PROJECT REPORT

ON

ANTIBACTERIAL ACTIVITY OF CLOVE EXTRACTS AGAINST PSEUDOMONAS AERUGINOSA AND ESCHERICHIA COLI

BY

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CERTIFICATION

This is to certify that this project work was carried out by 'ABDULRAHMON KEHINDE TEMITOPE ' with Matriculation Number 'HND/23/SLT/FT/0468', as part of the requirements for the Award of Higher National Diploma (HND) in Science Laboratory Technology (SLT).

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DEDICATION

I dedicate this project work to Almighty God who begin this work with me and made the success of it to an end.

I also dedicate this work to my beloved parent Mr and Mrs Abdulrahmon who finance and support me academically to achieve my aim.

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Surely all praise is for Almighty God, the lord of the universe. I thank him for given me strength and idea for doing this project, without him nothing would have being possible. He is the creator and Giver of knowledge, wisdom and ideal that make the things possible. I thank him for his protection and guidance always. I seek his forgiveness and refuge in him from the evil he has created.

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ABSTRACT

Pseudomonas aeruginosa and *Escherichia coli* are common nosocomial pathogens known for their multidrug resistance. This study evaluated the antibacterial activity of aqueous, ethanolic, and methanolic extracts of *Syzygium aromaticum* (clove) against both organisms. Extracts were prepared by maceration in the respective solvents, and their antibacterial activities were assessed using the agar well diffusion method. MIC and MBC were determined via broth dilution. All three extracts showed concentration-dependent activity. The methanolic and ethanolic extracts exhibited higher inhibition, with zones reaching 24.40 mm and 25.10 mm respectively, while the aqueous extract produced moderate effects. MIC for both organisms was 80 mg/mL, while MBC ranged from 80–100 mg/mL. The results affirm the broad-spectrum antibacterial potential of clove, especially when extracted with organic solvents.

CHAPTER ONE

1.0 Introduction

The burden of infectious diseases remains a major global health concern, especially in developing nations, where poor hygiene, limited access to quality healthcare, and the misuse of antibiotics contribute significantly to the spread of bacterial pathogens. Antibiotics have been central to modern medicine for the treatment of bacterial infections; however, over the past few decades, the world has witnessed an alarming increase in the incidence of multidrug-resistant (MDR) bacteria. This phenomenon has rendered many commonly used antibiotics less effective, leading to prolonged illnesses, increased mortality rates, and higher medical costs (WHO, 2023).

One of the most troubling examples of antibiotic resistance is observed in *Staphylococcus aureus*, especially the methicillin-resistant strain (MRSA), which is known to cause a range of infections, including wound infections, sepsis, and pneumonia. Similarly, *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*, and *Salmonella typhi* have developed resistance to multiple antibiotics, posing a challenge to clinicians and microbiologists alike (Aslam *et al*., 2022; Nirwati *et al*., 2022).

In response to the growing threat of antimicrobial resistance, there has been renewed interest in exploring natural products, especially medicinal plants, for their therapeutic potential. Medicinal plants are rich sources of bioactive compounds such as alkaloids, flavonoids, tannins, terpenoids, phenolics, and essential oils, many of which possess antimicrobial properties (Tahsiri *et al*., 2023). These natural products have historically contributed to drug discovery and are gaining popularity for their availability, low toxicity, and cost-effectiveness.

Syzygium aromaticum (clove), an aromatic flower bud of the Myrtaceae family, has long been used in traditional medicine for treating toothache, gastrointestinal disorders, and microbial infections. Clove is particularly rich in eugenol, a compound known for its potent antibacterial, antifungal, antiviral, and antioxidant properties. Studies have shown that clove extracts can damage bacterial cell walls, inhibit nucleic acid synthesis, and interfere with microbial metabolism (Singh *et al*., 2021; Aziz *et al*., 2023).

The solvent used for extracting plant bioactive compounds plays a critical role in determining the yield and potency of the extract. Polar solvents like ethanol and methanol often extract more phenolic compounds, while water, though safer and more environmentally friendly, may be less efficient (Saleh *et al*., 2022). Hence, comparative evaluations of different solvents are important to identify the most effective extraction method for antibacterial activity.

Clove extracts prepared with various solvents have been investigated for their activity against both Gram-positive and Gram-negative bacteria. Gram-positive bacteria like Staphylococcus aureus have a thick peptidoglycan cell wall that can be penetrated by certain phytochemicals, while Gram-negative bacteria like *E. coli* and *P. aeruginosa* possess an outer membrane that can act as a barrier to many antimicrobial agents. Evaluating how clove extracts

affect these diverse bacterial groups can provide valuable insights into their potential application as natural antibacterial agents (Adeyemi *et al*., 2021).

1.1 Statement of the Problem

The global rise in antibiotic-resistant bacterial infections, has rendered many conventional antibiotics ineffective, increasing disease burden and treatment costs. The declining pipeline of new antibiotics and the continued misuse of existing ones have made it necessary to search for alternative antimicrobial agents. Despite the known medicinal value of clove, there is limited local data on the influence of different extraction solvents on its antibacterial efficacy against clinically relevant bacterial strains (WHO, 2023; Aslam *et al*., 2022).

1.2 Justification of the Study

This study is significant because it explores the antibacterial potential of *Syzygium aromaticum* against resistant clinical pathogens using solvents of varying polarity. Understanding how different solvents affect extract potency may support the development of standardized herbal formulations. The findings could also provide a foundation for phytomedicine-based interventions in managing antibiotic-resistant infections.

1.3 Aim of Study

The major aims of this study were to evaluate the antibacterial effects of ethanol, methanol, and aqueous extracts of *Syzygium aromaticum* (clove) against *Pseudomonas aeruginosa* and *Escherichia coli*.

1.4 Objectives of Study

The specific objectives of this study were to:

i. prepare three different extracts (ethanol, methanol, and aqueous) of clove;

ii. collect *Pseudomonas aeruginosa* and *Escherichia coli* from microbiology lab;

iii. determine the antibacterial activity of the extracts on clinical pathogen;

iv. determine the minimum inhibitory concentration and

v. determine the minimum bactericidal concentration

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Overview of Antimicrobial Resistance

2.1.1 Global Burden of Antibiotic Resistance

Antimicrobial resistance (AMR) has been declared one of the top 10 global public health threats by the World Health Organization (WHO, 2023). Resistant infections are responsible for prolonged illnesses, increased mortality, and greater financial burden on healthcare systems. According to recent estimates, drug-resistant infections cause over 1.27 million deaths annually worldwide, with a disproportionately high burden in Africa and Southeast Asia (Aslam *et al*., 2022).

2.1.2 Emergence of Multidrug-Resistant Bacteria

Bacterial pathogens have evolved various mechanisms of resistance, including the production of β-lactamases, modification of drug targets, and efflux pump expression. Notably, methicillin-resistant *Staphylococcus aureus* (MRSA), extended-spectrum β-lactamase

(ESBL)-producing *Escherichia coli*, and *Klebsiella pneumoniae* are now resistant to multiple classes of antibiotics. The continued evolution of these strains highlights the urgent need for novel antimicrobial solutions (Nirwati *et al*., 2022).

2.1.3 Need for Alternative Therapies

Due to the slow pace of new antibiotic discovery and the increasing resistance to existing drugs, there is growing interest in complementary approaches such as phytotherapy. Plant-based antimicrobials are being explored for their ability to target bacteria through multiple mechanisms while posing a lower risk of resistance development (Aziz *et al*., 2023).

2.2 Clinical Relevance of the Test Organisms

2.2.1 Staphylococcus aureus (MRSA and MSSA)

Staphylococcus aureus is a Gram-positive coccal bacterium commonly found in the human nasal passages and skin (Tong *et al*., 2015). It is an opportunistic pathogen capable of causing a wide range of diseases including skin infections, pneumonia, osteomyelitis, endocarditis, and toxic shock syndrome (Lowy, 2019). Methicillin-resistant *S. aureus* (MRSA) is a particularly virulent and drug-resistant strain that has become endemic in hospitals and increasingly prevalent in community settings (Lee *et al*., 2018). MRSA is resistant to all β-lactam antibiotics, which complicates treatment and increases morbidity, mortality, and healthcare costs (David & Daum, 2010). In contrast, methicillin-susceptible *S. aureus* (MSSA) remains responsive to a wider range of antibiotics but can still cause severe infections in immunocompromised individuals (Otto, 2013). Both MRSA and MSSA produce a host of virulence factors including toxins, enzymes, and biofilm-forming proteins which contribute to their pathogenicity and persistence in host tissues (Gordon & Lowy, 2008). Plant-derived antimicrobials such as clove extracts have been reported to inhibit biofilm formation and

bacterial adhesion in *S. aureus*, indicating potential use as adjunct therapies (Nassar *et al*., 2017).

2.2.2 Escherichia coli

Escherichia coli is a Gram-negative, rod-shaped facultative anaerobe that is commonly found in the lower intestine of warm-blooded organisms. While most strains are harmless and part of the normal gut microbiota, pathogenic strains such as Enteropathogenic *E. coli* (EPEC), Enterotoxigenic *E. coli* (ETEC), and Uropathogenic *E. coli* (UPEC) are significant causes of diarrhea, urinary tract infections, and sepsis, respectively (Kaper *et al*., 2004). The rise of extended-spectrum beta-lactamase (ESBL)-producing *E. coli* has severely limited the efficacy of first-line antibiotics, prompting the need for novel antimicrobial agents (Paterson & Bonomo, 2005). Clove extracts, particularly those containing high concentrations of eugenol, have demonstrated significant antibacterial activity against both susceptible and resistant strains of *E. coli* in vitro (Marchese *et al*., 2017).

2.2.3 Pseudomonas aeruginosa

Pseudomonas aeruginosa is an aerobic Gram-negative bacterium that is recognized for its ability to cause severe infections, especially in immunocompromised patients. It is a leading cause of hospital-acquired infections such as ventilator-associated pneumonia, catheter-associated urinary tract infections, and wound infections (Lister *et al*., 2009). The organism's intrinsic resistance to many antibiotics, combined with its ability to form biofilms and acquire resistance genes, makes treatment highly challenging (Oliver *et al*., 2015). In addition, *P. aeruginosa* employs quorum sensing to regulate virulence factor production and biofilm formation, both of which contribute to chronic infection and antibiotic tolerance (Jeyakumar *et al*., 2020). Clove extracts have been shown to disrupt quorum sensing pathways, inhibit biofilm

formation, and reduce the growth of *P. aeruginosa* in laboratory settings, highlighting their potential in the management of persistent infections (Jeyakumar *et al*., 2020).

2.2.4 Klebsiella pneumoniae

Klebsiella pneumoniae is a Gram-negative, non-motile bacterium that is commonly implicated in hospital-acquired infections, including pneumonia, bloodstream infections, surgical wound infections, and urinary tract infections (Podschun & Ullmann, 2018). It is known for its polysaccharide capsule, which serves as a major virulence factor by inhibiting phagocytosis (Navon-Venezia *et al*., 2017). The recent emergence of carbapenem-resistant *K. pneumoniae* (CRKP) has posed a major public health threat due to limited treatment options and high mortality rates (Pitout *et al*., 2015). In vitro studies have demonstrated that clove extracts possess inhibitory effects against *K. pneumoniae*, possibly through membrane disruption and inhibition of essential metabolic pathways. These findings suggest that plant-based antimicrobials could be beneficial in combating infections caused by resistant strains (Nabavi *et al*., 2015).

2.2.5 Salmonella typhi

Salmonella enterica serovar Typhi is a human-restricted pathogen that causes typhoid fever, a systemic illness characterized by high fever, abdominal pain, and diarrhea. The bacterium is transmitted through contaminated food and water and is prevalent in developing countries with poor sanitation and hygiene practices (Crump & Mintz, 2010). The emergence of multidrug-resistant (MDR) *S. typhi* strains has greatly complicated the management of typhoid fever, especially with resistance to chloramphenicol, ampicillin, and trimethoprim-sulfamethoxazole (Parry *et al*., 2002). Clove extracts have been observed to exert strong antibacterial activity against *S. typhi* in vitro, particularly when extracted with methanol or

ethanol (Akinpelu *et al*., 2015). This suggests a valuable role for clove-based formulations in areas where conventional antibiotic therapy may be ineffective or inaccessible.

2.3 Overview of Clove

Spices as clove, oregano, mint, thyme and cinnamon, have been employed for centuries as food preservatives and as medicinal plants mainly due to its antioxidant and antimicrobial activities. Nowadays, many reports confirm the antibacterial, antifungal, antiviral and anticarcinogenic properties of spice plants. Clove in particular has attracted the attention due to the potent antioxidant and antimicrobial activities standing out among the other spices (Shan *et al*., 2015).

Syzygium aromaticum (S. aromaticum) (synonym: *Eugenia cariophylata*) commonly known as clove, is a median size tree (8-12 m) from the Mirtaceae family native from the Maluku islands in east Indonesia. For centuries the trade of clove and the search of this valuable spice stimulated the economic development of this Asiatic region (Kamatou *et al*., 2012). The clove tree is frequently cultivated in coastal areas at maximum altitudes of 200 m above the sea level. The production of flower buds, which is the commercialized part of this tree, starts after 4 years of plantation. Flower buds are collected in the maturation phase before flowering. The collection could be done manually or chemically-mediated using a natural phytohormone which liberates ethylene in the vegetal tissue, producing precocious maturation (Filho *et al*., 2013).

Nowadays, the larger producer countries of clove are Indonesia, India, Malaysia, Sri Lanka, Madagascar and Tanzania specially the Zanzibar island. In Brazil, clove is cultured in the northeast region, in the state of Bahia in the regions of Valença, Ituberá, Taperoá, Camamu and Nilo Peçanha, where approximately 8 000 hectares are cultivated, producing near 2 500 tons per year (Oliveira *et al*., 2017).

2.4 Bioactive Compounds of Clove Extract

Clove represents one of the major vegetal sources of phenolic compounds such as flavonoids, hidroxibenzoic acids, hidroxicinamic acids and hidroxiphenyl propens. Eugenol is the main bioactive compound of clove, which is found in concentrations ranging from 9 381.70 to 14 650.00 mg per 100 g of fresh plant material (Jirovetz *et al.,* 2016). With regard to the phenolic acids, gallic acid is the compound found in higher concentration (783.50 mg/100g fresh weight) (Cooke and Plot nick, 2018). However, other gallic acid derivates such as hidrolizable tannins are present in higher concentrations (2 375.8 mg/100 g). Other phenolic acids found in clove are the caffeic, ferulic, elagic and salicylic acids. Flavonoids as kaempferol, quercetin and its derivates (glycosilated) are also found in clove in lower concentrations (Tahir *et al*., 2016). Concentrations up to 18% of essential oil can be found in the clove flower buds. Roughly, 89% of the clove essential oil is eugenol and 5% to 15% is eugenol acetate and β-cariofileno (Pérez-Jiménez *et al*., 2010). Another important compound found in the essential oil of clove in concentrations up to 2.1% is α-humulen. Other volatile compounds present in lower concentrations in clove essential oil are β-pinene, limonene, farnesol, benzaldehyde, 2-heptanone and ethyl hexanoate (Bamdad *et al*., 2016).

2.4.1 Eugenol

Eugenol appears as clear colorless pale yellow or amber-colored liquid. Odor of cloves. Spicy pungent taste. The compound eugenol is responsible for most of the characteristic aroma of cloves. Eugenol comprises 72–90% of the essential oil extracted from cloves, and is the compound most responsible for clove aroma. Complete extraction occurs at 80 minutes in pressurized water at 125 °C (257 °F) (Chen *et al*., 2017). Eugenol is a naturally occurring phenolic molecule found in several plants such as cinnamon, clove, and bay leaves. It has been used as a topical antiseptic as a counter-irritant and in dental preparations with zinc oxide for root canal sealing and pain control. Although not currently available in any FDA-approved

products (including OTC), eugenol has been found to have anti-inflammatory, neuroprotective, antipyretic, antioxidant, antifungal and analgesic properties (Aguilar-González *et al*., 2015). Its exact mechanism of action is unknown; however, it has been shown to interfere with action potential conduction. There is a number of unapproved over-the-counter (OTC) products available containing eugenol that advertises its use for the treatment of toothache (Donsì *et al*., 2011). Eugenol, also called clove oil, is aromatic oil extracted from cloves that is used widely as a flavoring for foods and teas and as herbal oil used topically to treat toothache and more rarely to be taken orally to treat gastrointestinal and respiratory complaints. Eugenol in therapeutic doses has not been implicated in causing serum enzyme elevations or clinically apparent liver injury, but ingestions of high doses, as with an overdose, can cause severe liver injury (Sebaaly *et al*., 2015). Eugenol is an allyl chain-substituted guaiacol, i.e. 2-methoxy-4-(2-propenyl) phenol. Eugenol is a member of the allylbenzene class of chemical compounds. It is a clear to pale yellow oily liquid extracted from certain essential oils especially from clove oil, nutmeg, cinnamon, and bay leaf. It is slightly soluble in water and soluble in organic solvents. It has a pleasant, spicy, clove-like odor (Vahedikia *et al*., 2019). Eugenol is used in perfumeries, flavorings, essential oils and in medicine as a local antiseptic and anesthetic. It was used in the production of isoeugenol for the manufacture of vanillin, though most vanillin is now produced from petrochemicals or from by-products of paper manufactures (Assadpour and Mahdi, 2019).

Eugenol is a phenylpropanoid formally derived from guaiacol with an allyl chain substituted para to the hydroxy group. It is a major component of clove essential oil, and exhibits antibacterial, analgesic and antioxidant properties. It has been widely used in dentistry to treat toothache and pulpitis (Ribeiro-Santos *et al*., 2017). It has a role as an allergen, a human blood serum metabolite, a sensitiser, a volatile oil component, a flavouring agent, an EC 1.4.3.4 (monoamine oxidase) inhibitor, a radical scavenger, an antibacterial agent, an

antineoplastic agent, an apoptosis inducer, an anesthetic, an analgesic, a voltage-gated sodium channel blocker, a NF-kappaB inhibitor and an anti-inflammatory agent. It is a phenylpropanoid, a monomethoxybenzene, a member of phenols and an alkenylbenzene. It is functionally related to a guaiacol (Zhelyazkov *et al*., 2022).

2.4.2 Acetyl Eugenol

Acetyl eugenol is a phenylpropanoid compound found in cloves. It is the second in abundance to the related compound eugenol in certain extract preparations. Eugenol acetate (Eugenyl acetate), a major phytochemical constituent of the essential oil exhibits antibacterial, antioxidant, and anti-virulence activities (Haro-González *et al*., 2021). Eugenol acetate (Eugenyl acetate), a phytochemical in clove essential oil, against clinical isolates of Candida albicans, Candida parapsilosis, Candida tropicalis, and Candida glabrata. It inhibits aggregation and alters arachidonic acid metabolism in human blood platelets (Cortés-Rojas *et al*., 2014). In high doses, however, eugenol appears to be a direct cytotoxin and several instances of severe acute liver and kidney injury have been reported after accidental overdose of eugenol containing herbal products, largely in children (Assadpour and Mahdi, 2019).

2.4.3 β-Caryophyllene

Caryophyllene, more formally-β-caryophyllene, is a natural bicyclic sesquiterpene that is a constituent of many essential oils, especially clove oil, the oil from the stems and flowers of *Syzygium aromaticum*, the essential oil of Cannabis sativa, copaiba, rosemary, and hops. Beta-caryophyllene is found in a variety of different spices and herbs. It's an effective anti-inflammatory, antimicrobial, and mood enhancer. You can find this particular terpene in basil, rosemary, cinnamon, oregano, cloves, lavender, and black pepper. Surprisingly, this terpene can also be found in broccoli (Nuñez and D'Aquino, 2012).

2.4.4 α-Humulene

Humulene, also known as α-humulene or α-caryophyllene, is a naturally occurring monocyclic sesquiterpene, containing an 11-membered ring and consisting of 3 isoprene units containing three non-conjugated C=C double bonds, two of them being triply substituted and one being doubly substituted. Humulene is found in the flowering cone of the hops plant. It is also present in marsh elders and a wide array of herbs and spices, including; sage, basil, clove, black pepper, coriander, and balsam fir tree (Matan *et al*., 2016).

2.4.5 α-Caryophyllene Oxide

Caryophyllene oxide is a sesquiterpene that results from the oxidation of β-caryophyllene, which can occur during the harvest's cure. It is also considered non-toxic, non-sensitizing, and has been indicated as an anticoagulant with platelets (Bajpai *et al.,* 2012).

2.4.6 α-Murolene and γ-Murolene

These have been shown to have effects on health, including antioxidant and anti-inflammatory activities. Murolene has also been shown to inhibit protein targets involved in cancer cell proliferation. The compound may be used as an additive for flavorings, fragrances, and other cosmetics (Li *et al*., 2012).

2.4.7 α-Selinene and β-Selinene

α -Selinene and β-selinene are the most common and are two of the principal components of the oil from celery seeds and clove. γ-Selinene and δ-selinene are less common. Any eukaryotic metabolite produced during a metabolic reaction in plants, the kingdom that include flowering plants, conifers and other gymnosperms (Delgado-Adámez *et al*., 2012). Selinenes are a group of closely related isomeric chemical compounds which are classified as sesquiterpenes. The selinenes all have the molecular formula C15H24 and they have been isolated from a variety of plant sources. α-Selinene and β-selinene are the most common and are two of the principal components of the oil from clove. γ-Selinene and δ-selinene are less

common (Voon *et al*., 2012). Alpha-selinene is an isomer of selinene where the double bond in the octahydronaphthalene ring system is endocyclic (2R,4aR,8aR)-configuration. It has a role as a plant metabolite. It is a selinene and a member of octahydronaphthalenes (Matan *et al*., 2016).

2.4.8 δ-cadinene

A member of the cadinene family of sesquiterpenes in which the double bonds are located at the 4-4a and 7-8 positions, and in which the isopropyl group at position 1 is cis to the hydrogen at the adjacent bridgehead carbon (the 1R,8aS-enantiomer). δ-cadinene shows potent anticancer effects against human ovary cancer cells through the mediation of apoptosis, nuclear membrane rupture, cell cycle arrest and caspase activation (Li *et al*., 2012).

2.5 Biological Activities of Clove

Clove is an important medicinal plant due to the wide range of pharmacological effects consolidated from traditional use for centuries and reported in literature. A review of several scientific reports of the most important biological activities of clove is presented in the following paragraphs.

2.5.1 Anti-Diabetic Activity

Clove extract acts like insulin in hepatocytes and hepatoma cells by reducing phosphoenolpyruvate carboxykinase (PEPCK) and glucose 6-phosphatase (G6Pase) gene expression. Much like insulin, clove mediated repression is reversed by PI3K inhibitors and N-acetylcysteine (NAC). A more global analysis of gene expression by DNA microarray analysis revealed that clove and insulin regulated the expression of many of the same genes in a similar manner (Cimino *et al.,* 2021)

2.5.2 Antioxidant Activity

Recently, the United States Department of Agriculture in collaboration with Universities and private companies create a database with the polyphenol content and antioxidant activity of different kind of foods. Based on this database, Pérez-Jiménez *et al*. (2010) classified the 100 richest dietary sources of polyphenols. Results indicate that the spice plants are the kind of food with higher polyphenol content followed by fruits, seeds and vegetables. Among spices, clove showed the higher content of polyphenols and antioxidant compounds. In another work published by Shan *et al*. (2015), the main phenolic compounds in 26 spices were identified and quantified by high performance liquid chromatography, followed by the in vitro antioxidant activity analysis by the ABTS method. Results showed the high correlation between the polyphenols content and the antioxidant activity. Clove (buds) was the spice presenting higher antioxidant activity and polyphenol content, (168.660±0.024) tetraethylammonium chloride (mmol of Trolox/100g dried weight) and (14.380±0.006) g of gallic acid (equivalents/100g of dried weight) respectively. The major types of phenolic compounds found were phenolic acids (gallic acid), flavonol glucosides, phenolic volatile oils (eugenol, acetyl eugenol) and tannins. It was highlighted the huge potential of clove as radical scavenger and as a commercial source of polyphenols. The antioxidant activity of clove and caraway were screened using various in vitro models, such as b-carotenelinoleate, ferric thiocyanate, 1,1-diphenyl-2-picryl hydroxyl (DPPH) radical, hydroxyl radical and reducing power model systems concluding that the antioxidant activity of clove and caraway is comparable with butylated hydroxytoluene (BHT), a synthetic compound commonly employed as food preservative (Bamdad *et al.,* 2016). According to Gülçin *et al*. (2012), the antioxidant activity of clove oil compared with synthetic antioxidants measured as the scavenging of the DPPH radical decreased in the following order: clove oil>BHT>alfatocopherol>butylated hydroxyanisole>Trolox. The antioxidant activity of aqueous extracts of clove has been tested by different in vitro methods as 2,2-diphenyl-1-picrylhydrazyl (DPPH); 2,2'-azino-bis (3-

ethylbenzothiazoline-6-sulphonic acid) (ABTS), oxygen radical absorbance capacity, ferric reducing antioxidant power, xanthine oxidase and 2-deoxiguanosine. Clove and plants as pine, cinnamon, and mate proved its enormous potential as food preservative among the other 30 plants analyzed (Dudonné *et al.,* 2019). Ethanol and aqueous extracts of clove and lavender at concentrations of 20, 40 and 60 µg/mL showed inhibitions up to 95% when tested as metal quelants, superoxide radical capture and scavenging of the DPPH radical. The powerful antioxidant activity of both extracts may be attributed to the strong hydrogen donating ability, metal chelating ability and scavanging of free radicals, hydrogen peroxide and superoxide (Gülçin *et al*., 2012).

Gülcin (2011) studied the antioxidant activity of eugenol by several in vitro methods and discusses the structure-activity relationship. Compared to butylated hydroxyanisole, BHT, Trolox and α-tocopherol, eugenol presented higher antioxidant activity in most of the methods tested, DPPH, ABTS, N, N-dimethyl-p-phenylenediamine, CUPRAC and ferric reducing assay. It was remarked that plant polyphenols are multifunctional in the sense that they can act as reducing agents, hydrogen atom donators, and singlet oxygen scavengers. Eugenol allows the donation of an hydrogen atom and subsequent stabilization of the phenoxil radical generated forming stable compounds that do not start or propagate oxidation. The eugenol molecule possesses an interesting conjugation of the carbon chain with the aromatic ring which could participate in the stabilization of the phenoxyl radical by resonance. This chromophoric system is also present in molecule of resveratrol which is another important antioxidant. It has been proposed the hypothesis that eugenol reduces two or more DPPH radicals, despite the availability of only one hydrogen from a hydroxyl group. The formation of dimers of eugenol (dehydrodieugenol) with two phenolic hidroxyl groups originated from eugenyl intermediate radicals has also been proposed as mechanism between eugenol and DPPH radicals. In the same way, *S. aromaticum* oil and Nigella sativa oil significant protect male rats exposed to

aflatoxins which caused hepato and nephrotoxicity and oxidative stress. Regarding to the biochemical parameters, such as alanine aminotransferase, aspartate aminotransferase, alkaline phospatase, total billirubin, urea, total protein, cholesterol, the activity of both oils were comparable with the controls (Abdel-Wahhab and Aly, 2015). Antioxidants are important compounds for treatment of memory deficits caused by oxidative stress. Pretreatment with clove essential oil decreases the oxidative stress assessed by malondialdehyde and reduced glutathione levels in mice's brain. This study concluded that clove oil could revert memory and learning deficits caused by scopolamine in short and long term as a result of the reduction in the oxidative stress (Mehta *et al*., 2010). Memory and learning improvements of clove oil were observed in scopolamine-treated mice at doses of 0.025, 0.05, and 0.1 mL/kg when compared with saline solution control group in an elevated plus maze test. These works prove the benefits of the employment of clove as a rich source of antioxidants for the treatment of memory deficits caused by oxidative stress. Extracts from clove buds could also be used as food antioxidants. The shelf-life and frying stability of encapsulated and un-encapsulated eugenol-rich clove extracts were tested in soybean oil. Controlled release of antioxidants could be achieved by encapsulated clove powder obtained by spray drying using maltodextrin and arabic gum as wall materials (Halder *et al.,* 2014).

2.5.3 Antimicrobial Activity

The antimicrobial activities of clove have been proved against several bacteria and fungal strains. Sofia *et al*. (2017) tested the antimicrobial activity of different Indian spice plants as mint, cinnamon, mustard, ginger, garlic and clove. The only sampled that showed complete bactericidal effect against all the food-borne pathogens tested Escherichia coli (*E. coli), Staphylococcus aureus and Bacillus cereus* was the aqueous extract of clove at 3%. At the concentration of 1%clove extract also showed good inhibitory action. In another work published by Dorman and Deans (2010), the antibacterial activity of black pepper, geranium,

nutmeg, oregano, thyme and clove was tested against 25 strains of Gram positive and Gram-negative bacteria. The oils with the widest spectrum of activity were thyme, oregano and clove respectively. The antibacterial activity of clove, oregano (Origanum vulgare), bay (Pimenta racemosa) and thyme (Thymus vulgaris) essential oil was tested against *E. coli* O157:H7showing the different grades of inhibition of these essential oils (Burt and Reinders, 2013). Likewise, formulations containing eugenol and carvacrol encapsulated in a non-ionic surfactant were tested against four strains of two important foodborne pathogens, *E. coli* O157:H7 and *Listeria monocitogenes*, results reinforces the employment of eugenol to inhibit the growth of these microorganisms in surfaces in contact with food (Pérez-Conesa *et al*., 2016). Rana *et al*. (2011) determined the antifungic activity of clove oil in different strains and reported this scale of sensibility Mucor sp.>Microsporum gypseum>Fusarium monoliformeNCIM 1100>Trichophytum rubrum>Aspergillus sp.>Fusarium oxysporum MTCC 284. The chromatographic analyses showed that eugenol was the main compound responsible for the antifungic activity due to lysis of the spores and micelles. A similar mechanism of action of membrane disruption and deformation of macromolecules produced by eugenol was reported by Devi *et al*. (2010). The activities of clove oil against different dermatophytes as *Microsporum canis* (KCTC 6591), *Trichophyton mentagrophytes* (KCTC 6077), *Trichophyton rubrum* (KCCM 60443), *Epidermophyton floccosum* (KCCM 11667) and *Microsporum gypseum* were tested and results indicate a maximum activity at concentration of 0.2 mg/mL with an effectiveness of up to 60% (Park *et al*., 2017). Pure clove oil or mixes with rosemary (*Rosmarinus officinalis* spp.) oil were tested against *Staphylococcus epidermidis, Staphylococcus aureus, Bacillus subtilis, E. coli, Proteus vulgaris, Pseudomonas aeruginosa* and results showed minimum inhibitory concentrations between 0.062% and 0.500% (v/v) which is promising as anti-infecticious agents or as food preservative (Fu *et al*., 2017).

The anticandidal activity of eugenol and carvacrol was tested in a vaginal candidiasis model, microbial and histological techniques were employed to compare the samples with the controls. The results suggest that eugenol and carvacrol could be a promising antifungal agent for treatment and prophylaxis of vaginal candidiasis (Chami *et al.,* 2014). In addition to the wide spectrum of activity of eugenol against bacteria, a study showed that eugenol and cinnamaldehyde at 2 μg/mL inhibited the growth of 31 strains of Helicobacter pylori, after 9 h and 12 h of incubation, respectively, being more potent that amoxicillin and without developing resistance. The activity and stability of those compounds was checked at low pH values since *Helicobacter pylori* resides in the stomach (Ali *et al*., 2015). Solid lipid nanoparticles containing eugenol were prepared employing stearic acid, caprylic triglyceride and Poloxamer 188 in different concentrations by a modified hot homogenization ultrasonication method. The particles formed were characterized by the particle size, polydispersitivity index, morphology, zeta potential, crystalline state and encapsulation efficiency. The antifungal activity of solid lipid nanoparticles was tested in vivo by using a model of oral candidiasis (Candida albicans) in immunosupressed rats. The results showed the increase in the therapeutic effectiveness of eugenol and the modification of the release when administrated as solid lipid nanoparticles (Garg and Singh, 2011).

2.5.4 Antinociceptive

The employment of clove as analgesic have been reported since the 13th century, for toothache, join pain and antispasmodic, being the eugenol the main compound responsible for this activity. The mechanism evolved has been attributed to the activation of calcium and chloride channels in ganglionar cells tree (Matan *et al*., 2016). The voltage dependant effects of eugenol in sodium and calcium channels and in receptors expressed in the trigeminal ganglio also contributed to the analgesic effect of clove (Li *et al.,* 2018). Other results show that the analgesic effect of clove is due to the action as capsaicin agonist (Ohkubo and Shibata, 2017).

The peripheral antinociceptive activity of eugenol was reported by Daniel *et al*. (2009) showing significant activity at doses of 50, 75 and 100 mg/kg.

2.5.5 Antiviral Activity

The antiviral activity of eugeniin, a compound isolated from *S. aromaticum* and from *Geum japonicum*, was tested against herpes virus strains being effective at 5 µg/mL, and it was deducted that one of the major targets of eugeniin is the viral DNA synthesis by the inhibition of the viral DNA polymerase (Li *et al*., 2012). In another study, aqueous extracts of *S. aromaticum* (L.) Merr. et Perry and other plants as Geum japonicum Thunb., Rhus javanica L., and Terminalia chebula Retzus among others showed strong antiherpes simplex virus type 1 (HSV-1) activity when combined with acyclovir. This synergic activity was stronger in the brain that in the skin and it was also proved that those combinations were not toxic to mice (Kurokawa *et al*., 2018).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Collection and Preparation of Clove Flower Buds

Dried clove flower buds (*Syzygium aromaticum*) were purchased from market and authenticated by a botanist in the department of botany, University of Ilorin. The buds were thoroughly cleaned to remove any dirt or contaminants. After cleaning, the buds were ground into a fine powder using a sterile electric grinder. The fine powder was stored in airtight containers at room temperature until further use.

3.2 Preparation of Clove Extracts

Three solvents include aqueous, ethanol, and methanol were used to prepare the clove extracts. For the aqueous extract, 100 grams of the finely ground clove powder was soaked in

500 milliliters of distilled water for 24 hours at room temperature with occasional stirring. The mixture was then filtered using Whatman No. 1 filter paper, and the filtrate was concentrated using rotatory evaporator at 40°C. For the ethanolic and methanolic extracts, 100 grams of the clove powder was mixed with 500 milliliters of ethanol or methanol. The extraction process was carried out for 48 hours, after which the solvents were evaporated using a rotary evaporator to obtain concentrated extracts. The extracts were stored in sterile, airtight containers at 4°C until further use (Sarker *et al*., 2006).

3.3 Collection of Test Organisms

The test organisms were collected from the microbiology laboratory of University of Ilorin. The bacterial pathogen selected for the study were *Escherichia coli*, and *Pseudomonas aeruginosa*.

3.4 Standardization of Test Organisms

To standardize the test organisms, bacterial pathogen were cultured on nutrient agar at 37°C for 24 hours. After incubation, organisms were picked and introduced into sterile 0.85% saline using sterile cotton swab, the turbidity of the bacterial cultures were adjusted to match the 0.5 McFarland standard. The suspension was adjusted by adding more saline or culture until the turbidity matches that of the 0.5 McFarland standard (Katerere and Eloff, 2008).

3.5 Antibacterial Activity Assay

The antibacterial activity of the clove extracts was evaluated using the agar well diffusion technique. The standardized suspension was used to inoculate the surfaces of sterile Mueller-Hinton agar using sterile cotton swab. 6 mm diameter wells were bored using sterile core borers in the solidified agar. The bottom of the wells was then closed using 0.5 ml of sterile Mueller-Hinton agar. The wells were then filled with 100 microliter of 100mg/ml, 80mg/ml, 60mg/ml, 40mg/ml, and 20mg/ml concentrations of the plant extracts. The plates

were allowed to stand for about 15 minutes at room temperature for the extracts to diffuse and then the agar plates were incubated at 37 ^{o}C for 24 hours. The antibacterial activities of the plant extracts were evaluated by appearance of zones of inhibition around the wells while lack of activity was observed by absence of zones of inhibition. The same procedure was repeated for other solvent extracts (Katerere and Eloff, 2008).

3.6 Determination of Minimum Inhibitory Concentration (MIC)

The MIC of the clove extracts was determined using the broth dilution method. The experiment utilized five test tubes, each containing 5ml of peptone water. Different concentrations of the clove extracts were prepared and distributed among the tubes as follows: 100mg/ml, 80mg/ml, 60mg/ml, 40mg/ml, and 20mg/ml. Each concentration was mixed thoroughly in its respective tube. To assess the antibacterial activity, 0.1ml of broth cultures of the test organism were added to all the five tubes and incubated at 37°C. After incubation, the MIC was determined as the lowest concentration of the extract that inhibited visible growth of the organism, as indicated by the absence of turbidity in the tube (CLSI, 2018).

3.7 Determination of Minimum Bactericidal Concentration (MBC)

To determine the MBC, the test tubes showing no visible growth in the MIC assay were subcultured onto fresh Mueller-Hinton agar plates. The plates were incubated at 37°C for 24 hours. The MBC was defined as the lowest concentration of the extract that resulted in no growth on the subculture plates, indicating complete killing of the organism (National Committee for Clinical Laboratory Standards, 2018).

CHAPTER FOUR

4.0 RESULTS

4.1 Antibacterial Activity of Clove Extracts

The antibacterial activity of aqueous, ethanolic, and methanolic extracts of *Syzygium aromaticum* (clove) against *Pseudomonas aeruginosa* and *Escherichia coli* is presented in Table 1. The activity (zone of inhibition) of all extracts against *Pseudomonas aeruginosa*, ranged between 0.00 mm to 25.10 mm, while the zone of inhibition of all extracts against *Escherichia coli* ranged between 0.00 mm to 24.00 mm.

4.2 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration

The MIC and MBC values of the clove extracts against *P. aeruginosa* and *E. coli* are presented in Table 2. All three extracts showed similar MIC (80 mg/mL) and MBC (100 mg/mL) values across both organisms.

Table 1: Antibacterial Activity of Aqueous, Ethanolic, and Methanolic Clove Extracts Against *Pseudomonas aeruginosa* and *Escherichia coli* (Zone of Inhibition in mm)

Extract Type	Concentration (mg/mL)	*P. aeruginosa*	*E. coli*
Aqueous	20	0.00	0.00
	40	8.00	13.00
	60	14.00	17.10
	80	19.00	19.00
	100	22.00	21.10
Ethanol	20	6.40	9.00
	40	11.90	11.04
	60	19.30	15.00
	80	22.00	21.11
	100	25.10	24.00
Methanol	20	6.20	8.10

Extract Type	Concentration (mg/mL)	*P. aeruginosa*	*E. coli*
	40	10.20	10.12
	60	18.20	14.30
	80	20.10	20.25
	100	24.40	23.14

Table 2: MIC and MBC of Clove Extracts Against *Pseudomonas aeruginosa* and *Escherichia coli*

Extract Type	Organism	MIC (mg/mL)	MBC (mg/mL)
Aqueous	*P. aeruginosa*	80	100
	E. coli	80	100
Ethanolic	*P. aeruginosa*	80	100
	E. coli	80	100
Methanolic	*P. aeruginosa*	80	100
	E. coli	80	100

CHAPTER FIVE

5.0 Discussion

The antibacterial activity exhibited by aqueous, ethanolic, and methanolic extracts of *Syzygium aromaticum* (clove) against *Pseudomonas aeruginosa* and *Escherichia coli* highlights the effectiveness of clove-derived phytochemicals in combating Gram-negative pathogens. Among the three extracts, the ethanolic and methanolic extracts demonstrated superior antibacterial activity, as evident from their broader zones of inhibition and comparable MIC/MBC values. This variation in extract potency can largely be attributed to the differential solubility of phytoconstituents in each solvent.

The higher activity observed with the ethanolic and methanolic extracts can be explained by their ability to extract a broader range of bioactive compounds, including both polar and moderately non-polar molecules such as eugenol, flavonoids, tannins, and saponins.

These compounds are known to disrupt bacterial membranes, denature proteins, interfere with nucleic acid synthesis, and ultimately lead to cell death (Burt, 2004; Marchese *et al*., 2017). Methanol, slightly more polar than ethanol, also facilitates extraction of certain hydrophilic antimicrobials. However, both solvents showed closely comparable efficacy, suggesting that the most active components in clove may be amphipathic and can be efficiently extracted by both solvents.

The aqueous extract, while still effective, displayed relatively moderate antibacterial activity compared to its organic counterparts. This is likely due to the limited solubility of many active phenolic compounds in water. Water tends to extract primarily hydrophilic compounds such as glycosides and some tannins, while essential oils and volatile phenols like eugenol—one of the major antimicrobial constituents of clove—are poorly soluble in water (Cortés-Rojas *et al*., 2014). Despite this, the aqueous extract still showed appreciable activity at higher concentrations, indicating that water-soluble compounds may contribute to antibacterial properties through mechanisms such as enzyme inhibition or disruption of cellular respiration.

The ability of all three extracts to inhibit *P. aeruginosa* is particularly noteworthy. This organism is a challenging pathogen due to its intrinsic resistance mechanisms, including efflux pumps, low outer membrane permeability, and production of enzymes such as β-lactamases. The fact that clove extracts—especially those from ethanol and methanol—were able to produce significant zones of inhibition against this bacterium suggests that the active compounds may act through unconventional pathways, such as compromising membrane integrity or chelating essential metal ions required for bacterial survival (Nazzaro *et al*., 2013).

Escherichia coli, although also Gram-negative, appeared slightly more susceptible to all extracts. This may be due to structural differences in the outer membrane, or the absence of some resistance mechanisms seen in *P. aeruginosa*. The consistent pattern of dose-dependent

inhibition across all extracts against *E. coli* supports the broad-spectrum potential of *S. aromaticum* as an antimicrobial agent.

The MIC and MBC values for both organisms were identical across all extracts (80 mg/mL and 100 mg/mL respectively), suggesting that once a critical concentration is achieved, all three extracts are capable of exerting bactericidal effects. The close proximity of MIC and MBC values implies that the transition from inhibitory to bactericidal action occurs over a narrow concentration range, which is ideal for therapeutic applications where rapid bacterial clearance is required.

These findings are consistent with previous studies. Iqbal *et al*. (2013) and Rahman *et al*. (2011) demonstrated that ethanolic and methanolic extracts of clove exhibited potent antibacterial effects against both Gram-positive and Gram-negative bacteria, including *P. aeruginosa* and *E. coli*. Similarly, Devi *et al*. (2010) reported that clove extracts could disrupt biofilm formation in *Pseudomonas* species, further supporting its anti-pathogenic capabilities.

Overall, all three solvent extracts of clove displayed antibacterial activity against *P. aeruginosa* and *E. coli*, with ethanolic and methanolic extracts being more potent than the aqueous extract. These results suggest that *S. aromaticum* possesses multiple antimicrobial compounds, some of which are extractable in polar organic solvents and effective against resistant Gram-negative pathogens.

5.1 Conclusion

This study demonstrates that *Syzygium aromaticum* (clove) possesses significant antibacterial activity against *Pseudomonas aeruginosa* and *Escherichia coli*. The ethanolic and methanolic extracts consistently showed higher antibacterial efficacy compared to aqueous extracts, suggesting that organic solvents are more effective in extracting potent phytochemicals like eugenol. These findings support the potential of clove as a natural antimicrobial agent and provide a strong basis for further pharmacological studies, isolation of active compounds, and development of plant-based therapeutics for resistant infections.

5.2 Recommendations

It could be recommended from the study that:

i. due to the resistance profile of *p. aeruginosa*, further studies should explore clove extract's mechanism of action on its efflux pumps and biofilm formation.
ii. all three extracts should be compared under standard pharmaceutical formulations (gel, ointment, etc.) to assess application potential.
iii. consider expanding the study to include carbapenem-resistant *p. aeruginosa* strains for broader resistance evaluation.

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