, Z.Q. and Athar, A.M. (2012). Inhibitory Effect of *Allium sativum* and *Zingiber officinale* Extracts on Clinically Important Drug Resistant Pathogenic Bacteria. *Annual of Clinical Microbiol. Antimicrobials*. 11: 8.

Gupta, S. (1994). Prospects and Perspective of Natural Products in Medicine. *Indian Journal of Pharmacology*. 26: 1-12.

Guptha, S. and Ravishankar, S. (2005). A Comparison of the Antimicrobial Activity of Garlic, Ginger, Carrot and Turmeric Pastes against *E. coli* in Laboratory Buffer and Ground Beef. *Foodborne Patho. Dis*. 2: 330-340.

Gupta, S. K. and Sharma, A. (2014). Medicinal Properties of *Zingiber officinale Roscoe*-A Review. *Journal of Pharmacy and Biological Sciences*. 9(5): 124-129.

Gurib-Fakim, A. (2006). Medicinal Plants: Traditions of Yesterday and Drugs of Tomorrow. Molecular Aspects of Medicine. 27(1): 1–93

Han, J., Lawson, L. and Han, G. (1995). A Spectrophotometric Method for Quantitative Determination of Allicin and Total Garlic Thiosulfinates. *Annals of Biochemistry*. 225: 157-160.

Harbone, J.B. (1998). Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 3rd Ed, Chapman and Hall, London UK.

Harborne, J.B. (1999). Classes and Functions of Secondary Products from Plants. In: Walton, N.J. and Brown, D.E. (Eds). Chemicals from Plants. London: Imperial College Press. 1- 25.

Harold, M. (2004). On Food and Cooking: The Science and Lore of the Kitchen, Scribner, New York, NY, USA, 2nd edition.

Harris, L.G., Foster, S.J. and Richards. R.G. (2002). An Introduction to *Staphylococcus aureus*, and Techniques for Identifying and Quantifying *S. aureus* Adhesins in Relation to Adhension of Biomaterials. *European Cells and Materials*. 4: 39-60

Hassan, H.A., Rasheed- Raauf, A.M., AbdRazik, B.M. and Rasool- Hassan, B.A. (2012).Chemical Composition and Antimicrobial Activity of The Crude Extracts

Isolated from *Zingiber officinale* by Different Solvents. *Pharmaceut*. *Anal*. *Acta*. 3: 9

Heinrich, M., Barnes, J., Gibbons, S. and Williamson, E.M. (2004). Fundamentals of pharmacognosy and phytotherapy. 1st ed. Edinbrugh: Churchill Livingstone.245–252.

Hong-Xi, X.U. and Song, F. L. (2001). Activity of Plant Flavonoids against Antibiotic Resistant Bacteria. *Phytother, Res*. 15: 39-41.

Huang, J., Bachem, C., Jacobsen, E. and Visser, R. (2001). Molecular Analysis of Differentially Expressed Genes During Postharvest Deterioration in Cassava (*Manihot Esculenta Crantz*) Tuberous Roots. *Euphytica*. 120(1): 85-93

Hufford, C.D., Jia, Y., Clark, A.M. and Roger, R.D. (1993). Antimicrobial Compounds from *Petalostamum purpureum*. *Journal of Natural Product.* 56: 1878-1889.

Hughes, B.G. and Lawson D.L. (1991). Antimicrobial Effects of *Allium sativum L*. (garlic), *Allium ampeloprasum* (elephant garlic), and *Allium cepa* (onion), Garlic Compounds and Commercial Garlic Supplement Products. *Phytother Res*. 5: 154-158.

Huzaifa, U., Labaran, I., Bello, A.B. and Olatunde, A.(2007). Phytochemical Screening of Aqueous Extract of Garlic (*Allium sativum*) Bulbs. *Report and Opinion*. 6(8):1-4.

Huzaifa, U., Labaran, I., Bello, A.B. and Olatunde A. (2014). Phytochemical Screening of Aqueous Extract of Garlic (*Allium sativum*) bulbs. *Report and Opinion*. 6 (8): 10-11

Idowu, O., Iwelema, E.O., Aderogba, M.A., Akinpelu, B.A. and Ogundami A.O. (2008). Antinoceptive, Anti-Inflammatory and Anti-Oxidane Activities of Eleagnine: An Alkaloid Isolated from *Chrysophyllum Albidum* Seed Cotyledon. *Journal of Biological Sciences*. 6: 1029-1034.

Igwenyi, I.O., Offor, C.E., Ajah, D.A., Nwankwo, O.C., Ukaomah, J.I. and Aja, P.M. (2011). Chemical Compositions of *Ipomea aquatic* (Green Kangkong). *International Journal of Pharmacy and Biology*. 4:594-598.

Imanishi, N., Andoh, T. and Mantani, N. (2006). Macrophage Mediated Inhibitory Effect of *Zingiber officinale Rosc*., A Traditional Oriental Herbal Medicine, on the Growth of Influenza virus. *Am J Clin Med*.34: 157-169.

Iqbal, Z., Lateef, M., Akhtar, M., Ghayur, M. and Gilani, A.(2006). In vivo Antihelminthic Activity of Ginger against Gastrointestinal Nematodes of Sheep. *J Ethnopharmacol*. 106: 285-287.

Irshad, S., Mahmood, M. And Perveen, F. S. (2012). In-Vitro Anti-Bacterial Activities of Three Medicinal Plants Using Agar Well Diffusion Method. *Research Journal of Biology*. 2(1): 1-8.

Jageta, G., Baliga, M., Venkatesh, P. and Ulloor, J. (2003). Influence of Ginger Rhizome (*Zingiber officinale Rosc*.) on Survival, Glutathione and Lipid Peroxidation in Mice after Whole Body Irradiation. *Radiat Res*.160:584-592.

James, M., Nannapaneni, R. and Johnson, M. (1999). Identification and Characterization of Two Bacteriocin Producing Bacteria Isolated from Garlic and Ginger Root. *J. Food Prot*. 62: 899- 907.

Jisika, M., Ohigashi, H., Nogaka, H., Tada, T. and Hirota, M. (1992). Bitter Steroid Glycosides, Vernon Sides A1, A2, and A3 and Related B1 from the Possible Medicinal Plant *Vernonia amygdalina* used by Wild Chimp. *Tetrahedron*. 48: 625-630

Johnson, M., Maridass, M. and Irudayaraj, V. (2008). Preliminary Phytochemical and AntiBacterial Studies on *Passiflora edulis*. *Ethnobotanical Leaflets.* 12: 425-432.

Just, M.J., Recio, M.C., Giner, R.M., Cueller, M.U., Manez, S., Billia, A.R. and Rios, J.L. (1998). Antinflammatory Activity of Unusual Lurpine Saponins from *Bupleurum Fruticescens*. *Thieme-E Journals*. 64: 404-407.

Kahn, G. (1996). History of Garlic. In: Koch, H. P. and Lawson, L. D. Eds. Garlic: The Science and Therapeutic Application of *Allium sativum L*. and Related Species: 25-36 Williams and Wilkins New York, NY.

Kapoor, A. (1997). Antifungal Activities of Fresh Juice and Aqueous Extracts of Turmeric and Ginger (*Zingiber officinale*). *J Phytological Res*. 10: 59-63.

Kar, A. (2007). Narcotic Analgesics (Opiate Analgesics). In: Medicinal Chemistry, 4th Ed. New Age International publishers, Ltd, New Delhi. 304-341.

Karuna, S.B., Rajendrakumar, C.S. and Reddy, A.R. (2000). Aldose Reductase in Rice (*Oryza sativa L*.): Stress Response and Developmental Specificity. *Plant Science*. 160(1): 149- 157.

Khulbe, K. and Sati, S.C. (2009). Antibacterial Activity of *BoenIninghausenia albiflora Reichb*. (Rutaceae). *Afr. J. Biotechnol*. 8(22): 6346-6348.

Kirby-Bauer, A. (1996). Antimicrobial Sensitivity Testing by Agar Diffusion Method. *J Clin Pathol*. 44: 493

Kohner, P.C., Rosenblatt, J.E. and Cockerill, F.R. (1994). Comparison of Agar Dilution, Broth Dilution and Disk Diffusion Testing of Ampicillin against *Haemophilus* spp. by using In- house and Commercially Prepared Media. *J. Clin. Microbiol*. 32: 1594 -96.

Kokwaro, J.O. (2009). Medicinal Plants of East Africa. Nairobi: University Press.

Krishnaiah, D., Devi,T., Bono, A. and Sarbatly, R. (2009). Studies on Phytochemical Constituent of Six Malaysian Medicinal Plants. *Journal of Medicinal Plant Research*. 3: 67-72.

Kumar, G.S., Jayaveera, K.N., Kumar, C.K., Sanjay, U.P., Swamy, B.M. and Kumar. D.V. (2007). Antimicrobial Effects of Indian Medicinal Plants against Acne-Inducing Bacteria. *Tropical Journal of Pharmaceutical Research*. 6 (2): 717–723.

Kumar,G.V.P. and Subrahmanyam, S.N. (2013). Phytochemical Analysis, *In-Vitro* Screening for Antimicrobial and Anthelmintic Activity of Combined Hydroalcoholic Seed Extracts of Four Selected Folklore Indian Medicinal Plants. *Der Pharmacia Lettre*. 5(1): 168-176.

Labu, Zubair, Rahman and Mustafijur (2019). Proven Health Benefits of Garlic- *A Review*.

Langner, E., Greifenberg, S. and Gruenwald, J.(1998). Ginger: History and Use, Advances in Therapy. 15 (1): 25–44.

Leandro, L.M. and Vargas, F.S. (2012).Chemistry And Biological Activities of Terpenoids from Copaiba (*Coparfera* SPP). *Oleoresins*. 17: 3866-3889.

Lemar, K.M., Passa, O., Aon, M.A., Cortassa, S. and Muller, C.T. (2005). Allyl Alcohol and Garlic (*Allium sativum*) Extract Produce Oxidative Stress in *Candida albicans*. *Microbiology*. 151: 3257-3265.

Lewis, N. (2014). African Traditional Herbal Research Clinic.Respiratory Diseases 2013, Newsletter. 9 (1): 1.

Li, H., Liu, Y., Luo, D., Ma, Y., Zhang, J., Li, M., Yao, L., Shi, X., Liu, X., and Yang, K. (2019). Ginger for Health Care: An Overview of Systematic Reviews. *Complement. Ther. Med. 2019.* 45: 114-123.

Mahady, G., Rendland, S., Yun, G., Lu, Z. and Stoia, A.(2003). Ginger (*Zingiber officinale Rosc*.) and the Gingerols Inhibit the Growth of Cag A+ strains of *Helicobacter pylori*. *Anticancer Res*.23: 3699-3702.

Mahmoodi, M., Islami, M.R., Asadi-Karam, G.R., Khaksari, M., Sahebghadam, L. A., Hajizadeh, M.R. and Mirzaee, M.R. (2006). Study of the Effects of Raw Garlic Consumption on the Level of Lipids and Other Blood Biochemical Factors in Hyperlipidemic Individuals. *Pak J Pharm Sci*.19 (4): 295-298

Malu, S.P., Obochi, G.O., Tawo, E.N. and Nyong, B.E. (2008). Antibacterial Activity and Medicinal Properties of Ginger (*Zingiber officinale*). *Global J Pure Appl Sci*.15: 365–368.

Maobe, M.A.G., Gatebe, E., Gitu, L. and Rotich, H. (2013). Preliminary Phytochemical Screening of Eight Selected Medicinal Herbs Used for the Treatment of Diabetes, Malaria and *Pneumonia* in Kisii Region, Southwest Kenya. *European Journal of Applied Sciences*. 5(10): 1-6.

Massar I. and Jinan, S. (2012). Study of the Inhibitory Effect of the Ethanolic Extract of *Corianrum sativum*, *Vitis vinifera*, and *Zingiber officinale* on the Growth of *Staph. aureus* from Milk of Cows Infected with Clinical *Mastitis*. *Bas.J.Vet.Res*.11: 93-107.

Mathabe, M.C., Nikolova, R.V., Lall, N. and Nyazema, N.Z. (2006). Antibacterial Activities of Medicinal Plants used for the Treatment of Diarrhea in Limpopo Province, South Africa. *Journal of Ethnopharmacology*.105: 286-293.

Mcdevit, J.T., Schneider, D.M., Katiyar, S.K. and Edlind, T.D. (1996). Berberinea Candidate for Treatment of Diarrhoea in AIDS Patients Abstract 175. In Program and Abstracts of the 36 InterScience Conference on Antimicrobial Agents and Chemotherapy. *American Society for Microbiology.* Washington D.C.

Mcmahon, J.B., Currens, M.J., Gulakowski, R.J, Buckheit, W.J., lackman- Smith, C., Hallock, Y.F. and M.R. Boyd. (1995). Michellamine B, a Novel Plant Alkaloid, Inhibits Human Immune Deficiency Virus Induced Cell Killing by at Least Two Distinct Mechanisms. *Antimicrobial Agents Chemotherapy*. 39: 484-488.

Mahesh, T. and Menon, P.V. (2004). Quercetin Alleviates Oxidative Stress in Streptozotocin Induced Diabetic Rats. *Phytother. Res*. 18: 123-127

Mei-chin Yin. (2003). Meat Science. 63: 23–28

Mendoza, L., Wilkens, M. and Urzua, A. (1997). Anti-Microbial Study of Resinous Exudates of Diterpenoids and Flavonoids Isolated from Some *Chilean pseudognaphalium* (Asteraceae). *Journal of Ethnopharmacology*. 58: 85-88.

Mothana, R.A. and Lindequist, U. (2005). Antimicrobial Activity of Some Medicinal Plants of the Island Soqotra. *Journal of Ethnopharmacology.* 96(1-2): 177-181.

Nagosh, C., Shiota, S. and Kuroda, T.(2006). Synergistic Effect of [10]-gingerol and Aminoglycosides against Vancomycin-Resistant Enterococci (VRE). *Biol Pharm Bull*. 29: 443-447.

Naqvi, S.A.H., Khan, M.S.Y. and Vohora, S.B. (1991). Antibacterial, Antifungal and Anthelmintic Investigations on Indian Medicinal Plants. *Fitoterapia*. 62: 221-228.

Nascinento, G. G. F., Locatelli, J. I., Freitas, P. C. and Silval, G. L. (2000). Antibacterial Activity of Plant Extracts and Phytochemichals on Antibiotic Resistant Bacteria. *Brazilian Journal of Microbiology.* 31: 247-256.

Newman, D.J., Cragg, G.M. and Snader, K.M. (2003). Natural Products as Sources of New Drugs over the Period 1981-2002. *Journal of Natural Product*. 66: 1022-1037.

Newman, D.J. and Cragg, G.M. (2004). Marine Natural Products and Related Compounds in Clinical and Advanced Preclinical Trials. *Journal of Natural Product*. 67: 1216-1238.

Nguelefack, T. B., Watcho, P., Wansi, S. L. and Kamany, A. (2005). Effects of the Methanolic Leaf Extract of *Alchornea cordifolia* (*Schum*. & *Thonn*.) Muell. Arg. on Different Gastric Ulcer Models in Rats. *Cameroon Journal of Experimental Biology*.1(1).

Nielsen, P. and Rios, R. (2000). Inhibition of Fungal Growth on Bread by Volatile Compounds from Spices and Herbs and Mustard Essential oil. *Inter. J. Food Micro*. 60: 219-229.

Nitta, T., Arai, T., Takamatsu, H., Inatomi, Y., Murata, H., Iinuma, M., Tanaka, T., Ito, T., Asai, F., Ibrahim, I., Nakanishi, T. and Watabe, K. (2002). Antibacterial Activity of Extracts Prepared from Tropical and Subtropical Plants on Methicillin-Resistant *Staphylococcus aureus*. *Journal of Health Science.* 48(3): 273-276.

Njoku, P.C. and Akumefula, M.I. (2007). Phytochemical and Nutrient Evaluation of *Spondias Mombin* Leaves. *Pakistan Journal of Nutrition*.6 (6): 613-615

Nok, A.J., Williams S. and Onyenekwe, P.C. (1996). *Allium sativum* Induced Death of African Trypanosomes. *Parasitol. Res*. 82: 634-637

Norajit, K., Laohakunjit, N. and Kerdchoenchuen, O. (2007). Antibacterial Effect of Five Zingiberaceae Essential oils Molecules. 12: 2047-2060.

Noumi, E. And Yomi. A. (2001). Medicinal Plants Used for Intestinal Diseases in Mbalmayo Region, Centre Province, Cameroon.*Fitoterapia* 72: 246-254.

Nwankwo, I.U. and Osaro-Mathew, R.C. (2014). Assessment of the Phytochemical Components of *Mangifera indica* (leaf) and Musa *paradisiaca* (roots) Extracts and their Antibacterial Activity against Some Common Pathogenic Bacteria. *Journal of Pharmacy and Biological Sciences*. 9: 8-11

Nweze, L.A., Okafor, J.L. and Nwoku, O. (2004). Antimicrobial Activities of Methanolic Extracts of *Treme guineenes* (*Schumm* and *thom*) and *Morinda lucida Benth* used in Nigeria Traditional Herbal Medicinal Practice. *Biol. Res.* 2: 33-48.

Obadoni, B.O. and Ochuku, P.O. (2001). Phytochemical Studies and Comperative Efficacy of the Crude Extracts of Some Haemostatic Plants in Edo and Delta States of Nigeria. *Global Journal Pure and Applied Science.* 8:203-208.

Ody. (1997). Complete Guide to Medical Herbs (2nd edition). London.

Ogu, G.I.,Tanimowo, W.O., Nwachukwu, P.U and Igere, B.E. (2012). Antimicrobial and Phytochemical Evaluation of Leaf, Stem Bark and Root Extract of *Cyathula Prostrate* (*L*) Blume against Some Human Pathogens. *J. Intercult. Ethnopharmacol*.1: 35-43

Ohae. (1999). Complete Guide to Medical Herbs (2nd edition). London.

Okigbo, R.N., Anuagasi, C.L., Amadi, J.E. and Ukpabi, U.J. (2009b). Potential Inhibitory Effects of Some African Tuberous Plant Extracts on *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*. *International Journal of Integrative Biology*. 6(2): 91-98.

Okpekon, T., Yolou, S. Gleye, C. Roblot, F. Loiseau, P. Bories, C. Grellier, F. Frappier, F. Laurens, A. and Hocquemiller. R. (2004). Antiparasitic Activities of Medicinal Plants Used in Ivory Coast. *Journal of Ethnopharmacology*. 90: 91-97.

Omulokoli, E., Khan, B. and Chabra, S.C. (1997). Anti-Plasmodial Activity of Four Kenyan Medicinal Plants. *Journal of Ethnopharmacology*. 56: 133-137.

Onyeagba, R. A., Ugbogu, O. C., Okeke, C.U. and Iroakasi O. (2004). Studies on the Antimicrobial Effects of Garlic (*Allium sativum Linn*), Ginger (*Zingiber officinale Roscoe*) and Lime (*Citrus aurantifolia Linn*). *Afr. J. Biotechnol*. 3(10): 552-554.

Osabor, V.N., Bassey, F.I. and Umoh, U.U. (2015). Phytochemical Screening and Quantitative Evaluation of Nutritional Values of *Zingiber officinale* (Ginger). *American Chemical Science Journal.* 8(4): 1-6.

Osagie, A.U. and Eka, O.U. (1998). Mineral Elements in Plant Foods. In: Nutritional Quality of Plant Foods. Ambik Press, Benin City, Edo State, Nigeria. 8(14): 43- 86

Palombo, E.A. (2006). Phytochemicals from Traditional Medicinal Plants Used in the Treatment of Diarrhoea: Modes of Action and Effects on Intestinal Function. *Phytotherapy Research*. 20: 717–724.

Parekh, J. and Chanda, S.V. (2007). In-Vitro Antimicrobial Activity and Phytochemical Analysis of Some Indian Medicinal Plants. *Turkish Journal of Biology*. 31: 53–58.

Peggy. (2006). Antimicrobial Activity of Medicinal plants. *African Journal of biochemistry*. 12(3): 379-399.

Pichersky, E. and Gershenzon, J. (2002). The Formation and Function of Plant Volatiles: Perfumes for Pollinator Attraction and Defense. *Current Opinion in Plant Biology*. 5: 237–243.

Piera, F.A., Souza, C.F., Costa, J., Barreto, M.A., Espescheit, I., Silva, V. and Moreira, M.A. (2011). Inhibition of *E.coli* from Mastitic milk by Agrarias. Londrina. 32: 1929-1934.

Pietta, P. (2000). Flavonoids as Antioxidants. *Journal of Natural Products*. 63(7): 1035–1042.

Prohp, T.P. and Onoagbe, I.O. (2012). Acute Toxicity and Dose Response Studies of Aqueous and Ethanol Extracts of *Triplochton scleroxylon K. Schum* (Sterculiaceae). *Intern. J. Appl. Biol. Pharm. Technol*. 3 (1): 400 – 409.

Punopas, K. (2012).The study of Antibacterial Activity of Some Medicinal Plants in Larriaceae Family. Msc Suranaree University of Technology.

Qidwai, W., Alim, S. R., Dhanani, R. H., Jehangir, S., Nasrullah, A. and Raza, A. (2003). Use of Folk Remedies among Patients in Karachi Pakistan. *Journal of Ayub Medical College*, Abbottabad. 15 (2): 31–33.

Rahman, A. and Choudhary, M.I. (1995). *Nat. prod. Rep*. 12: 361-379

Rahman, A. (2010). Stress and Coping Attitudes of Cancer and Cardiac Patients as a Function of Personality and Socio-Demographic Factors in Bangladesh. An unpublished Ph.D. Dissertation. Department of Psychology, Rajshahi University, Rajshahi, Bangladesh;

Rahman, K. (2010). Bioactive Foods in Promoting Health: Chapter 16 - Garlic and Heart Health, Ed. Elsevier. 235-244.

Raja, Z.C, Ahmed, S.,Imen, H. and Nabiha, B. (2014). Chemical Composition, Antibacterial and Antioxidant Activities of Tunisian Garlic (*Allium sativum*) Essential Oil and Ethanol Extract. *Mediterranean Journal of Chemistry*. 3(4): 947-956

Rajabather, K. (1994). Chinopectic and Nutrition. Designer Food for Health of it. Townsend Letters for Doctors. 1170-1172.

Ranpal, S. (2009). An Assessment of Status and Antibacterial Properties of *Dactylorhiza hatagirea* in Annapurna Conservation Area. Bsc Forestry of Tribhuvan University.

Roa, R.R., Babu, R.M. and Rao, M.R.V. (1995). Saponins as Anti-carcinogens. *The J. Nutr*. 125: 717-724

Roa, V.R. and. Arora, R.K. (2004). Rationale for Conservation of Medicinal Plants Medicinal. *Plants Research in Asia*. 1: 7-22.

Regasa, B. (2014). Aetiology of Bacterial Pathogens from Adult Patients with Community-Acquired *Pneumonia* in Arba Minch Hospital, South Ethiopia. *Science Journal of Clinical Medicine*. 3(3): 33-36.

.Rocha, A.B., Lopes, R.M. and Schwartsmann, G. (2001). Natural Products in Anticancer Therapy. *Current Opinion in Pharmacology*. 1: 364-369.

Ross, I. (1999). Medicinal Plants of the World: Chemical Constituents, Traditional and Modern Medicinal Uses. 1st Edn., Humana Press, Totowa.

Ross, Z. M., Maslin, D. J. And Hill, D. J. (2000). The Effect of Steam Distilled Garlic Oil on Lactic Acid and other Enteric Bacteria. 4th Symposium on European Microbiological Societies. *FEMS Microbiol. Rev*. 12(G43): 137.

Ross, Z., O'Gara, E. and Hill, D. (2001). Antimicrobial Properties of Garlic Oil against Human Enteric Bacteria: Evaluation of Methodologies and Comparisons with Garlic Oil Sulphides and Garlic Powder. *Appl Environ Microbiol*. 67(1): 475-480

Rukarigira, E. (2001). Sustainable Development International.4: 179-184

Sacchetti, G., Maietti, S., Muzzoli, M., Scaglianti, M., Manfredini, S. and Radice, M. (2005). Comparative Evaluation of 11 Essential Oils of Different Origin as

Functional Antioxidants, Antiradicals and Antimicrobials in Foods. *Food Chemistry*. 91(4): 621-632

Sakanaka, S., Shimura, N. and Kim, M. (1992). Preventive Effect of Green Tea Polyphenols against Dental Caries in Conventional Rats. *Bioscience Biotechnology Biochemistry*. 56: 592-594.

Samuelsson, G. (2004). Drugs of Natural Origin: A Textbook of Pharmacognosy. 5th ed. Stockholm: Swedish Pharmaceutical Press.

Santos, A.O., Ueda-Nakumura, T., Dias Fiho, B.P., Veiga, V.F., Pinto, A.C. and Nakamwa. C.V. (2008). Antimicrobial activity of Brazilian Copaipa oils obtained from different species of the *Copalifera* genes. Mem Inst. Oswaldo Cruz. 103: 277-281.

Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K.M. and Latha. L.Y. (2011). Extraction, Isolation and Characterization of Bioactive Compounds from Plants Extracts. *African Journal of Traditional Complementary Alternative Medicines*. 8 (1): 1–10.

Schnitzler, P., Koch, C. and Reichling, J.(2007). Susceptibility of Drug-Resistant Clinical Herpes Simplex Virus Type 1 Strains to Essential oils of Ginger, Thyme, Hyssop, and Sandalwood. *Antimicrob Agents Chemother*.51: 1859-1862.

Senthilkumar, P.K. and Reetha, D. (2009). Regular Article Screening of Antimicrobial Properties of Certain Indian Medicinal Plants. *Journal of Phytology*. 1(3): 193–198

Shams-Ghahfarokhi, M., Shokoohamiri, M.R., Amirrajab, N., Moghadasi, B., Ghajari, Zeini, F., Sadeghi, G. and Razzaghi-Abyaneh, M. (2006). In-vitro Antifungal Activities of *Allium cepa*, *Allium sativum* and Ketoconazole against Some Pathogenic Yeasts and Dermatophytes. *Fitoterapia*. 77(4): 321- 323.

Shobana, S., Vidhya, V. and Ramya, M. (2009). Antibacterial Activity of Garlic Varieties (*Ophioscordon* and *Sativum*) on Enteric Pathogens. *In Current Res. J. Bio. Sc*. 1: 123-126.

Shukla, Y.N., Dubey, S., Srivastava, A., Jain, S.P. and Kumar. S. (2003). Antibacterial Activity and Some Chemical Constituents of *Adansonia digitata Linn*. *Indian Drugs*. 40 (3): 186-187.

Shukla, Y. and Singh, M. (2007). Cancer Preventive Properties of Ginger: A Brief Review. *Food and Chemical Toxicology*. 45(5): 683–690.

Singh, S.B. and Barrett. J.F. (2006). Empirical Antibacterial Drug Discovery-Foundation in Natural Products. *Biochemical Pharmacology*.71 (7): 1006–1015.

Singh, G., Kapoor, I., Singh, P. and Captslan, C. (2008). Chemistry, Antioxidant and Antimicrobial Investigations on Essential oil and oleoresins of *Zingiber officinale*. *In Food and Chemical Toxicology*. 46: 3295-3302.

Sivam, G.P., Lampe, J.W., Ulness, B., Swanzy, S.R. and Potter, J.D. (1997).*Helicobacter pylori*-In vitro Susceptibility to Garlic (*Allium sativum*) Extract. *Nutr. Cancer* 27: 118-121.

Sofowora, A.O. (1993). Medicinal Plants and Traditional Medicine in Africa. University of Ife Press 2nd Ed. 320

Sood, A., Kaur P. and Gupta R. (2012). Phytochemical Screening and Antimicrobial Assay of Various Seeds Extract of Cucurbitaceae Family. *Int. J. Applied Biol. Pharmaceut. Technol*. 3: 401-409

Sparg, S.G., Light, M.E. and Van Staden .J. (2004). Biological Activities and Distribution of Plant Saponins. *Journal of Ethnopharmacology*. 94 (2–3): 219–243.

Sri Balasubashini, M., Rukkumani, R., Viswanathan, P., Menon, P.V. (2004). Ferulic Acid Alleviates Lipid Peroxidation in Diabetic Rats. *Phytother. Res*. 18: 310-314

Srinivasan D., Sangeetha, S., Lakshmanaperumalsamy, P. (2009). In vitro Antibacterial Activity and Stability of Garlic Extract at Different pH and Temperature. *Electronic Journal of Biology.* 5(1): 5-10

Srivastava, J., Chandra, H., Nautiyal, A.R. and Kalra, S.J.S. (2013). Antimicrobial Resistance (AMR) and Plant Derived Antimicrobials (PDAms) as an Alternative Drug line to Control Infections. *Biotech*.4: 451–60.

Stern, J.L., Hagerman, A.F., Steinberg, P.D. and Mason, P.K. (1996). Phlorotannin Protein Interactions. *Journal of Chemical Ecology*. 22: 1887-1899.

Strika, I., Basic, A. and Halilovic, N. (2016). Antimicrobial Effects of Garlic (*Allium sativum L.*). *Bulletin of the Chemists and Technologists of Bosnia and Herzegovina*.47: 17-20.

Suhad A., Iman, A., Jabbar, I. and Hamssah, E. (2012). Study the Antibacterial Activity of *Zingiber officinale* Roots against Some of Pathogenic Bacteria. *Al- Mustansiriya J. Sci*. 23: 63-70.

Suresh, B., Sriram, S., Dhanaraj, S.A. and Chinnaswamy, K. (1997). Anti-Candidal Activity of *Sactolina Chinnaswamy* Volatile Oil. *Journal of Ethnopharmacology*. 55: 151-159.

Takemori, C.K., Hodding, J.H. and Kraus, D.M (1999). Mophine Pediatric Dosage, Handbook, 6th Ed Lexi-Comp Inc, Hudson (Cleveland). 6.

Tanko, Y., Yaro, A.H., Isa, A.I., Yerima, M., Saleh, M.I.A. and Mohammed, A. (2007). Toxicological and Hypoglycemic Studies on the Leaves of *Cissampelos mucronata* (Menispermaceae) on Blood Glucose Levels of Streptozocin Induced Diabetic Wistar Rats. *Journal of medicinal plants research*. 1(5): 113-116

Tanko, Y., Yerima, M., Mahdi, M.A, Yaro, A.H., Musa, K.Y. and Mohammed. A. (2008). Hypoglycemic Activity of Methanolic Stem Bark of Adansonnia Digitata Extract on

Blood Glucose Levels of Streptozocin Induced Diabetic Wistar Rats. *International Journal of Applied Research in Natural products*.1(2): 32-36.

Tapiero, H. (2004). *Biomedicine & Pharmacotherapy Journal*. 183–193.

Trease, G.E and Evans, W.C. (2002). Phytochemicals. In: Pharmacognosy. 15th ed. Saunders Publishers, London. 391-393.

Tsao, S.M. and Yin, M.C. (2001). In vitro Activity of Garlic Oil and Four Diallyl Sulphides against Antibiotic Resistant *Pseudomonas aeruginosa* and *Klebsiella pneumoniae. J. Antimicrob. Chemother*. 47: 665-670.

Tsuchiya, H., Sato, M., Miyazaki, T., Tanigaki, S., Ohyama, M., Tanaka, T. and Linuma, M. (1996). Comparative Study on the Antibacterial Activity of Phytoflavours against methicillin Resistant *S. aureus*. *Journal of Ethnopharmacology*. 50: 27-34.

Tsy, J.L.P., Lumaret, R., Mayne, D., Vall, A.O.M., Abutaba, Y.I.M., Sagna, M., Raoseta. S.O.R. and Danthu. P. (2009). Chloroplast DNA Phylogeography Suggests a West African Centre of Origin for *Baobab*, *Adansonia digitata L*. (Bombacoideae, Malvaceae). *Molecular Ecology*. 18: 1707–1715.

Tyler, V. E. (1994). The Therapeutic Use of Phytomedicinals Pharmaceutical Products. Press, New York, NY, USA.

Tynecka, Z., Syczesniak, Z. and Gelowniak. (1993). The Effect of Various Environmental Conditions on the Antimicrobial Activity of *Allium Ursinum*. *Planta Med*. 59.

Umerie, S.C., Umeh, S.O. and Nwobi, S.C. (2007). Phytochemical Analysis and Analgesic Studies on *Scoparia dulcis* (Scrophulariaceae). *Natur Appl Sci J*. 8: 66-72

Usman, H., Abdulrahman, F.I. and Usman, A. (2009). Qualitative Phytochemical Screening and In-vitro Antimicrobial Effects of Methanol Stem Bark Extract of *Ficus thonningii* (Moraceae). *Afr. J. Trad. CAM*. 6 (3): 289 – 295.

Van-wyk, B.E. and Gericke. N. (2000). People's Plants—A Guide to Useful Plants of Southern Africa. Pretoria, South Africa: Briza Publications.

Van-Burden and Robinson (1981). Phytochemical Determination In Plant Species. Food and Agricultural Institution of the United Nations and Internal Life Science Institute, Washington DC.15 -19.

Venugopal, P.V. and Venugopal, T.V. (1994). Antidermatophytic Activity of Neem (*Azadirachta indica*) leaves In vitro. *Indian J. Pharmacol*. 26: 141-143.

Venugopal, P.V. and Venugopal, T.V. (1995). Antidermatophytic Activity of Garlic (*Allium sativum*) In vitro. *Int. J. Dermatol*. 34: 278-279.

Vijayakumar, M. R., Bharathidasan, L. Prince- VijayaKumar, M. (2015). Antimicrobial Activity of *Carica Papaya L. International Journal of Arts and Science Research*. 2(2): 37 – 43.

Vuddhakul, V., Bhooponga, P., Hayeebilana, F. and Subhadhirasakulb, S. (2007) Inhibitory Activity of Thai Condiments on Pandemic Strain of *Vibrio Parahaemolyticus*. *Food Microbiol*. 24: 413-418.

Waag, T., Gelhaus, C., Rath, J., Stich, A., Leippe, M., Schirmeister, T. (2010). Allicin and Derivate are Cysteine Protease Inhibitors with Antiparasitic Activitiy. *Bioorg Med Chem Lett*. *20*, 5541-5543.

Way, W.L., Fields, H.L and Schumacher, M.A. (2001). Opoid Analgesics and Antagonists. In: Basic and Clinical Pharmacology. 8th Ed, Katzung, BG McGraw-Hill Companies, Inc. New York, pp: 512-531.

Wet, H., Dludla, P.M., Mkhaliphi, S.E. and Shabalala. S.N.M. (2008). An Ethnopharmacological Investigation of Home Grown Plants Used for Treating Diarrhoea and Wound Infections in the Mbazwana Area, Maputaland. South African. *Journal of Botany*.74: 365–366.

White, B. (2007). Antimicrobial Activity of Ginger against Different Microorganisms. *Physician*.75:1689-1691.

WHO, (1999). Monographs on selected medicinal plants. 1: 16-32.

Yang, B., Zhao, M. and Jiang. Y. (2009). Anti-Glycated Activity of Polysaccharides of Longan (*Dimocarpus Longan Lour*.) Fruit Pericarp Treated by Ultrasonic Wave. *Food Chemistry*. 114: 629–633.

Yeh, G.Y., Davis, R.B. and Phillips, R.S. (2006). Use of Complementary Therapies in Patients with Cardiovascular Disease. *The American Journal of Cardiology*. 98(5): 673-680.

Yin, R.K. (2003). Case Study Research: Design and Methods. Sage.Thousand Oaks, California. An open access and academic publisher.5:1-2

Zaidan, M.R., Noor, A., Badrul, A.R., Adlin, A., Norazah, A. and Zakiah, I. (2005). In Vitro Screening of Five Local Medicinal Plants for Antibacterial Activity Using Disc Diffusion *Method. Trop. Biomed*. 22(2):165-790.