COMBINED ANTIMICROBIAL ACTIVITIES OF *Euphorbia heterophyllus* AND *Zingiber officinale* EXTRACTS ON SOME CLINICAL ISOLATES OF BACTERIA AND FUNGI

BY

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CERTIFICATION

This is to certify that this project work was carried out by CHIMEH JENNIFER ONYEKA with the matriculation number (MCB/11/0332) of the Department of Microbiology, Federal University, Oye-Ekiti in partial fulfillment of the requirement for the award of B.Sc. degree in Microbiology.

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DEDICATION

This dissertation is dedicated to God almighty for his sustenance, provision, strength, understanding, wisdom and the knowledge he has given to me to successfully complete this work. I also thank my parents for their love and support both financially and morally.

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ABSTRACT

The antimicrobial activity of the aqueous and methanolic extracts of two (Nigerian) medicinal plants *Euphorbia heterophylla* and *Zingiber officinale* were analyzed using agar well diffusion method against three clinical isolates; *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*. The diameters of their zones of inhibition, their minimum inhibitory concentration (MIC) as well as minimum bactericidal and fungicidal concentration (MBC/MFC) were examined. There were varied rates of inhibition. The diameter of their zones of inhibition ranged from 12mm-29mm at 0.5mg/ml of the crude extract. The MIC ranges were between 6.5mg/ml for the methanolic ginger extracts and 50mg/ml for cold methanol plant extract. The MBC and MFC ranged from 25mg/ml to 100mg/ml while the range for the combination was from 12mm-25mm. This study showed that these plants have antimicrobial activities against these reference organisms and is indicative of their possible medicinal use.

CHAPTER ONE

1.0. INTRODUCTION

Since the ancient times, man has been using natural resources such as vegetables, for various purposes, mainly food and medicine. In this constant man-environment interaction, the need has become an important factor in the development of folk medicine. For thousands of years, plants have been used as the basis of many traditional medicine systems throughout the world. They continue to provide the mankind with new remedies. Every region has its own history of traditional medicine. Such practices are traditional because they are deeply rooted in a specific socio-cultural context, which varies from one community to another. Each community has its own particular approach to health and disease even at the level of ethno-pathogenic perceptions of diseases and therapeutic behavior (Rukangira, 2001).

In Africa, herbal medicine gained popularity as alternative and complementary therapies, largely as a result of cultural traditions and excessive cost of modern medicines. Traditional remedies made from plants play various important roles in the health of millions of people. Low income people such as subsistence farmers, people of small isolated villages and native communities use folk medicine for treatment of common infections (Rojas *et al.*, 2006).

The interest in medicinal plants has grown considerably in recent years because they have been a valuable source of products for maintaining human health, becoming potential candidates for many applications in the pharmaceutical industry. Medicinal plants are also one of the best sources to obtain a variety of drugs (Santos *et al.*, 2010).

Nigeria, with her rich and diverse flora, is one of the countries where people rely much on the use of traditional knowledge and medicinal plants to treat various diseases. *Euphorbia*

heterophylla (E. heterophylla) is a medicinal plant which belongs to the family of **Euphorbiaceae**. It is commonly called *Nono-kunchiya* in Hausa, *Egele* in Ibo and *Adimeru/ Tebaje* in Yoruba, in Nigeria various dialect. It is also referred to as 'Mexican fire plant', 'Milk weed' and 'Spurge weed' in English. The toxicity of this plant, especially the root and latex is recognized in Africa. Despite the toxicity hazard of this plant, it has various medicinal properties which include the following;

The leaves are used as purgative, in the treatment of gonorrhoea, respiratory tract infection, malaria, eczema, asthma and wart cure by traditional medicine. Studies have shown that the Ethanol extract and water free extract of *Euphorbia heterophylla* leave contain some wound healing properties (Omale and Emmanuel, 2010). The leaf is known to possess antibacterial activity (Falodun *et al*., 2003).

Toxicity is documented in most of the genus *Euphorbia* with individual sensitivity to latex. *Zingiber offinale* (Ginger) is a native plant in the Southeast Asia but is grown in many tropical regions of the world. It is a medicinal plant that has been widely used all over the world, since antiquity, for a wide array of unrelated ailments including arthritis, cramps, rheumatism, sprains, sore throats, muscular aches, pains, constipation, vomiting, hypertension, indigestion, dementia, fever and infectious diseases (Ali *et al*., 2008). Ginger has direct anti-microbial activity and thus can be used in treatment of bacterial infections (Tan and Vanitha, 2004). Ginger belongs to Zingiberaceae family (Sharma *et al*., 2010). The Zingiberaceous plants have strong aromatic and medicinal properties and are characterized by their tuberous or non-tuberous rhizomes (Chen *et al*., 2008). Ginger is relatively inexpensive, available the year round, well tolerated and universally accepted by the most people. The plant is reported to have antibacterial, anti-oxidant, anti- protozoa, anti-fungal,

anti-emetic, anti-rhinoviral, anti- inflammatory and anti-insecticidal activity (Ficker *et al.*, 2003). Ginger also has been found to have the following phytochemicals: Tannins, alkaloids, saponins, flavonoids, phenols, steroids, glycosides and anthraquinones.

The aim of the study is to confirm the combined antibacterial activity of aqueous extract of *E. heterophylla* and *Zingiber officinale* (*Z. officinale*).

1.1. OBJECTIVES OF THIS RESEARCH

The objectives of this research were to:

1. obtain the methanolic extracts of *Euphorbia heterophylla*,
2. obtain the methanolic and aqueous extracts of *Zingiber officinale*,
3. investigate the phytochemical components of the extracts,
4. evaluate the antibacterial activities of *E. heterophylla* and *Z. officinale* alone and in synergy on the selected bacteria and fungi,
5. compare the efficacy of the extracts with conventional antibiotics,
6. carry out the minimum inhibitory concentration and minimum bactericidal and fungicidal concentration tests.

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1. ANTIMICROBIAL AGENTS

Man is in constant contact with a large number of different micro-organisms which temporarily or permanently inhibits his body creating temporary or permanent community. A systematic survey of antimicrobial agents shows that they are general nomenclature for all drugs or chemical substances that act on micro-organisms either to kill or suppress their growth (Laport *et al.*, 2009). Among the antimicrobial agents are antibacterial drugs, antiviral agents, antifungal agents and antiparasitic drugs. Agents that kill micro-organisms are called 'cidal agents' while those that inhibit their growth are known as 'static agents'.

They can be further categorized based on their target specificity. "Narrow-spectrum" antimicrobial agents target specific types of bacteria where as "Broad-spectrum" antimicrobial agents are effective against a wide range of bacteria.

Antimicrobial agents can be gotten from three main basic sources;

1. **Natural Sources**: Few antimicrobial agents occur naturally, they can be gotten or extracted from animals or plants and can be produced by micro-organisms' biochemical pathways (Gullo *et al.*, 2006). An example is antibiotic like Polymyxin gotten from *Streptomyces* species of bacteria.
2. **Synthetic Sources**: So many antimicrobial agents have been produced synthetically. They include antibiotics like chloramphenicol and chemical

preservatives, as well as other chemotherapeutic drugs used in treating various diseases.

3. **Semi-synthetic Sources**: These antimicrobial agents can be produced naturally but still requires synthetic processes to finish up its production. These chemical processes enhance/ modify the products (Li and Vederas, 2009).

Factors that influence the rate and extent of antimicrobial action of a substance include;

1. **Temperature**: An increase in the temperature at which the chemical acts often enhances its activity, as long as it is within its range and does not denature it.
2. **pH of the environment**: The changes in pH may affect the activities of the antimicrobial agents by affecting the rate of growth of microbial cells and physiochemical states of their surfaces (Sivonen, 2002).
3. **Concentration or intensity of an antimicrobial agent**: Often, but not always, the more concentrated a chemical agent or intense a physical agent, the more rapidly micro-organisms are destroyed.
4. **Duration of exposure**: The longer populations of micro-organisms are exposed to a microbial agent, the more organisms are killed.
5. **Local environment**: Depending on what situation or what atmosphere you are in, will depend on how effective your agent will be. This affects the efficacy of the drugs. For example, antimicrobials that can kill a micro-organism in vitro may only inhibit its growth invivo (using the body for example).
6. **Interfering substances in the environment**: Organic matters in the environment can influence antimicrobial agents thereby reducing its effectiveness.

2.1.1. Mechanism of action of antimicrobial agents

There are five main mechanisms by which antimicrobial agents act. They include;

1. **Inhibition of cell metabolism**: Antimicrobial agents involved in the inhibition of cell metabolism are known as '**Antimetabolites**'. These compounds inhibit the metabolism of a micro-organism, but not the metabolism of the host. They do this by inhibiting an enzyme-catalyzed reaction which is present in the bacterial cell, but not in animal cells. The best known examples of antimicrobial agents acting in this way are the sulfonamides (Dibrov *et al.*, 2002).
2. **Inhibition of bacteria cell wall synthesis**: The inhibition of cell wall synthesis in bacteria leads to cell lysis and death. Agents operating in this way include penicillin and cephalosporins.
3. **Interactions with the plasma membrane**: Some antimicrobial agents interact with the plasma membrane of bacteria cells affecting membrane permeability. This brings about fatal results for the cell. Polymyxins and tyrothricin operate in this way.
4. **Disruption of protein synthesis**: Disruption of protein synthesis means that essential enzymes required for the cells survival can no longer be made. Agents which disrupt protein synthesis include the rifamycins, aminoglycosides, tetracyclines and chloramphenicol.
5. **Inhibition of nucleic acid transcription and replication**: The inhibition of nucleic acid function prevents cell division and/or the synthesis of essential enzymes. Agents acting in this way include nalidixic acid and proflavin.

2.1.2. Natural antibiotic properties of plant secondary metabolites

The plant chemicals are classified as primary or secondary metabolites.

- **Primary metabolites:** Primary metabolites are widely distributed in nature, occurring in one form or another in virtually all organisms. In higher plants, such compounds are often concentrated in seeds and vegetative storage organs and are needed for physiological development because of their role in basic cell metabolism. Primary metabolites obtained from higher plants for commercial use are high volume-low value bulk chemicals (e.g. vegetable oils, fatty acids, carbohydrates etc.).
- **Secondary metabolites:** Plants generally produce many secondary metabolites which are biosynthetically derived from primary metabolites and constitute an important source of microbicides, pesticides and many pharmaceutical drugs. For a long period of time medicinal plants or their secondary metabolites have been directly or indirectly playing an important role in the human society to combat diseases (Wink *et al.*, 2005). Secondary metabolites (compounds) have no apparent function in a plant's primary metabolism, but often have an ecological role like being pollinator attractants, representing chemical adaptations to environmental stresses, or serving as chemical defense against micro-organisms, insects, higher predators and even other plants (allelochemics). Secondary metabolites are frequently accumulated by plants in smaller quantities than the primary metabolites (Karuppusamy *et al.*, 2009).

In contrast to primary metabolites, secondary metabolites are synthesized in specialized cell types and at distinct developmental stages, making their extraction and purification difficult. As a result, secondary metabolites that are used commercially as biologically active compounds, are generally high value-low volume products than the primary metabolites (e.g. steroids, quinines, alkaloids, terpenoids and flavonoids), which are used in drug manufacture by the pharmaceutical industries. These are generally obtained from plant materials by steam distillation or by extraction with organic or aqueous solvents and the molecular weights are generally less than 2000. Some biologically active plant compounds have found application as drug entities or as model compounds for drug synthesis and semi-synthesis.

A survey of current pharmaceutical use revealed that, of the total prescription drugs dispensed, 25% are plant derived (Ogundipe *et al.*, 1998). Plant compounds are highly varied in structure; many are aromatic substances most of which are phenols or their oxygen-substituted derivatives, others are aliphatic substances. However increased attention has been given to extracts and biologically active compounds isolated from plant species used in herbal medicine because synthetic drugs are seen to have side effects and there is more antimicrobial resistance from pathogenic micro-organisms against synthetic antibiotics.

New compounds inhibiting microorganisms such as Benzoin and Emetine have been isolated from plants (Cox, 1994). Of the various Pharmaceuticals used in modern medicine, Aspirin, Atropine, Ephedrine, Digoxin, Morphine, Quinine, Reserpine and Tubocurarine serve as examples of drugs discovered through

observations of indigenous medical practices (Gilani and Atta-ur-Rhaman, 2005). Eloff (1999) stated that the antimicrobial compounds from plants may inhibit bacteria by a different mechanism than the presently used antibiotics and may have clinical value in the treatment of resistant microbial strains. Plant constituents may be isolated and used directly as therapeutic agents or as starting materials for drug synthesis or they may serve as models for pharmacologically active compounds in drug synthesis. The general research methods includes proper selection of medicinal plants, preparation of crude extracts, biological screening, detailed chemo pharmacological investigations, toxicological and clinical studies, standardization and use of active moiety as the lead molecule for drug design (Wink *et al*., 2005).

Types of secondary metabolites:

I. Alkaloids

Alkaloids are naturally occurring chemical compounds containing basic nitrogen atoms and are produced by a large variety of organisms including bacteria, fungi, plants, and animals. Many alkaloids are toxic and often have a pharmacological effect, which makes them to be used as medications and recreational drugs. Some alkaloids have a bitter taste (Victor, 1999).

II. Flavonoids

Flavonoids are derived from 2-phenylchromen-4-one (2-phenyl-1-4-benzopyrone) and are commonly known for their antioxidant activities. Flavonoids, which are widely distributed in plants, fulfill many functions including producing yellow, red or blue pigmentation in flowers and protection from attacks by microbes and insects.

Compared to other active plant compounds, they are low in toxicity. Flavonoids are referred to as nature's biological response modifiers because of their inherent ability to modify the body's reaction to allergens, viruses and carcinogens. They show anti-allergic, anti-inflammatory, antimicrobial and anticancer activity (Spencer, 2008).

III. Saponins

Saponins are the glycosides of 27 carbon atom steroids, or 30 carbon atom triterpenes in plants. They are found in various plant parts; leaves, stems, roots, bulbs, flowers and fruits. They are characterized by their bitter taste and their ability to haemolyze red blood cells. They are used medically as expectorant, emetic and for the treatment of excessive salivation, epilepsy, chlorosis and migraines. They are used in Ayurvedic medicine as a treatment for eczema, psoriasis and for removing freckles. Saponins are believed to be useful in the human diet for controlling cholesterol. Digitalis-type saponins strengthen the heart muscle causing the heart to pump more efficiently (Oakenfull and Fenwick, 1990). Saponins also inhibit cancer tumor growth in animals, particularly, lung and blood cancers, without killing normal cells. Saponins are the plant's immune system acting as an antibiotic to protect the plant against microbes and fungus (Chakraborty, 1993).

IV. Anthraquinones

Anthraquinones are aromatic organic compounds and is a derivative of anthracene. It has the appearance of a yellow or light-gray to gray-green, solid, crystalline powder. It is fairly stable under normal conditions. Anthraquinones naturally occur in some plants, fungi, lichen and insects, wherein they serve as a basic skeleton for their

pigments. Anthraquinones are used in the production of dyes and are also used as a laxative (Samp, 2008).

V. Cardiac glycosides

Cardiac glycosides are drugs used in the treatment of congestive heart failure and cardiac arrhythmia. These glycosides are found as secondary metabolites in several plants and in some animals. Some of these compounds are used as arrowhead poisons in hunting (Filippos *et al.*, 2007).

VI. Tannins

Tannins are astringents, bitter plant polyphenols that either bind and precipitate or shrink proteins. The astringency from the tannins is that which causes the dry and puckering feeling in the mouth following the consumption of red wine, strong tea or unripened fruits. They may be employed medicinally in antidiarrhoeal, homeostatic and antihaemorrhoidal compounds. The anti-inflammatory effects of tannins help control all indications of gastritis, esophagitis, enteritis and irritating bowel disorders. It also controls irritation in the small intestine.

2.2. MEDICINAL PLANTS

Medicinal plants are plants which contain substances that could be used for therapeutic purposes and precursors for the synthesis of useful drugs (Abolaji *et al.*, 2007). Medicinal plants are of great importance to the health of individuals and communities in general. Traditional medicine is an important part of African cultures and local medicinal systems vary between different cultural groups and regions (Makhubu, 2006). In developing countries like Nigeria, a vast number of people live in extreme poverty and some are suffering and dying for want of safe water and medicine they have no alternative for primary health care (WHO, 1995).Therefore, the need to use medicinal plants as alternatives to orthodox medicines in the provision of primary health care cannot be over-emphasized. Moreover, herbal medicines have received much attention as sources of lead compounds since they are considered as time tested and relatively safe for both human use and environment friendly. They are also cheap, easily available and affordable (Ernst, 2005). The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. There is therefore the need to look inwards to search for herbal medicinal plants with the aim of validating the ethno-medicinal use and characterization of compounds which will be added to the potential lists of drugs (Omale and Emmanuel, 2010). Medicinal plants play a significant role in providing primary health care services to rural people and are used by about 80% of the marginal communities in the world (Prajapati and Prajapati, 2002). Each medicinal plant species has its own nutrient composition besides housing pharmacologically important phytochemicals. These nutrients are essential for the physiological functions of the human body. Such nutrients and biochemicals like carbohydrates, fats and proteins play an

important role in satisfying human wants and needs for energy and other life processes (Dingman, 2002). Many medicinal plants are used by marginal communities to cure various diseases (Adnan and Holscher, 2010). As various medicinal plant species are used either in the form of extract or decoction by the local people in different regions, therefore, evaluating their marginal significance can help to understand the worth of these plants species in different ecological conditions. Some of these medicinal plants serve as both food and medicine. *Euphorbia heterophylla* is one of such plants. (Omale and Emmanuel, 2010).

2.2.1. ANTIMICROBIAL ACTIVITY OF MEDICINAL PLANTS

Medicinal plants have always been considered as a source for healthy life for people. Therapeutically, properties of medical plants are very useful in healing various diseases and the advantage of these medicinal plants are natural (Kalemba and Kunicka, 2003). In many parts of the world, medicinal plants have been used for its antibacterial, antifungal and antiviral activities for hundreds of years (Ali and Blunden, 2005).

Researchers are increasingly turning their attention to natural products and looking for new leads to develop better drugs against cancer, as well as viral and microbial infections (Ibrahim and Moody, 2009). Several synthetic antibiotics are employed in the treatment of infections and communicable diseases. Harmful microorganisms can be controlled with drugs and this has resulted in the emergence of multiple drug resistant bacteria and it has created alarming clinical situations in the treatment of infections. In general, bacteria have the genetic ability to transmit and acquire resistance to synthetic drugs which are utilized as therapeutic agents (Murray and Nagesh, 2009). Therefore, actions must be taken to reduce this problem, such as to minimize the use of antibiotics, develop research of resistance among microorganism and to continue studies to develop new antibiotic and immune modulating compounds with diverse chemical structures and novel mechanisms of action, either synthetic or natural to control pathogenic microorganisms because there has also been an alarming increase in the incidence of new and re-emerging infectious diseases (Ikenebomeh and Metitiri, 2005).

Antimicrobial studies have shown that Gram-negative bacteria show a higher resistance to plant extracts than Gram-positive bacteria. This may be due to the variation in the cell

wall structures of Gram-positive and Gram-negative bacteria. More specifically, Gram-negative bacteria have an outer membrane that is composed of high density lipopolysaccharides that serves as a barrier to many environmental substances including antibiotics (Paz *et al.*, 2001). Although hundreds of plant species have been tested for their antimicrobial properties, the vast majority of plants have not been adequately evaluated (Onwuliri and Dawang, 2006; Mahesh and Satish, 2008). The Nigerian flora offers great possibilities for the discovery of new compounds with important medicinal applications in combating infection and strengthening the immune system. The antimicrobial compounds found in plants may prevent bacterial infections by different mechanisms than the commercial antibiotics and therefore may have clinical value in treating various resistant strains of microorganism. The indiscriminate use of antibiotics has resulted in many bacterial pathogens rapidly becoming resistant to a number of originally discovered antimicrobial drugs (Barbour *et al.*, 2004). There is, thus, a continuous search for new antibiotics, and medicinal plants may offer a new source of antibacterial agents. This is indeed very important because *Candida albicans, Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli* are some of the important human pathogens that have developed resistance to antimicrobials (Barbour *et al.*, 2004).

2.2.2. MEDICINAL PLANTS USED IN THIS STUDY

Euphorbia heterophylla *(E. heterophylla)*

Euphorbia plants are widespread in nature ranging from herbs and shrubs to trees in tropical and temperate regions all over the world. It is a local medicinal plant commonly known as "**spurge weed**" (Mosango, 2008) .The family, Euphorbiaceae comprises of 280 genera and 730 species with the largest genus, *Euphorbia* having about 1600 species. Generally, they have characteristic milky latex. *E. heterophylla* leaf is used in traditional medical practices as laxative, anti-gonorrhoeal, migraine and heart cures. The plant latex has been used as fish poison and insecticides (Falodun *et al.*, 2003). The leaves of *Euphorbia heterophylla* are commonly used as a lactogenic agent by taking a decoction of it or massaging the breast with the poultice to induce milk flow (Dokori, 1998). The leaves of *E. heterophylla* have been reported to contain quercetin (Falodun *et al.*, 2003). Diterpenoids have also been reported in the root of *Euphorbia heterophylla* (Rowan and Onwukaeme, 2001). The skin irritant, tumour-promoting and anti-tumour /cancer and recently anti-HIV activities of *Euphorbia species* have also been reported in *Euphorbia heterophylla* leaf (Williams *et al.*, 1995).

Phytochemical study of several species of the Euphorbiaceae family has led to the isolation of several secondary metabolites with high biological activity, especially, antimicrobial, antiinflammatory, antidiarrhoeal, antidysenteric, antioxidant, antinociceptive, antifungal etc. The Euphorbiaceae family possesses the following secondary metabolites: Flavonoids, alkaloids, saponins, terpenoids, steroids, tannins, carbohydrates and coumarins.

***Zingiber offinale* (Ginger)**

Ginger is the rhizome of the plant *Zingiber offinale*, consumed as a delicacy, medicine, or spice. It lends its name to its genus and family (Zingiberaceae). Other notable members of this plants family are turmeric, cardamom, and galangal (NPGS/GRIN, 2011). Preliminary research indicates that nine compounds found in ginger may bind to human serotonin receptors, which may explain ginger's extensive effects on the gastro-intestinal tract and suggesting a mechanism for its effects on anxiety (Nievergelt *et al.*, 2010). Many scientists have reported antimicrobial properties of several plants. The antimicrobial, anti- tumour, anti-inflammatory and anti-necrotic (Omoya and Akharaiyi, 2012) activities have been reported from the use of plants extracts. The most well-known member of Zingiber (ginger) is *Zingiber officinale* (*Z. officinale*). In many parts of the world, *Z. officinale* has medicinal and culinary values (Omoya and Akharaiyi, 2012). The volatile oil gingerol and other pungent principles not only give ginger its pungent aroma, but are the most medically powerful because they inhibit prostaglandin and leukotriene formation, which are products that influence blood flow and inflammation, (Longe *et al.*, 2005). Ginger has been found to be more effective than placebo in multiple studies for treating nausea caused by seasickness, morning sickness and chemotherapy (Ernst and Pittler, 2000). Ginger compounds are active against a form of diarrhoea which is the leading cause of infant death in developing countries. Zingerone is likely to be the active constituent against enterotoxigenic *Escherichia coli* heat-labile enterotoxin-induced diarrhoea (Chen *et al.*, 2007).

2.2.3. MICRO-ORGANISMS USED IN THIS RESEARCH

Microorganisms are very diverse and even though their different cells look similar in morphology and produce similar colonies, it becomes necessary to identify the organisms by their biochemical characteristics that help to properly classify the disease causing organisms that are pathogenic to humans, animals and plants.

I. ***Staphylococcus aureus (S. aureus)***

This is a common colonizer of human skin and mucosa. *S. aureus* can cause mainly opportunistic diseases. Prescott *et al.*, (2005) stated that *S. aureus* is the most important human staphylococcal pathogen and causes boils, abscesses, wound infections, pneumonia, and toxic shock syndrome amongst other diseases. *S. aureus* is also a pathogen frequently reported to produce food poisoning, which leads to cramps and severe vomiting. Most strains of this bacterium are sensitive to many antibiotics, and infections can be effectively treated (Abbas and Kenneth, 2004).

II. ***Pseudomonas aeruginosa (P. aeruginosa)***

P. aeruginosa is an opportunistic pathogen that exploits some break in the host defenses to initiate an infection. It is a common environmental microorganism present in water and soil and is notorious for its resistance to antibiotics. It is therefore, a particularly dangerous and dreaded pathogen (Prescott *et al.*, 2005). The bacterium is naturally resistant to many antibiotics due to the impermeable characteristics of its outer membrane. Moreover, its tendency to colonize surfaces in a biofilm form makes the cells impervious to therapeutic concentrations of antibiotics (Okemo *et al.*, 2001).

III. *Candida albicans (C. albicans)*

This is the most common organism implicated in fungal infections, and is found in the human digestive tract, mouth, and genital region (Eggima *et al.*, 2003). Under normal circumstances, levels of *Candida* are controlled by beneficial bacteria. However, if the bacteria-fungus balance is upset by the use of antibiotics or if the immune system is compromised, an overgrowth of *Candida* can occur, resulting in infection (Braunwald *et al.*, 2001). Fungal overgrowth is encouraged by certain pH levels and the availability of sugar. People with the right conditions for fungal infection, such as a high sugar diet, are at higher risk. Also, Candida infections can be a nosocomial infection to vulnerable people with depressed immune systems who are in the hospital, where the fungus is commonly found on the hands of care givers.

Specific objectives of the study are to determine the inhibitory effect of *E. heterophylla* and *Z. officinale* on the growth of *Staphylococcus aureus* (*S. aureus*), *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Candida albicans* (*C. albicans*). The work will also compare the inhibitory effect of different concentrations of the extracts on the growth of *S. aureus*, *P. aeruginosa* and *C. albicans* as well as the inhibitory effect of the extracts and some standard antibiotics on the growth of *S. aureus*, *P. aeruginosa* and *C. albicans*.

CHAPTER 3

3.0. MATERIALS AND METHODS

3.1. MATERIALS

The stem and leaves of *Euphorbia heterophyllus*, rhizome of *Zingiber officinale* methanol, distilled water, conical flasks, incubator, autoclave, normal saline, petri dishes, aluminum foil, test tubes, filter paper, cotton wool, test tube rack, beaker, glass stirring rod, measuring cylinder, Bunsen burner, weighing balance, grinding machine, muslin cloth, inoculating loop, bijou bottles, swab sticks, nutrient agar, Mueller-Hinton Agar, antibiotics sensitivity discs.

3.2. METHODS

3.2.1. COLLECTION AND PREPARATION OF PLANT MATERIALS

Fresh leaves of *E. heterophylla* were collected during the rainy season in the month of May from the Federal University, Oye-Ekiti, Ekiti State, Nigeria. Fresh rhizomes of *Zingiber officinale* was gotten from a market seller in Trade fair market, Lagos state, Nigeria. The plants were taxonomically authenticated and voucher samples were deposited in the Department herbarium. The leaves and rhizomes were air dried at ambient temperature for several days until well dried. The dried leaves and rhizomes were reduced to fine powder using laboratory mortar and pestle, and the powder stored in an air- tight container until needed.

3.2.2. SAMPLE EXTRACTION

Extraction of plant materials using methanol

- Cold Methanolic Extraction

Powdered leaves (50g) was weighed and percolated in 500 ml of methanol contained in 500 ml conical flask and was agitated manually several times over a period of 24 hours. It was filtered and the filtrate evaporated to dryness using Soxhlet extraction apparatus. The same was done for the powdered ginger rhizome.

- Hot Methanolic Extraction

Powdered leaves (50g) was weighed and percolated in 500 ml of methanol contained in 500 ml conical flask. The conical flask was fixed directly on the heating mantle and solution was allowed to boil for 6hours at a temperature of 70°C. After heating, it was allowed to cool before filtration. The filtrate was evaporated to dryness over a water bath at 80°C until a gelatinous form of the extract was gotten. The same was done for the powdered ginger.

Aqueous extraction of plant materials

- Cold Aqueous Extraction

Fresh *Zingiber officinale* were air-dried at room temperature. Thereafter, 50g of the powdered rhizome was weighed and percolated in 500ml of distilled water contained in a conical flask. The flask was agitated manually several times over a period of 24 hours. The extract was filtered using Whatman No. 1 filter paper and the filtrate collected in a

clean beaker was concentrated by evaporation over a water bath at 80°C till a gelatinous form is achieved.

- Hot Aqueous Extraction

Powdered ginger (50g) was weighed and percolated in 500 ml of distilled water contained in 500 ml conical flask. The conical flask was fixed directly on the heating mantle and solution was allowed to boil for 6hours at a temperature of 70°C. After heating, it was allowed to cool before filtration. The filtrate was evaporated to dryness over a water bath at 80°C until a gelatinous form of the extract was gotten.

3.2.3. COLLECTION AND IDENTIFICATION OF TEST ORGANISMS

Reference clinical isolates were obtained from the Drug Discovery Unit, Microbiology Laboratory, Federal University, Oye-Ekiti, Ekiti State. They were stored on agar slants, in the refrigerator. The test organisms used were: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans*.

3.3. PHYTOCHEMICAL SCREENING

Qualitative phytochemical analysis of *Euphorbia heterophylla* and *Zingiber officinale* extracts was carried out using standard procedures to identify the constituents as described by Harbone (2001), Trease and Evans (2005) and Sofowora (2008).

I. Test for alkaloids (Mayer's test)

To a few ml of the filtrates, a drop of Mayer's reagent was added by the side of the test tube. A creamy or white precipitate indicated positive test (Harbone, 2001).

II. Test for saponins (Froath forming test)

The extract was diluted with distilled water and made up to 20ml. The suspension was shaken in a graduated cylinder for 15minutes. A thick (2cm) layer of foam indicated the presence of saponins.

III. Test for Phenolic compounds

The extract was diluted to 5ml with distilled water. To this a few drops of neutral 5% ferric chloride solution was added. A dark green colour indicated the presence of phenolic compounds.

IV. Test for tannins

About 0.5mg of dried powdered samples was boiled in 20ml of distilled water in test tube then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or blue black colour (Van-Burden and Robinson, 2002).

V. Test for flavonoids

To 5ml dilute ammonia solution, a portion of extract was added, followed by addition of concentrated sulphuric acid. Appearance of yellow colour indicates the presence of flavonoids (Boham and Kocipai-Abyazan 1995).

VI. Test for steroids

To 2ml filtered extract, 2ml acetic anhydride and 1ml concentrated sulphuric acid was added. Green colour indicated the presence of steroids.

VII. Test for cardiac glycoside

To 2ml filtered extract, 1ml of glacial acetic acid, 2ml ferric chloride and 2ml of concentrated sulphuric acid was added. The brown colour indicated the presence of glycoside.

VIII. Test for anthraquinones

Borntrager's test was used for the detection of anthraquinones. 5g of the each plant extracts were shaken with 10ml benzene, filtered and 5ml of 10% ammonia solution added to the filtrate. The mixture was shaken and the pink, red or violet colour indicated the presence of anthraquinones.

3.4. DETERMINATION OF ANTIMICROBIAL ACTIVITY OF THE EXTRACTS

The antimicrobial activity of the plant extracts on the test organisms was determined using the agar well diffusion method. Mueller Hinton Agar (MHA); for bacteria and Yeast Extract Agar (YEA); for fungi, were prepared respectively according to the manufacturers specifications. The prepared agars were poured aseptically into different petri dishes and were left to solidify before inoculating with the broth culture of the test organisms using spreading method with the use of sterile swab sticks. The inoculated medium was allowed to dry for about thirty minutes (30minutes) so as for the organisms to adhere firmly to the agar surface. This was followed by the boring of holes on the agar with the aid of a sterile cock borer of 7mm in diameter.

Different concentrations of the extracts were prepared. These concentrations were added to the wells (0.5mg/ml each) using syringe/needle. Distilled water was added into one of the wells which served as the control and the plates were incubated at 37°C for 18-24hours. The sensitivity of the test organisms to the extracts is indicated by a clear zone of inhibition around the well containing the extracts. The zones of inhibition (diameter in mm) were measured on the agar surface.

3.5. MINIMUM INHIBITORY CONCENTRATION (MIC)

Sterile Mueller Hinton broth and Yeast Extract broth were used to prepare the different extract concentrations. 9ml of each extract concentration was introduced into sterile test tubes. 1ml of standardized bacterial/fungal broth culture of test clinical bacterial and fungal isolate was added to each set of the extract concentrations.

The control test tube was inoculated with sterile distilled water. The concentrations used were 100mg/ml, 50mg/ml, 25.0mg/ml, 12.5mg/ml, 6.25mg/ml and 3.125mg/ml. All tubes were cotton plugged and incubated at 37°C for 24hours. The MIC was taken as the lowest inoculated extract concentration that did not permit any visible growth when compared with the turbidity of test tube containing sterile Mueller Hinton broth and inoculated with water (Rojas *et al*., 2006).

3.6. MINIMUM BACTERICIDAL/FUNGICIDAL CONCENTRATION (MBC/MFC)

The content of all the MIC tubes with no visible growth were placed out on sterile MHA (for bacteria) and YEA (for fungi) and then incubated at 37°C for 24hours. The MBC/MFC was taken to be the lowest inoculated extract concentration that did not produce bacterial and fungi colonies when platted out on a sterile Mueller Hinton agar (Rojas *et al*., 2006).

3.7. ANTIBIOTICS SENSITIVITY TEST (AST)

The antibiotic sensitivity test was carried out by using commercial antibiotic disc as the control. The antibiotic discs were placed on the solidified agar medium that had been inoculated with the test organisms.

The plates were then incubated at 37°C for 24hours and observed for clear zones around the discs, which were indicative of inhibition. The diameters of clear zones were measured in millimeters.

CHAPTER FOUR

4.0. RESULTS AND DISCUSSION

4.1. RESULTS

4.2. PHYTOCHEMICAL SCREENING OF PLANT EXTRACTS

The result of these tests confirmed the presence of some phytochemical groups (tannin, alkaloids, saponins and some anthraquinones) in the extracts.

Table 1 shows the results obtained from the phytochemical analysis/screening of the combination of both *Euphorbia heterophylla* and *Zingiber officinale* extracts.

4.3. ANTIMICROBIAL ACTIVITIES OF THE EXTRACTS OF *Euphorbia heterophylla* AND *Zingiber officinale* AGAINST TEST ORGANISMS.

Figure 1 shows the inhibitory activities of the methanolic and aqueous extracts of both plant and ginger on the test organisms at 50mg/ml 25mg/ml, 12.5mg/ml and 6.25mg/ml concentrations. Plates 1-9 also show the results of the antimicrobial activities of the extracts against the test organisms.

4.4. MINIMUM INHIBITORY CONCENTRATION (MIC) OF *Euphorbia heterophylla* AND *Zingiber officinale* EXTRACTS AGAINST TEST ORGANISMS

The minimum inhibitory concentration of the plant and ginger extracts against the inhibited test organisms was determined using various concentrations of the extract. Figure 2 shows the minimum inhibitory concentration of both plant extracts against the test organisms.

4.5. MINIMUM BACTERIOCIDAL AND FUNGICIDAL CONCENTRATION (MBC/MFC) OF *Euphorbia heterophylla* AND *Zingiber officinale* EXTRACTS AGAINST TEST ORGANISMS

Figure 3 shows the minimum bactericidal concentration of both plant and ginger extracts against the test organisms.

4.6. ANTIBIOTICS SENSITIVITY TEST AGAINST TEST ORGANISMS

Table 2 shows the inhibitory effects of standard antibiotic discs on the test organisms. Plates 10-12 also show the results of the antibiotics sensitivity test against the test organisms.

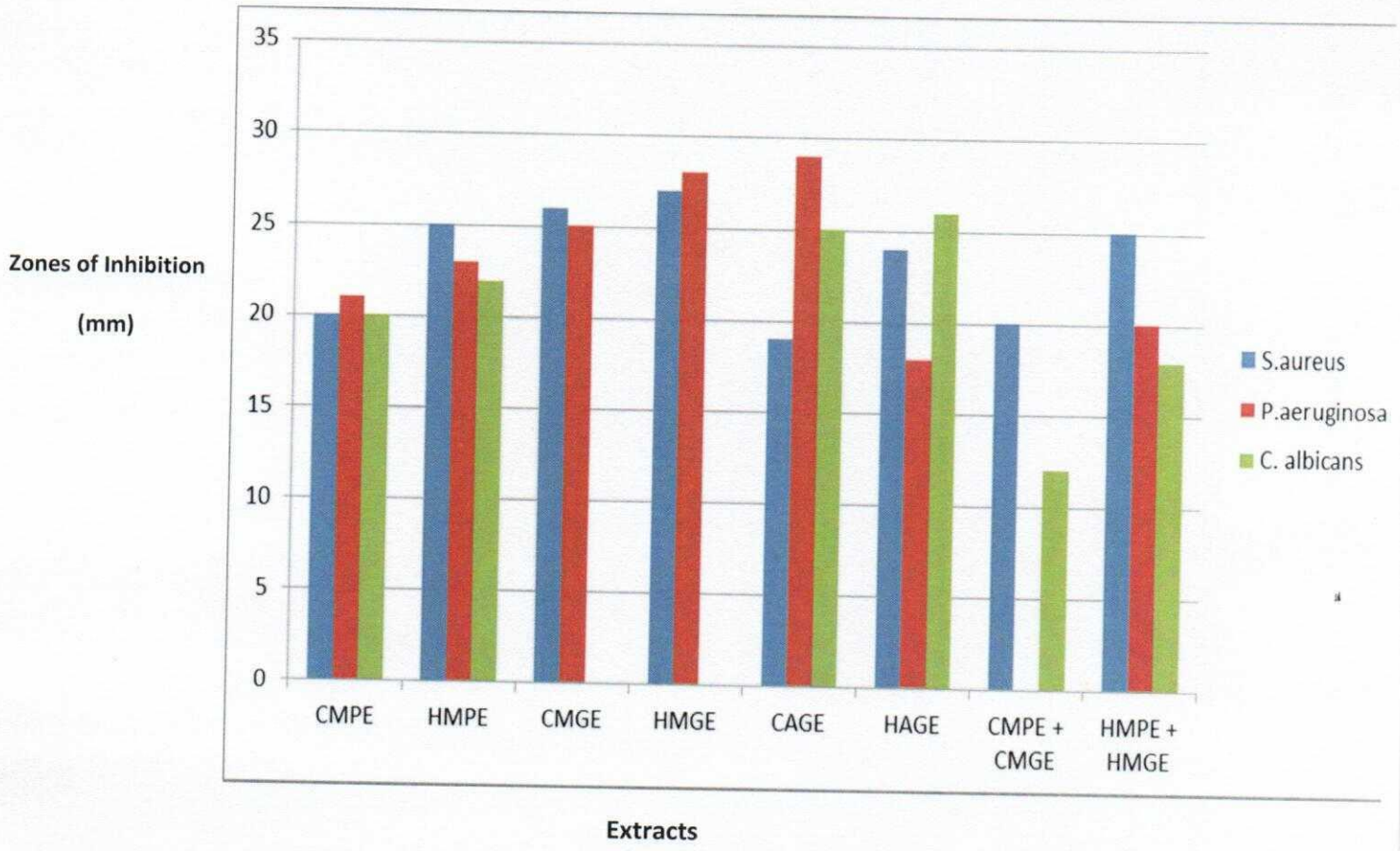
Table 1: QUALITATIVE ANALYSIS OF THE PHYTOCHEMICALS *Euphorbia heterophylla* And *Zingiber officinale* EXTRACTS.

Phytochemical groups (Chemical constituents)	CMEE	HMEE	CAZE	HAZE	CMZE	HMZE
Tannins	+	–	++	++	++	++
Alkaloids	+	+	+	++	+	++
Saponins	+	+	–	–	–	–
Flavonoids	+	++	++	++	++	++
Phenol	+	++	+	++	+	++
Steroids	–	+	–	+	–	+
Glycosides	+	+	–	+	–	+
Anthraquinones	+	+	+	+	+	+

KEY:

1. Cold Methanolic Euphorbia Extract (CMEE)
2. Hot Methanolic Euphorbia Extract (HMEE)
3. Cold Aqueous Zingiber Extract (CAZE)
4. Hot Aqueous Zingiber Extract (HAZE)
5. Cold Methanolic Zingiber Extract (CMZE)
6. Hot Methanolic Zingiber Extract (HMZE)
7. + (Present)
8. + + (Abundant in quantity)
9. – (Absent).

FIGURE 1: ANTIMICROBIAL ACTIVITY OF *Euphorbia heterophylla* AND *Zingiber officinale* EXTRACTS AGAINST TEST ORGANISMS.



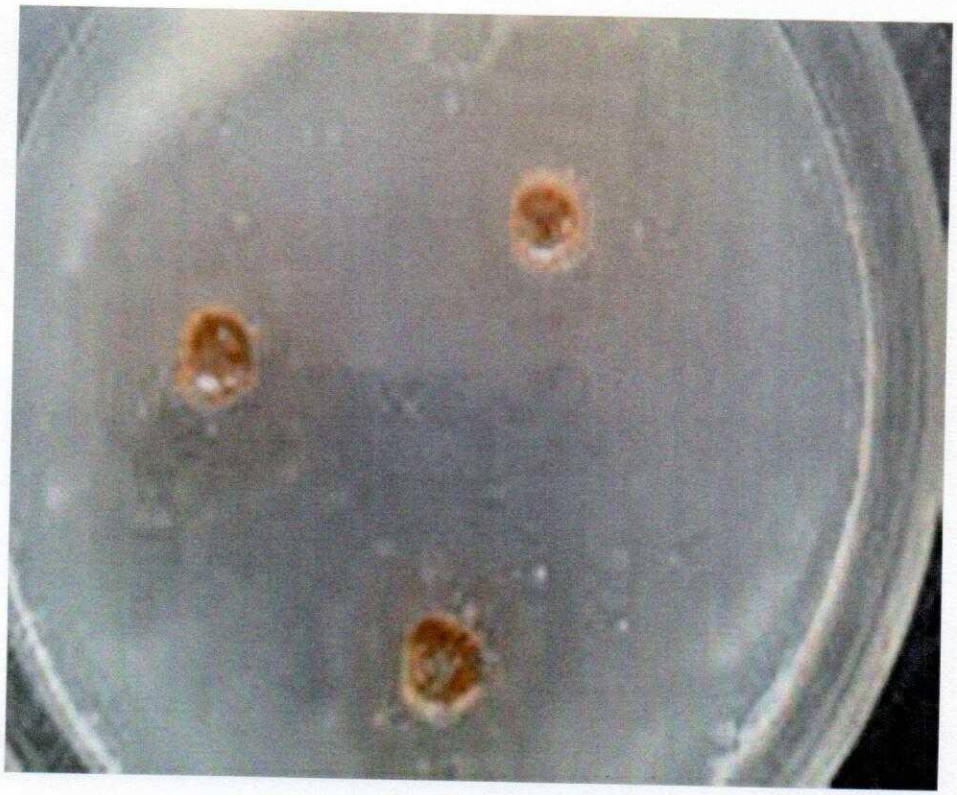


Plate 1: Zones of inhibition of cold methanolic *Euphorbia* extract, hot methanolic *Euphorbia* extract and cold methanolic *Zingiber* extract against *Staphylococcus aureus*

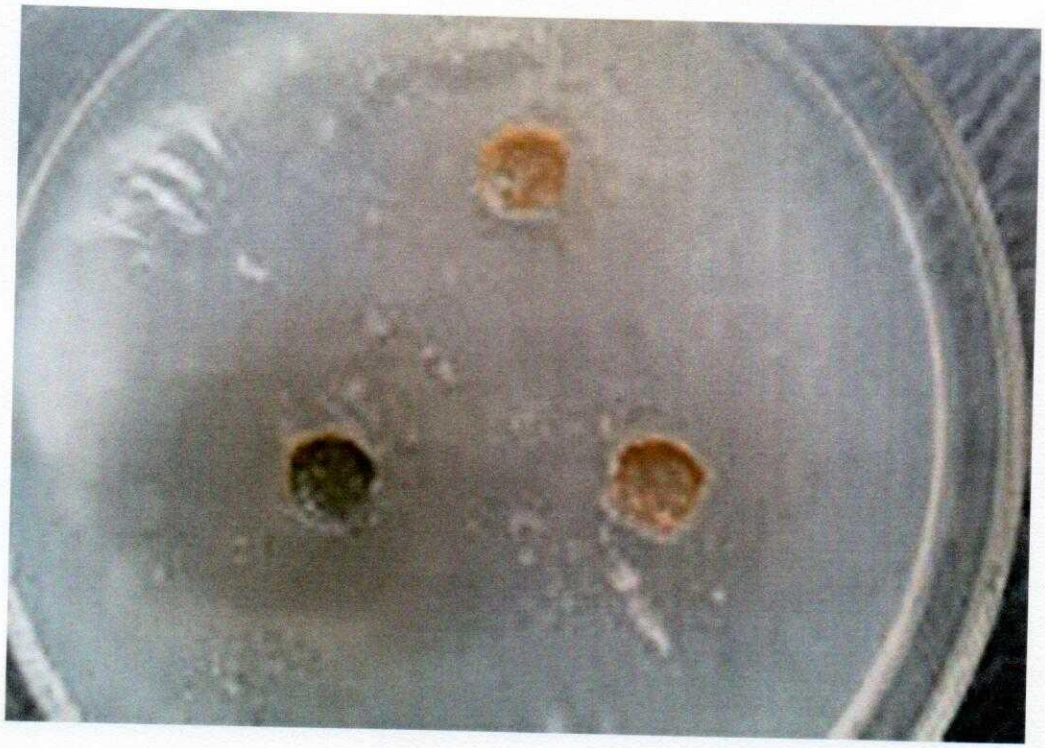


Plate 2: Zones of inhibition of hot methanolic *Zingiber* extract, cold aqueous *Zingiber* extract and hot aqueous *Zingiber* extract against *Staphylococcus aureus*

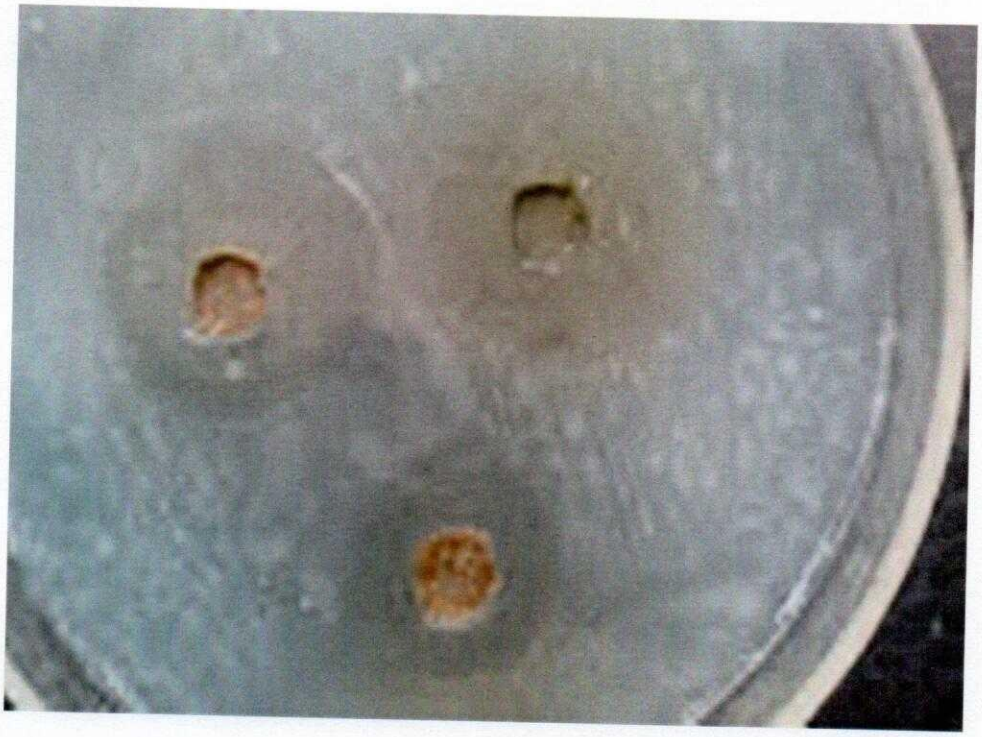


Plate 3: Zones of inhibition of cold methanolic *Euphorbia* extract, hot methanolic *Euphorbia* extract and cold methanolic *Zingiber* extracts against *Pseudomonas aeruginosa*

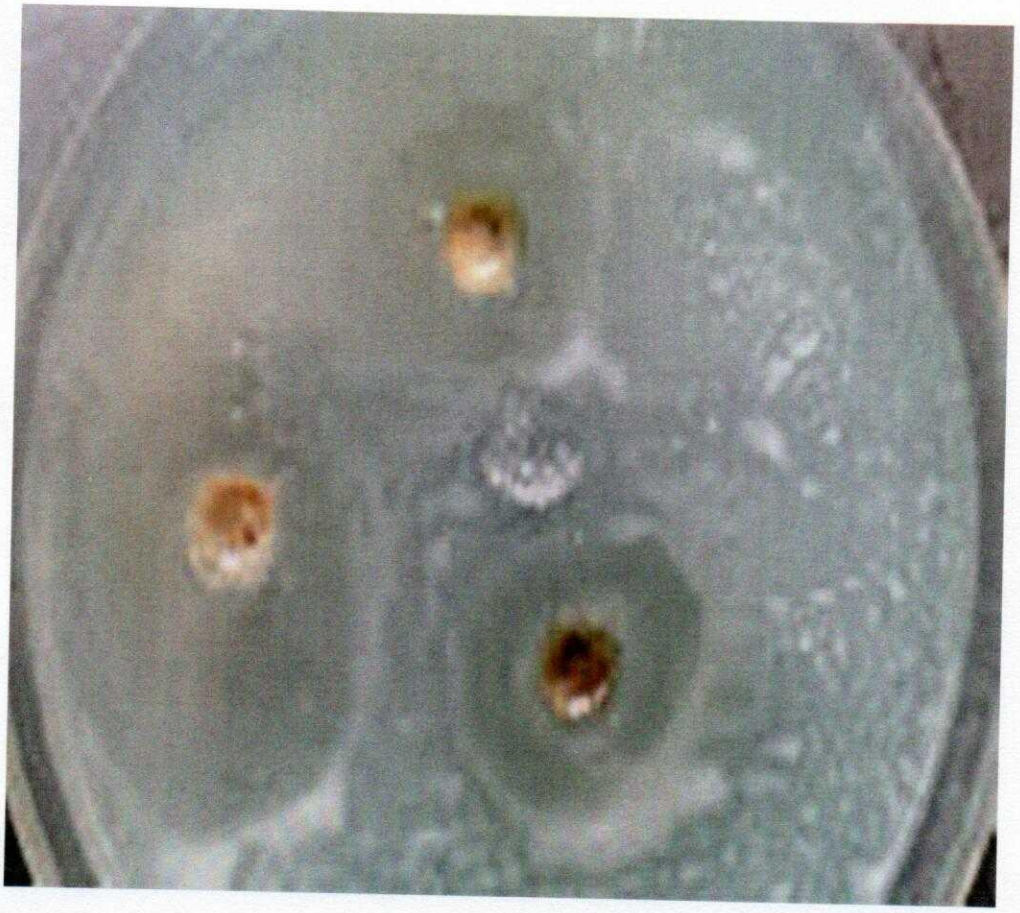


Plate 4: Zones of inhibition of hot methanolic *Zingiber* extracts, cold aqueous *Zingiber* extracts and hot aqueous *Zingiber* extracts on *Pseudomonas aeruginosa*

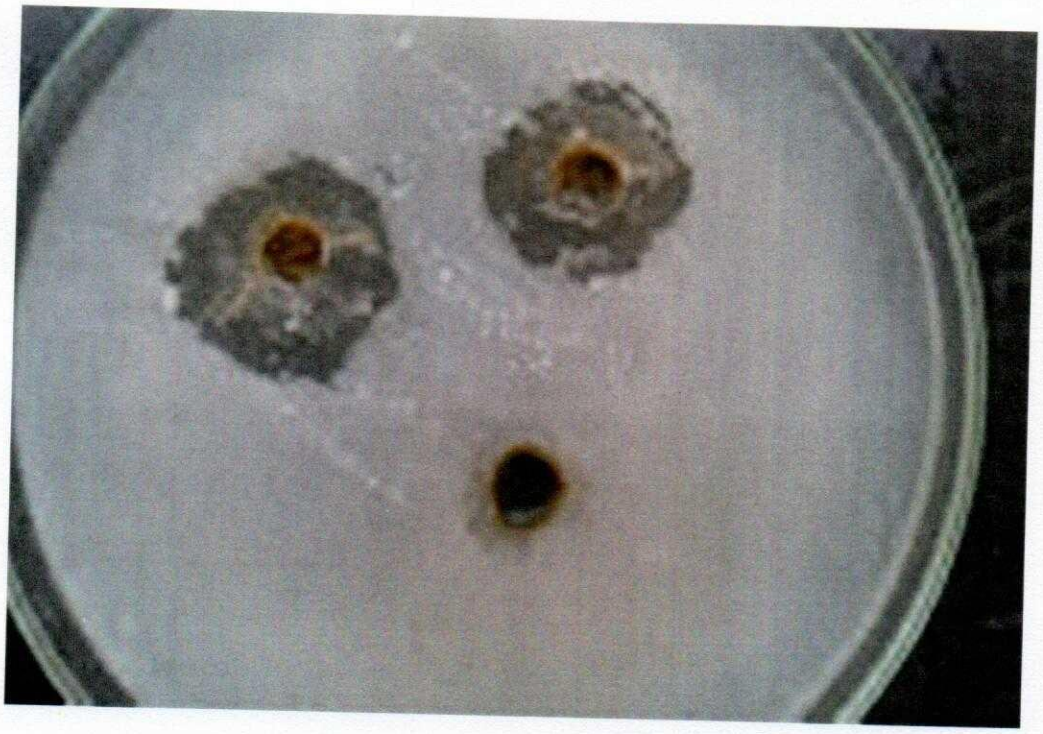


Plate 5: Zones of inhibition of cold methanolic *Euphorbia* extract, hot methanolic *Euphorbia* extract and cold methanolic *Zingiber* extracts on *Candida albicans*

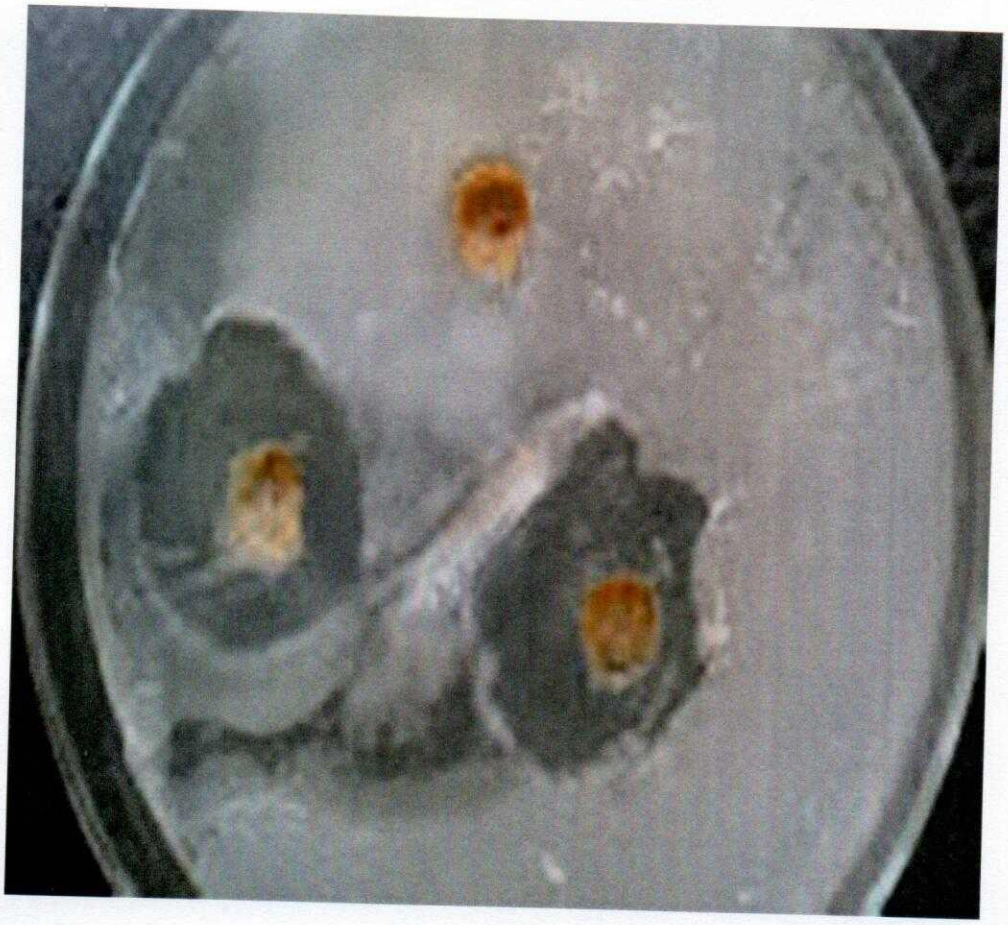


Plate 6: Zones of inhibition of hot methanolic *Zingiber* extracts, cold aqueous *Zingiber* extracts and hot aqueous *Zingiber* extracts on *Candida albicans*

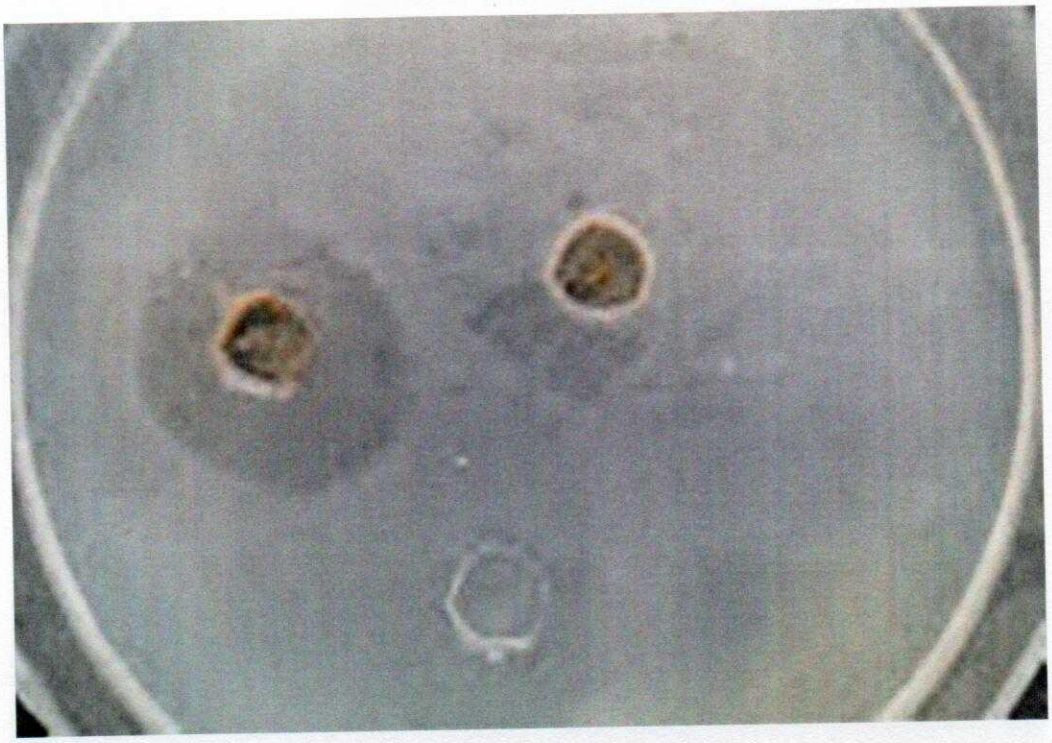


Plate 7: Combination of cold methanolic *Euphorbia/Zingiber* extracts and the combination of the hot methanolic *Euphorbia/Zingiber* extracts on *Staphylococcus aureus*

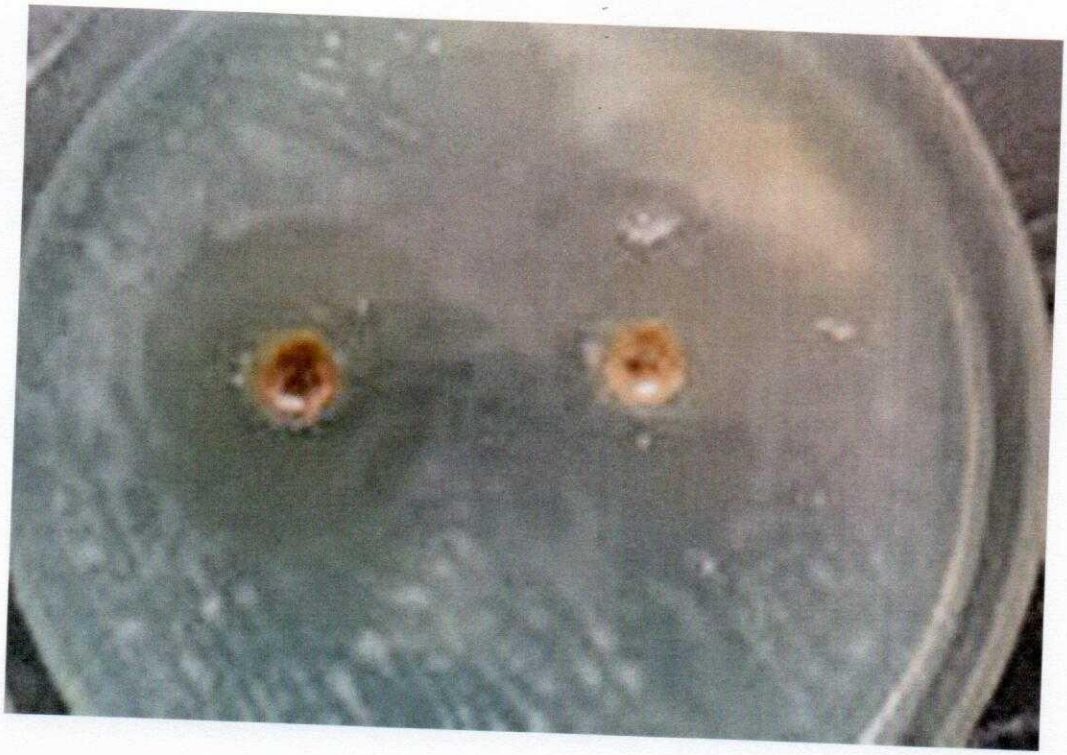


Plate 8: Combination of cold methanolic *Euphorbia/Zingiber* extracts and the combination of the hot methanolic *Euphorbia/Zingiber* extracts on *Pseudomonas aeruginosa*

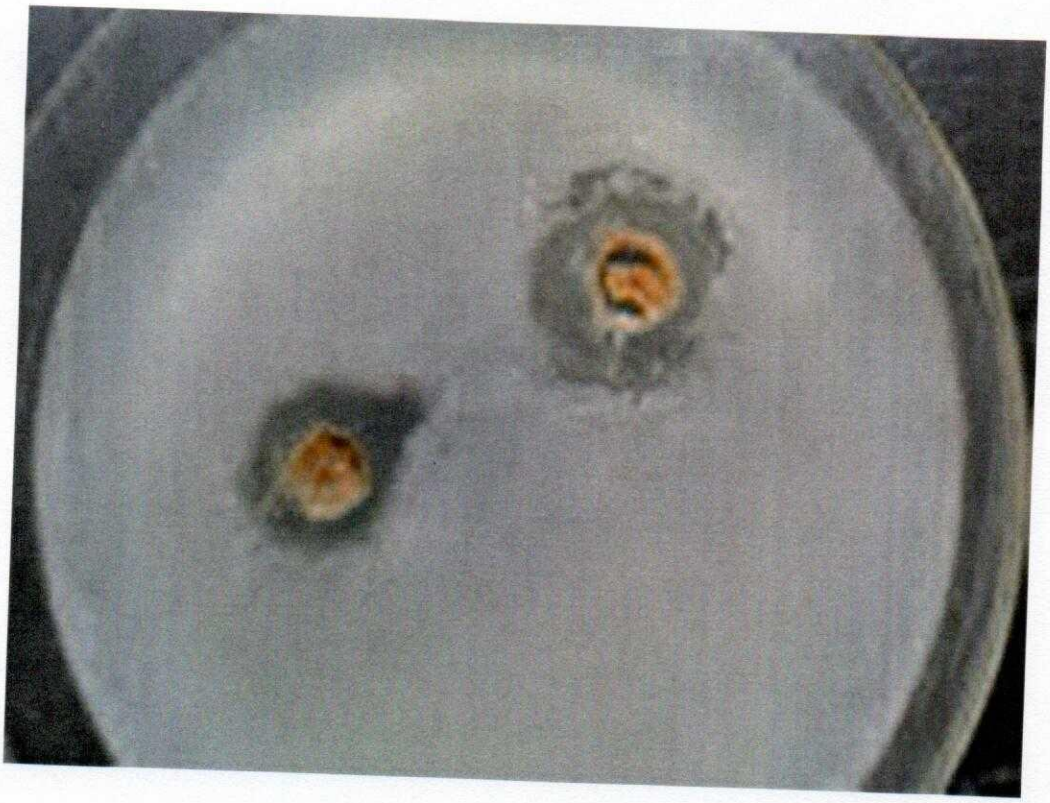


Plate 9: Combination of cold methanolic *Euphorbia/Zingiber* extracts and the combination of the hot methanolic Euphorbia/Zingiber extracts on *Candida albicans*

FIGURE 2: MINIMUM INHIBITORY CONCENTRATION OF *Euphorbia heterophylla* AND *Zingiber officinale* EXTRACTS AGAINST TEST ORGANISMS

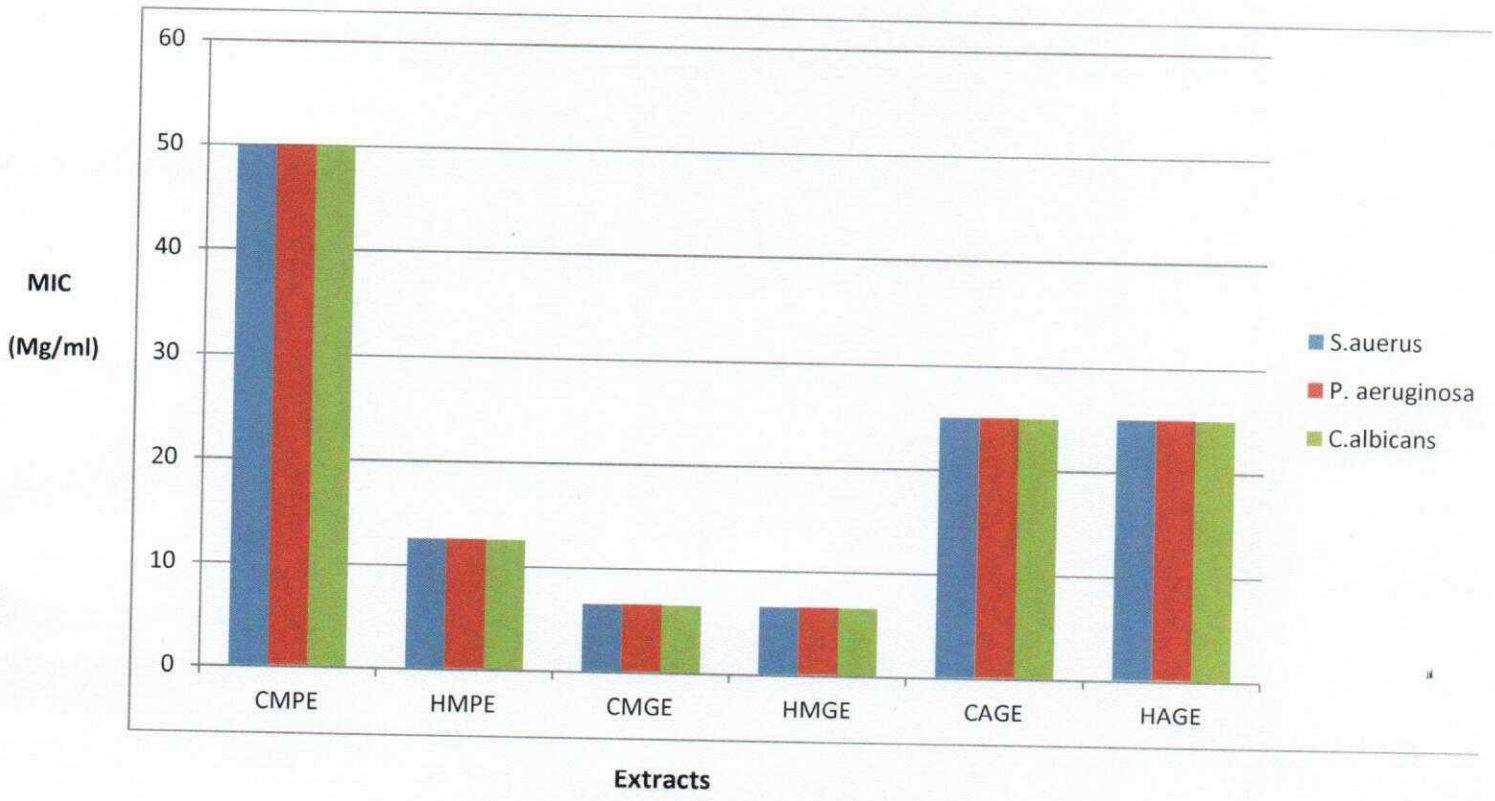


FIGURE 3: MINIMUM BACTERICIDAL AND FUNGICIDAL CONCENTRATION OF *Euphorbia heterophylla* AND *Zingiber officinale* AGAINST TEST ORGANISMS

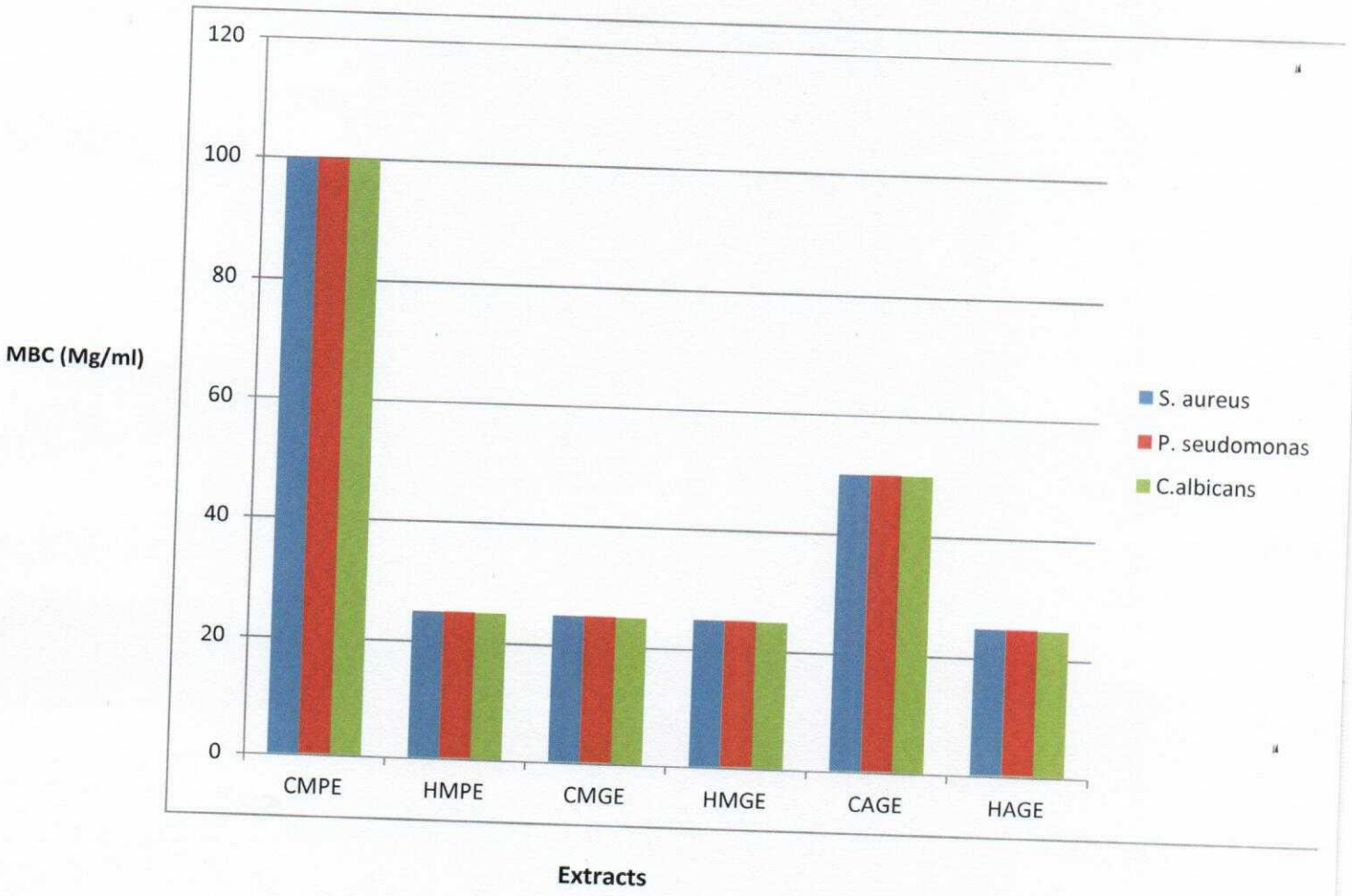


TABLE 2: ANTIBIOTICS SENSITIVITY TEST AGAINST TEST ORGANISMS

1. GRAM POSITIVE ORGANISM

TEST ORGANISM	ZONES OF INHIBITION (mm)									
	PEF	GN	APX	Z	AM	R	CPX	S	SXT	E
Staphylococcus aureus	15	NI	NI	NI	NI	12	16	NI	NI	10

2. GRAM NEGATIVE ORGANISM

TEST ORGANISM	ZONES OF INHIBITION (mm)									
	SXT	CH	SP	CFX	AM	AU	CN	PEF	CFX	S
Pseudomonas aeruginosa	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI

3. **FUNGI**

TEST ORGANISM	**ZONES OF INHIBITION (mm)**	
	KETOCONAZOLE	**NYSTATIN**
Candida albicans	NI	30

KEY:

NI= No inhibition

PEF= Pefloxacin (10µg), GN= Gentmycin (10µg), APX= Ampiclox (30µg)

Z= Zinnacet (20µg), AM= Amoxacillin (30µg), R= Rocephin (30µg)

CPX= Ciprofloxacin (10µg), S= Streptomycin (30µg), SXT= Septrin (30µg)

E= Erythromycin (19µg), CH= Chloramphenicol (30µg), SP= Sparfloxacin (10µg)

AU= Augmentin (10µg), OFX= Tanvid (10µg)

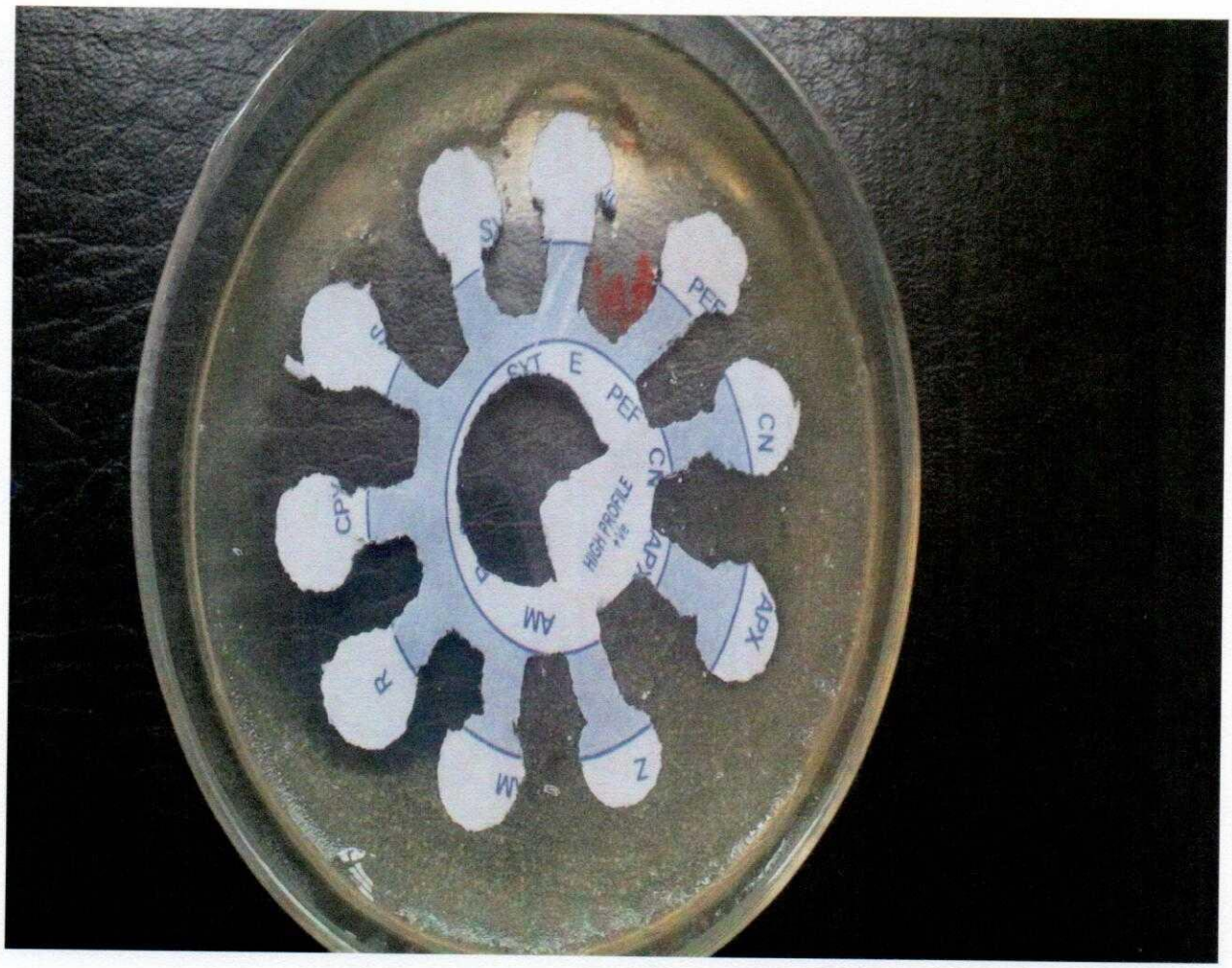


Plate 10: Positive antibiotic disc on *Staphylococcus aureus* showing some zones of inhibition

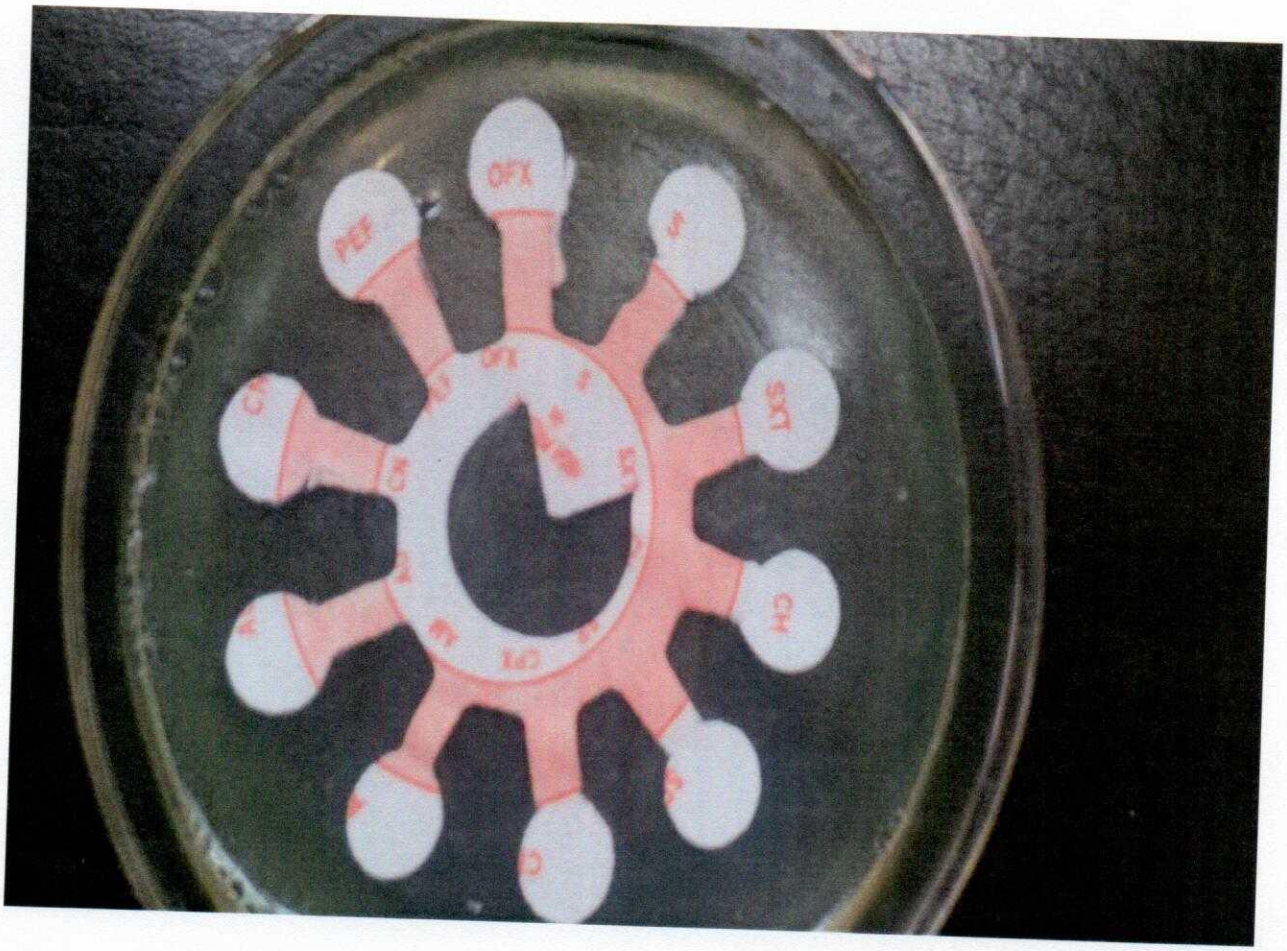


Plate 11: Negative antibiotic disc on *Pseudomonas aeruginosa* showing no zone of inhibition

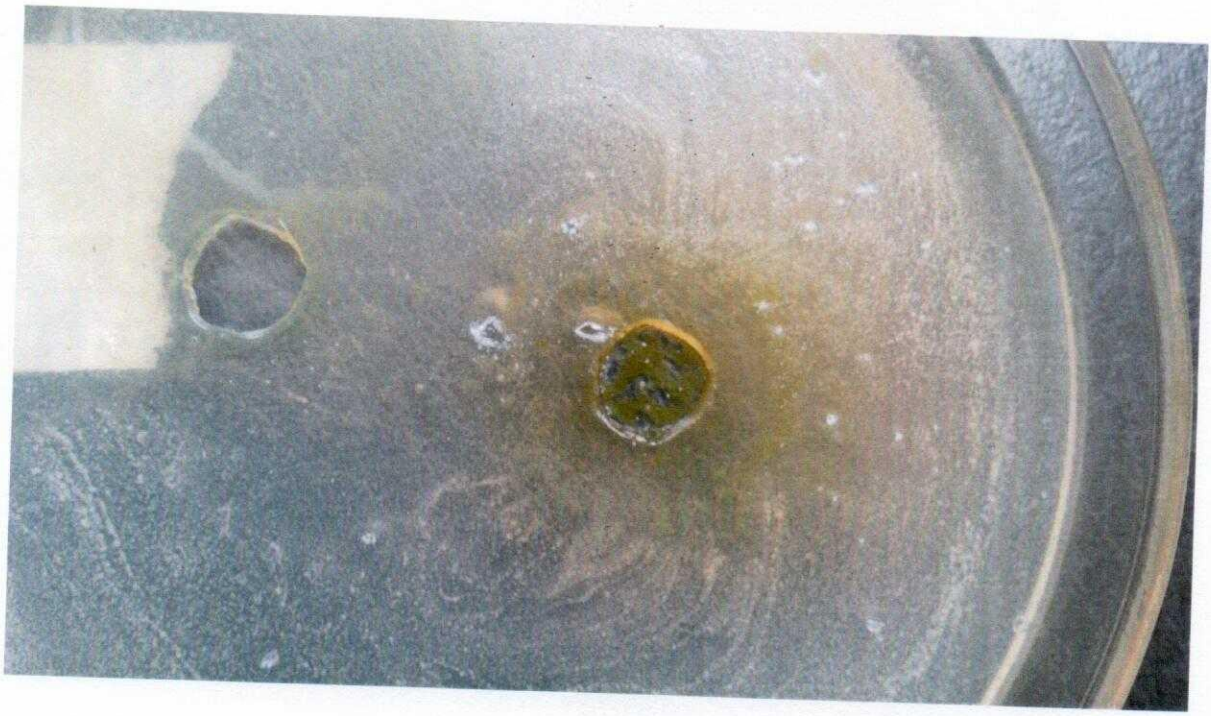


Plate 12: Ketoconazole and Nystatin on *Candida albicans* showing zone of inhibition

4.5. DISCUSSION

Plants and herbs have been found to have medicinal and therapeutic importance in the prevention, palliation and treatment or cure of diseases and ailments. This knowledge has been passed down from one generation to another either verbally or in writing (Sofowora, 2008).The universal role of plants in the treatment of diseases is exemplified by their employment in all major systems of medicine (Okeniyi *et al*., 2007).

The phytochemical screening *Euphorbia heterophylla* and *Zingiber officinale* indicated the presence of alkaloids, flavonoids, saponins, phenols, anthraquinones, steroids and glycosides (Table 1). The presence of these compounds may be responsible for the antimicrobial activities of the extracts of these plants on the organisms. The phytochemical components of *Euphorbia heterophylla* and *Zingiber officinale* have been established in previous studies. Several studies have linked the presence of these bioactive compounds in plant materials to antimicrobial activity.

The presence of these secondary metabolites in plants, produce some biological activity in man and animals and it is responsible for their use as herbs. These compounds also serve to protect the plant against infection by microorganisms and predation by insects and herbivores. While some give plants their odours and/or flavours and some are still responsible for their pigments (El-Mahmood *et al*., 2008). In some cases, the activity has been associated with specific compounds or classes of compounds. These active constituents can be used to search for bioactive lead compounds that could be used in the partial synthesis of more useful drugs (El-Mahmood *et al*., 2010).

The result of the antimicrobial activity of the extracts showed that the cold methanolic extract of *Euphorbia heterophylla* produced diameters of zones of inhibition of 20mm for both *Staphylococcus aureus* and *Candida albicans* respectively and 21mm for *Pseudomonas aeruginosa.* The hot methanolic extract *of Euphorbia heterophylla* also produced diameters of zones of inhibition of 25, 23 and 22mm respectively for *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans* respectively.

This shows that the cold and the hot methanolic extracts from the *E. heterophylla* were effective against all the test organisms. This is in line with Ekundayo and Ezeogu (2013).

Both the CMZE and the HMZE were effective against the bacteria but not against the yeast. It could mean that these extract had solely antibacterial but not antifungal effect/potency.

The aqueous extracts from *Z. officinale* were effective against all the test organisms though to different degrees and with no obvious pattern. This could mean that the potency of ginger when used with water (either hot or cold) has strong antimicrobial activities against these micro-organisms. Comparing the activities of the *Zingiber* aqueous extract with that of the methanolic extract, one could also say that perhaps in the process of methanolic extraction or by the methanol itself, the antifungal potency of the extract was lost. This was unexpected because the synergy was expected to be more effective than the extracts alone. The combination of the cold methanolic *Euphorbia* and *Zingiber* extracts was effective against *Staphylococcus aureus* and *Candida albicans* with 20mm and 12mm zones of inhibition while it had no inhibitory effect against *Pseudomonas aeruginosa*. The combination of the hot methanolic *Euphorbia* and *Zingiber* extracts was effective for all organisms producing diameters of zones of inhibition of 25, 20 and 18mm for *Staphylococcus aureus*,

Pseudomonas aeruginosa and *Candida albicans* respectively. These results indicate that both the *Euphorbia* and *Zingiber* extracts are still effective when combined but its result was not better than the hot methanolic *Euphorbia* alone or the hot methanolic *Zingiber* which did not inhibit *Candida*.

The result for the minimum inhibitory concentration showed that the cold methanolic *Euphorbia* extract had the highest inhibitory concentration at 50mg/ml for *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans* while the lowest inhibitory concentration was at 6.5mg/ml for the three test organisms each for the cold and hot methanolic *Zingiber* extracts and the hot methanolic *Euphorbia* extracts. The MIC results indicate that both the cold and hot methanolic *Zingiber* extracts were the most potent as they were able to inhibit the growth of all these test organisms more than all the other extracts.

Also, the results for the minimum bactericidal concentrations showed that the cold methanolic *Euphorbia* extract killed these organisms at the highest concentration of 100mg/ml, followed by the cold aqueous *Zingiber* extracts killing the test organisms at a concentration of 50mg/l. The least concentrations were from the hot methanolic *Euphorbia* extract, cold methanolic *Zingiber* extract, hot methanolic *Zingiber* extract and aqueous *Zingiber* extract, killing the test organisms at 25mg/ml.

This indicated that the extracts of both plants has similar potency on *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*, this is similar to the findings of the National Library of Medicine at the National Institutes of Health.

The result of the antibiotics sensitivity test showed that Pefloxacin, Rocephin, Ciprofloxacin and Erythromycin had inhibitory effects on *Staphylococcus aureus* but the micro-organism

was insensitive to Gentamycin, Ampiclox, Zinnacet, Amoxacillin, Streptomycin and Septrin. This could be due the varying selective toxicity of the antibiotics and the nature of the organism involved. *Pseudomonas aeruginosa* was insensitive to all the antibiotics. *Candida albicans* was sensitive to Nystatin but not to Ketoconazole.

The results obtained in this study contribute to the scientific validation for the use of these medicinal plants in traditional medicine and serve as a guide for selection of plants with antimicrobial activity for further phytochemical work on isolation and identification of the active compounds. Furthermore, these results show the potential of some of these medicinal plants for development of standardized culturally acceptable herbal medicines for local use as broad spectrum antimicrobial agents.

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CHAPTER FIVE

5.0. CONCLUSION AND RECOMMENDATION

5.1. CONCLUSION

The combination of these extracts did not work in synergy to the level expected compared to the extracts when used alone. This study revealed that the cold aqueous extract from *Zingiber* was more potent against *Pseudomonas* than any other extract, while the hot aqueous extract showed less potency against *Pseudomonas* and the cold methanolic combination was totally ineffective against it. The hot methanolic *Zingiber* extract was more effective for *Staphylococcus* while the cold aqueous *Zingiber* extract was least effective for it. For *Candida*, both cold and hot methanolic *Zingiber* extracts totally had no potency against it, while the combined cold methanolic extracts showed less potency against it. The hot aqueous *Zingiber* extract showed the highest potency against it.

All these extracts are from natural sources and thus have been seen to have little or no adverse effect on man when used unlike conventional antibiotics. The use of medicinal plants as sources of antibiotics and in drug production would be of great benefit to our health and mankind in general.

5.2. RECOMMENDATION

The results of this invitro study suggests that the extracts of both *Euphorbia heterophylla* and *Zingiber officinale* possess compounds with antimicrobial properties but further research can be carried out on their extracts to isolate and identify the active substances present in them. Toxicity can also be carried out to reveal the side effects, pharmacokinetic properties and diffusion in different body parts. Further studies also can be carried out to determine the precise dosage for administration in form of tablets and capsules.

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APPENDIX

APPENDIX 1: ANTIMICROBIAL ACTIVITIES OF *Euphorbia heterophylla* AND *Zingiber officinale* EXTRACTS AGAINST THE TEST ORGANISMS

TEST ORGANISMS	ZONES OF INHIBITION (mm)							
	CMEE	HMEE	CMZE	HMZE	CAZE	HAZE	CMEE + CMZE	HMEE + HMZE
Staphylococcus aureus	20	25	26	27	19	24	20	25
Pseudomonas aeruginosa	21	23	25	28	29	18	NI	20
Candida albicans	20	22	NI	NI	25	26	12	18

APPENDIX 2: MINIMUM INHIBITORY CONCENTRATION OF *Euphorbia heterophylla* AND *Zingiber officinale* EXTRACTS AGAINST THE TEST ORGANISMS

TEST ORGANISMS	(Mg/ml)					
	CMEE	HMEE	CMZE	HMZE	CAZE	HAZE
Staphylococcus aureus	50	12.5	6.5	6.5	25	25
Pseudomonas aeruginosa	50	12.5	6.5	6.5	25	25
Candida albicans	50	12.5	6.5	6.5	25	25

APPENDIX 3: MINIMUM BACTERICIDAL AND FUNGICIDAL CONCENTRATIONS OF *Euphorbia heterophylla* AND *Zingiber officinale* EXTRACTS AGAINST THE TEST ORGANISMS

TEST ORGANISMS	(Mg/ml)					
	CMEE	HMEE	CMZE	HMZE	CAZE	HAZE
Staphylococcus aureus	100	25	25	25	50	25
Pseudomonas aeruginosa	100	25	25	25	50	25
Candida albicans	100	25	25	25	50	25