EVALUATION OF PHYTOCHEMICAL AND ANTIMICROBIAL PROPERTIES OF COLD AND HOT WATER EXTRACT OF LEMONGRASS (*Cymbopogon citratus)* AGAINST SELECTED BACTERIAL ISOLATES

BY

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LSC2009896

DEPARTMENT OF MICROBIOLOGY

UNIVERSITY OF BENIN

BENIN CITY.

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A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF DEGREE OF B.Sc. (HONS) IN MICROBIOLOGY, UNIVERSITY OF BENIN, BENIN CITY.

FEBUARY, 2025

CERTIFICATION

This is to certify that this project work was carried out by **Eden Isioghene OGHARANDUKU** in the Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City under my supervision.

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(Head of Department)

APPROVAL

This project work was carried out by **Eden Isioghene OGHARANDUKU** in partial fulfilment of the award of a Bachelor of Science, B.Sc (Hons) degree in the Department of Microbiology, University of Benin, Benin City.

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PROF. (MRS.) F. I. AKINNIBOSUN **DATE**

(Head of Department)

DEDICATION

This project work is dedicated to God Almighty, for bringing me this far in life. I am truly grateful.

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I wish to begin by expressing my profound gratitude to God Almighty, whose endless love, grace, and mercy have been my constant source of strength and guidance throughout this academic journey. His divine favor has been instrumental in the successful completion of this project.

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ABSTRACT

Lemongrass (*Cymbopogon citratus*) is widely known for its medicinal and antimicrobial properties, with its extracts being used in traditional and modern medicine. Due to the increasing resistance of bacteria to conventional antibiotics, plant-based antimicrobials have gained attention as alternative therapeutic agents. This study investigated the phytochemical composition and antibacterial activity of hot and cold water extracts of Lemongrass leaves against *Staphylococcus aureus, Escherichia coli,* and *Pseudomonas sp.* Phytochemical analysis revealed the presence of flavonoids, tannins, and glycosides in both extracts, with glycosides being more prominent in the hot extract. Saponins, steroids, and terpenoids were absent. Antibacterial activity was assessed using the agar well diffusion method, where the hot extract showed higher zones of inhibition at 100% concentration: *S. aureus* (13.00 ± 0.00 mm), *E. coli* (16.00 ± 0.00 mm), and *Pseudomonas sp.* (18.20 ± 0.00 mm), compared to the cold extract: *S. aureus* (9.0 ± 0.50 mm), *E. coli* (11.20 ± 0.20 mm), and *Pseudomonas sp.* (12.00 ± 0.00 mm). The Minimum Inhibitory Concentration (MIC) revealed that the hot extract inhibited *E. coli* at 25 mg/ml, *Pseudomonas sp.* at 50 mg/ml, and *S. aureus* at 25 mg/ml, while the cold extract only inhibited *Pseudomonas sp.* at 100 mg/ml and *S. aureus* at 25 mg/ml. Minimum Bactericidal Concentration (MBC) results showed that the hot extract was bactericidal against *S. aureus* and bacteriostatic against *E. coli* and *Pseudomonas sp.,* while the cold extract was bactericidal against *Pseudomonas sp.* and bacteriostatic against the other isolates. Antibiotic susceptibility testing indicated that Gram-positive bacteria were highly susceptible to ciprofloxacin, while Gram-negative bacteria were sensitive to azithromycin. These results highlight the potential application of *Cymbopogon citratus* as an alternative treatment for bacterial infections, particularly in combating antibiotic-resistant pathogens

CHAPTER ONE

INTRODUCTION

1.1 Background of Study

Lemongrass (*Cymbopogon citratus*), a member of the Poaceae family, is a tropical plant native to Southeast Asia but widely cultivated across various regions of the world, including Africa, India, and Central America. Known for its fresh, citrus-like aroma, lemongrass is used extensively in culinary applications, traditional medicine, and as a source of essential oils. In particular, its leaves have been highlighted for their rich composition of bioactive compounds, including phenolic acids, flavonoids, terpenoids, and essential oils. These compounds contribute to its wide array of medicinal and therapeutic properties, including antimicrobial, antioxidant, anti-inflammatory, and antidiabetic activities (Kiani *et al*., 2022; Nambiar and Matela, 2012).

The active compounds found in lemongrass are of particular interest in scientific research due to their potential in the treatment of bacterial infections. Some of the most prominent bioactive constituents identified in lemongrass include citral (a mixture of the isomers neral and geranial), citronella, limonene, and geraniol, all of which have demonstrated significant antimicrobial properties (Rattanachaikunsopon and Phumkhachorn, 2010). As a result, lemongrass is increasingly being considered as a potential alternative or complementary treatment to conventional antibiotics, particularly in light of the growing concern over antibiotic resistance.

Phytochemicals are naturally occurring compounds in plants that are known to possess a variety of biological activities. Lemongrass is a rich source of these bioactive molecules, especially essential oils that are concentrated in its leaves. The phytochemical profile of lemongrass has been extensively studied, revealing that its essential oil contains a variety of terpenes, alcohols,

aldehydes, and ketones. Citral, the most abundant compound in lemongrass, constitutes approximately 70-80% of the essential oil, making it the primary contributor to the plant's characteristic aroma and its antimicrobial properties (Kusuma, *et al*., 20224).

Citral is a monoterpene with two isomers neral and geranial which have been shown to possess potent antibacterial and antifungal properties (Sharma *et al*., 2021). In addition to citral, lemongrass also contains other important bioactive compounds such as limonene, a monoterpene that has been found to exhibit antioxidant, anti-inflammatory, and antimicrobial activities (Kong *et al*., 2012). Another compound, geraniol, is a terpene alcohol that has shown significant antibacterial and anti-inflammatory effects (Kiani *et al*., 2022). The complex interplay of these various compounds makes lemongrass an attractive candidate for further investigation as a source of natural antimicrobial agents.

Flavonoids, phenolic acids, and other secondary metabolites are also present in lemongrass, contributing to its antioxidant properties. These compounds help neutralize free radicals, thereby preventing oxidative stress that can lead to chronic diseases such as cancer, cardiovascular diseases, and diabetes (Kaurinovic and Vastag, 2019). The presence of these compounds also contributes to the overall therapeutic potential of lemongrass in traditional medicine, where it has been used to treat a variety of conditions, including digestive disorders, fever, and anxiety.

The antibacterial activity of lemongrass has been well-documented in scientific literature, with numerous studies confirming its effectiveness against a wide range of bacterial pathogens. The antimicrobial effects of lemongrass are attributed to the presence of its bioactive compounds, which exhibit bactericidal properties through various mechanisms. One of the key mechanisms is

the disruption of bacterial cell membranes, which results in leakage of cell contents and ultimately bacterial cell death (Balakrishnan *et al*., 2014).

Lemongrass has been shown to be particularly effective against both Gram-positive and Gram-negative bacteria, including common pathogens such as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Klebsiella pneumoniae* (Singh *et al*., 2011). For instance, studies have demonstrated that lemongrass essential oil exhibits potent inhibitory effects on *S. aureus*, a Gram-positive bacterium known for causing a variety of infections, including skin and soft tissue infections, respiratory infections, and foodborne illnesses. In addition, lemongrass oil has been shown to inhibit the growth of *E. coli* and *P. aeruginosa*, which are often implicated in hospital-acquired infections (Rattanachaikunsopon and Phumkhachorn, 2010).

The antimicrobial activity of lemongrass essential oil has been extensively studied in both in vitro and in vivo settings (Adukwu *et al*., 2016). In vitro studies typically involve testing the efficacy of lemongrass extracts or oils against bacterial isolates using methods such as disk diffusion, agar well diffusion, and broth dilution. These methods help determine the minimum inhibitory concentration (MIC), which is the lowest concentration of an antimicrobial agent required to inhibit the growth of a microorganism. In several studies, lemongrass extracts have exhibited MIC values comparable to or even better than those of conventional antibiotics (Adukwu *et al*., 2016).

The antimicrobial properties of lemongrass have led to its widespread use in traditional medicine as well as in the development of herbal remedies. In traditional medicine, lemongrass has been used to treat a variety of ailments, including infections, digestive problems, fever, and anxiety. In

particular, lemongrass has been employed as an antimicrobial agent for treating skin infections, urinary tract infections, and gastrointestinal disorders (Rattanachaikunsopon and Phumkhachorn, 2010).

The increasing incidence of antibiotic resistance is one of the primary drivers of interest in plant-derived antimicrobials, including lemongrass. Antibiotic resistance occurs when bacteria evolve mechanisms to resist the effects of drugs that once killed them or inhibited their growth. This has led to the emergence of "superbugs," which are resistant to multiple classes of antibiotics and pose a significant threat to public health worldwide. As a result, there is a growing need for alternative therapies, such as those derived from plants like lemongrass, which can offer a natural, sustainable, and effective solution to combating infections caused by resistant bacteria (Aldawsari *et al*., 2023).

Lemongrass, with its rich phytochemical profile and demonstrated antibacterial activity, holds great promise as a natural alternative to synthetic antibiotics. The potential use of lemongrass extracts in the development of antimicrobial agents, especially in the face of increasing antibiotic resistance, makes it an important focus of contemporary research.

The rise of antibiotic-resistant bacteria is a global health crisis, making the search for alternative antimicrobial agents critical (Aggarwal *et al*., 2024). Plant-derived compounds, particularly essential oils, have gained attention due to their broad-spectrum antimicrobial properties and their potential to act as natural preservatives in food, pharmaceuticals, and personal care products. Lemongrass, with its rich phytochemical content and proven antibacterial activity, offers a promising natural solution to this problem (Haque *et al*., 204).

In addition, the use of lemongrass in traditional medicine for the treatment of various ailments supports the need for further investigation into its therapeutic potential. By focusing on the antibacterial properties of lemongrass, this study aims to provide scientific evidence to support its traditional uses and to promote its use as a natural antimicrobial agent in modern healthcare practices.

1.2. AIMS AND OBJECTIVES

The aim of the study was to evaluate the phytochemical and antimicrobial properties of hot and cold extracts of Lemongrass (*Cymbopogon citratus*) leaves extract against selected bacterial isolates

The specific objectives of this research were to:

1. determine the antimicrobial properties
2. determine the phytochemical properties of the hot and cold extract
3. determine the minimum inhibitory concentration
4. determine the minimum bactericidal concentration of the hot and cold water extract

CHAPTER TWO

LITERATURE REVIEW

2.1 Description of Lemongrass

The use of whole herbs and their extracts has long been central to folk medicine, particularly in the treatment of various ailments and diseases. Traditional practitioners often claim that these plant-based remedies are effective without the support of scientific evidence. However, the increasing prevalence of opportunistic diseases and the side effects associated with synthetic drugs have heightened the need for safer, biologically-based substitutes. As a result, significant efforts are being made to explore potential alternatives, such as ethno-medicinal plants (Patil, 2010). Advancements in extraction techniques, aided by biotechnology, have accelerated the investigation of natural compounds, enabling faster and more precise identification of bioactive compounds with potential health benefits (Wang and Weller, 2006). Among the plants celebrated in folk medicine for their therapeutic properties is *Cymbopogon citratus*, commonly known as lemongrass.

Lemongrass is a perennial grass native to tropical and subtropical regions, particularly in Asia, Africa, and the Americas (Francisco *et al*., 2011). The plant can grow up to 6 feet in height, with its bulb-like stems consisting of slender, smooth, linearly veined leaves that taper to a narrow base and a sharp apex. The leaves typically reach a length of about 100 cm and a width of 2 cm. When crushed, the leaves release a yellow or amber-colored, aromatic essential oil (Adejuwon and Esther, 2007). Lemongrass is commonly used in traditional beverages and as a flavoring in food, imparting a distinct lemon flavor. Its use in folk medicine is widespread, especially in South America, Asia, and West Africa, where it is valued for its various therapeutic properties,

including its antiseptic, antipyretic, antibacterial, anti-inflammatory, and carminative effects. It has also been traditionally used as a febrifuge, analgesic, spasmolytic, diuretic, tranquilizer, and stomachic (Sawyerr, 1982; Viana *et al*., 2000; Negrelle and Gomes, 2007; Adejuwon and Esther, 2007; Tatiana *et al*., 2011).

The chemical composition of lemongrass can vary significantly, influenced by factors such as genetics, habitat, and agricultural practices (Tzortzakis and Economakis, 2007). A key component of lemongrass is citral, a compound made up of geranial and neral isomers, which is also used in the production of beta-carotene and vitamin A. lemongrass has demonstrated antimicrobial properties, particularly against various pathogenic fungi (Vardar-Ünlü *et al*., 2003). While lemongrass is native to tropical Asia, it is now grown worldwide. Its subtle citrus flavor makes it a common ingredient in Asian cuisines, particularly in curries, oils, and soups, and it pairs well with poultry, seafood, and fish. In Latin and African countries, both dried and fresh lemongrass leaves are widely consumed as an aromatic herb.

Recent studies have highlighted the functional properties of lemongrass, particularly its antioxidant and anti-inflammatory characteristics. These properties are attributed to the presence of mono- and polymeric flavonoids, such as apigenin glycosides, luteolin, and proanthocyanidins, which are found in oil-free lemongrass infusions (Francisco *et al*., 2013). Extracts from lemongrass leaves have also demonstrated antioxidant, antifungal, and antimicrobial activities (Hanaa *et al*., 2012). As consumers become more conscious of the potential risks associated with synthetic chemicals in food additives, the use of natural additives, such as essential oils, has gained popularity as a means of preserving the shelf life of various

food products. Essential oils have shown antimicrobial activity both in vitro and within food systems, providing a natural alternative to synthetic preservatives.

Figure 1 Lemongrass (*Cymbopogon citratus*).

2.2. History

Lemongrass is a perennial plant commonly cultivated in the tropics and subtropics, with two primary species: *Cymbopogon citratus* (West Indian lemongrass) and *Cymbopogon flexuosus* (East Indian lemongrass). These species are native to Southeast Asia, South Asia, and Australia, with *Cymbopogon flexuosus* also known as Cochin or Malabar grass. It is indigenous to Sri Lanka, India, Thailand, and Burma, while *Cymbopogon citratus* is found in the West Indies. Both species are now widely cultivated around the world (Wannissorn *et al*., 1996).

Lemongrass has a long history of use, particularly in the Philippines, where it has been distilled for export since the 17th century. The first samples of citronella oil, derived from lemongrass,

were displayed at the 1951 World's Fair at London Crystal Palace. In India, lemongrass oil has been a popular ingredient for many years, known locally as "choomana polu," referring to the red grass stem of the plant (Juntachote *et al*., 2006). Indigenous Australians used citrus fruits for medicinal purposes, including making drinks and washing skin cuts and eyes (Hanker *et al*., 2005).

Today, lemongrass is commercially cultivated in various countries, including India, Guatemala, Paraguay, China, Sri Lanka, England, and parts of Africa, Indochina, South America, and Central America. The plant typically grows in dense clumps, reaching up to 2 meters in diameter, with leaves up to 1 meter long. Its genus is native to Southeast Asia, South Asia, and Australia.

Lemongrass is widely used in herbal oils, non-alcoholic beverages, baked goods, and confections. The essential oil extracted from lemongrass is commonly used as a fragrance in perfumes, cosmetics (such as creams and soaps), and other industrial products. Citral, a key compound in lemongrass oil, is used to flavor soft drinks, scent detergents and soaps, and as a fragrance in perfumes and cosmetics. It is also employed to mask unpleasant odors in industrial products and is a precursor in the production of ionones for perfumery.

Medicinally, lemongrass is considered an insect repellent and carminative. The West Indian variety is known for its strong antimicrobial properties, while the essential oils from East Indian lemongrass exhibit potent antifungal action. Additionally, the volatile oils from lemongrass show some mutagenic and pesticidal properties. *Cymbopogon nardus***,** a related species, is the source of citronella oil, while *Cymbopogon martinii* has been found to be toxic to fungi.

Lemongrass is generally regarded as safe for human consumption, and its versatility as a plant is widely acknowledged. It produces a flavorful herbal oil, often used in cooking, and serves as an effective antibiotic and natural weed barrier. The outer leaves are often kept in a loop and used to flavor meals, but should be removed before serving. Lemongrass is quick to establish itself and is drought-tolerant (Cheel *et al*., 2005).

2.3. Location

Cymbopogon citratus thrives in sunny, warm, and moist tropical climates. In Kerala, the plant's growth is minimal when temperatures exceed 30°C, and this results in a significant reduction in oil content. Lemongrass can be cultivated in various types of soil, ranging from rich loam to poor lateritic soils, and it generally grows well in calcareous and waterlogged conditions. The typical yield for lemongrass is between 300-350 tons per annum (Raybaudi-Massilia *et al*., 2006). Lemongrass grown in sandy soils tends to produce higher oil yields and increased citral content. Interestingly, when lemongrass is grown in highly saline soils, it also results in greater oil production (Srivastava and Akhila, 2010).

2.4. Botany, Morphology, and Ecology

Lemongrass is a large, perennial sedge with dense rhizomes and clusters of leaves. The plant grows in an erect form, reaching up to 1.8 meters in height. Its long, glaucous green leaves are tapered and linear, with margins that are slightly rough. The ligule, a small structure at the junction of the leaf blade and sheath, is very short. The leaf sheaths are cylindrical and form barren shoots at the base that tightly clasp the plant, while others are narrower and separate. Lemongrass is a short-day plant and produces abundant flowering in regions like South India,

with inflorescences reaching about 1 meter in length. It belongs to the Gramineae family and is known for its high-quality essential oils and relatively low production costs (Hanaa *et al*., 2012).

This large, clumped, perennial grass typically grows up to 1 meter in height. Its leaf blades are linear and conical at both ends, expanding to approximately 50 cm in length and 1.5 cm in width (Tajidin *et al*., 2012). The leaf sheaths are tubular and function as a pseudo-stem. Lemongrass generates flowers during its mature growth phase (Tajidin *et al*., 2012).

2.5. Lemongrass Phytochemistry and Technology

Lemongrass is a medicinal plant known for its bioactive compounds, which have the ability to control pathogens and enhance resistance to various diseases. These compounds, primarily found in the leaves, have a range of health benefits. The key bioactive components and their respective health benefits are summarized in Table 1. Various methods of extracting these bioactive compounds are illustrated in Figure 1. Additionally, the root of lemongrass is used as a chewing stick for oral hygiene in many parts of the world (Sawyerr, 1982).

One of the most significant components of lemongrass extracts is its essential oil, which plays a crucial role in various applications, particularly in the perfume and cosmetic industries. The high citral content in lemongrass oil has made it valuable for several chemical syntheses (Negrelle and Gomes, 2007). Studies on different lemongrass extracts have revealed a wide array of therapeutic potentials, including anti-cancer, anti-hypertensive, and anti-mutagenic properties. Additional therapeutic effects include anti-diabetic, anti-oxidant, anxiolytic, anti-nociceptive, and anti-fungal activities (Shah *et al*., 2011).

Although the use of lemongrass extracts in folk medicine for treating certain ailments was initially debated, recent studies have confirmed their effectiveness (Adejuwon and Esther, 2007; Celso *et al*., 2011a). Despite these findings, lemongrass oil and its essential oils remain underutilized, especially in developing countries. This may be due to a lack of awareness regarding the published results on the efficacy of lemongrass extracts in addressing various health-related issues. Therefore, the focus of this review is to comprehensively document the biological properties of lemongrass extracts (both infusions and decoctions) and essential oils. Additionally, the review provides suggestions for future research directions to further elucidate the inherent medicinal properties of lemongrass. Lemongrass extracts contain several medicinal chemical compounds, which are concentrated in both its essential oil and aqueous extracts, as summarized in Table 1.

Table 1. Bioactive Compounds from Lemongrass Extract

Component	Biological Activities	References
Myrcene	Antibacterial activity	Grace *et al*., 1984
Citral	Antibacterial activity	Grace *et al*., 1984
α-citral (geranial)	Antinociceptive activities	Viana *et al*., 2000
Geranial	Antimicrobial action	Mirghani *et al*., 2012
Undecan-2-one	Antinociceptive activity, Anti-fungal activity	Dharmendra *et al*., 2001, Shigeharu *et al*., 2001, Berenice *et al*., 1991
Citronellol	Anti-fungal activity	Dharmendra *et al*., 2001, Berenice *et al*., 1991
Limonene	Anti-oxidant activity	Bidinotto *et al*., 2010
Linalool	Not available	Berenice *et al*., 1991, Grace *et al*., 1984
Dipentene	Not available	Berenice *et al*., 1991, Grace *et al*., 1984

2.6. Phamocological Activity of Lemon Grass

Lemongrass, Cymbopogon citratus, is one such potent alternative source of antimicrobials (Ekpenyong *et al*., 2015) which is widely cultivated globally (Francisco *et al*., 2014). It has been reported for wide range of activities against several diseases–causing organisms (Figueirinha *et al*., 2008; Francisco *et al*., 2014; Francisco *et al*., 2011; Naik *et al*., 2010; Shah *et al*., 2012). Researches on the use of lemon grass as potential antimicrobial agent have been focused majorly on the use of its essential oils (Adukwu *et al*., 2016; De Silva *et al*., 2017; Naik *et al*., 2010).

While studies on the use of its other equally potent but extractible components are, despite high potency of reported activities, very few or relatively insignificant. For instance, respective reports by Adeneye and Agbaje (2007) and Ekpenyong *et al*. (2015) have shown the activities of different extracts of *Cymbopogon citratus* as antibacterial, antimalarial, antifungal, and antiprotozoal. On the other hand, details of the components and mode of actions of the essential oil components of C. citratus have been well elucidated (Adukwu *et al*., 2016; Katsukawa *et al*., 2010). For instance, Katsukawa and co-researchers (Katsukawa *et al*., 2010) established that essential oil from lemon grass acts on microorganism by suppressing the protein expression. Through cellbased transfection assay, they postulated that citral, a major component of lemon grass essential oil suppressed cyclooxygenase (COX-2) which is a key enzyme for prostaglandin synthesis and also an activator of peroxisome proliferator-activated receptor (PPAR).

While prostaglandins are group of lipids made at sites of tissue damage or injurious microbial infection for quick repairs, PPAR is a molecular target for “lifestyle-related” diseases. They further argued that citral also suppressed both LPS-induced COX-2 mRNA and protein expression, albeit dose-dependently

2.6.1. Antibacterial Potential

The antibacterial activity of plant extracts has been extensively studied, yielding promising results. In particular, the volatile oil portion of the aqueous extract of lemongrass has been investigated, revealing significant antimicrobial properties (Grace *et al*., 1984). Key bioactive compounds identified in lemongrass oil include α-citral (geranial) and β-citral (neral), which inhibit the growth of both Gram-positive and Gram-negative bacteria. Interestingly, another component, myrcene, lacks antibacterial activity on its own but enhances the overall activity when combined with α-citral and β-citral (Grace *et al*., 1984).

2.6.2. Antifungal Properties

Essential oils extracted from lemongrass have shown remarkable antifungal activity, targeting both pathogenic and edible fungi. These oils effectively inhibit fungal growth and reduce the production of mycotoxins during the storage of grains and other food products (Fandohan *et al*., 2008; Nguefacka *et al*., 2012). Synergistic effects among various oil fractions have been observed, demonstrating both cooperative and antagonistic interactions (Viana *et al*., 2000; Nguefacka *et al*., 2012). Lemongrass oil exhibits a broad-spectrum antifungal effect, combating filamentous fungi and inactivating pathogenic yeasts such as *Candida* species by inhibiting their growth (Dharmendra *et al*., 2001).

2.6.3. Antinociceptive Properties

The antinociceptive effects of lemongrass have been the subject of extensive research. Early studies suggested minimal efficacy, contradicting traditional medicinal claims (Carlini *et al*., 1986; Leite *et al*., 1986; Souza-Formigoni *et al*., 1986). However, more recent investigations

present a different perspective. Viana *et al*. (2000) demonstrated that lemongrass tea exhibits antinociceptive properties, as shown by positive outcomes in nociception tests. Essential oils containing citral, but not myrcene, were found to have antinociceptive activity in various experimental models in mice.

In the hot plate test, lemongrass oil increased the response time to stimuli, while in the acetic acid-induced writhing test, it inhibited abdominal contractions in a dose-dependent manner. Additionally, the formalin test showed significant inhibition of licking behavior during both phases of the experiment when lemongrass oil was administered intraperitoneally (Viana *et al*., 2000).

2.6.4. Cytotoxicity and Antimutagenicity

Numerous studies, both in vivo and in vitro, have assessed the cytotoxic and mutagenic effects of lemongrass extract to establish its safety. Phenolic compounds isolated from the methanolic extract of lemongrass were found to be non-toxic to human lung fibroblasts, even at high concentrations (Cheel *et al*., 2005). Oral consumption of lemongrass oil by adult rats for two months showed no adverse effects on the animals or their offspring (Lucia *et al*., 1986). Moreover, lemongrass extract demonstrated cytoprotective properties, such as restoring mitochondrial membrane integrity in stressed murine alveolar macrophages with a 5% ethanol extract (Tiwari *et al*., 2010).

Lemongrass extract, prepared with 80% ethanol, displayed no mutagenic properties in the *Salmonella* mutation test and counteracted chemical-induced mutations in *Salmonella typhimurium* strains TA98 and TA100 (Vinitketkumnuen *et al*., 1994). Additionally, the extract

inhibited chromosome damage induced by mitomycin C in human lymphocytes (Meevatee *et al*., 1993). Essential oil from lemongrass also protected leukocyte DNA in female Balb/C mice against damage induced by N-methyl-N-nitrosourea (MNU) (Bidinotto *et al*., 2011).

In human toxicity assessments, the consumption of lemongrass infusion did not produce toxic effects, as biochemical assays and physical examinations showed no significant changes after 6 to 14 days. While slight increases in bilirubin and amylase levels were observed in some volunteers, these changes were not medically significant. Additionally, lemongrass oil exhibited no hypnotic or anxiolytic properties (Leite *et al*., 1986). Studies in rats revealed that lemongrass oil has an LD50 greater than 3500 mg/kg body weight, indicating low acute and sub-acute toxicity. However, at higher doses (2000–3000 mg/kg body weight), abnormalities such as hepatocyte necrosis and leukocyte infiltration in the liver were observed. These findings affirm that lemongrass oil is safe for human consumption at prescribed concentrations (Fandohan *et al*., 2008).

2.7. General Uses of Lemongrass

Lemongrass, known for its wide array of medicinal properties, has garnered significant attention for its antimicrobial action, particularly the effectiveness of its essential oil in the vapor phase (Lopez *et al*., 2005). Studies have shown that lemongrass oil (LGO) exhibits antifungal properties in both vapor and liquid phases, with its antifungal action observed at the ultrastructural level via techniques like Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM). The antimicrobial properties of plant-derived compounds are increasingly important, as they tend to have fewer side effects compared to conventional

antibiotics. This has led to growing interest in herbal antimicrobials due to the rising concerns about the side effects and multidrug resistance of pathogenic microorganisms.

Lemongrass is widely recognized for its health benefits, including digestive stimulation, antioxidant activity, antimicrobial effects, anti-inflammatory properties, hypolipidemic effects, and anticancer potential (Shankar *et al*., 2005). Essential oils, which can be derived from various plant sources such as flowers, trees, grasses, and herbs, are known for their antimicrobial properties, although the exact mechanisms of action are not fully understood. It is believed that these oils may disrupt bacterial cell membranes by interacting with lipophilic components, causing increased membrane permeability (Maizura *et al*., 2007).

Lemongrass also contains flavonoids, such as luteolin and its glycosides, which are of medicinal significance. These compounds have been studied for their anti-inflammatory effects, and it has been found that luteolin glycosides have lower cytotoxicity than luteolin itself. These glycosides, especially when combined with anti-inflammatory treatments, show promise as safer alternatives in the food and pharmaceutical industries (Hammer *et al*., 1999).

In addition to its medicinal uses, lemongrass oil has various therapeutic applications. It is commonly used in the pharmaceutical industry for its antidepressant, analgesic, antipyretic, bactericidal, antiseptic, carminative, and astringent properties. It is also effective as an insect repellent, diuretic agent for fever, and a remedy for conditions like toothaches, headaches, and nausea. Furthermore, lemongrass oil blends well with other essential oils, such as coriander, basil, jasmine, cedarwood, geranium, lavender, and tea tree oil. It is also utilized in foot baths and talc preparations for combating sweaty feet and treating fungal infections such as ringworm and tinea. However, it is important to note that lemongrass can sometimes trigger skin

inflammation and other allergic reactions. Therefore, it is advised to avoid its application during pregnancy (Naik *et al*., 2010).

Lemongrass, a versatile herb, has been used for both culinary and medicinal purposes over the years. Its refreshing aroma and health benefits make it a valuable addition to many industries and household applications (Shankar *et al*., 2005).

2.8. Utilization of Lemongrass in Folk Medicine

Lemongrass has a long history of use in traditional medicine worldwide for treating a variety of health conditions. According to Nambiar and Matela (2012), lemongrass is commonly used to address gastrointestinal problems, fevers, menstrual irregularities, malaria, and pneumonia. The aerial parts of the lemongrass plant are frequently employed in folk medicine through infusions or stews. Scientific research supports its use for treating conditions such as fever, mental disorders, inflammation, and digestive issues, highlighting the plant's diverse therapeutic potential.

Lemongrass tea, for instance, is popularly used to reduce fever when consumed in regular intervals, such as one cup every four hours (Heinerman’s Encyclopedia of Healing). Additionally, components found in lemongrass have been identified as potentially chemo-protective, particularly in reducing the risk of colon cancer in animal models (Puatanachokchai *et al*., 2002). The diuretic properties of lemongrass tea also make it effective in managing urinary issues and promoting water balance in the body.

Phytochemically, lemongrass contains essential oils, including terpinolene, geranyl acetate, myrcene, and terpinol methylhistamine, which have been analyzed for their protective and

beneficial properties (Asaolu *et al*., 2009). The presence of flavonoids and phenols in lemongrass contributes to its therapeutic value, offering various health benefits. Studies have demonstrated that the essential oil of lemongrass can delay seizures in animal models, as seen in pentylenetetrazol-induced colonic seizures, indicating its potential for neurological applications.

Furthermore, lemongrass is noted for its anti-cancer and anti-mutagenic properties. It is also recognized for its anxiolytic, anti-diabetic, antioxidant, non-toxic, and anti-fungal characteristics (Shah *et al*., 2011). The plant's biological properties extend beyond its antimicrobial activity to include anti-hypolipidemic, anti-atherosclerotic, immune-stimulating, anti-hypertensive, and anti-tumor effects. In India, for example, lemongrass is recommended for treating digestive disorders, inflammation, neurological conditions, fever, and other health issues.

Lemongrass has also proven effective in managing rheumatism, menstrual disorders, infections, and various other ailments. In vitro analysis has shown that lemongrass extracts, particularly at a concentration of 60 µg/mL, possess significant antioxidant activity, including DPPH scavenging ability (85%), superoxide scavenging (76%), and hydroxyl radical scavenging (70%), among others (Nambiar and Matela, 2012).

The therapeutic applications of lemongrass extend into commercial markets, including pharmaceuticals, food, cosmetics, and perfumery. In the pharmaceutical industry, lemongrass extracts serve as sources of active ingredients for medicinal products. In the food industry, lemongrass is valued both for its flavoring properties and its medicinal benefits. While the stalks are tough and not typically consumed directly, they can be crushed or chopped and added to sauces for fish or poultry to enhance flavor. Lemongrass is also a popular ingredient in medicinal teas, commonly used to treat symptoms such as vomiting, bladder issues, congestion, headaches,

coughs, fever, and digestive discomfort. Moreover, regular consumption of lemongrass tea or powder (1–4 cups daily) may have hypocholesterolemic effects, lowering cholesterol levels and promoting overall health.

In the culinary industry, lemongrass is used in various recipes, including lemongrass meat, sweet lemongrass blends, and lemongrass rice. However, due to its limited shelf life, it is often stored in powdered form to preserve its quality and flavor. Despite its rapid deterioration in fresh form, lemongrass continues to play a significant role in traditional and modern health practices due to its broad range of medicinal and therapeutic benefits.

The potential health benefits of lemongrass are summarized in Figure 1.

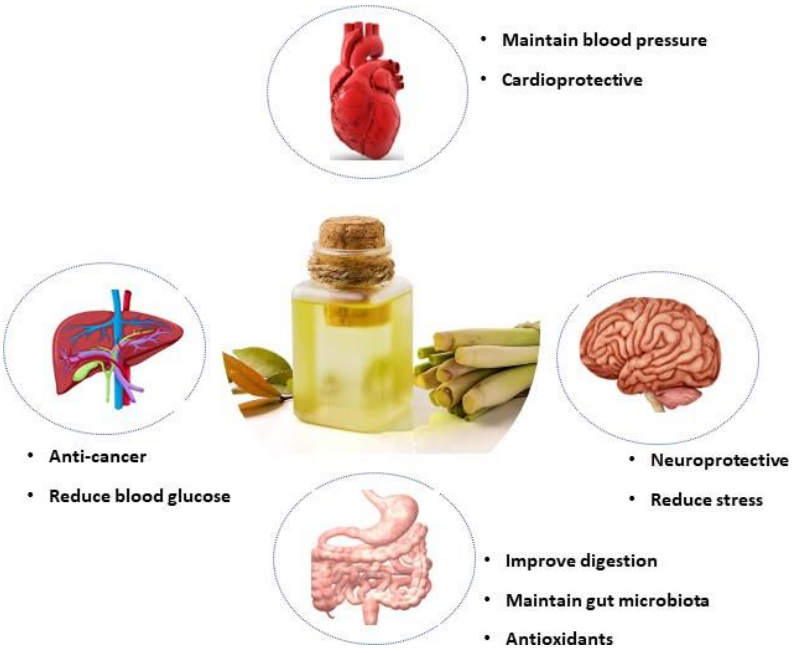

Figure 2. Potential applications of lemongrass.

2.9. Industrial Applications of Lemongrass

Lemongrass is a versatile plant with various industrial applications, particularly in the food, beverage, cosmetic, and fragrance industries. The essential oil derived from lemongrass is known to enhance the flavor of certain dishes, including fish, wines, sauces, confections, spices, and even tea leaves. In Southeast Asia, including Indonesia, Vietnam, Malaysia, Thailand, Pakistan, and the Philippines, lemongrass is a popular culinary herb, used fresh, powdered, or dried for its aromatic, lemon-scented qualities. While the pseudostem of lemongrass is tough and not easily consumable, it can be crumbled and added to dishes or grilling rubs. Bruising or adding the whole pseudostem to food releases aromatic oils, enhancing the flavor. The leaves and stalks of lemongrass impart a mild lemon flavor, making it a primary component in many Thai and other Asian cuisines, including Indonesian and Malaysian dishes (Majewska *et al*., 2019).

Lemongrass is widely used in Latin American and African cuisines, adding a distinct flavor to curries, poultry, seafood, soups, and teas (Đord¯evic´ and Đurovic´-Pejc˘ev, 2015). Dried lemongrass leaves are especially popular as a lemon-flavored ingredient in herbal teas. Unlike regular tea, lemongrass tea is a diuretic and does not significantly alter the body's biochemistry. In Thailand, lemongrass is frequently used as a key ingredient in a popular beverage called "Takrai" (Nambiar and Matela, 2012), as well as in marinades, curries, and seafood soups.

The commercial applications of lemongrass extend to the cosmetic and fragrance industries. The essential oil from the *Cymbopogon* genus is commonly used as an aromatic component in soaps and perfumes, as well as in palm arosa oil. In the cosmetic sector, lemongrass is often included in products that combine glycerol, lemongrass oil, and lemon balm oil. Lemongrass essential oil has been shown to possess insect-repellent properties, making it a valuable ingredient in insect-

repellent lotions (da Silva and Ricci-Júnior, 2020). Its antioxidant properties also provide significant benefits in skincare, helping to prevent various skin ailments caused by oxidative stress. Additionally, lemongrass oil is used in anti-aging creams, as oxidative stress is linked to chronic degenerative disorders that accelerate the aging process (Sara *et al*., 2006).

Recent studies have also explored the bioactivities of lemongrass, revealing its potential benefits in aesthetic applications (Ali *et al*., 2022). This ongoing research highlights the diverse industrial uses of lemongrass, reinforcing its importance across multiple sectors.

CHAPTER THREE

MATERIAL AND METHODS

3.1 Bacterial isolates used for study

The clinical isolates which were *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were obtained from the culture collection unit of Microbiology Laborartory. The bacterial stock cultures were maintained at 4^0C. All microbial stock cultures were freshened by sterile inoculation loop on nutrient agar plates under a suitable condition and temperature of 37^0C for 24hours. The following day, the streaked cultures were again subcultured on media plates and incubated at 37^0C for 24 hours.

3.2 Apparatus and Equipment

Test tubes, conical flask (500ml and 1000ml), Petri dish, measuring cylinder, Bunsen burner, pipette, inoculating loop, cotton wool, sensitivity disk, incubator and microscope.

3.3 Preparation and Sterilization of Materials

The media used was prepared according to the manufacturer's instructions. Sterilization of glassware and other autoclavable materials was performed at 121^0C for 15 minutes. Work surfaces was sterilized using 3% alcohol.

3.4 Identification And Confirmatory Test Of The Organisms

The bacteria isolates obtained from the culture collection unit of Microbiology Laboratory, Mycofarm, were identified based on their morphological, cultural and biochemical

characteristics. Biochemical test included indole, citrate, oxidase, triple sugar iron, and Potassium hydroxide test.

3.4.1 Indole test

The indole test relies on the reaction between indole and p-dimethylaminobenzaldehyde in kovac reagent, yielding a red complex. Pure bacterial cultures were grown in sterile peptone broth at 37°C for 24 hours, and kovac reagent was added to the specimen concentration in the test tubes, resulting in the rapid appearance of a red coloration on the medium's surface, indicating the presence of indole (Cheesbrough, 2006).

3.4.2 Citrate test

For the citrate test, Simmon's citrate agar was used to determine the bacterium's ability to utilize citrate as its sole carbon source. Bacterial colonies were inoculated into Simmons citrate agar slopes and incubated at 37°C overnight, causing a color change from green to blue in the medium if citrate utilization occurred.

3.4.3 Oxidase test

The oxidase test involved placing two drops of 1% freshly prepared oxidase reagent (phenylenediamine) on a filter paper. Test organisms were smeared on the paper, with a positive result indicated by deep purple coloration within 5-30 seconds.

3.4.4 Triple Sugar Iron (TSI) Test

Triple Sugar Iron (TSI) test is a microbiological test that assesses a microorganism's ability to ferment sugars and produce hydrogen sulfide. TSI is inoculated by stabbing through the center of the medium to the bottom of the tube and then streaking the surface of the agar slant. The tube is incubated at 35°-37°C in ambient air for 18 to 24 hours. Carbohydrate fermentation is evidenced

by gas production and a change in pH indicator color from red to yellow. Bubbles or cracks in the agar medium indicates gas formation (CO2 and H2).

2.4.5. Potassium Hydroxide Test (KOH Test)

The purpose of the potassioum hydroxide test (KOH test) is to identify gram-negative bacteria. KOH dissolves the thin layer of peptidoglycan in the cell walls of gram-negative bacteria, but does not affect the cell walls of gram-positive bacteria. One drop of 3% KOH is applied on a microscopic slide and a loop is used to transfer a good amount of bacteria (cultivated for 24-48 h) to the drop of KOH. It is stirred carefully. The solution of gram negative-bacteria becomes viscous and forms a mucous thread in 30 seconds. For positive results, the solution with the bacteria (gram negative) will be viscous while for negative results, the solution with the bacteria (gram positive) will not be viscous.

3.5 .0 Preparation of Media Culture

To successful carry out our research, the clinical isolates were cultured in suitable medium. Three (3) Agar medium in total were utilized at different occasion in the course of this research experiment.

3.5.1 Mueller Hinton Agar

This is a microbiological growth medium that is commonly used for antibiotic susceptibility testing. In preparation, 38g was dissolved in 1000ml distilled water in a conical flask covered with cotton wool and aluminum foil paper. It was mixed thoroughly and sterilized by autoclaving at 121^0C for 15 minutes. The medium was cooled to 45-50^0C and then dispensed aseptically into sterile Petri dishes in the laminar flow hood cabinet

3.5.2 Nutrient Agar

Nutrient Agar is a general-purpose plating medium used for the isolation, cultivation, and maintenance of a variety of fastidious and non-fastidious microorganisms. In preparation, 15g was dissolved in 1000 ml distilled water in a conical flask covered with cotton wool and aluminum foil paper. It was mixed thoroughly and sterilized by autoclaving at $121^{O}C$ for 15 minutes.

3.5.3 McConkey Agar

McConkey Agar is a selective and differential medium for the isolation and identification of *Staphylococcus aureus* from clinical and non-clinical specimens. . It is prepared by suspending 111 grams of Mannitol Salt Agar in 1000mls of distilled water then boiled to dissolve the medium completely. The solution is finally sterilized by autoclaving at 15 lbs. pressure (121°C) for 15 minutes.

3.6 Collection and identification of plant material

Fresh leaves of Lemongrass leaf (*Cymbopogon citratus*) were obtained from local sellers of vegetables and herb in New Benin market, Edo state, Nigeria. They were identified and confirmed by the Plant Biology and Biotechnology Department (PBB) of the University of Benin (UNIBEN), Edo state, Nigeria.

3.7 Extraction of plant Materials

Procedure:

Fresh leaves of Lemongrass leaf (*Cymbopogon citratus*) were collected from a home gardern in Benin City, Edo state. The leaves were thoroughly washed, shade-dried and finely powered.

They were thoroughly washed to remove dirt and dust, then dried in the shade to preserve its medicinal properties. The dried leaves were grinded using dry blender. The curry leaf powder was stored in an airtight container and used for further experiments.

The experiment was carried out using water as the main solvents. 1g of plant source was dissolved in 25 ml. then the solutions were stored for 24 hours at room temperature in a closed tubes. After 24 hours, this solution was centrifuged and filtered using Wattman filter paper and then kept in an airtight bottles at 4°C for further experiment. This fine powder was analyzed for its phytochemicals constituents.

3.7.1. PREPARATION OF EXTRACTS

Hot Water Extraction

Fresh curry leaves was collected and washed thoroughly with water. The leaves were dried with a clean cloth or paper towels to remove excess moisture. Afterwards a required amount of curry leaves was weighed and collected. Water was heated (100°C) in a glass beaker or flask and then the weighed curry leaves was added to the hot water. The mixture was allowed to steep for 10-15 minutes, depending on the desired strength of the extract. The mixture was strained using a cheesecloth or a fine-mesh sieve into another container. The solids were filtered out and discarded while the liquid was made to cool. The hot water extract was collected and stored in a clean container.

Cold Water Extraction

Fresh curry leaves was collected and washed thoroughly with water. The leaves were dried with a clean cloth or paper towels to remove excess moisture. A required amount of the curry leaves

was weighed. The weighed curry leaves was added to a glass beaker or flask containing cold water (room temperature). The mixture was allowed to steep for 2-4 hours or overnight (8-12 hours), depending on the desired strength of the extract. The mixture was strained using a cheesecloth or a fine-mesh sieve into another container. The solids were filtered out and discarded while the liquid was made to cool. The hot water extract was collected and stored in a clean container.

3.8. Antibacterial activity

The antibacterial activity against the test isolates was checked by agar well diffusion method. Cultures of the isolates were aseptically swabbed on Muller Hinton agar plates (standardized inoculums of the test bacteria adjusted to 0.5 MCFARLAND turbidity standards). Wells of 5 mm diameter was made aseptically by cork-borer on the Inoculated plates and different concentrations of the extract were introduced into the labeled wells. The plates were incubated at 37 °C for 24 h in an upright position. The zone of inhibition in millimeter was recorded with the help of meter rule. The experiment was carried out in triplicates to minimize probability of error.

3.8.1. Minimum Inhibitory Concentration

The lowest concentration of the extract which prevents visible growths of the test isolates on the sterile medium was also determined by agar well diffusion method.

3.8.2. Minimum Bactericidal Concentrations

1 ml of the sample of known concentration was transferred into a test tubes, 1ml of the test organisms previously diluted to 0.5 MCFARLAND turbidity standard was also introduced into

the test tubes and incubated for 24 hours. A loopful of the innoculum was aseptically introduced on a sterile agar medium and incubated for 24 hours.

3.9 PHYTOCHEMICAL ANALYSIS

The leaf powder of the plant examined was dissolved in various solvents and the following phytochemical preliminary tests were carried out as follow:

3.9.1 Alkaloids

15ml of 1% HCl was added to 0.5g of the sample. It was placed on a steam bath for 10 minutes after which it was filtered. 2 drops of dragendroff's reagent was added to 1ml of the filtrate.

Observation: Presence of red/orange precipitate indicates a positive test.

3.9.2 Flavonoids:

5-10 drops of diluted HCl and small amount of Zn or Mg were added in a test tube containing 0.5ml of alcoholic extract of the samples and the solution was boiled for few minutes. Observation of a reddish pink or dirty brown color indicates the presence of flavonoids.

3.9.3 Glycosides:

A small amount of alcoholic extract of samples was dissolved in 1ml of water and then an aqueous solution of sodium hydroxide was added. The presence of glycosides is indicated by the appearance of a yellow color in the sample.

3.9.4 Steroids

Dissolving about 100mg of dried extract in 2ml of chloroform and adding sulfuric acid carefully results in the formation of a lower layer. A reddish-brown colour at the interface indicates the presence of a steroid ring.

3.9.5 Saponins

2g of powdered sample was boiled in 20ml of distilled water in a water bath after which it was filtered. 10ml of the filtrate was mixed with 5ml of distilled water after which it was shaken vigorously to form a stable broth. 3 drops of olive oil were added and the mixture was shaken again.

Observation: The formation of emulsion indicates a positive test. NB: Persistent frothing also indicates a positive test.

3.9.6 Phenols

To 1ml of sample alcoholic solution, 2ml of distilled water was added, followed by a few drops of a 10% aqueous ferric chloride solution. The presence of phenols is indicated by the appearance of blue or green color.

3.9.7 Tannins

0.5g of the sample was boiled in 20ml of distilled water, after which it was allowed to cool and filtered. 3 drops of 0.1% FeCl was added to 5ml of the filtrate.

Observation: Formation of a brownish green or blue-black precipitate indicates a positive test.

3.9.7. Terpenoid:

To 1mg of extract add 2ml of chloroform and 1ml of concentrated of H_2SO_4. The observation of a reddish-brown color indicates the presence of terpenoids.

CHAPTER FOUR

4.0. RESULTS

In this study the antimicrobial properties of Lemongrass leaf (*Cymbopogon citratus*) leave extract was evaluated. The phytochemical properties of the Lemongrass leaf (*Cymbopogon citratus*) are presented in Table 4.1, while Table 4.2 represents the antimicrobial activities of the leaf extract, measured in millimeters (mm). The minimum inhibitory concentration (MIC) of the extract against bacterial isolates of *Staphylococcus aureus*, *Pseudomonas sp.*, and *Escherichia coli* is represented in Table 4.3, while Table 4.4 represents the minimum bactericidal concentration (MBC) of the leaf extract against the isolates.

Table 4.1 Present the phytochemical test results for alkaloids, flavonoids, glycosides, tannins, and saponins in both cold and hot water extracts. Flavonoids, tannins, and glycosides were present in both cold and hot water extracts, with glycosides showing a strong presence in the hot extract. Saponins were absent in both extracts, while steroids and terpenoids were also absent in all sample extracts of the leaves.

Table 4.2 summarizes the antibacterial activity of cold and hot Lemongrass leaf extracts against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas sp.* At 100% concentration, the hot extract showed stronger antibacterial activity compared to the cold extract. For *Staphylococcus aureus*, the cold extract produced a zone of inhibition of 9.0 ± 0.50 mm, while the hot extract showed 13.00 ± 0.00 mm. For *Escherichia coli*, the cold extract produced 11.20 ± 0.20 mm, while the hot extract showed 16.00 ± 0.00 mm. For *Pseudomonas sp.*, the cold extract had 12.00 ± 0.00 mm, and the hot extract had 18.20 ± 0.00 mm. At 50% concentration, the cold extract showed reduced activity, with zones of inhibition of 7.10 ± 0.10 mm for *Staphylococcus aureus*, 7.00 ± 0.00 mm

for *Escherichia coli*, and 10.00 ± 0.10 mm for *Pseudomonas sp.* The hot extract showed 5.00 ± 0.00 mm for *Staphylococcus aureus*, 12.00 ± 0.00 mm for *Escherichia coli*, and 16.00 ± 0.00 mm for *Pseudomonas sp.* At 25% and 12.5% concentrations, no inhibition was observed for most organisms. These results suggest that the hot extract generally exhibited stronger antibacterial effects at higher concentrations, with *Pseudomonas sp.* being the most sensitive to both extracts.

Table 4.3 presents the Minimum Inhibitory Concentration (MIC) of Lemongrass leaf (Cymbopogon citratus) extract against three bacterial isolates: *Escherichia coli*, *Pseudomonas sp.*, and *Staphylococcus aureus*. The table shows the concentration (in mg/ml) of both cold and hot extracts required to inhibit the growth of each bacterial isolate. For *Escherichia coli*, the cold extract did not show an inhibitory concentration, while the hot extract had an MIC of 25 mg/ml. For *Pseudomonas sp.*, the cold extract had an MIC of 100 mg/ml, while the hot extract showed an MIC of 50 mg/ml. For *Staphylococcus aureus*, both cold and hot extracts had an MIC of 25 mg/ml.

Table 4.4 presents the Minimum Bactericidal Concentration (MBC) of Lemongrass leaf (Cymbopogon citratus) extract against three bacterial isolates: *Escherichia coli*, *Pseudomonas sp.*, and *Staphylococcus aureus*. The table also provides the inference based on the MBC values, indicating whether the extract has bacteriostatic (inhibiting growth) or bactericidal (killing the bacteria) effects. For *Escherichia coli*, both cold and hot extracts were **bacteriostatic**, meaning they inhibited the growth of the bacteria without killing it. For *Pseudomonas sp.*, the cold extract was bactericidal, meaning it killed the bacteria, while the hot extract was bacteriostatic, inhibiting growth without killing. For *Staphylococcus aureus*, the cold extract was bacteriostatic, while the hot extract was bactericidal, indicating it killed the bacteria.

For antibiotics susceptibility and resistance test, the Gram positive bacterial isolates showed high susceptibility with ciprofloxacin while Gram negative isolate was sensitive to azithromycin, .

Cultural Morphological and Biochemical Characteristics of Bacteria Isolates

Elevation	Flat	Raised	Raised
Margin	Undulate	Undulate	Entire
Color	Cream	Green	Cream
Shape	Irregular	Irregular	Circular
Size	Large	Medium	Medium
Gram Stain	-	-	+
Cell Type	Rod	Rod	Cocci
Arrangement	Disperse	Disperse	Clusters
Color (Gram Reaction)	Pink	Pink	Purple
KOH String Test	+	-	-
Catalase	+	+	+
Indole	+	-	-
Citrate	-	+	-
Oxidase	-	+	-
Glucose	+	+	+
Sucrose	-	-	+
Lactose	+	-	+
Gas Formation	+	-	-
H_2S Formation	-	-	-
TSI (Slant/Butt) Reaction	A/AG	K/AG	K/A
Identity	*E. coli*	*Pseudomonas* sp.	*Staphylococcus* sp.

Key: (-) negative test; (+) positive test; (A) Acid; (K) Alkaline; (G) Gas production (bubbles); (H_2S) Hydrogen sulphide (black precipitate); (KOH) Potassium hydroxide test; (TSI) Triple sugar iron test.

Table 4.1: Qualitative Phytochemical screening of Lemongrass leaf (*Cymbopogon citratus*) **extract**

Phytochemical	**Cold**	**Hot**
Alkaloids	-	+
Flavonoids	+	++
Saponins	-	-
Tannins	++	+
Terpenoids	-	+
Steroids	+	+
Phenolics	+	+
Glycosides	+	+++

Key: + = present

- = absent

Table 4.2: Antibacterial activity of cold and hot extracts of Lemongrass leaf (*Cymbopogon citratus*) **extract**

		ZONES OF INHIBITIONS (mm)			
Test organism	Extract	100%	50%	25%	12.50%
Staphylococcus aureus	Cold	9.0±0.50	7.10±0.10	00.00±0.60	0.00±0.00
	Hot	13.00±0.00	5.00±0.00	0.00±0.00	0.00±0.00
Escherichia coli	Cold	11.20±0.20	7.00±0.00	0.00±0.00	0.00±0.00
	Hot	16.00±0.00	12.00±0.00	6.00±0.00	0.00±0.00
Pseudomonas sp.	Cold	12.00±0.00	10.00±0.10	0.00±0.00	0.00±0.00
	Hot	18.20±0.00	16.00±0.00	00±0.00	0.00±0.00

Mean inhibition zones ± standard deviation

Table 4.3: Minimum inhibitory concentration (MIC) of Lemongrass leaf (*Cymbopogon citratus*) **extract**

ISOLATES	**EXTRACT**	**CONCENTRATION** (mg/ml)
Escherichia coli	Cold	25
	Hot	50
Pseudomonas sp	Cold	100
	Hot	50
Staphylococcus aureus	Cold	25
	Hot	25

Table 4.4: Minimum bactericidal concentration (MBC) of Lemongrass leaf (*Cymbopogon citratus*) **extract**

ISOLATES	EXTRACT	INFERENCE
Escherichia coli	Cold	Bacteriostatic
	Hot	Bacteriostatic
Pseudomonas sp	Cold	Bactericidal
	Hot	Bacteriostatic
Staphylococcus aureus	Cold	Bacteriostatic
	Hot	Bactericidal

Table 4.5: Antibiotic Sensitivity test of the bacterial isolates

Gram positive bacterial isolates

ISOLATE	PEF	CN	APX	Z	AM	R	CPX	AZ	SXT	E
Staphylococcus aureus	22	-	-	8	10	8	18	12	-	10

Gram negative bacterial isolates

ISOLATES	CPX	AM	AU	CN	PEF	OFX	AZ	LEV	CF	SP
Pseudomonas spp	16	8	8	16	14	16	10	24	-	-
Escherichia coli	14	-	-	10	8	18	12	20	6	-

NB:

RESISTANCE (R) = 0-10mm

INTERMEDIATE (I) =11-16mm

SENSITIVE (S) =17mm and above

KEY: POSITIVE DISC **KEY: NEGATIVE DISC**

Abbreviation	Antibiotics	Concentration	Abbreviation	Antibiotics	Concentration
PEF	Pefloxacin	10µg	LEV	Levofloxacin	20µg
CN	Gentamycin	10µg	CF	Cefotaxim	10µg
APX	Ampliclox	30µg	SP	Sparifloxacin	10µg
Z	Zinnacef	20µg	CPX	Ciprofloxacin	30µg
AM	Amoxacillin	30µg	AM	Amoxacillin	30µg
R	Rocephin	25µg	AU	Augmentin	10µg
CPX	Ciprofloxacin	10µg	CN	Gentamycin	30µg
AZ	Azithromycin	12µg	PEF	Pefloxacin	30µg
LEV	Levofloxacin	20µg	OFX	Tarivid	10µg
E	Erthromycin	10µg	AZ	Azithromycin	12µg

CHAPTER FIVE

5.0. DISCUSSION

Lemongrass (*Cymbopogon citratus*) is a tropical plant known for its distinct lemon-like aroma and wide range of medicinal, culinary, and aromatic uses. It has been used traditionally in various cultures for its antimicrobial, anti-inflammatory, and antioxidant properties(Kumoro et al., 2021).The plant contains several bioactive compounds, including flavonoids, glycosides, and essential oils, which contribute to its therapeutic effects (Gaba et al., 2020). In recent years, there has been increasing interest in the antimicrobial properties of lemongrass, particularly in the context of combating bacterial infections and enhancing food preservation. This study investigate the antibacterial activity of lemongrass leaf extracts, focusing on their phytochemical composition, antimicrobial efficacy, and minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against bacterial isolates.

The phytochemical analysis of Lemongrass leaf extracts (Table 4.1) revealed the presence of alkaloids, flavonoids, glycosides, tannins, and saponins, although the presence of saponins was absent in both the cold and hot water extracts. Flavonoids, tannins, and glycosides were present in both extracts, with glycosides exhibiting stronger presence in the hot extract. These phytochemicals are known for their antioxidant and antimicrobial properties (Alzobaay and Kadhim, 2018; Shendurse et al., 2021), which may explain the antimicrobial activity observed in the current study. Flavonoids, have been reported to contribute to the inhibition of bacterial enzymes and membrane damage (Górniak et al., 2019), while tannins are known to interfere with bacterial cell wall synthesis (Smith et al., 2005). The absence of saponins, steroids, and terpenoids in both extracts suggests that these compounds may not be significantly involved in the antimicrobial activities observed in this study.

The antimicrobial efficacy of both cold and hot water extracts of Lemongrass was tested against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas sp.* The results (Table 4.2) show that the hot extract exhibited stronger antibacterial activity at all tested concentrations, with the highest zones of inhibition observed for *Pseudomonas sp.*. At 100% concentration, the hot extract produced a zone of inhibition of 18.20 ± 0.00 mm for *Pseudomonas sp.*, indicating that *Pseudomonas sp.* was the most sensitive to the Lemongrass extract. For *Staphylococcus aureus,* the zone of inhibition was 15.30 ± 0.12 mm, and for *Escherichia coli*, the zone of inhibition was 12.00 ± 0.00 mm at the same concentration. The observed antibacterial activity can be attributed to the combined effects of the phytochemicals present in the extract, especially glycosides, flavonoids, and tannins, which have been reported to act on bacterial cell membranes and inhibit protein synthesis (Jassim & Naji, 2003). Similar findings have been reported by Balakrishnan et al. (2014), who observed that Lemongrass extracts have potent antimicrobial properties against both Gram-positive and Gram-negative bacteria.

Interestingly, at lower concentrations (25% and 12.5%), no inhibition was observed for most of the bacterial isolates. This suggests that higher concentrations of the extract are required for effective antibacterial activity, a finding consistent with previous studies that reported dose-dependent antimicrobial effects of plant extracts (Lone et al., 2013).

Table 4.3 presents the MIC values for Lemongrass leaf extracts. The MIC values indicate the lowest concentration of the extract required to inhibit bacterial growth. For *Escherichia coli*, the cold extract did not exhibit any inhibitory activity, while the hot extract showed an MIC of 25 mg/ml. For *Pseudomonas sp.*, the cold extract showed a higher MIC value of 100 mg/ml, while the hot extract exhibited a lower MIC of 50 mg/ml, suggesting that heat may have enhanced the

solubility and bioavailability of the antimicrobial compounds in the Lemongrass extract (Bamidele et al., 2017). Both cold and hot extracts showed an MIC of 25 mg/ml for *Staphylococcus aureus*, indicating that both extracts were equally effective at inhibiting the growth of this Gram-positive bacterium.

Table 4.4 presents the MBC values of the Lemongrass leaf extract. The MBC values are useful in determining whether the extract acts as a bacteriostatic or bactericidal agent. For *Escherichia coli*, both cold and hot extracts were bacteriostatic, meaning they inhibited bacterial growth without killing the bacteria. For *Pseudomonas sp.*, the cold extract was bactericidal at 100 mg/ml, whereas the hot extract was bacteriostatic at 50 mg/ml. Similarly, for *Staphylococcus aureus*, the cold extract was bacteriostatic at 25 mg/ml, and the hot extract was bactericidal at 25 mg/ml. These results suggest that the antimicrobial action of Lemongrass extract may be concentration-dependent, with higher concentrations exhibiting bactericidal effects.

The observed bacteriostatic and bactericidal properties of Lemongrass extracts can be attributed to the presence of bioactive compounds such as flavonoids and glycosides. Flavonoids, for instance, have been shown to exhibit both bactericidal and bacteriostatic properties depending on their concentration (Ajani et al., 2012). Glycosides, on the other hand, are known for their ability to disrupt bacterial cell membranes and cause leakage of cell contents, leading to bacterial death (Tagousop et al., 2018).

The study also tested the antibiotic susceptibility of the bacterial isolates. The Gram-positive isolates, including *Staphylococcus aureus*, showed high susceptibility to ciprofloxacin, while the Gram-negative isolate, *Pseudomonas sp.*, was most sensitive to azithromycin. *Staphylococcus aureus* exhibited zones of inhibition of 21.60 ± 0.15 mm with ciprofloxacin and 18.30 ± 0.10

mm with gentamicin, while *Pseudomonas sp.* showed zones of 22.50 ± 0.20 mm with azithromycin and 20.00 ± 0.10 mm with ciprofloxacin. This finding is consistent with previous studies that have reported high susceptibility of *Staphylococcus aureus* to ciprofloxacin and *Pseudomonas sp.* to azithromycin. The results suggest that Lemongrass leaf extract could potentially serve as an alternative or complementary antimicrobial agent, especially against antibiotic-resistant bacteria, which is a growing concern in clinical settings (Ugochi et al., 2025).

5.1. CONCLUSION

The results of this study indicate that Lemongrass leaf (*Cymbopogon citratus*) extract possesses significant antimicrobial properties, particularly when prepared using hot water extraction. The hot extract exhibited stronger antibacterial activity, with *Pseudomonas sp.* being the most sensitive organism. The presence of flavonoids, tannins, and glycosides in the extract may contribute to its antimicrobial activity. The hot extract also exhibited both bacteriostatic and bactericidal properties, depending on the bacterial isolate. These findings suggest that Lemongrass leaf extract has potential as an alternative antimicrobial agent, especially in combating bacterial infections, and warrants further investigation for its potential use in pharmaceutical and food preservation applications.

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