ANTIMICROBIAL PROPERTIES OF GINGER RHIZOME (*Zingiber officinale*) *AGAINST Escherichia coli, Staphylococcus aureus* AND *Candida albican*

BY

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CERTIFICATION

We, the undersigned hereby certify that this project written by **AGBAMHON OMONON CHRISTABEL AST/2382060601, AGBAI IVIE GIFT AST/238200129** and **AGBONIFO OSATOHANMWEN AST/2382052033,** is adequate in scope and quality and is submitted to the Department of Biological Sciences, Auchi Polytechnic, Auchi in partial fulfillment of the requirement for the award of Higher National Diploma (HND) in Biology/Microbiology.

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DEDICATION

This project is specially dedicated to God Almighty who has been my source of help and giver of all wisdom, knowledge, understanding and for making this project a success.

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I give thanks to God Almighty the maker of the universe for our life and also making us to accomplish my goal in life, also for giving us the strength and determination to put this project together.

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Abstract

Ginger rhizome (Zingiber officinale), is an essential plant based product consumed as spice and can be used as naturopathy due to its potential antimicrobial activity against various pathogenic microbes and is also used in various food industries and traditional medicine. In this study, the antimicrobial potential of ginger was analyzed against Gram -ve Escherichia coli and Gram +ve Staphylococcus aureus and Candida albican . Analysis of the results of sensitivity tests (disc diffusion assays) indicated each of the microorganisms to be inhibited. The formation of zones of inhibition indicated that the spice tested was effective as an antimicrobial agent when screened. Zones of inhibition of ethanolic extract of ginger on the tested microorganism Staphylococcus aureus was 10mm, Escherichia coli 15mm and Candida albican 2mm while that of methanolic of same extract on same microorganism only Staphylococcus aureus shows 15mm zone of inhibition. Analysis of the results concluded that the active compounds present in the Ginger were effective against tested micro-organisms and may be used to treat infections. This could be an alternative treatment to antibiotics for one or all of the microbial species investigated and in so doing allow the healing powers of spices to be acknowledged. The observations of such inhibition zones were comparatively significant and demonstrated the potential use of these Ginger as antimicrobial agents with an efficacy that can be compared to that of the already recognized and widely used antibiotic, neomycin.

Keywords: Ginger, antimicrobial, zone of inhibition, antibiotics, extraction, microorganism

CHAPTER ONE

1.0 INTRODUCTION

1.2 BACKGROUND OF THE STUDY

The increased usage of antibiotics has induced microorganisms to acquire resistance factors which have become a burning predicament (Abimbola*et al*., 2017). 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 (Khulbe& Sati, 2009). The use of higher plants and their extracts for treating the infectious diseases has long been practiced in many parts of the world (Sofowora, 2016). 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 (Apata, 2015). Ginger (*Zingiber officinale*) 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, 2018).

Ginger ***(Zingiber officanale)*** is one of the important spice crops that is preferred for its therapeutic values. An herbaceous perennial plant, of Zingiberaceae family (Sharma, 2010). The Zingiberaceous plants possess high

medicinal and aromatic characteristics and are categorized as non-tuberous or tuberous rhizomes (Chen, 2018). It is propagated through rhizome, leafy stem, 30-90 cm in height. It is widely used in food beverages, confectionaries and medicines. Ginger may act as an antimicrobial agent and hence could be used for treating number of bacterial diseases (Tan &Vanitha, 2014). Ginger is relatively cheaper because of its easy availability, universal acceptability and is tolerated by most people. Fresh ginger contains 80.9% moisture, 12.3% carbohydrates, 2.4% fiber, 2.3% protein, 0.9% fat and 1.2% minerals (calcium, iron and phosphorous). It also comprises of vitamins such as riboflavin, niacin, thiamine and ascorbic acid. With variety, type, curing methods, agronomic circumstances, storage and drying conditions, the composition varies (Govindarajan&Connell, 1982). Traditionally, it is used to treat the headache, cold, muscular and rheumatic disorders (Yang et al., 2009). Various studies also examined the phytochemical composition of their rhizome, revealing zingiberene, gingerol, shogaol and their derivatives as the chief constituents (Sivasothy*et al.*, 2011). Reported pharmacological activities on ginger involve antioxidant, anti-inflammatory, antimicrobial, antinociceptive and hepatoprotective (Abdel *et al.,* 2013; Mostafa&Singab, 2016) this project investigates the antimicrobial properties of ginger rhizome against *Escherichia coli*, *Staphylococcus aureus* and *Candida albican.*

Escherichia coli, also known as *E. coli,* is a Gram-negative, facultative anaerobic, rod-shaped, coliform bacterium of the genus *Escherichia* that is commonly found in the lower intestine of warm-blooded organisms.

Staphylococcus aureus is a genus of Gram-positive bacteria in the family *Staphylococcaceae* from the order *Bacillales.* Under the microscope, they appear spherical, and form in grape-like clusters. *Staphylococcus* species are facultative anaerobic organisms.

Candida albican is a genus of yeasts and is the most common cause of fungal infections worldwide. Many species are harmless commensals or endosymbionts of hosts including humans; however, when mucosal barriers

1.3 objectives of the study

The objective of this study is to determine the antimicrobial properties of ginger rhizomeagainst Escherichia coli, Staphylococcus *aureus* and *Candida albican.*

1.4 Statement of the problem

Antimicrobial Resistance (AMR) occurs when bacteria, viruses, fungi and parasites change over time and no longer respond to medicines making infections harder to treat and increasing the risk of disease spread, severe illness and death.

As a result of drug resistance, antibiotics and other antimicrobial medicines become ineffective and infections become increasingly difficult or impossible to treat.

Ginger has longed been used as naturopathy due to their potential antimicrobial activity against different microbes hence the need for these research to determine it antimicrobial propertiesagainst *Escherichia coli, Staphylococcus aureus and Candida albican.*

1.5 Scope of the study

The study entails the determination of the antimicrobial properties of Ginger (*Zingiber officinale*) against *Escherichia coli*, *Staphylococcus aureus and Candida albican.*

1.6Significances of the Study

The significant of these study is to determine the antimicrobial properties of Ginger (*Zingiber officinale*)against Escherichia coli, *Staphylococcus aureus* and *candida albican* cannot be over emphasized. The investigation will be of relevance to academic communities as an attempt will be made to broaden their academic knowledge about the research work. Also creating alternative medicine for pharmaceutical industry in which further study can be carried out.

1.7 Definition of terms

Isolation - The field of biology describes "isolation" as a process by which two species that could otherwise produce hybrid offspring are prevented from doing so. There are five isolation processes that prevent two species from interbreeding: ecological, temporal, behavioral, mechanical/chemical and geographical.

Microbial - Microbes are tiny living things that are found all around us and are too small to be seen by the naked eye. They live in water, soil, and in the air. ... Some microbes make us sick, others are important for our health. The most common types are bacteria, viruses and fungi.

Antimicrobial - An antimicrobial is an agent that kills microorganisms or stops their growth. Antimicrobial medicines can be grouped according to the microorganisms they act primarily against. For example, antibiotics are used against bacteria, and antifungals are used against fungi.

Zone of Inhibition- zone of inhibition: This is an area of media where bacteria are unable to grow, due to presence of a drug that impedes their growth. Minimum inhibitory concentration: This is the lowest concentration of an antimicrobial drug that prevents visible growth of a microorganism after overnight incubation with media.

Staphylococcus aureus: is a genus of Gram-positive bacteria in the family *Staphylococcaceae* from the order *Bacillales.*

***Escherichia coli*:** also known as *E. coli*, is a Gram-negative, facultative anaerobic, rod-shaped, coliform bacterium of the genus *Escherichia*

***Candida albican*:** is a genus of yeasts and is the most common cause of fungal infections worldwide.

CHAPTER TWO

LITERATURE REVIEW

2.0 INTRODUCTION

Ginger has both gingerols and shogaols that are rich in antimicrobialproperties and active constituents of ginger inhibit the replication of colon bacteria hence helps in resolving stomach infection and other health outcomes. There is evidence that harmful microbes can contribute to the formation of chronic conditions, such as cancer and coronary heart disease. As pathogens become more resistant to developed drugs, the use of antibiotics and vaccines can be non-effective. Ginger has been shown to play a vital role as an antimicrobial agent. Several active components have been shown to be active *against E coli, Salmonella typhi, Candida albicans*(Abimbola*et al*., 2017).

At Indian Institute of Spices Research (IISR), Kozhikode, Kerala, scientists have developed three improved varieties of ginger, namely- Varadha, Mahima and Rajitha, which are suitable for growing all over India. It is grown mainly as a rain fed crop in Kerala and in north-eastern parts of India. It can also be grown as an irrigated crop (Sasikumar, 2016). Ginger is known as *Adrak*in Hindi, *Ingi*in Tamil, *Inchi*in Malayalam, *Allamu*in Telugu *shounth*in kashmiri and *Shunthi*in Kannada. About 1,500-1,800 kgs of seed rhizomes are required for planting in one hectare (Sasikumar, 2016).

Kamrul*et al.*(2016) examine the antimicrobial activity of ginger (zingiberofficinale) extracts against food-borne pathogenic bacteria. Their study was conducted to determine the antimicrobial activity of soybean oil extract of dried ginger powder, using agar diffusion assay, against 24 isolates of food borne pathogens including *Escherichia coli, Pseudomonas aruginosa, Staphylococcus aureus, Vibrio cholerae, Klebsiella spp. and Salmonella spp.*Their studyshowed the potent antimicrobial activity of the ginger extract against the all tested bacterial pathogens. Soybean oil extract of ginger showed highest zone of inhibition (11.67±1.53mm) against *Salmonella spp.* and lowest zone of inhibition (8.0±1.73mm) against *Escherichia coli.* Ginger extract also showed lower zone of inhibition (8.67±2.52mm) against *Staphylococcus aureus* compared to the Gram-negative bacteria. Soybean oil extract of ginger at boiling temperature has potential antimicrobial activity and could be used in food preparation to get the synergistic effect of soybean and ginger.

Wail *et al.*(2018) highlight the antibacterial properties of ginger rhizome, based on the published data. They found that, out of 40 published papers on the antibacterial properties of ginger rhizome, 2 reported negative results, while 38 exhibited positive results against all or some of the tested bacteria. Even though, most of the positive results were not a competitor to the tested antibiotics (as positive controls). However, there were wide differences and contradictions

between the positive results themselves even against the same bacterial species, indicating that the efficacy of this plant product is greatly affected by many reasons such as the method of extraction, antibacterial assay conditions, genetic variations among bacterial strains and its sources. Also, the source of plant sample is an important factor, since plants affected by geographic variations, environmental conditions and physiological factors which influence its bioactive phytochemical compounds. Accordingly, this mini-review suggests that the antibacterial properties of ginger rhizome have yet to be adequately explored using advanced multidisciplinary approach (in vitro and in vivo).

Mozhgan A *et al.*,(2016) investigate antimicrobial activity of Hydro-alcoholic extracts ofginger (*Zingiber officinale*) and Mallow (*Malva sylvestris*) against P*seudomonas aeroginosa, Staphylococcus aureus,* Meticillin resistant *Staphylococcus aureus* and Listeria monocytogenesis as a number of important factors and infectious microorganisms. Antimicrobial effects of Hydro-alcoholic extracts of ginger (*Zingiber officinale*) and Mallow (*Malva sylvestris*) was evaluated by micro-plate dilution and disk diffusion methods. Minimal inhibitory concentration of ginger extract for *Staphylococcus aureus*, Meticillin resistant *Staphylococcus aureus, Pseudomonas aeroginosa* and *Listeria monocytogenesis* were 52, 52, 416 1nd 52 µg/ml and MBC of this extract were 104, 104, 416 and 104 µg/ml, respectively. Growth inhibition zone of ginger extract for these bacteria were 16, 9, 7 and 8 mm, respectively. Mallow extract

had no antibacterial effects on studied bacteria. Obtained results showed ginger extract can be introduced as bioactive antibiotic in traditional medicine, microbiology and pharmacology sciences.

Emanuel *et al.*,(2020) carried out to investigate on the bacteria pathogens found in digestive tract infections and assess antimicrobial activities of ginger extract to on the identified bacteria. Different types of bacteria were isolated from stool samples of digestive tract infection patients by using various methods such culture, biochemical test and antimicrobial activity of ginger extracts was analyzed at INES-Ruhengeri in clinical microbiology laboratory. Ginger has shown to have an antibacterial activity on bacteria isolated from digestive tract infected patients. It can be used as a medicine to treat these infections. Number of researches should be done to be sure on this reality of antibacterial activity of ginger

Shafia (2021), examined the antimicrobial activity of Ginger against various pathogenic microbes used in various food industries and traditional medicine. In their study, the antimicrobial potential of ginger was analyzed against Gram -ve*Escherichia coli* and Gram +ve*Enterococcus faecalis and Staphylococcus aureus.* Analysis of the results of sensitivity tests (disc diffusion assays) indicated each of the microorganisms to be completely inhibited. The formation of zones of inhibition indicated that the spice tested was effective as an antimicrobial agent when screened. Zones of

inhibition (greater than 30 mm in diameter were obtained during positive disc diffusion assaying. Analysis of the results concluded that the active compounds present in the Ginger were effective against tested micro-organisms and may be used to treat bacterial infections. This could be an alternative treatment to antibiotics for one or all of the microbial species investigated and in so doing allow the healing powers of spices to be acknowledged. The observations of such inhibition zones were comparatively significant and demonstrated the potential use of these Ginger as antimicrobial agents with an efficacy that can be compared to that of the already recognized and widely used antibiotic, neomycin.

Miloš(2014), investigated the antibacterial and anti-biofilm activity of ethanolic extract from the rhizome of ginger (*Zingiber officinale)* were evaluated. In vitro antibacterial activity was investigated bymicrodilution method. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) have been determined. The values were in the range from 0.0024 to > 20 mg/ml. The most sensitive bacteria were Gram-positive bacteria: *Staphylococcus aureus* and *Staphylococcus aureus* ATCC 25923. Anti-biofilm activity was tested by crystal violet assay. *Pseudomonas aeruginosa* ATCC 27853, *Proteus mirabilis* and *Escherichia coli* ATCC 25922 were used as the test organisms. Ethanolic extract showed the best result on Proteus mirabilis biofilm where biofilm inhibitory concentration (BIC50) was 19 mg/ml.

Mohaddese (2019) reviewed the chemical composition and biological activities of *Z. officinale* (ginger) essential oil. Ginger oil is extracted from *Z. officinale rhizomes*. The antibacterial, antifungal, analgesic, anti-inflammatory, anti-ulcer, immunomodulatory, relaxant, and warming effects of ginger oil have been confirmed in experimental and preclinical studies. The safety issues of ginger oils are well documented and are generally regarded as safe. Due to wide pharmacological effects of ginger oil, attention to ginger oil as an ingredient of natural formulations in management of gastrointestinal and respiratory diseases is valuable.

Malu*et al*. (2018) examined the antibacterial activity and medicinal properties of ginger extracts were studied. Ginger extracts were obtained using solvents, n-hexane, ethyl acetate, ethanolicsoxhlet and water. The extracts were assayed for antibacterial activity and bacterial growth inhibition activity. The results showed that all the extracts except the water extract have antibacterial activity and that the inhibition of bacterial growth was dose dependent. The results alsoshowed that ginger extracts possesses antibacterial properties and could be used for the treatment of bacterial infections.

2.1 TAXONOMICAL CLASSIFICATION OF GINGER (*Zingiberofficinale*)

Kingdom:	Plantae(Plants)
Subkingdom:	Tracheobionta-Vascular plants,
Superdivision:	Spermatophyta-Seed plants,
Division:	Magnoliophyta-Flowering plants,
Class:	Liliopsida-Monocotyledons,
Subclass:	*Zingiberidae,*
Order:	*Zingiberales,*
Family:	*Zingiberaceae*
Genus:	*Zingiber mill*
Species:	*Zingiberofficinale*

(Dhanik*et al.* 2017).

Ginger, (*Zingiber officinale*), herbaceous perennial plant of the family *Zingiberaceae,* probably native to southeastern Asia, or its pungent aromatic rhizome (underground stem) used as a spice, flavouring, food, and medicine.

2.2.1 Physical Description of Ginger (*Zingiber officinale*)

The leafy stems of ginger grow about 1 metre (about 3 feet) high. The leaves are 15 to 30 cm (6 to 12 inches) long, elongate, alternate in two vertical rows, and arise from sheaths enwrapping the stem. The flowers are in dense conelike spikes about 2.5 cm (1 inch) thick and 5 to 8 cm (2 to 3 inches) long that are

composed of overlapping green bracts, which may be edged with yellow. Each bract encloses a single small yellow green and purple flower (Dhanik*et al.* 2017).

2.3 PROPERTIES OF GINGER (*Zingiber officinale*)

In 13th century, ginger culinary properties were discovered and soon it was widespread across the globe including Europe and was indicated for several diseases including travel sickness and flatulence. It is cultivated from Asia to Africa and used everywhere as a cooking spice (Kala *et al.,* 2016). It is also useful in case of chills. In India, it is widely consumed in dose of 8-10 g as a flavouring agent with the following properties;

- Potency: spicy
- Taste: bitter
- Properties: light, adhesive and thick

2.3.1 FORMS OF AVAILABILITY OF GINGER (*Zingiber officinale*)

- Fresh green root
- Preserved ginger in brine or syrup
- Dry ginger spice

Fresh ginger is usually consumed in the area where it is produced, although it is possible to transport fresh roots internationally. Both mature and immature rhizomes are consumed as a fresh vegetable. Preserved ginger is only made from immature rhizomes. Most preserved ginger is exported. Dried ginger spice

is produced from the mature rhizome. As the rhizome matures the flavour and aroma become much stronger (Kala *et al.*, 2016)..

2.4BIOCHEMISTRY OF GINGER

Ginger standards have been well documented in USP (United State Pharmacopoeia) and National formulary (Kala *et al.* 2016). The chemistry of ginger is well documented with the respect to the oleoresin and volatile oil. There is stringent criteria for the usage of medical grade (should contain 1.5% or more volatile oil). The studies have identified more than 400 different compounds in ginger and major constituents are as follows:

- Carbohydrates- about 70%
- Lipid- about 8% which includes free fatty acids.
- Volatile oils- about 3% consist mainly of the sesquiterpenes, beta-bisabolene
- In addition, raw fibres, vitamins and minerals are also present in ginger.

Ginger also contains amadaldehyde, paradole, gingerdiols, gingerdiacetates, gingerenones, 6- gingersulfonic acid, diterpense, gingerglycolipids A, B and C

2.5 GINGER NUTRITIONAL COMPOSITION AND CHEMICAL CONSTITUENTS

The main area under ginger covering is related to Nigeria 56.23% of the total global area followed by India (23.6%), China (4.47%), Indonesia (3.37%),

and Bangladesh (2.32%) (Dhanik et al. 2017). Minerals content of ginger for ginger root (Ground) consists of Calcium (114 mg per 100 g), Iron (19.8 mg per 100 g), Magnesium (214 mg per 100 g), Manganese (33.3 mg per 100 g), Phosphorus (168 mg per 100 g), Potassium (1320 mg per 100 g), Sodium (27 mg per 100 g), and Zink (3.64 mg per 100 g), and minerals contents for ginger root (Raw) are Calcium (16 mg per 100 g), Iron (0.6 mg per 100 g), Magnesium (43 mg per 100 g), Phosphorus (34 mg per 100 g), Potassium (415 mg per 100 g), Sodium (13 mg per 100 g), and Zink (0.34 mg per 100 g) (The United States Department of Agriculture 2013). It was found that ginger contained 1.5%-3% essential oil, 2–12% fixed oil, 40–70% starch, 6–20% protein, 3–8% fibre, 8% ash, 9–12% water, pungent principles, other saccharides, cellulose, colouring matter and trace minerals (Chan et al. 2019). Ginger is called by different names in different parts of the world such as Zingiberis rhizome, Shenjiany, Cochin, Asia ginger, Africa ginger and Jamaican ginger (Peter 2017). Kala et al. (2016) stated that ginger oil also used as food-flavouring agent in soft drink, as spices in bakery products, in confectionary items, pickles, sauces

2.6 THERAPEUTIC PROPERTIES OF GINGER(*Zingiber officinale*)

2.6.1 Cardiovascular effects: Large number of studies showed that the important constituents of ginger namely gingerol and shogaol classes of compounds might have many therapeutic effects including anti-inflammatory, antioxidant, and hypo-cholesterolemic effects (Kumar et al., 2015).

2.6.2 Gastrointestinal effect of ginger: Ginger is very useful in the treatment of several gastrointestinal diseases including peptic and duodenal ulcer. Ulcer is generally caused due to imbalance between defensive and offensive factors like acid, pepsin and *Helicobacter pylori*; and in this case, ginger is useful due to its anti-inflammatory properties (Kumar et al., 2015).

2.6.3Antiemetic effect of ginger: Ginger shows strong antiemetic property by enhancing intestinal motility and inhibiting serotonin receptors. It stimulates peripheral anti-cholinergic and ant-histaminic receptors and antagonises 5-hydroxytreptamine receptors in the GIT (Duarte, 2016).

2.6.4Ginger anti-nausea effect due to chemotherapy: Chemotherapy is known to cause severe nausea and vomiting. It has been proved that ginger is effective in preventing nausea and vomiting caused by chemotherapy. Gingerols the key ingredients responsible for the activity have shown pharmacological effect (Duarte, 2016).

2.6.5 Hematologic (platelets) effects of ginger: Scientific evidence is still pending; however it was found that ginger is having anti-thrombotic and strong antiinflammatory effect due to increased fibrinolytic activity when same has been taken at about 5 g. It was found that Gingerols and Paradol have good anti-platelet and COX-I inhibitor properties (Kumar et al., 2015)

2.6.6Anti- Inflammatory effect: Ginger is showing anti-inflammatory effect by suppression of PG synthesis and also interference in cytokine signaling

2.6.7Antimicrobial: Due to phenolic compounds, ginger has shown excellent antimicrobial properties and effective in controlling virus, bacteria, fungal disease. Example: Antiviral, Antibacterial and Antifungal (Duarte, 2016).

2.7. USES OF GINGER (*Zingiber officinale*)

The spice has a slightly biting taste and is used, usually dried and ground, to flavour breads, sauces, curry dishes, confections, pickles, and ginger ale. The fresh rhizome, green ginger, is used in cooking. The peeled rhizomes may be preserved by boiling in syrup. In Japan and elsewhere, slices of ginger are eaten between dishes or courses to clear the palate. Ginger is used medically to treat flatulence and colic(Abimbola*et al.*, 2017).

Ginger contains about 2 percent essential oil; the principal component is zingiberene and the pungent principle of the spice is zingerone. The oil is distilled from rhizomes for use in the food and perfume industries.

2.8 SIDE EFFECT OF GINGER

It can cause mild side effects including

- Heartburn,
- Diarrhea,
- Burping, and
- General stomach discomfort.

Taking higher doses of 5 grams daily increases the risk for side effects.

When applied to the skin: Ginger is possibly safe when used short-term. It might cause skin irritation for some people (Abimbola*et al.*, 2017).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

The materials used for this research work includes; Petri dishes, Distilled water, Beaker, Conical flask, inoculating loop, Autoclave, incubator, Bunsen Burner, Cotton wool, Aluminum Foil Paper, Test tube, Nutrient agar, Sabouraud Dextrose Agar (SDA), Beakers, Measuring Cylinder, Spatula, Ethanol, Methanol, *Ginger rhizome*

3.2 Sterilization of Materials

All glass wares were first washed with detergent and rinsed with distilled water, wrapped with aluminum foil after drying and sterilized by dry heat method in the oven at a temperature of 160^{o}c for 2-3hrs.

3.3 Method

3.3.1 Sample Collection

Fresh ginger rhizome, were purchased from Uchi market in Auchi Etsako West Local Government Area of Edo State and was transported to the laboratory. The fresh *Ginger rhizome*, were oven dried at 60^{o}c for 48 hours. The Oven dried Ginger rhizome were grinded using an electric blender into a fine powder.

3.4 Preparation of Extract

Ginger rhizome extract were isolated as described by funk *et al*. (2010) with some modifications. About 50g of dried powdered of Ginger rhizome was mixed in 500ml of each of the solvent (ethanol and methanol) used and kept at room temperature for 48 hours. During this period, shaking of the flask was performed daily. The solvent soluble compound, were filtered using whatman filter paper No. 1. The filtered solutions were concentrated using a rotary evaporator.

3.5 Screening of Micro organism

A total of three human pathogens (*Staphylococcus aureus, Escherichia coli* and *Candida albicans*) were used in this study. The organisms were collected from the cottage hospital of Federal Polytechnic Auchi where they were isolated and identified.

3.6 Sensitivity Test (Agar Well Diffusion Method)

The micro organisms seeded on prepared Nutrients Agar and SDA (Sabouraud Dextrose Agar) were poured into sterilized petri dishes at 45^0C. the plates were then allowed to colonize by the pathogenic organisms. Using a flamed cork borer of 7mm, well were punched into the seeded plates. 1ml of the ginger rhizome extract were then poured and left for 24 hours (bacteria) and 72 hours (fungi) respectively. The diametric zones of inhibition were measured in millimeter.

3.7 Phytochemical Screening

Phytochemical analysis were studied by the following methods; glycosides were estimated by killer test, terpenoids by Salkowski test, flavonoids with the use of lead acetate, saponnins by foam test, tannins by ferric chloride test, alkanoids by Hager's test, carbohydrates by molish test, protein by biuret test (Simanjuntak, 2020).

CHAPTER FOUR

RESULT AND DISCUSSION

4.1 Result

The tables below show the result of the antimicrobial activities of the extract of ginger rhizome on the tested organisms

Table 1: Antimicrobial activity of ethanolic *Ginger rhizome* extract on the tested microorganisms

Tested Microorganism	Zone of Inhibition Observed (mm)
Staphylococcus aureus	10mm
Escherichia coli	15mm
Candida albican	2mm

The above table shows that the ethanolic ginger rhizome extract has antimicrobial effect on tested micro organisms (*Staphylococcus aureus, Escherichia coli* and *Candida albicans*). From the result, it were also observed that the zone of inhibition were higher in *Escherichia coli* followed by *Staphylococcus aureus* and *Candida albicans*

Table 2: Antimicrobial Activity of Methanolic Ginger rhizome extract on the tested organism.

Tested Microorganism	Zone of Inhibition Observed (mm)
Staphylococcus aureus	15mm
Escherichia coli	No zone of inhibition
Candida albican	No zone of inhibition

Table 2 Shows that the methanolic ginger rhizome extract has antimicrobial effect on *Staphylococcus aureus* except for *Escherichia Coli* and *Candida albican* has no zone of inhibition.

Table 3: Phytochemical analysis of *Ginger rhizome* extract

Photochemicals properties	Ethanolic extracts of Ginger rhizome	Methanolic extract of Ginger rhizome
Alkaloids	+++	+++
Tannins	+++	-
Glycosides	-	-
Saponins	+++	-
Steroids	_	+
Flavonnoids	-	++

Terpenoids	-	+
Carbohydrates	-	+

Key:

+++ = Abundantly Present

++ = Moderately Present

+ = Fairly Present

- = Absent

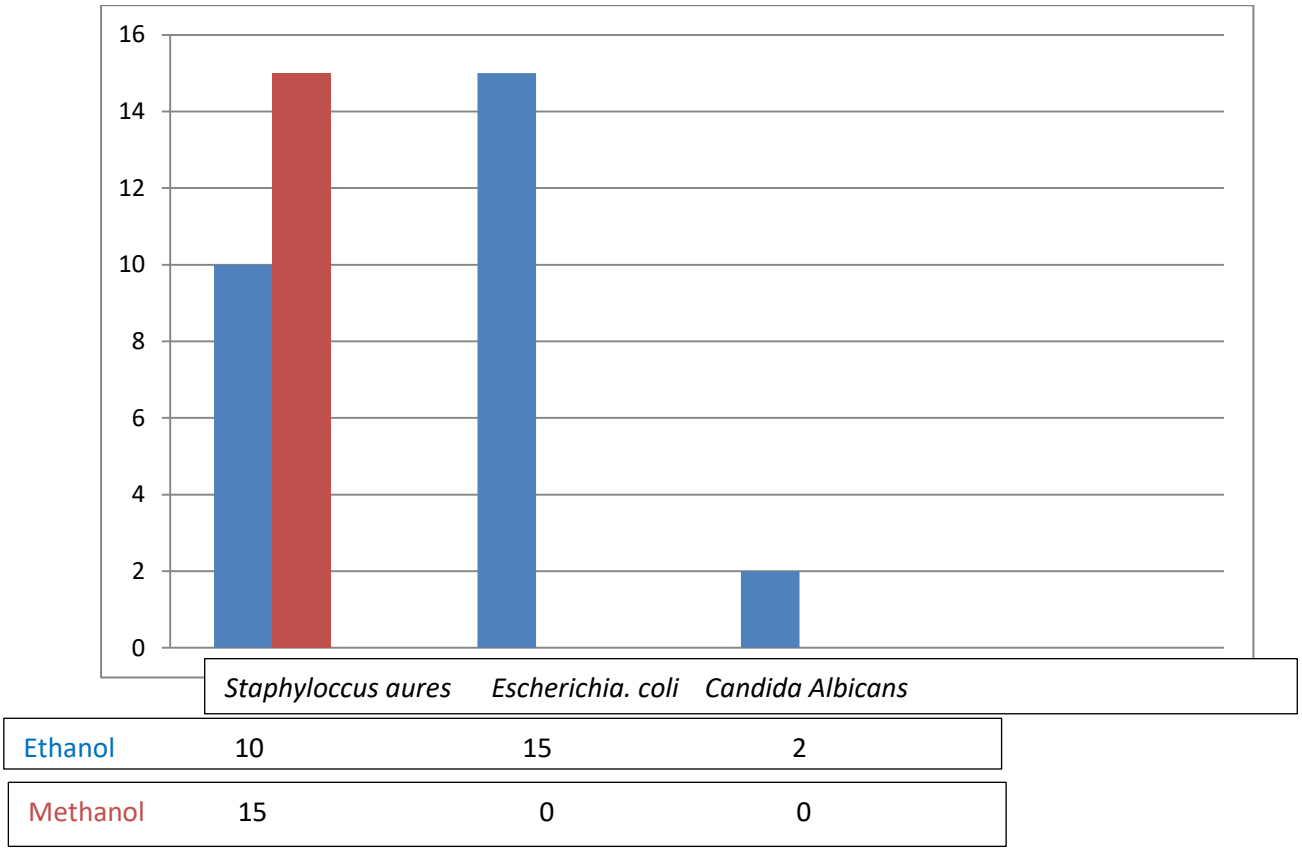

Figure 1. Diagrammatical representation of antimicrobial activities of Ginger rhizome

4.2 Discussion

Based on the result in this study (antimicrobial activities of ginger rhizome extract), it was observed from table 1 & 2 that ethanolic and methanolic ginger rhizome extracts has antimicrobial effect on tested microorganisms except for *Escherichia Coli* and *Candida albican* with no zone for methanolic ginger rhizome extract.

Davis and Stout (2016) explain the criteria for antibacterial inhibition consistency of $\geq$ 20mm is very strong, 10 to 20mm is strong, 5 to 10mm is moderate and $\leq$ 5mm is weak.

Based on the phytochemical screening, ginger rhizome extract contain alkaniod, flavonoid saponin, tannin and terpenoid/steroid compound. The mechanism of action of alkanoid as an antimicrobial, is by inhibiting the synthesis of nucleic acids, because it can inhibit the enzymes dihydrofolate reductase and topoisimerase. Alkanoid can disrupt the constituent components of peptidoglycan on bacterial cell death. another mechanism of antibacterial alkaloids is that the alkaloid compounds is known as a DNA accelerator and inihibits bacterial cell poisimerase enzymes (Simanjuntak, 2020).

Flavonoids provide bacteriolytic effects, inhibit protein synthesis, DNA synthesis, RNA and damage cell membrane permeability (Dzoyem, 2013). Flovonoids have antibacterial activity because of the ability of flavonoid to interact with cell membrane and affect cell membrane bioactivity and it has been reported that flavonoid are able to reduce fluidity of bacteria cell

membrane that is directly related to damage to cytoplasmic membranes or indirect damage through autolysis/weakening of the cell wall consequently osmotic lysis (Simanjuntak, 2020).

The mechanism of action of saponin as an antibacterial and antifungi causes lysis of the bacterial cell and leakage of AKP (Alkaline Phosphate), an increase in saponin concentration causes the protein to dissolve, causing intercellular compound to diffuse through the outer membrane and cell wall. This causes the cytoplasm to leak out of the cell resulting in cell death.

Tannin is a water soluble polyphenol that can precipitate proteins. Tannin has been reported to prevent the development of microorganisms by precipitating microbial proteins and making nutrient proteins unavailable to bacterial (Dzoyem, 2013). Tannin act as an antimicrobial with less iron, hydrogen bonds or specific interactions with vital proteins such as enzymes in microbial cells. Herbs that have a tannin component astringent and are used in the treatment of intestinal disorder such as diarrhea and dymenorrhea (Dharmananda, 2018).

Terpenoids have broad antimicrobial activity against filametous bacteria, yeast and fungi. Terpenoids are antimicrobial because they can damage yeast cell membrane or damage lipid membrane synthesis that effect on membrane permeability resulting in cell leakage component (Dzoyem, 2013).

These findings reveals that ginger rhizome extract can be used for therapeutic and pharmaceutical purpose.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

The ginger rhizome extract has as group secondary metabolities including alkaloid, flavonoids, saponin, tannin and terpenoids. The extract has effective antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus* and *Candida albican* with exception of the methanolic extract that could not inhibit *Escherichia coli* and *Candida albican*. This result also confirms that the use of ethanol as good extraction medium for the active ingredients in ginger rhizome against micro-organisms.

5.2 RECOMMENDATION

This present study is based on current and past research done on the therapeutic effect of *Ginger rhizome* against human pathogenic organisms. It was found that *Ginger rhizome* is useful for many therapeutic purpose. Therefore, its recommended for use by pharmaceutical companies and other related agencies.

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