ANTIMICROBIAL ACTIVITIES OF CLOVES AGAINST *ESCHERICHIA COLI, STAPHYLOCOCCUS AUREUS AND CANDIDA ALBICANS*

BY

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CERTIFICATION

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DEDICATION

We dedicated this book to the Almighty God, thank you for the guidance, strength power of mind, protection and skills and for giving us a healthy life. All of these we offer to you.

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ABSTRACT

This study was carried out to investigate the antimicrobial activities of cloves against *Escherichia coli, Staphylococcus aureus and Candida albicans* with the exception of the aqueous extract that could not inhibit *Staphylococcus aureus.* This result also confirms the use of ethanol and methanol as good extraction medium for the active ingredients in clove extract against microorganism. It is recommended that cloves extract should be used for its therapeutic and pharmaceutical purpose as it could form a good antimicrobial agent. Due to limited choice of antibiotics, medicinal plant extracts have gained interest because of their known antimicrobial nature

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CHAPTER ONE

1.0 Introduction

In Nigeria, a high proportion of the rural and urban population resort to natural food ingredients, particularly because of their availability. Spices are a large group of such natural ingredients, and include dried seeds, fruits, roots, rhizomes, barks, leaves, flowers and any other vegetative substances used in a very small quantity as food additives to colour, flavour or preserve food (Birt, 2006). Spices are fragrant, aromatic and pleasant. The bulk of the spices consist of carbohydrates such as cellulose, starch, pentosans and mucilage, and some amount of protein and minerals (Ogutimein *et al.*, 2009).

Only very small fractions of dry matter of the spices such as the phytochemicals are responsible for the flavouring, colouring, preservative and health-promoting characteristics (Cowan, 2019). These phytochemicals are plant metabolites (Sofowurra, 2013) which act as natural defense systems for host plants, and also provide characteristic colour, aroma and flavour in specific plant parts. They are a group of non-nutrient compounds that are biologically active when consumed by human. Many phytochemicals are health-promoting and are of many disease preventive (Rowland, 2009; Birt, 2006). Both epidemiological and clinical studies have proven that phytochemicals present in cereals, fruits and vegetables are mainly responsible for reduced incidence of chronic and degenerative diseases among populations whose diets are high in these foods (Shahidi,2006). As a result there has been an increased search for phytochemical constituents that possess antioxidant and antimicrobial potency in recent time (Jayaprakasha and Jaganmohan, 2000, Birt, 2006).

1.2 Background of the Study (Description of Cloves)

Cloves are the aromatic dried buds of a tree (Syzygium aromaticum) used as a spice in virtually all the world's cuisine. The dried clove bud contains carbohydrates, fixed oil, steam-

volatile oil, resins, tannins, proteins, cellulose, pentosans and mineral elements. A few non-volatiles have been isolated from clove, which include tannins, sterols, triterpenes and flavonoids. (Gopalakrishnan *et al.,* 2008). Volatile constituents yields different types of volatile oil [oil extracted from i. leaves, ii. the stem, iii. the buds and iv. the fruit, which are Eugenol (70- 85%), eugenyl acetate (15%) and p-caryophyllene (5-12%), together make up 99% of the oil (Balch and Phillis 2000).

Clove oil (Syzygium aromaticum) is widely used as a perfume and food flavouring (Zheng *et al.,* 2012; Kalemba and Kunicka, 2003), as a medicine for the treatment of asthma, rheumatoid arthritis, acne, warts, scars and various allergic disorders (Kim *et al.,* 2018), as an analgesic, anti spasmodic, and as a general antiseptic in medical dental practices (Cai and Wu, 2006). Clove bud oil, has been used for a long time by dentists as a dressing in dentistry for minor wounds, as an analgesic in painful and infective diseases of the oral cavity and pharynx as well as general hygiene. Importantly, Lee and Shibamoto (2001) reported that clove oil might also be used as an anti-carcinogenic agent due to its antioxidant properties.

Their results also suggested that clove oil might be of use as a potential chemo preventative agent (Zheng *et al.,* 2005). Research has shown that clove oil is an effective mosquito repellent (Trongtokit *et al.,* 2005). However, clove oil is toxic to human cells (Prashar *et al.,* 2006). If ingested or injected in sufficient quantity, it has been shown to cause life threatening complications, including Acute Respiratory Distress Syndrome, Fulminant Hepatic Failure and Central Nervous System (Prashar *et al.,* 2006).

1.3 Scope of the study

The scope of this study was to evaluate the potentials of four local Nigerian spices for food preservation and promotion of good health.

1.4 Purpose of the study

Evaluate antimicrobial properties of the four spices against strains of *Escherichia coli*, *Salmonella typhii* and *Staphylococcuss aureus* in nutrient broth, that cloves should be used at times instead of consumption of a lot of antibiotic drugs which may lead to inhibited some medically important bacteria proves that the plant might have some potential as an alternative source of antibacterial substances.

The public should be enlightened on the use of herbs for the treatment of ailments and infections. The use of plants should not be indiscriminate, or based on rumours. resistance if taken excessively. The fact that the extract of these medicinal plants.

1.5 Significant of the Study

This study will provide detailed information on phytochemical compositions, antioxidant and antimicrobial of these four spices for broader application in foods and other relevant areas. The spices, with information on phytochemical, antioxidant and antimicrobial properties, would attract international recorgnitions that can earn Nigeria huge revenue. This would create employment for many Nigerians who would propagate and process the spices.

The antimicrobial activity of clove and clove bud oil were investigated by agar well diffusion method against five dental caries causing microorganisms namely Streptococcus mutans, Staphylococcus aureus, Lactobacillus acidophilus (bacteria), Candida albicans and Saccharomyces cerevisiae (yeast). The results indicated that clove and clove oil have a potent antimicrobial activity against the tested dental caries causing microorganisms. The highest antimicrobial activity of clove was found against *Saccharomyces cerevisiae* (25.32mm) in methanolic extract and an MIC of 50mg/ml and that of clove oil was found against Streptococcus mutans (34.32mm) with a MIC value of 3.125mg/ml.

1.6 Objective of this Study

The objective of this study was to assess

1. The in vitro antibacterial activity of different extracts of cloves against selected food borne pathogens and spoilage bacteria,
2. The minimum inhibitory concentration (MIC) against each bacterium,
3. The effect of pH and temperature on the antibacterial activity of their EOs, and
4. Application of clove EOs to inactivate L. monocytogenes in ground chicken meat.

1.7 Aims of the Study

The aim of this study research contains

1. The evaluation of the antibacterial activity of aqueous extracts isolated from Clove (Syzygiumaromaticum) against gram-positive Staphylococcus aureus.
2. Due to limited choice of antibiotics, medicinal plant extracts have gained interest because of their known antimicrobial nature.
3. Medicinal plants are the richest bio resource of drugs for traditional systems of medicine, nutraceuticals, food supplements,

1.7 Justification for the study

The paucity of knowledge of the phytochemical constituents, antioxidant and antimicrobial properties of these indigenous herbs and spices has resulted in their neglect and underutilization. It is envisaged that the result of this study will initiate the exploitation of the preservative, nutraceutical and therapeutic potentials of these culinary herbs and spices.

CHAPTER TWO

2.0 Literature Review

2.1 Scholarly Reviews On Anti Microbial Activities Of Cloves

Clove (*Syzgium aromaticum*) is one of the most valuable spices that have been used traditionally as food preservative and for many therapeutic purposes. Cloves is native of Indonesia but it has also been cultured in several parts of the world including Pakistian. This plant represents one of the richest sources of phenolic constituents as augenol, and eugenol, and eugenol acetate and possess great potential for pharmaceutical, cosmetic, food and agricultural applications. This article includes main studies reporting the phytochemical profile and pharmacological activites of cloves and eugenol. The antioxidant and antimicrobial activities of cloves are higher than many fruits, vegetables and offer spices. Toxicological studies are also mentioned. The different studies reviewed in current work authenticate the traditional use of clove as food preservative and medicinal plant. Key words *Syzgium aromaticum,* eugenol, antioxidant (Shahid Hussain 2011)

Cloves are native to the Maluku islands in Indonesia where it has grown for thousand of years without needing to be planted by people by people. The first clove tree was planted around the 16-17th century during the spice trade wars when the Dutch East India Company wanted a monopoly on the clove crop (Thangeslvabai *et al.,* 2010). Clove is one of the most ancient and valuable spices of the orient and holds a unique position in the international spices trade. Native to Moluccas, the so called ‘ Spices islands in the East Indian archipelago, this spice was first introduced in India around 1800 A.D. by the East India Company (Gordon 2009).

Harbone (2010), examined the extraction of cloves oil by steam distillation the dried flower bids were grinded and 120g weighed out, placed in a round bottom flask and water was added approximately three-quarter full. Heat was applied from the heating mantle and as the water in the flask boiled, steam carrying the volatile oil rose through the neck of the flask. Distillation was continued until there was no more difference in successive readings of the oil volume. The oil was drained off and dried over anhydrous sulphate (BDH). The density of the oil was determined according to the weight volume ratio (wlv)

Hili *et al.,* (2015) carried out to investigate on the bacteria pathogens found in digestive tract infections and assess antimicrobial activities of clove 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 on cloves extracts was analyzed at INES-Ruhen geri in clinical microbiology laboratory. Cloves has shown to have an antibacterial activity on bacteria isolated from digestives tract of infected patients. It can be used as a medicine to treat these infections.

Shan *et al.,* (2017) examined the anti microbial activity of cloves. *Syzygium aromaticum* extracts against food borne pathogenic bacteria. Their study was conducted to determine the antibactreria activity of cloves extract using the agar diffusion assay against food pathogens (*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa*). The agar diffusion test revealed inhibition zone of clove sample compare ethanolic extract , methanolic extract was showing best result against gram positive culture *staphylococcus aureus* (*staphylococcus aureus* (MTCC 2940) and two gram negative cultures pseudomonas aeruginosa (MTCC2453) and *Escherichia coli* (MTCC739)

Abed (2014), examined the invitro antibacterial activity of the essential oil of cloves and the effect of temperature on antibacterial activity. Essential of cloves (*Syzygium aromaticum*) was obtained by steam distillation using a Cleaver- type system. The oil obtained was completely evaporated, leaving the absolute essential oil. The oil was analyzed for the determination of antibacterial activity against *staphylococcus aureus*, *Escherichia coli*. The effect of temperature on the antibacterial activity of essential oil of cloves against Escherichia coli, staphylococcus auerus was determined. The essential oils for cloves showed antibacterial activity after treatment at 100c for 30mins suggesting that the high temperature does not affect the activity of E.O

Optik (2018), investigated the physical, optical and antimicrobial properties of syzygium aromaticum (cloves). Copper nanoparticles (CUNPS) were prepared using syzygium aromaticum (cloves) bud extract through simple and eco-friendly route. The synthesized nanoparticles were subjected to structural, morphological, optical and antimicrobial studies. The high crystalline nature of CUNPS with a face centered cubic phase is evident from the x-ray diffraction (XRD) pattern. morphological studies were used to study the shape and size of the synthesized nanoparticles. Energy dispersive spectroscopy (EDS) attests the high intense metallic peak of copper (cu), oxygen (O), chlorine (cl) and phosphorus (p) elements due to the capping action of bio molecules of bud extract in (CUNPS) formation. The positive test results of zone of inhibitions of 8mm and 6mm were attained against *Bacillus spp* and *penicillium spp* respectively.

Maati (2016) investigated the phenolic extracts of cloves (*syzygium aromaticum*) with novel antioxidant and antibacterial activities. Ethanol (80), Ethyl acetate, and water were used to extract bioactive phtyochemicals from cloves, scanning and transmission electron microscopy was applied to study the effects of ethanol extracts on the morphology and membranes of tested bacterial cells. Cloves extracts exhibited antibacterial activities against the growth of *Staphylococcus aureus* and

Escherichia coli in concentration range from 50 to 100 ug/mL .the results indicated that the extracts with stronger antibacterial capacity also had higher phenolic content. Scanning and transmission electron microscopy showed that ethanol extract damaged the morphology and membranes of the tested bacterial cells.

Rana *et al.*, (2014),determined the antifungic activity of clove oil in different strains and reported this scale of sensibility > Microsporum gypseum > Fusarium monoliformeNCIM110 > Fusarium oxysporiumMTCC284. The chromatographic analyses showed that eugenol was the main compound responsible for the antifungic activity due to lysis of the spores and micelles.

The company's spice garden in Courtland in Tamil Nadu was then established to cultivate clove and nutmeg as the principal spice crops. Induced by the success of the cultivation in Courtallam, cultivation of clove was extended during the period after 1850 A.D. to Nilgiris (Burliar) in Tamil Nadu, Southern regions of Travancore and also to Cochin State on the slope of Western Ghats

Clove (*Syzygium aromaticum*), tropical evergreen tree of the family Myrtaceae and its small reddish brown flower buds used as a spice. Cloves were important in the earliest spice trade and are believed to be indigenous to the Moluccas, or Spice Islands, of Indonesia. Strong of aroma and hot and pungent in taste, cloves are used to flavour many foods, particularly meats and bakery products; in Europe and the United States (Nevas, 2014).

2.2 Taxonomy

Scientific classification

Kingdom	Plantae
Division	Magnoliophyta
Class:	Magnoliopsida

Order: Myrtales

Family: Myrtaceae

Genus: Syzygium

Species: *S. aromaticum*

Source: (Merr & Perry 2010)

2.3 Spices

Spices are indispensable components of cuisines used mainly for flavouring to improve palatability of food (Okigbo, 2007; Okafor, 2008). A spice is a dried seed, fruit, root, bark, flower, leave or any vegetative substance used in a very small quantity as food additive to colour, flavour or preserve food. The United States Food and Drug Administration (FDA) defined spices as aromatic vegetative substances used for seasoning of food and from which no portion of any volatile oil or flavouring principles have been removed, and are free from artificial colouring matters, adulterants and impurities (Farrel, 2012). Spices are ‘‘Generally Recognized As Safe’’ (GRAS) by the FDA, at least at concentrations commonly found in foods. (Cervenka 2016)

Spices contribute very minimal nutrients to menu because they are used a very small amount. The bulk of the major components of spice materials consist of carbohydrate, protein and little minerals. Tannins, resins, pigments, volatile, essential and fixed oils which contribute to flavouring occur in traces and constitute only a small fraction of the dry matter (Cowan, 2019). Some well known spices of commerce include red pepper, onions, sage, ginger, nutmeg, clove, cinnamon, mustard, curry, turmeric, rosemary and garlic. Spices add flavour, relish and pungency to diets. Most spices are fragrant, aromatic and pleasant. Spices in food also exert such secondary effects as salt and sugar reduction, prevention of spoilage and improvement of texture (Ravindran *et al.*, 2012).

The use of spices as colorants and preservatives is becoming fashion in the food industry, and among these spices, *Syzygium aromaticum* is of great important (Gassara, Kouassi, Brar, & Belkacemi, 2017). Compared with sodium benzoate, potassium sorbate and other chemical food preservatives, clove oil, the main volatile constituent of S. aromaticum, expresses various advantages on antimicrobial activity, aromas, and safety, and is an ideal substitute of chemical food preservatives. Eugenol, the most important composition of clove oil, has been accepted as food preservatives by China, the United States, the European Union, and other countries and regions. Otherwise, many oral care product products containing clove oil have appeared on the market in China, these products express beneficial effects on halitosis, dental plaque, oral bacteria, and allergic pain of tooth (Zhang, Chen, & He, 2009).

2.4 Health Benefits

2.4.1 Cloves can help regulate your hunger levels

Cloves are kind of magic because they can play a role in keeping your stomach from growling between meals. "For a spice, cloves have an impressive amount of fiber a nutrient that can help regulate your hunger levels," says nutritionist "One teaspoon of cloves alone provides close to a gram of fiber.

2.4.2 Cloves can keep your sugar levels in check

Anyone who is watching their blood-sugar levels will be happy to know one great strategy for keeping things in order is to simply add cloves into their meals. "Cloves provide manganese, a mineral that can help regulate blood sugar levels," Gorin (2009)

2.4.3 Some notable health benefits of cloves include:

Reduced Inflammation: Cloves include multiple compounds that are linked to anti-inflammatory properties. Eugenol is the most important of these compounds. Eugenol has been shown to reduce the

inflammatory response in the body, reducing the risk of diseases such as arthritis and helping to manage symptoms.

Fewer Free Radicals: Eugenol is also a potent antioxidant. Cloves are full of antioxidants. These compounds help your body to fight free radicals, which damage your cells and can lead to disease. By removing free radicals from your system, the antioxidants found in cloves can help reduce your risk of developing heart disease, diabetes, and certain cancers.

Reduced Ulcers: There is some evidence that cloves can help protect your stomach from ulcers. Most ulcers are caused by thinning in the layers of mucus that protect your stomach lining. Preliminary studies show that eaten in large quantities, cloves can thicken this mucus, lowering your risk of developing ulcers and helping existing ulcers heal.

Improved Liver Function: Cloves may also promote better liver function. Some trials have shown that the eugenol found in cloves can help reduce signs of **liver cirrhosis** and fatty liver disease. It may also improve general liver function.

2.5 Medicinal properties and Health benefits of Cloves

1. The active principles in the clove are known to have antioxidant, antiseptic, local anesthetic, anti-inflammatory, rubefacient (warming and soothing), carminative and anti-flatulent properties.
2. The spice contains health benefiting essential oils such as **eugenol.** It is a phenylpropanoids class of chemical compound which gives pleasant, sweet aromatic fragrances to the clove-bud. Eugenol has local anesthetic and antiseptic properties, hence, useful in dental care essentials as well as in treatment procedures.
3. The active principles in the cloves increase the gut motility and digestion power through improved gastrointestinal enzyme secretions. Thus, it helps relieve indigestion and constipation problems.

4. The spice also contains a good amount of minerals like potassium, manganese, iron, selenium and magnesium. Potassium is an essential electrolyte of cell and body fluids that helps control heart rate and blood pressure. The human body uses manganese as a co-factor for the antioxidant enzyme, superoxide dismutase.
5. Further, the spice buds contain very good amounts of vitamin A and beta-carotene levels. These compounds are known to have antioxidant properties. The body also requires vitamin-A for maintaining healthy mucosa, skin and for healthy vision. Consumption of natural foods rich in flavonoids helps to protect the body from lung and oral cavity cancers.

2.6 Uses of Cloves

Clove is very aromatic and fine flavoured and imparts warming qualities. In all Indian homes, it is used as a culinary spice as the flavour blends well with both sweet and savoury dishes. Clove is used for flavouring pickle, curries, ketchup and sauces. It is highly valued in medicine as a carminative, aromatic and stimulant. Clove has stimulating properties and is one of the ingredients of betel chewing.

In Jawa, clove is used in preparation of a special brand of cigarette for smoking. The essential oil which is obtained by distilling clove with water or steam, has even more uses. It is used medicinally in several ways. The chief constituent of the oil eugenol, is extracted and used as an imitation carnation in perfumes (Enzo 2012).

2.7 Side Effects

Clove extract taken by mouth in food amounts not enough is known about the safety of taking clove by mouth in larger medicinal amounts. Clove oil or cream containing clove flower is when applied to the skin. However, frequent and repeated application of clove oil in the mouth or on the gums can sometimes cause damage to the gums, tooth pulp, skin, and mucous membranes. (Snyder 2017).

Inhaling smoke from clove cigarettes or injecting clove oil into the veins is and can cause side effects such as breathing problems and lung infections. Dried clove can also cause mouth sensitivity and irritation, as well as damage to dental tissues (Snyder 2017).

2.8 Special Precautions & Warnings

Children: In children, clove oil can cause severe side effects such as seizures, liver damage, and fluid imbalances.

Pregnancy and breast-feeding: There is not enough reliable information about the safety of taking clove in medicinal doses if you are pregnant or breast-feeding. Stay on the safe side and avoid use.

Bleeding disorders: Clove oil contains a chemical called eugenol that seems to slow blood clotting. There is a concern that taking clove oil might cause bleeding in people with bleeding disorders.

Surgery: Clove oil contains a chemical called eugenol that seems to slow blood clotting. There is a concern that it might cause bleeding during or after surgery. Stop using clove at least 2 weeks before a scheduled surgery.

2.9 Production and Traditional Use of S. aromaticum

Clove *S. aromaticum* is a Syzygium gaertn genus plant of Myrtaceae family originated in the Moluccas Island of Indonesia. This plant is introduced into Guangdong Province of China from Philippines at 200 to 206 BC, and is used in Egypt and India since 1st and 2nd century AD. Since 15th century, clove is supplied to European countries such as Portugal (1500s), Netherlands (1600s), France (1700s), and England (1792) by the Arab sailors. Nowadays, statistics data of the Food and Agriculture Organization (FAO) suggests that almost all the production of clove is mainly concentrated in Asia (74.0%) and Africa (26.0%), and that of the Oceania and Latin America is less than 1.0% (Kamatou, Vermaak, & Viljoen, 2012).

(A): Clove dried flower buds, (B) Clove plant, (C) Cloves flowering, (D) Fruits and seeds.

2.10 Cloves have antibacterial properties

Sure, chemical-free mouthwash (and fruit and green tea) is a great way to improve your oral hygiene—but it might work even better with the addition of cloves. "Clove oil has been investigated as an antibacterial agent" Gorin says. "In one study, a mouth rinse containing clove, basil, and tea tree oil was found to help fight plaque and bacteria in the mouth." That's also why you'll find clove oil in plenty of popular (Orwa 2009).

Cloves are a great source of beta-carotene, which helps give them their rich brown color. The carotene family of pigments are important antioxidants and pro vitamins. Carotene pigments can convert into vitamin A, an important nutrient for keeping your eyes healthy (Pandey 2011).

2.11 Soil and Climate Required for Growing Cloves:

Deep and rich loams with high humus content are best suited for clove cultivation. In India, clove has developed well in the open sandy loams and the laterite soils of South Kerala region. But the best growth is seen in black loams of the semi forest regions. Clove abhors water logging and, therefore, perfect drainage is essential.

Clove is strictly a tropical plant and it requires a warm humid climate. Although there has been a general belief that clove requires proximity to sea for the proper development and cropping,

experience in India has shown that the trees do well in the submontane regions and have been found to perform better than those in other areas (Nanasombat 2015)

2.12 Antimicrobial Activity

Eugenol exhibits antimicrobial activity toward fungi, as well as a widely range of gram-positive and gram-negative bacteria. Eugenol is naturally occurring essential oil i.e, hydroxyphenyl propene belonging to family mytraceae responsible for human infection diseases, Oral cavity eugenol disease, food-borne pathogens eugenol demonstrates bioactive compounds with broad range anti-microbial activity against both plank-tonic and sessile cells carrying foodborne micro-organisms and human diseases (Marchese *et al*, 2017).

Antimicrobial activity of a plant or a substance can be defined as its ability to kill or inhibit the growth, metabolism, and replication of a microorganism, ultimately leading to its death. The discovery of the antimicrobial effects of certain plants, chemicals, and substances (synthesized or found in nature) has been of great impact and importance in microbiology and medicine. It has led to the development of antibiotics, and alternative herbal remedies for the treatment and control of contamination and infection caused by microorganisms (Aneja and Joshi 2009)

2.12.1 Antimicrobial properties of cloves

Clove oil has biological activities, such as antibacterial, antifungal, insecticidal and antioxidant properties, and is used traditionally as a savoring agent and antimicrobial material in food. In addition, clove oil is used as an antiseptic in oral infections (Aneja and Joshi 2009)

2.12.2 Anti-Oxidant Activity:

Antioxidant activity above essential oil separated significant eugenol compounds and its derivative are determineology using two the free radical scavenging assays in vitro-model involving di-phenyl-1-picryl hydrazyl (DDPH)and nitric oxides. The percentage inhibition was measured and

reports were represented using ascorbic acid as normal in a term of IC50 val – uses (Concentration when compared at which free radicals is inhibition by 50 per cent) (Kaur *et al.,* 2019).

This activity is evaluated with DPPH. Radical scavenging activity is the very common method to determine the activity. Capturing of free radical by eugenol is expressed as IC50, Expresses the concentration needed to capture redicals as a medium of 50 per cent. As a result it shows strongly decreases anti-oxidant activity (Da Silva *et al.,* 2018).

2.12.3 Antidiabetic Activity:

There work is hypo-the sized to evaluate the anti-hyperglycemic ability are eugenol via defining activity are enzymes the involved in streptozotocin (STZ)-induced glucose metabolized in a diabetics rats. Increases in the activity of major carbohydrate metabolized enzymes such as hexo-kinase, pyruvate-kinase, glucose-6- phosphate-dehydrogenase, glucose-6-phosphatase, fructose-1, 6-bis-phosphatase, and liver biomarkers (AST, ALT, and ALP), creatine-kinase, and blood urea nitrogen's in serum, and diabetes rat blood have appreciably returned to nearly normal rates via eugenol administration.

Eugenol dosing to improve body weight for diabetic rats and hepatic glycogen quality exemplify eugenol's anti-hyperglycemic ability in diabetic rats. Current findings indicate that eugenol in experimental diabetes may potentially boost activities of enzyme of glucose metabolized, and the prudent are extend from a scope the eugenol used in a trial of alleviate the adverse effect of diabetes (Srinivasan *et al.,* 2013).

2.12.4 Anti-Inflammatory Activity

Essential oil open breathing channels, serving as an expectorant to treat many of the upper respiratory disorders including colds, eye sties, bronchitis, sinus disorders, S cough and asthma. In

traditional clinical medicine, clove was used to relieve nasal hindrance and musculoskeletal discomfort that indicates its anti-inflammatory function and the action is attributed to COX-2 inhibition. When inhaled, the aromatic oil could help to alleviate such breathing problems such as cough, colds, asthma, bronchitis, and sinusitis (Mittal. *et al.*, 2014).

2.12.5 Antiviral Activity

Plaque reduction assays investigated antiviral activity and the anti - viral mechanism of action was determined by administering the drugs to uninfected cells, infected viruses or herpesvirus-infected cells. Phenylpropanoids inhibited about 60–80% HSV infection and 40–98% sesquiterpenes that prevented herpes virus infection. Anise essential oil exhibited anti-HSV-1 activity on all isolated compounds by direct inactivation of free virus particles. Tested medications bind with particles of the herpesvirus, inactivating viral infectivity. The antiviral potential of the βcaryophyllene suggests that here in essential oils phenylpropanoids and sesquiterpenes contribute to their antiviral function as opposed to HSV (Astani *et al.*, 2009).

2.12.6 Anticancer Activity

Showed cytotoxic effects of antimutagenic activity against cancer cell lines. Clove oil extracts demonstrated cytotoxic activity against cervical cancer Hela cells, MCF-7 cells and MDA-MB-231 cells for breast adeno-carcinoma, DU-145 cells for prostate-cancer, TE-13 cells for oesophageal-cancer. Cytotoxicity of methanol extract from clove buds was also reported against melanin formation, and it was found that aqueous clove infusion had a promising function in limiting the carcinogenesis process in 9, 10- dimethyl benz (a) anthracene-convinced skin carcinoma. There were also studies of anti-mutagenic activities of clove seeds extract (Mbaveng et al,. 2017).

2.12.7 Anti Bacterial Activity

This research was conducted againsts gram-positives and gramnegatives bacteria, pathogenic fish bacteria isolated from Korea's cultivated olive flounder. CEO includes 7 chemical compounds including 83.63 percent eugenol disc - diffusion assay, micro, mbc test indicates that ceo eugenol inhibits growth gram positive and gram negative bacteria (Pathirana *et al.,* 2019).

2.12.8 Antifungal Activity

Antifungal clove activity is increased if collected by isomerizing double bond or adding nitro group on the aromatic ring. Based on the relationship between fungicides, chemical structure and mechanism of action (MOA), activity for commercial fungicide BC-1000 is highly documented. As a result, the compound shows potential impact and can be used to design the natural compound's new and efficient balance (Olia *et al.,* 2019).

2.12.9 Anesthetic Activity

Anesthetic Activity in study, methyl-Isoeugenol show effects and was only active when higher dose is given. Isoeugenol as compound to methylisoeugenol shows similar activity and potency between eugenol and methyleugenol.eugenol is a allyl compound and is more active than propenyl isomer (iso eugenol) (Dallmeier *et al.,* 2018).

2.13 Phytochemicals

Phytochemicals consist of a large group of naturally occurring non nutrient, biologically active compounds found in plants. As implied by the prefix “phyto” in the name, phytochemicals are basically produced only by plants. Phytochemicals act as natural defense system for the host plants and in addition provide colour, aroma and flavour. Plants use phytochemicals as natural protection from bacteria, fungi and viruses (Ramanthan *et al.,* 2019; Duyff, 2015). More than 4000 of these compounds have been discovered and it is expected that scientists will yet discover many more phytochemicals in plant foods such as fruits, vegetables, legumes, cereals, herbs and spices (Rowland, 2019; American Institute of Cancer Research, 2010).

Phytochemicals give hot pepper the burning sensation, onions and garlic the pungent flavour and tomatoes their red colour. Phytochemicals can have profound physiological effects, act as antioxidants, mimic body hormones and suppress development of diseases in the body (Milner, 2012; Lesschaeve and Noble, 2015; Hayes, 2015). Phytochemials are needed in daily meals for proper healthy living.

2.14 Pharmacological Activities of Clove oil

Pharmacologically, clove and its main constituents possess antimicrobial, antioxidant, anti-inflammatory, analgesic, anticancer, and anesthetic effects. Moreover, they showed insecticidal, mosquito repellant, aphrodisiac, and antipyretic activities

Clove is a pharmacologically active medicinal plant that includes Anti-oxidant, anti-pyretic, anti-viral, anti-microbial, anti-diabetic, anti-inflammatory, analgesic, anti-platelet, anti-stress, anti-disease, and anti-carcinogenic in cervical cancer. The clove is available in three types of essential oil (Thangaselvabai *et al.*, 2010).

CHAPTER THREE

3.0 Materials and Methods

3.1 Materials

Materials used for this research work are as follow:

Autoclave, Incubator, Conical flask, Beakers, Measuring grinder, Spatula ethanol, Methanol and Distilled water

3.2 Methods

3.2.1 Sample Collection

Cloves were purchased from Uchi market in Auchi Etsako West Local Government Area of Edo State. The dried cloves were grinded into powder using an electric blender

3.3 Preparation of Extract

100g of dried cloves was soaked in 300ml of each of the solvent (ethanol and methanol) used and kept at room temperature for 48hiours. During this period, shaking of the flask was filtered using what man filtered paper No.1. The filtered solutions were using a rotary evaporator.

3.4 Photochemical Screening

Photochemical analysis were studied by the following methods:

Glycosides were estimated by Killer-kiliani Test, Terperiods by Salkowski Test, tannins by ferric chloride test, alkanoids by Hager's Test, carbohydrate by Molish Test, protein by burette test (Hajoori *et al., 2014)*

3.5 Screening of Microorganism

The test organisms were collected from the stock cultures of cottage Hospital of Auchi Polytechnic, Auchi, Edo State, Nigeria. The microorganisms are *Staphylococcus aureus, Escherichia coli and Candida albicans*

3.6 Sensitivity test (Agar well Diffusion Method)

The microorganisms seeded on prepared nutrients Agar and SDA (Sabourald Dextrose Agar) were poured to colonize by the pathogenic organism. Using a flamed cork borer of 1mm, wells were punched into the seeded plates 1ml of the cloves extracts were then poured and left for 24hours (bacteria) and 72 hours (fungi) respectively. The plates were incubated at 37^{o}c and 25^{o}c for 24hours and 48hours respectively. The diametric zones of inhibition were measured in millimeter

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

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

Table 1: Antimicrobial activities of ethanolic cloves extract on tested microorganism

Tested Microorganisms	**Zone of Inhibition (mm)**
Staphylococcus aureus,	12
Escherichia coli	10
candida albicans	18

The above table shows that the ethanolic cloves extract has antimicrobial effect on tested microorganisms (*Staphylococcus aureus, Escherichia coli and Candida albicans*) from the result, it is also observed that zone of inhibition is higher in *Candida albicans* than *Staphylococcus aureus, Escherichia coli and Candida albicans*

Table 2: Antimicrobial activities of methanolic cloves extract on test microorganisms

Tested Microorganisms	**Zone of Inhibition (mm)**
Staphylococcus aureus,	20
Escherichia coli	21
Candida albicans	26

Table 2: Shows that the methanolic cloves extract has antimicrobial effect on tested microorganism *(Staphylococcus aureus, Escherichia coli and Candida albicans)* from the result, it is observed that the zone of inhibition is higher in *Candida albicans*, followed by *Escherichia coli Staphylococcus aureus.*

The result from table 3 shows the aqueous cloves extract has antimicrobial effect on tested microorganisms (*Escherichia coli and Candida albicans)* excepte for Staphylococcus aures which has no zone of inhibition. For aqueous cloves extract, the zone of inhibiton is higher in *Escherichia coli.*

Table 3: Photochemical analysis of cloves extract

S/N	Photochemical	Methanolic Extract	Ethanolic Extract
1	Carbohydrates		
	a. Molisch's test	+	+
	b. Benedict's test	+	+
	c. Fehling's test	-	+
2	Protein		
	a. Xanthropotic test	-	+
	b. Biuret test	-	-
3	Lipid		
	a. Solubility test		

	b. Glycerol test c. Sudan III test	- - -	- - -
4	Alkaloids a. Mayer's test b. Dragen droff's test c. Wagner's test	 + + +	 + + +
5	Saponins a. Foam test	 -	 -
6	Flavonoids test	+	+
7	Resins test	-	+
8	Tannins test a. Gelatin Test b. Lead Acetate Test c. Ferric chloride Test	 + + +	 + + +
9	Sterols a. Salkowski Test	 +	 +
10	Cardiac Glucosides Test a. Keller-Killiani Test	 -	 -
11	Triterpeness Test	+	-
12	Anthraquinones	-	-

Keys: Negative Sign (-) = Absent Positive Sign (+) Present

Antimicrobial activities of cloves extract

4.2 Discussion

The methanolic and ethanolic solutions of the extract were found to have potent antimicrobial activity against all the test organisms.

Cloves extract exhibited a broad spectrum antimicrobial activity with a minimum zone diameter of 10mm for *Escherihia coli* isolate and a maximum zione diameter of 26mm for *Candia albicans.*

Cloves extract are known to posses some antimicrobial activities and are used in various food preparations as flavor enhancers and in herbal medicine. Briozzo, *et al.,* 2015. Though the modes of action of the extract are not known, they are antimicrobial agents include thymol, terpenes eugenol, flavones, glycosides of phenoliv monoterpenoids and aliphatic alcohols among other elements (Deans and Ritchie, 2012).These substances acting alone of antimicrobial activity exhibited for both bacteria and fungi. Cloves extract posses antimicrobial activity against *Staphylococcus aureus, Escherichia coli* and *Cadida albicans.* These findings agree with the work of others such as (Gislene *et al.,* Hammer *et al.,* and Hili *et al.,* 2010).

In this study, *Candida albicans* was found to be most susceptible to cloves extract. Similar findings were reported by others such as (Hili *et al.,* Panizzi *et al.,* and Piccaglia *et al.,* 2012). Therapeutic agent with high killing propensity for fungi are few. The reason why yeast is more

susceptible to the extracts than bacteria is unclear but it may be that at any given time these extracts may break up structural integrity of Candida .albicans faster than they dissociate bacteria. In this study, the incubation time and temperature were the same for bacterial and fungi.

The inhibition zones obtained with cloves extract against *Staphylococcus aureus, Escherichia coli* and *Candida albicans* were greater than those obtained by other workers such as (Cosention *et al.,* and Singh Sangwan 2014). The differences may be due to the methods used in solubilizing the extracts to obtain hydrophilic molecules.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.0 Conclusion

The cloves extract has a group of secondary metabolictes including alkaloid, flavonoids, saponin, tannin and tripterpenoids. The extract has effective antimicrobial activity against *E.coli, Staphylococcus aureus* and Candida albicans with exception of the aqueous extract that could not inhibit Staphylococcus aureus. This result also confirms that use of ethano and methnol as good extraction medium for the active ingredients I cloves extract against microorganism

5.2 Recommendation

From the result of this study, it can be recommended that ethanol and methanol should be used for cloves extraction for effective result. Also it is recommended that cloves extract should be used for its therapeutic and pharmaceutical purpose as it could form a good antimicrobial agent

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