ANTIBACTERIAL EFFECT OF *Zingiber officinale* ON *Staphylococcus aureus*

B.Sc. RESEARCH PROJECT

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CERTIFICATION

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DEDICATION

I dedicate this work to God Almighty for his immeasurable love and grace. May His name be praised forever Amen.

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ABSTRACT

The growing population concern about health has recently led to the development of natural antimicrobials to control diseases. Medicinal plants and spices are one of the most commonly used natural antimicrobial agents in food and have been used traditionally for thousands of years by many cultures for controlling common health. *Zingiber officinale* has long been used as naturopathy due to their potential antimicrobial activity against different microbial pathogens. This study was conducted to determine the antimicrobial activity of *Zingiber officinale* rhizome on *Staphylococcus aureus*. The present study showed the potent antimicrobial activity of the rhizome extract against the tested bacterial pathogen, *Staphylococcus aureus* (P1023) which was collected from the Department of Microbiology, University College Hospital, Ibadan, Oyo State. Using the disc method, 0.03mg/disc – 2.80mg/disc tested showed that only 1.40mg/disc and 2.80mg/disc inhibited the growth of the test organism, which gave a corresponding zone of inhibition of 14mm and 17mm, respectively. The minimum inhibitory concentration (MIC) result showed that the least concentration of the ginger extracts tested where no growth was recorded was with 125mg/ml while 175mg/ml concentration proved to be the minimum bactericidal concentration (MBC).

CHAPTER ONE

INTRODUCTION

Staphylococcus aureus has been recognized for a long time as one of the leading cause of hospital infections all over the world. It is the most common cause of nosocomial wound infections. Most of it strains are opportunistic pathogens that colonize individuals without symptoms, for either short or extended period of time, causing disease when the immune system becomes compromised. Before the antibiotic era, diseases caused by *Staphylococcus aureus* had high mortality rates. The introduction of benzyl penicillin into chemotherapy in the early 1940s found *Staphylococcus aureus* fully susceptible but in 1950s, the number of the isolates with resistance to penicillin increased rapidly. The mechanism of penicillin resistance involved the acquisition of a plasmid-borne penicillinase capable of degrading the antibiotic before it reached it cellular targets (Maida *et al.*, 2010; Amita *et al.*, 2003).

Staphylococcus aureus is a facultative anaerobic, gram positive bacterium that appears in form of cocci clusters. It is a member of the *Firmicutes*, belonging to the *Staphylococcaceae* family. It is known to be non-motile, non-spore producer, positive to catalase and coagulase test and nitrate reduction (Masalha *et al.*, 2001). This bacterium is a part of the normal microbiota frequently found in the upper respiratory tract, on the skin and in the gut mucosa. It is also a major human pathogen that causes a wide range of clinical infections.

There has been a great swing from the use of antibiotics to the use of remedial plants and a relatively small percentage of about 500,000 kinds of plant are used as flavors, drugs and food (Ekwenye *et al.*, 2005).

Numerous species of plants have been used for centuries as remedies for human diseases such as ginger which has been used for a range of illnesses including diarrhea, stomach aches, nausea asthma, and respiratory disorder (Kaushik *et al.*, 2011) because ginger contain components of therapeutic values. Newly, the approval of traditional medicine as a reserve form of health care and the progress of microbial resistance to the existing antibiotics has directed researchers to explore antimicrobial activity of medicinal plants. Ginger belongs to *Zingiberaceae* family (Sharma *et al.*, 2010) and 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 (Ali *et al.*, 2008). It is antiplatelet, antibacterial, antifungal, antiviral, anti-inflammatory, has anti-oxidative activity and are known to be effective as an immuno-modulatory agent in human and animals. Ginger is relatively inexpensive due to their easy availability, universally acceptable and well tolerated by most people. The development of new antibiotics and plant based antimicrobial compounds are effective against the resistant organisms. Ginger a common substance found increasingly in the diets of the global population is known to have antibacterial effects and has been valued for thousands of years in Asian cultures (Weil, 2005).

JUSTIFICATION

Infectious diseases are directly responsible for 25% of death throughout the world. Despite the advances made in the field of infectious diseases, the diseases are debilitating factor and ultimately the cause of death for millions of people worldwide. (Mozhgan *et al.*, 2016).

Microbial pathogenicity and other infectious diseases have been controlled by the use of commercially available antimicrobial drugs (Humayun *et al.*, 2015) but the misuse and incomplete dosage of antibiotics used in treatment has induced microorganisms to acquire resistance factors which have become a burning predicament. However, antibiotic resistant bacteria are known to be a major health concern worldwide, in particular, *Staphylococcus aureus* which is recognized as the etiological agent of suppurative abscesses that asymptomatically colonizes 25% of humans as a member of the nostril and skin microbiota, when it resides with other bacteria. The use of antibiotics such as Penicillin and Methicillin in the mid-20th century initially proved effective against *Staphylococcus aureus*. These bacterial strain rapidly acquired resistance to these antibiotics that made the infections with Penicillin and Methicillin-resistant *Staphylococcus aureus* difficult to treat.

As a result, there is an urgent need to find the alternative of chemotherapeutic drugs in diseases treatment particularly those of plants origin which are easily available and have considerably less side effects. The use of higher plants and their extracts for treating the infectious diseases has long been practiced in many parts of the world. The plant derived medicines may be used in many different forms including: powder, liquid or mixtures which could be raw or boiled such as, liniments, ointments and incisions (Kamrul *et al.*, 2014). Ginger (*Zingiber officinale*), which is one of the medicinal plants will be used in this study because it is known to have medicinal properties that helps to reduce progressively increasing drug resistance of pathogens and low

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) could portend good chemotherapeutic substance which could be used for treatment of microbial infections caused by *Staphylococcus aureus.*

AIM & OBJECTIVES

Aim:

- To establish the MIC & MBC of *Zingiber officinale* rhizome extract on *Staphylococcus aureus*

Objective to:

- Prepare different concentrations of *Zingiber officinale* rhizome extract.
- Test the effect of the different concentration of rhizome extract on *S. aureus.*
- Determine the Minimum Inhibitory Concentration on the test organism.
- Determine the Minimum Bactericidal Concentration

CHAPTER TWO

LITERATURE REVIEW

2.1. What is Herbal Medicine?

Herbal medicine, like many natural or traditional therapies, has been around for many hundreds of years. Virtually every society or culture has adopted it in one form or another. Even today, in spite of the massive inroads achieved by conventional medicine and synthetic drug usage, around 80% of the world's population uses it to a greater or lesser extent (Wachtel *et al.*, 2011)

Herbal medicines are finished, labeled medicines containing pharmacologically active parts of a plant or plants, either in crude form or physically modified during processing. They are usually administered orally or topically to deal with chronic or acute clinical disorders and are available as fresh or dried plants, or as tablets, capsules, powders, teas, or extracts. They are based on certain parts of the medicinal plant–flower, leaf, stem, bark, or root. However, they are not single substances extracted by chemical techniques from the plant and subsequently purified or even structurally altered (Roy, 2010). Herbal medicine reflects the urge towards a more natural lifestyle, and a new found respect for natural or holistic healing. It deals with the underlying disharmony which manifests during the onset of a chronic or recurring disease (Vander, 2002).

Active metabolites of medicinal plants have low toxicity and are useful for treatment of infectious diseases (Cowan, 1999). Due to wide spreading of infectious diseases, Side effects of antibiotics and bacterial resistance to antibiotics, identification of medicinal plants and purification of their active compounds are useful in the treatment of diseases. Medicinal plants traditionally are one of the most valuable resources in medicine and can be used as synthetic drugs with plant origin. Medicinal plants are not only involved in the treatment of infectious

diseases but at the same time reduce a large number of antibiotic side effects (Seyyed *et al.*, 2010).

2.2. Advantages of Herbal Medicine over Alternative Drugs

Unlike with alternative medicine, the administration of herbal products, alone or as part of a broader complementary or traditional medical strategy is a good example of holism in action. Herbs are increasingly being used these days as therapeutic agents, or combined with minerals and vitamins in health enhancing supplements, or in invigorating tonics and teas. Alternative medicine is a practice of consuming a medicine without the use of drugs. The scope of herbal medicine is sometimes extended to include fungal and bee products, as well as minerals and shells. Each type of medicine has many strengths and weaknesses.

However, herbal products are not regulated for purity and potency. Thus, some of the adverse effects reported could be caused by impurities. The potency of herbal products may increase the possibility of adverse effects. Potential benefits and possible risks associated with consumption of herbal product should be considered so that conventional treatments can be made more safe and effective (Kaur *et al.*, 2013).

There are several reasons why people choose to use herbal medicinal products including:

• There is a perception that "natural equals safe". This is not necessarily true as many commonly used medicines have been derived from natural sources and possess potent adverse effects in addition to therapeutic benefits.

• There is a perception that "more" adverse reactions occur with conventional therapies.

• Self-medication with herbal medicinal products may provide a sense of control or psychological comfort for the patient. This is particularly evident in those patients for whom conventional medicines cannot provide any further benefits e.g. chronic conditions such as eczema, arthritis.

• Patients may have cultural, religious or ecological reasons for using herbal medicinal products.

There are a number of advantages associated with using herbal medicines as opposed to pharmaceutical products:

Reduce risk of side effects: most herbal medicines are well tolerated by patients with fewer unintended consequences than pharmaceutical drugs. Herbs typically have fewer side effects than traditional medicines, and may be safer to use over time.

Lower cost: herbs cost much less than prescription medications. They tend to be inexpensive compared to drugs.

Widespread availability: herbs are available without prescription. Many of them can be grown at homes or gardens and in some remote parts of the world they may be the only treatment available to the majority of people (Kathleen, 2006).

Although, these herbs have advantages, they also have their side effects especially when the appropriate dose is not taken and there is medication interaction.

2.3. History of *Zingiber officinale*

Zingiber officinale commonly known as Ginger belonging to the Family *Zingiberaceae*, (Chen, 2008) is a horizontal, branched, fleshy, aromatic white to yellow coloured perennial herb with leafy stem up to 60 cm. Leaves are 20cm long and 2 - 3 cm broad with sheathing bases, the blade

gradually tapering to a point. Flowers are rare, rather small, calyx superior, gamosepalous, three-toothed open splitting on one side, corolla of three subequal oblong to lanceolae, yellow green with purple ending flowers. The herb develops several lateral shoots in clumps, which begin to dry when the plant matures (Ross, 2005). The ginger plant has a long history of cultivation known to originate in China and then spread to India, South East Asia, West Africa and the Caribbean (McGee, 2004; Kuschener, 2003).

Ginger (Zingiber officinale) is used in Iranian traditional medicine for treatment of Colds, Fever, and Menstrual pain, Headaches, joints Pain, Nausea, bloat, Indigestion and vomiting (Shams *et al.*, 2012). Ginger is relatively inexpensive due to their easy availability, universally acceptable and well tolerated by the most people (Kamrul *et al.*, 2014). South East Asia is considered as home grown land for Ginger production (Ravindran *et al.*, 2004). By tradition, ginger farming is common in number of countries like Japan, China, Indonesia, Nigeria, India, Brazil, Sri Lanka and Jamaica Islands (Humayun *et al.*, 2015). Indian and Chinese used ginger to treat many diseases. For thousands of years it has been used to treat different diseases such as colds, nausea, arthritis, migraine, cancer, asthma, dementia, ulcerative colitis, diabetes and high blood pressure. It also has antioxidant, anti-inflammatory, analgesic properties in animals (Rasmussen, 2011; Oboh *et al.*, 2012).

2.4. Phytochemical and Nutritional Properties of *Zingiber officinale*

Fresh ginger contains 80.9% moisture, 2.3% protein, 0.9% fat, 1.2% minerals, 2.4% fibre and 12.3% carbohydrates. The minerals present in ginger are iron, calcium and phosphorous. It also contains vitamins such as thiamine, riboflavin, niacin and vitamin C (Shubha, 2015). The

composition varies with the type, variety, agronomic conditions, curing methods, drying and storage conditions (Govindarajan, 1982; Gugnani *et al.*, 1985).

Ginger is antiplatelet, antibacterial, antifungal, antiviral, antiworm, anti-inflammatory and has anti-oxidative activity. It effects in gastrointestinal and cardiovascular systems, antilipidemic and antihyperglycemic, anti-tumour properties and are known to be effective as an immuno modulatory agent in human and animals, including fish (Nya *et al.*, 2009; Apines-Amar *et al.*, 2012 and Talpur *et al.*, 2013).

The main pungent compounds in fresh ginger are gingerols whereas the pungency of dry ginger is shogaols, for example [6]-shogaol, which are dehydrated forms of [6]-gingerols (Wang *et al.*, 2011; Hoffman, 2007). The volatile oil components consists mainly of sesquiterpene hydrocarbons, predominantly zingeberene (35%), curcumene (18%) and farnesene (10%) (Govindarajan, 1982). Non-volatile pungent compounds include gingerols, shogaols, paradols and zingerone. Paradol is similar to gingerol and is formed from hydrogenation of shogoal (phenylalkanones). Ginger contains fats, waxes, carbohydrates, vitamins and minerals. Ginger rhizomes also contain a potent proteolytic enzyme called zingibain. A mixture of many terpenes and some non-terpenoid compounds make up the essential oil. However, its chief elements, sesquiterpene hydrocarbons, remain constant. The gingerols provide its distinctive taste and characteristic pharmacological effects. (Muhammed, *et al.*, 2007).

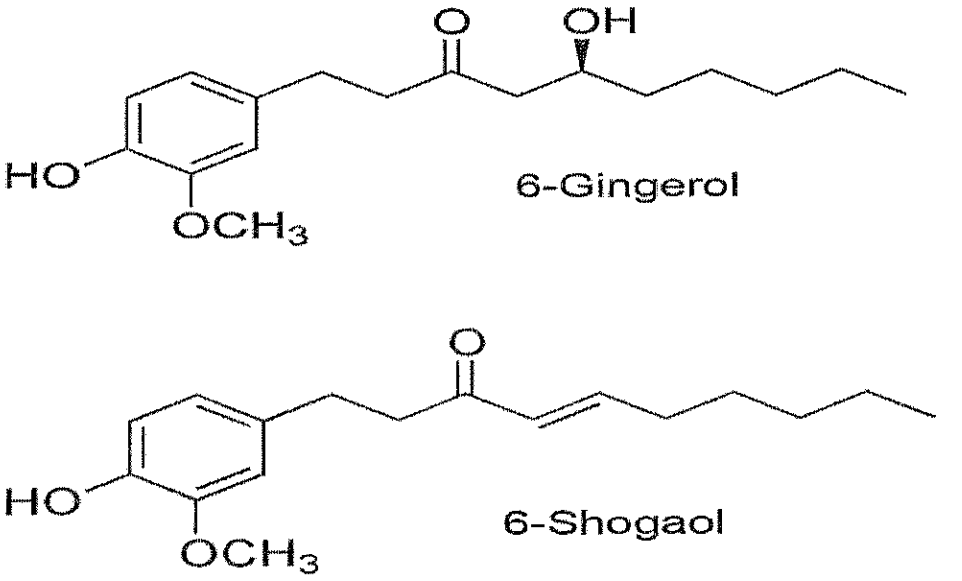

Figure 1: Chemical structures of major constituents of ginger (Schwertner *et al.*, 2006).

Table 1: Biological activities of ginger reported in some literatures

Active principles	Biological action	Mode of action	References
Gingerols, shogaols, Sesquiterpenes and Monoterpenes	For the treatment of nausea	By anticholinergic and antiserotonin action	(Bryer, 2005)
Ethanolic extract of Ginger	Hypolipidemic agent	By reducing triglycerides and cholesterol	(Bolanle, 2011)
[6]-gingerol	Anti-tumor property	Stimulation of apoptosis	(Surh *et al.*, 1998)
	Anti-ulcerative effects	Suppression of gastric contraction, increasing mucin secretion.	(Minaiyan *et al.*, 2006)
6-Shogaol	Anti-inflammory effect	Inhibition of pro-inflammatory cytokines and chemokines	(Penna *et al.*,2003)
	Anticancer activities	Inhibition of cell invasion reduction of matrix metalloproteinase-9 expression	(Ling *et al.*,2010)
Sesquiterpenes	Anti-viral effect		(Chang *et al.*,2013)

2.5. Uses of *Zingiber officinale*

Ginger is a minor chemical irritant, and has a sialagogue action, stimulating the production of saliva (O'Hara *et al.*, 1998). Mature ginger roots are fibrous and nearly dry. They can be cooked as an ingredient in many dishes; stewed in boiling water to make ginger tea to which honey is often added as a sweetener and sliced orange or lemon fruit may also be added. The juice of ginger roots is extremely potent and is often used as spice to flavour dishes such as seafood, mutton, snacks or stew. Powdered dry ginger roots (ginger powder) are typically used to add spiciness to ginger bread and other recipes. Ginger is also made into candy and used as flavoring for cookies, crackers and cakes as well as flavour in carbonated, non-alcoholic beverage (McGee, 2004).

Ginger 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, 2008). Ginger has direct anti-microbial activity and thus can be used in treatment of bacterial infections (Tan *et al.*, 2004). Supplementing ginger in fish diets may enhance disease resistance by reinforcing host innate immune functions that are necessary for protection against infectious diseases. Ginger may play diverse biological roles in anti-oxidative, anti-inflammatory, hypolipidemic, anti-carcinogenic, anti-nausea, antithrombotic, cardiovascular, and antibacterial processes (Kikuzaki *et al.*, 1993; Grzannar *et al.*,2005; Kadnur *et al.*, 2005; Stoilova *et al.*, 2007; Nicoll *et al.*, 2009).

Medicinal uses

Ginger is considered a safe herbal medicine with only few adverse side effects. More studies are required in animals and humans on the kinetics of ginger and its constituents and on the effects of their consumption over a long period of time (Amin *et al.*,2006).

Cardiovascular health

Ginger has been described as great heart tonic. It helps in preventing various heart diseases by reducing blood clotting that can lead to plaque formation or thrombosis. It can also open the blockage in the blood vessels thus decreasing peripheral vascular resistance and hence blood pressure. Ginger also may help to lower high cholesterol making the heart healthy (Akoachere *et al.,* 2002).

Antiplatelet activity

The aqueous extract of ginger inhibited platelet aggregation induced by ADP, epinephrine, collagen and arachidonic acid in vitro (Srivastava *et al.*, 1984)

Ginger acted by inhibiting thromboxane synthesis. It also inhibited prostacyclin synthesis in rat aorta. The antiplatelet action of 6-gingerol was also mainly due to the inhibition of thromboxane formation (Guh *et al.*, 1995).

Powerful antioxidant

Antioxidant helps to prevent all kind of disease and it also slower downs the aging process. There was a study of more than 120 plant foods, published in the Journal of Nutrition. In the report ginger was ranked number one among the five richest food sources of antioxidants, including berries, walnuts, sunflower seeds, and pomegranates. Test-tube and animal researches have shown that ginger inhibits the production of free radicals. Ginger also enhances the body's internal production of antioxidants (Srivastava *et al.*, 1992).

Help for cancer patients

There are a number of mechanisms that contribute to the chemopreventive effects of ginger based on in vitro and in vivo studies (Baliga *et al.*, 2011). A review on the current evidence for the cancer protective properties of ginger was conducted in 2007 by Shukla *et al*, who concluded that in a number of experimental models active components found in ginger did exhibited anti-cancer properties. Further investigations have solidified this conclusion by identifying mechanisms by which gingerols and shogaols can offer these protective benefits. 6-Gingerol has demonstrated an ability to inhibit the action of enzymes MMP-9 and MMP-2 (Lee *et al*, 2008), both in animal trials and in in laboratory studies with human cells. In cancer cells, these enzymes are associated with the cancer migration and invasion (Mendes *et al*, 2007), which takes part in late stage cancer development. Purified ginger constituents such as [6]-, [8]-, and [10]-shogaol showed much stronger growth inhibitory effects than gingerols on human lung cancer cells and human colon cancer cells (Sang *et al.*, 2009).

Inflammation

Inflammation is a natural response to a wound or area of infection. It increases the amount of immune cells (which produce toxins to kill pathogens) in the target area, and increases the rate of healing. However, diets high in pro-inflammatory agents (which can range from environmental toxins to omega-6 fatty acids), or lifestyles factors which can increase stress on joints can result in a persistent inflammation, also known as chronic inflammation, which is painful and damaging. This can lead to an increased risk of a number of diseases including cardiovascular disease, sore joints and cancer. Inflammation is primarily mediated by two groups of chemicals in the body- prostaglandins and leukotrienes, and elevated levels of both these compounds causes

inflammation. Prostaglandins are created by two enzymes- cyclooxygenase- 1 (COX-1) and cyclooxygenase-2 (COX-2), and leukotrienes are produced by an enzyme called 5-lipoxygenase. Gingerols have repeatedly been shown to inhibit these enzymes (Grzanna *et al.*, 2005; Lantz *et al.*, 2007) preventing the production of prostaglandins and leukotrienes, which will reduce inflammation. Shogaols were also shown to inhibit 5-lipoxygenase and COX-1, but appeared to have no effect on COX-2. Interestingly 10-gingerol has been shown to have more potent anti-inflammatory properties than other gingerols (Lantz *et al.*, 2007)

Toxicity

The toxicity of ginger is generally considered to be negligible. Oral LD 50 values in various animals of ginger oil exceed 5 gm/Kg. In vitro microbial assays have shown both mutagenicity and antimutagenicity for compounds isolated from ginger. The adverse reaction profile of ginger is benign, consonant with its use as a common spice and food (Chen et al., 2008).

It has been speculated that since there are a variety of chemical classes that these compounds can belong to, it is likely that ginger can eliminate symptoms associated with a variety of illnesses, such as arthritis, by interfering with the production and release of metabolic products from lipid membranes, peptides, proteins and amino acids.

Allergic reactions to ginger include heartburn, bloating, gas, belching and nausea (particularly if taken in powdered form). Unchewed fresh ginger may result in intestinal blockage, and individuals who have had ulcers, inflammatory bowel diseases or blocked intestines may react badly to large quantities of fresh ginger (Opdyke, 1974; O'Hara *et al*, 1998).

2.6. Description of *Staphylococcus aureus*

Staphylococcus aureus belongs to the family *Micrococcaceae* and is part of the genus Staphylococcus, which contains more than 30 species such as *S. epidermidis, S. saprophyticus* and *S. haemolyticus*. Among the staphylococcal species, *S. aureus* is the most virulent and pathogenic for humans. *S. aureus* is a 1 μm, Gram-positive cell that in the laboratory may be observed as single cells, in pairs or as grape-like irregular clusters. It is characterized as coagulase- and catalase positive, non-motile, non-spore-forming and as facultative anaerobic. It grows in yellow colonies on nutrient rich media and is referred to as the yellow staphylococci (Winn, 2006).

S. aureus was discovered in 1880 by the surgeon Sir Alexander Ogston. He observed grape-like clusters of bacteria when examining a purulent discharge from patients with post-operative wounds during microscopy. He named them *Staphylé*, the Greek expression for a bunch of grapes. In 1884, Rosenbach succeeded in isolating yellow bacterial colonies from abscesses and named them *Staphylococcus aureus*, "*aureus*" from the Latin word for golden.

S. aureus has the ability to adapt to different environments and it may colonize the human skin, nails, nares and mucus membranes and may thereby disseminate among recipient host populations via physical contact and aerosols (Lowy, 1998). Colonization with *S. aureus* is an important risk factor for subsequent *S. aureus* infection (Wertheim *et al.,* 2004; Eiff *et al.*, 2001). *S. aureus* causes a wide range of infections from a variety of skin, wound and deep tissue infections to more life-threatening conditions such as pneumonia, endocarditis, septic arthritis and septicemia. This bacterium is also one of the most common species in nosocomial infections. However, little is known about the virulence factors behind all these conditions. In addition, S.

aureus may also cause food poisoning, scalded-skin syndrome and toxic shock syndrome, through production of different toxins (Winn, 2006).

2.7. Medical Significance of *Staphylococcus aureus*

Staphylococcus aureus is transferred through skin to skin contact and more commonly cause infection in persons previously diseased or with weaker immune systems. The most common transfer happens from a health worker who has been in contact with an active strain of it. Other transfers occur from environmental sources or from other carriers of the bacteria. Possible outcomes from Staph infections include many hospital originated issues including pneumonia and wound infections. Simultaneously, it is a leading cause of bacteremia and infective endocarditis as well as osteoarticular, skin and soft tissue infection so also pleuropulmonary and devise-related infections. (Steven *et al.*,2015). The following describes some infections caused by *S. aureus*.

Scalded skin syndrome (Ritter disease)

An exfoliative toxin causes this relatively rare syndrome, which takes the form of superficial fragile blisters that burst, leaving a tender base. The patient is often febrile and, occasionally, has mucopurulent eye discharge. This Misdiagnosis of this syndrome delays treatment and allows exfoliation to progress, and corticosteroid therapy may potentiate bacterial superinfection. Although the mortality rate is low in children with this entity, most fatalities are associated with delay in diagnosis.

Folliculitis, furuncle, and carbuncle

These are increasingly severe staphylococcal skin infections. Folliculitis is a tender pustule that involves the hair follicle. A furuncle involves both the skin and the subcutaneous tissues in areas with hair follicles, such as the neck, axillae, and buttocks. They are actually small abscesses characterized by exuding purulent material from a single opening. A carbuncle is an aggregate of connected furuncles and has several pustular openings. Skin infections may be self-limited, but they can also disseminate hematogenously and cause life-threatening septicemia. [Jenkins et al., 2010).

Bone infections (osteomyelitis)

Children often present with sudden onset of fever and bony tenderness or a limp. The pain may be throbbing and severe; however, presentation in neonates can be subtle. Infants may appear well except for failure to move an extremity or pain on movement. Redness or swelling indicates that infection has spread into the subperiosteal space. Rupture of a focus of osteomyelitis into joint space can result in septic arthritis. This is often observed in neonates. Children with vertebral osteomyelitis present with back pain, and those younger than 3 years present with refusal to walk or with a limp. Occasionally, children with vertebral osteomyelitis present with incontinence. Children with discitis tend to present with less fever and often appear less ill than children with vertebral osteomyelitis (Spellberg, 2010).

Septic arthritis

Typical findings include decreased range of motion, warmth, erythema, and tenderness of the joint with constitutional symptoms and fever. Infants (in whom the hip is the most commonly involved joint) are an exception, as these signs may be absent. The child typically lies with the

involved joint abducted and externally rotated. Because pain fibers are located within the joint capsule, movements that compress the head of the femur into the acetabulum (eg, changing a diaper) cause pain. A portal of infection is almost never found, and the infection is nearly always unilateral. Patients with infection of the sacroiliac joint present with tenderness elicited during digital rectal examination and with pain during flexion, abduction, and external rotation of the hip (Jamal *et al.*,2011)

Endocarditis

The initial presentation of patients with *S, aureus* endocarditis is fever and malaise. However, the disease has a more rapid onset than that caused by less virulent pathogens. Notably, on initial presentation, the usual physical stigmata are absent. Endocarditis may also involve healthy valves (Jaromillo, 2011).

Toxic shock syndrome (TSS)

Staphylococcal TSS is a potentially life-threatening systemic bacterial intoxication. Case definition includes fever, diffuse macular erythema, and hypotension, with involvement of 3 or more organ systems. Emesis or diarrhea appears at the time of illness. Diarrhea is secretory and profuse, and is found in almost all patients with TSS but is uncommon in patients in septic shock. Severe myalgia or elevated creatine kinase (CK) levels are observed.The most striking aspect of the disease is the rapidity with which it can progress in a previously healthy individual of any age. This is especially true in postsurgical patients, particularly following nasal surgery, because this is an area commonly colonized with S aureus. Late-onset dermatologic findings include a red and pruritic maculopapular rash, desquamation of the fingers and toes, and telogen effluvium (Chou *et al.*, 2011).

Pneumonia

Cases of rapidly progressive and fatal staphylococcal pneumonia still occur, although they were much more common in the 1950s and early 1960s, when S aureus phage type 80/81 caused frequent disease in infants. Staphylococcal pneumonia most commonly occurs in infants, young children, and patients who are debilitated. This is a rapidly progressive disease. Patients with primary staphylococcal pneumonia present with a short prodrome of fever followed by rapid onset of respiratory distress, which may include tachypnea, retractions, and cyanosis. Patients may also have prominent GI tract symptoms. Staphylococcal pneumonia may also develop after influenza infection, which seems to occur preferentially among young adults (in whom mortality reaches 50%). Typically, the child seems to recover from a febrile illness only to once again develop an increasing fever and the symptoms mentioned above. In the CA-MRSA era, staphylococcal pneumonia is becoming more prevalent (Hulken *et al.*, 2011)

Thrombophlebitis

Usually occurring in a hospitalized patient, thrombophlebitis is characterized by fever, pain, and, occasionally, erythema at the insertion site of an intravenous catheter. Occasionally, pus is expressed. Severe suppurative thrombophlebitis may occur in burn patients, with fewer than half of diagnoses made while the patients are alive.

Deep tissue abscess and infection

Muscles (myositis and pyomyositis) and organs can become infected, including the parotid gland, eyes, liver, spleen, kidneys, and central nervous system. Deep abscesses also may occur. These infections typically cause fever with or without localizing pain (Vander *et al.*,2009).

CHAPTER THREE

MATERIALS & METHODOLOGY

3.1 Materials Used

Test tubes, Petri dishes, beaker, 95% ethanol, Whatman filter paper No. 1, bijou bottles, cotton wool, inoculating loop, autoclave, Cork borer, ruler, rotary evaporator, blender machine, incubator, distilled water, Nutrient agar, Muller Hinton Broth & Agar, *Zingiber officinale* rhizome, *Staphylococcus aureus* culture.

3.2 Source of the Plant material

Zingiber officinale rhizome (fresh sample) was purchased at Bodija, a local market in Ibadan, Oyo State and was identified by Mr Awoyemi of the Department of Plant Science and Biotechnology, Federal University, Oye Ekiti.

3.3 Source of the bacterial strain

The standard culture of *Staphylococcus aureus* (P1023) was collected from Microbiology laboratory, University College Hospital, Ibadan, Oyo State and maintained in a Nutrient medium.

3.4 Preparation of *Zingiber officinale* rhizome extract

- The rhizome was washed with water, deskinned, sliced into small pieces and sun dried for 10 days.
- After drying, it was ground into powder form using a clean electric blender.

- 100g of the powdered sample was weighed using a weighing balance and poured into measured 500ml of 95% ethanol in a conical flask percolated at room temperature, 28^0C (1:5 ratio) according to Santo *et al.*, 2017.
- The conical flask was corked with cotton wool and foil paper, and left to stand for 2 weeks with regular shaking.
- After 2 weeks, the suspension was filtered using Whatman No.1 filter paper into a conical flask.
- The filtrate was poured into round bottom flask of a rotary evaporator and concentrated at 40^0C.
- After concentrating the filtrate, the crude extract of 19.28g obtained was poured into a McCartney bottle.

3.5. Determination of absorbing power of disc

- 3mm cork borer was used to punch out discs from a Whatman No.1 filter paper and the discs were put in a bijou bottle.
- The discs were sterilized in a Hot air oven for 1 hour at 150^0C.
- 0.1ml of Dimethylsulphoxide was dispensed in a test tube and 7 discs absorbed the 0.1ml. Therefore, from the calculation 0.1ml ÷7, the absorbing power of each disc is 0.014.

3.6. Preparation of Disc

- According to the absorbing power, from the 200mg/ml concentration, using double-fold dilution, 100mg/ml, 50mg/ml and 25mg/ml concentrations were prepared.
- The potency of the discs were calculated as follows:

  For 25mg/ml: 1----------25mg

0.014--------25×0.014=0.35mg/disc

For 50mg/ml: 1----------50

0.014--------50×0.014=0.7mg/disc

For 100mg/ml: 1----------100

0.014--------100×0.014=1.4mg/disc

For 200mg/ml: 1----------200

0.014-------200×0.014=2.8mg/disc

- Three (3) discs were put into each of the different concentrations prepared and removed after soaking into a labeled sterile Petri dish to get dried.
- The extract was kept in the refrigerator (4^0C) for further analysis.

3.7. Testing of Bacterial strain

1. Gram's Staining

- From a young culture of the test organism, a smear was made on a grease-free slide and allow to air dry after which was heat-fixed.
- The smear was stained with crystal violet (primary stain) for 60 seconds, rinsed, flooded with Lugol's iodine to retain the primary stain for 45 seconds and then rinsed.
- 70% ethanol was used on the smear for discolouration under running water and finally flooded with safranin (counter stain) for 60 seconds and rinsed.
- The smear was allowed to air-dry, oiled with immersion oil and viewed under the microscope using oil objective lens. Purple Cocci observed under a pink background indicated the isolate to be Gram-positive.

2. Catalase test: A drop of hydrogen peroxide (3%) was put on a grease-free slide and a colony of the test organism was picked and emulsified on the solvent. Bubbles indicated the isolate to be catalase positive.

3.8. Bioassay

- The stored microorganism was cultured on a Nutrient agar and incubated at 37^0C for 24 hours.
- After incubation, 3 well isolated colonies of the young culture of the test organism were picked and emulsified into 3ml of physiological saline prepared by dissolving 0.85g of common salt in 100ml of distilled water. This was carried out under an aseptic condition.
- The turbidity of the suspension of the test organism was compared with 0.5M McFarland turbidity standard already prepared in the laboratory.
- Muller Hinton agar was prepared according to the manufacturer's specification (38g of the agar powder in 1000ml of distilled water), sterilized in an autoclave at 121^0C for 15minutes, cooled, poured into sterile Petri dishes and allowed to set.
- With the use of a sterile swab stick dipped into the suspension, the test organism was inoculated on the sterile prepared Muller Hinton agar media and left for about 5 minutes.
- Aseptically, the slightly dried discs of the four different concentrations of the ginger extracts (25mg/ml, 50mg/ml, 100mg/ml and 200mg/ml) were placed on 4 (four) labeled inoculated plates separately.
- After 15 minutes of the discs application, the plates were incubated at 37^0C for 24 hours. This was done in triplicate and the plates were observed for zone of inhibition.
- 10µg of Gentamicin was used as a positive control using the same method.

3.9. Minimum Inhibitory Concentration

- Muller hinton powder was weighed, dissolved in distilled water, 4ml of it broth was dispensed into 10 test tubes each.
- The test tubes were properly corked and sterilized in an autoclave.
- From a fresh sub-cultured plate, the organism was picked into a sterilized normal saline and then compared with MacFarland turbidity.
- 1g of the stock extract was dispensed into 5ml of dimethylsuphoxide (DMSO).
- After cooling the broth, 0.5ml of the organism was added to each test tubes
- According to the formular $C_1V_1=C_2V_2$, the volume of extract to be added based on each concentrations were calculated. The C_1 is the concentration of the stock extract which is 200mg/ml; C_2 is the concentration of the extracts to be used (25mg/ml, 50mg/ml, 75mg/ml, 100mg/ml, 125mg/ml, 150mg/ml, 175mg/ml and 200mg/ml); V_1 is the volume of the solvent while V_2 is the volume of extract to be added.
- The extract was added accordingly to the mixture of broth and the organism in 8 well labeled test tubes.
- The ninth test tube served as a positive control which contained the broth, organism and gentamicin
- The tenth test tube served as a negative control which contained only the broth and the extract without the organism.
- The test tubes were corked and incubated at 37^0C for 18 hours to check for turbidity.

3.10. Minimum Bactericidal Concentration

- After 24 hours of carrying out the MIC, Muller Hinton Agar was prepared and poured into four plates.
- From the MIC result, sterile swab sticks were dipped into test tubes labeled 125mg/ml to 200mg/ml which appeared to be clear and probably inhibitory
- Each swab stick was streaked on each plate aseptically.
- The plates were then incubated for 24 hours at 37C to know if there will be growth, to confirm the concentration that can kill the organism.

CHAPTER FOUR

RESULT AND DISCUSSION

4.1. RESULT

4.1.1. The bioassay was carried out to reveal the concentration of the stock that inhibited the growth of the test organism. Table 2 shows the result of the different concentrations prepared from stock extract of 66.6mg/ml (1:15) when tested on the test organism. The different concentrations prepared were not potent enough to inhibit the growth of the organism and when the stock was used directly, there was zone of inhibition of 10mm. Discovering that the concentration is not potent enough, the concentration of the stock extract was increased to 200mg/ml (1:5). From the four concentrations prepared (25mg/ml, 50mg/ml, 100mg/ml, 200mg/ml), there was no zone of inhibition from the 25mg/ml and 50mg/ml but the others appeared to be potent shown in table 3, which brought about the continuation of the work.

4.1.2. Minimum Inhibitory Concentration performed from the concentration of the stock extract of 200mg/ml shows that lower concentrations (25mg/ml, 50mg/ml, 75mg/ml, 100mg/ml) are not potent enough to inhibit the growth of the organism and therefore appeared turbid while higher concentration which showed no turbidity indicate they were potent to inhibit the organism reported in table 4. In order to know the concentration that will kill the organism, the work proceeded to knowing the minimum bactericidal concentration. The non-turbidity of the suspension started from the concentration of 125mg/ml which makes it to be the Minimum Inhibitory Concentration (MIC).

4.1.3. From MIC value 125mg/ml, minimum bactericidal concentration (MBC) test was done by streaking on fresh Muller Hinton agar. The concentration of 125mg/ml and 150mg/ml showed some growth of the organism. While the plates of the concentration of 175mg/ml and 200mg/ml appeared to have no growth. From this result, 175mg/ml is the minimum bactericidal concentration (MBC) value of the extract that can kill the test organism.

Table 2: Bioassay result of ginger extract of ratio 1:15

Concentrations of extract (mg/ml)	Disc potency (mg/disc)	Zone of inhibition (mm)
1.8	0.03	Nil
3.6	0.05	Nil
7.2	0.10	Nil
14.4	0.20	Nil

Table 3: Bioassay result of ginger extract of ratio 1:5

Concentration of extract (mg/ml)	Disc potency (mg/disc)	Zone of inhibition (mm)
25	0.35	Nil
50	0.70	Nil
100	1.40	14mm
200	2.80	17mm
gentamicin (control)	10µg/disc	26mm

Table 4: Minimum Inhibitory Concentration of ginger extract of ratio 1:5

Concentration of extract (mg/ml)	Turbidity
25	turbid
50	turbid
75	turbid
100	slightly turbid
125	non-turbid
150	non-turbid
175	non-turbid
200	non- turbid

Table 5: Minimum Bactericidal Concentration result

Concentration of the extract (mg/ml)	Growth of organism
125	+
150	+
175	-
200	-

'+' indicates "growth" on sub culturing

'-' indicates "no growth" on sub culturing

4.2. DISCUSSION

Medicinal plants are of great use in pharmaceutical, cosmetic, agricultural and food industry. The efficacy of some herbal products is beyond doubt, in which *Allium sativum* commonly known as 'garlic' and *Zingiber officinale* Roscoe, commonly known as 'ginger' are examples. Ginger contains natural organic materials beneficial to health and enhances resistance to infectious diseases by increasing non-specific and specific immune mechanisms (Shubha, 2015). The rhizome of ginger has shown to be effective in the control of a range of bacterial, viral, fungal and parasitic diseases in humans, poultry and aquaculture owing to its antimicrobial, antioxidant, growth promoter and as immune-stimulant properties to health (Shubha, 2015).

From both the bioassay and Minimum inhibitory concentration results, it was observed that concentrations of ginger extract ranging from 100mg/ml to 200mg/ml are potent in inhibiting *Staphylococcus aureus*. Meanwhile, lower concentrations of 25mg/ml to 75mg/ml were not potent enough which resulted in the non-efficacy of the ginger extract when used against *Staphylococcus aureus*. According to the work done by *Malu et al.* (2009), it was suggested that the inhibition of bacterial growth activity of the extracts is dose dependent, and that the extracts are active at high concentration and inactive at very low concentrations. The antibacterial and inhibitory activities of ginger extracts could be attributed to the chemical properties of ginger in which the main constituents of ginger are sesquiterpenoids with zingiberene. Other components include β- sesquiphellandrene, bisabolene and farnesene, which are sesquiterpenoids, and trace monoterpenoid fraction, β-sesquiphellandrene, cineol and citral (Kamrul *et al.*,2014).

Previous views of researchers have established that aqueous extract of ginger is not as effective as the ethanolic extract of ginger which brought about the extraction of ginger using ethanol (95%) in this study. Ethanolic extract of the ginger was prepared by using dry ginger which was

commercially available. In the present study, ethanolic extract of ginger was found to have inhibitory effect against *Staphylococcus aureus* which corroborates with the findings of Sebiomo *et al.* (2010) who observed that the aqueous extract of ginger leaf and root of 20g/100ml concentration potency was low to inhibit the growth of *Staphylococcus aureus*, however the ethanolic extract of ginger had significant effect with a high zone of inhibition. The research thus showed that ethanolic extract was more effective than the aqueous extract because of the bacterial sensitivity to the former (Sebiomo *et al.*, 2010). *Zingiber officinale* Roscoe produced marked inhibitory effect on *Staphylococcus aureus* and *Escherichia coli* with ethanolic, methanolic and hexanic extracts, while aqueous extracts did not have inhibitory effect on microorganisms tested, chloroform extract had weak inhibitory effects against *Staphylococcus aureus*. The results indicated that the plant have growth inhibitory effect in vitro against pathogenic bacteria.

Onyeagba *et al.* (2004) found the synergistic effect of ethanol extract of ginger and garlic against *Bacillus* spp. and *Staphylococcus aureus*. It was also reported that the antimicrobial activity of the ethanolic extract of ginger, lime and garlic had effect against broad range of bacteria including *Bacillus* spp., *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella* spp. (Onyeagba *et al.*, 2004). Previous studies have shown that soy bean extract of ginger has a good antimicrobial activity against food borne pathogens; *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Vibrio cholerae, Klebsiella spp. and Salmonella spp.* (Kamrul *et al.*, 2014).

In this present study of ethanolic extract of ginger, the concentration of 100mg/ml to 200mg/ml had zone of inhibition ranging from 14mm to 16mm and this is comparable to the research work of Karuppiah *et al.*(2012) that reported that the activity of ginger on *Staphyloccoccus aureus*

from the concentration 25ug/ml to 200ug/ml had zones of inhibition ranging from 9.3mm to 13.55mm (Karuppiah *et al.*, 2012).

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION.

Medicinal herbs contain a wide range of chemical compounds commonly referred to as phytochemicals. *Zingiber officinale* commonly known as ginger is an important plant with several medicinal, ethno-medicinal and nutritional values used in traditional medicine. This research work has brought about the establishment of the fact that ginger is effective against *Staphylococcus aureus* which is one of the major human pathogens that cause a wide range of clinical infections and has been found to resist some conventional drugs now a days because of indiscrimate use of antibiotics.

It is then recommended that more research work could be conducted on the activity of ginger on organisms responsible for fungal and parasitic infections. Also, since ginger is commonly used as spice in food, the biosafety assessment of this plant could be carried out to ascertain the appropriate proportion of concentration to be used in order to know if the side effect is dose dependent.

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