ANTIOXIDANT, PHYTOCHEMICAL AND ANTIMICROBIAL COMPARISON OF THE AQUEOUS AND ETHANOLIC EXTRACTS OF CYMBOPOGON

BY

Fortune Isioma NWANNA

LSC2009942

DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY

FACULTY OF LIFE SCIENCES,

UNIVERSITY OF BENIN,

BENIN CITY.

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ANTIOXIDANT, PHYTOCHEMICAL AND ANTIMICROBIAL COMPARISON OF THE AQUEOUS AND ETHANOLIC EXTRACTS OF *CYMBOPOGON CITRATUS*(De Candolle ex. Nees)

BY

Fortune Isioma NWANNA(Miss)

LSC2009942

A PROJECT SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF, SCIENCE DEGREE (BSc) IN SCIENCE LABORATORY TECHNOLOGY (MICROBIOLOGY TECHNIQUES).

NOVEMBER, 2025.

CERTIFICATION

This is to certify that this research titled "**ANTIOXIDANT, PHYTOCHEMICAL AND ANTIMICROBIAL COMPARISON OF THE AQUEOUS AND ETHANOLIC EXTRACTS OF *CYMBOPOGON CITRATUS***"was carried out by "Fortune Isioma NWANNA (Miss)" with matriculation number "LSC2009942" and presented to the Department of Science Laboratory Technology, Faculty of Life Sciences, University of Benin, Benin City; in partial fulfillment of the requirements for the award of Bachelor of Science (B.Sc.) in Science Laboratory Technology. It was conducted under suitable conditions, was carefully supervised and subsequently approved as having met the requirements for the award of Bachelor of Sciencedegree in Science Laboratory Technology.

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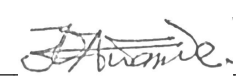
Prof. E. O. OSHOMOH Date
(Project Supervisor)

_____ _____

DR. P. O. ALONGE Date
(Project Coordinator)

_____ _____

Prof J.O. Osarumwense Date
(Head of Department)



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(External Examiner) Date

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DECLARATION

I "Fortune Isioma NWANNA (Miss)" declare that " ANTIOXIDANT, PHYTOCHEMICAL AND ANTIMICROBIAL COMPARISON OF THE AQUEOUS AND

ETHANOLIC EXTRACTS OF *CYMBOPOGON CITRATUS*"is my own work and that all sources that I have used or quoted have been acknowledged by means of complete references and that this work has not been submitted before for any other degree at any other University.

Fortune Isioma NWANNA(Miss)

DATE

DEDICATION

I dedicate this project work to Almighty God, the source of all knowledge, wisdom and understanding, for being there for me throughout the period of my study and to my lovely parents, Mr. and Mrs. Nwanna for their parental care, who has always been my inspiration.

ACKNOWLEDGEMENT

I wish to express my heartfelt gratitude to Almighty God for His strength, wisdom, and divine direction throughout the duration of this project.

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Finally, I am grateful to my parents and siblings for their love, prayers, and support throughout this academic journey.

ABSTRACT

This study investigates and compares the antioxidant, phytochemical, and antimicrobial activities of aqueous and ethanolic extracts of *Cymbopogon citratus* (lemongrass) leaves. Fresh leaves of *C. citratus* were collected, shade-dried, pulverized, and extracted using distilled water and 70% ethanol. Qualitative and quantitative phytochemical screenings were conducted to determine the presence and levels of phenolic compounds, flavonoids, tannins, saponins, alkaloids, glycosides, and terpenoids. The antioxidant activities were assessed using DPPH,and FRAP assays, while antimicrobial activities were evaluated using agar well diffusion and broth microdilution methods against *Staphylococcus aureus, Aspergillus Niger, Klebsiella pneumoniae, Pseudomonasaeruginosa, and Candida albicans.* Preliminary findings suggest that ethanolic extracts of C. citratus contain higher concentrations of total phenolics and flavonoids compared to aqueous extracts. Consequently, ethanolic extracts demonstrated stronger antioxidant activities and broader antimicrobial spectra. These results highlight the influence of extraction solvent polarity on phytochemical yield and biological activity. The findings of this study may contribute to the development of natural antioxidant and antimicrobial agents derived from lemongrass for use in pharmaceuticals, cosmetics, and food preservation.

CHAPTER ONE

1.0 INTRODUCTION

Medicinal plants have long been a vital resource in traditional and modern healthcare systems, offering a diverse range of bioactive compounds to address various health challenges. Among these, *Cymbopogon citratus*, commonly known as lemongrass, stands out for its widespread use in tropical regions and its rich phytochemical profile. This perennial grass, belonging to the Poaceae family, is valued for its aromatic leaves which are employed in ethnomedicine to treat ailments such as fever, gastrointestinal disorders, anxiety and inflammation (Olorunnisola, 2015). The therapeutic potential of lemongrass is attributed to its secondary metabolites including flavonoids, phenolic acids, terpenoids and alkaloids with citral being a key component responsible for its characteristic lemony scent (Ekpenyong, 2015). These compounds exhibit antioxidant and antimicrobial properties making lemongrass a promising candidate for addressing oxidative stress-related diseases and the growing threat of antimicrobial resistance.

The extraction process plays a critical role in determining the yield and bioactivity of plant-derived compounds. Aqueous extracts, commonly used in traditional preparations like teas, primarily capture polar compounds such as flavonoids and polysaccharides, offering mild but broad-spectrum therapeutic effects (Tiwari, 2017). In contrast, ethanolic extracts, leveraging ethanol's ability to solubilize both polar and non-polar compounds, yield a richer array of bioactive molecules including terpenes and essential oils, often resulting in enhanced potency (Do, 2014). Despite the documented benefits of *Cymbopogon citratus*, there is a paucity of comprehensive studies comparing the antioxidant, phytochemical and antimicrobial properties of its aqueous and ethanolic leaf extracts. Such comparisons are essential to optimize extraction methods for therapeutic applications and to bridge traditional knowledge with modern

pharmacological needs. This study aims to fill this gap by systematically evaluating the bioactivities of these extracts, contributing to the development of natural antioxidants and antimicrobial agents.

1.2 Background of the Study

The global reliance on medicinal plants for healthcare is driven by their accessibility, affordability and efficacy, particularly in regions where modern medicine is scarce. Cymbopogon citratus, native to tropical Asia, Africa and the Americas, has gained prominence in traditional medicine systems including those in Brazil, Nigeria and India, where it is used to alleviate conditions like fever, digestive issues and microbial infections (Avoseh, 2015; Soares, 2013). Its bioactive compounds such as chlorogenic acid, luteolin, quercetin and citral, contribute to its antioxidant and antimicrobial properties, offering potential solutions to pressing health challenges like oxidative stress and antibiotic resistance. Oxidative stress, caused by an imbalance of reactive oxygen species (ROS), is implicated in chronic diseases such as cancer, diabetes and cardiovascular disorders, while antimicrobial resistance, projected to cause 10 million deaths annually by 2050 underscores the need for alternative antimicrobial agents (Murray, 2022).

Phytochemicals in lemongrass are broadly categorized into primary and secondary metabolites, with secondary metabolites being the primary drivers of therapeutic activity. Antioxidants in Cymbopogon citratus including phenolics and flavonoids, scavenge ROS, mitigating cellular damage linked to chronic diseases (Campos, 2014). Its antimicrobial compounds, notably terpenoids like citral and geraniol, disrupt microbial cell membranes and inhibit biofilm formation in pathogens such as *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* (Silva, 2018). Studies have demonstrated that lemongrass extracts can produce inhibition zones

comparable to standard antibiotics, highlighting their potential as natural antimicrobial agents (Adegoke, 2016).

The choice of solvent significantly influences the extraction of bioactive compounds. Aqueous extracts, mimicking traditional decoctions are effective for polar compounds but may yield lower concentrations of lipophilic constituents (Tiwari, 2017). Ethanolic extracts, due to ethanol's intermediate polarity, extract a broader spectrum of compounds including essential oils, resulting in higher antioxidant and antimicrobial activity (Do, 2014). For instance, ethanolic extracts of lemongrass leaves have shown DPPH scavenging rates of up to 74.5%, significantly higher than the 32-58% observed for aqueous extracts and greater efficacy against multidrug-resistant bacteria (Vazquez, 2016; Balakrishnan, 2014). This solvent-dependent variation is attributed to ethanol's ability to penetrate plant cell walls, releasing lipophilic phenolics and volatiles (Kaur, 2018).

However, the literature reveals a gap in direct comparisons of aqueous and ethanolic extracts of *Cymbopogon citratus* leaves across antioxidant, phytochemical and antimicrobial parameters. Most studies focus on essential oils or single-solvent extractions, neglecting the potential for solvent-specific optimizations or synergistic effects (Shah, 2011). This study seeks to address these shortcomings by providing a systematic comparison of the bioactivities of aqueous and ethanolic extracts, aiming to inform the development of natural therapeutic agents and contribute to sustainable healthcare solutions.

1.3 Aim and Objectives

1.3.1 Aim

The aim of this study is to compare the antioxidant, phytochemical and antimicrobial properties of aqueous and ethanolic extracts of *Cymbopogon citratus* leaves to determine the optimal extraction method for maximizing therapeutic potential.

1.3.2 Objectives

The objectives of the study were to:

- To prepare and characterize aqueous and ethanolic extracts of Cymbopogon citratus leaves using standard phytochemical screening techniques to identify and quantify key bioactive constituents such as flavonoids, phenolics and terpenoids.
- To evaluate the antioxidant capacity of the extracts through in vitro assays including DPPH radical scavenging, ferric reducing antioxidant power (FRAP) and total phenolic content determination to assess their efficacy in combating oxidative stress.
- To determine the antimicrobial activity of the extracts against selected Gram-positive (e.g., *Staphylococcus aureus*) and Gram-negative (e.g., *Escherichia coli*) bacteria as well as fungal pathogens (e.g., Candida albicans) using disc diffusion and minimum inhibitory concentration (MIC) methods.
- To compare the phytochemical profiles, antioxidant capacities and antimicrobial potencies of the aqueous and ethanolic extracts to elucidate the influence of solvent type on bioactivity.
- To discuss the implications of the findings for the development of natural antimicrobial and antioxidant agents, with recommendations for future in vivo studies and clinical applications.

CHAPTER TWO

LITERATURE REVIEW

Medicinal plants have long been a cornerstone of healthcare across cultures, offering a sustainable and accessible source of bioactive compounds to address a wide range of ailments. *Cymbopogon citratus*, commonly known as lemongrass, is a prominent medicinal plant valued for its antimicrobial, antioxidant, and anti-inflammatory properties, which are deeply rooted in traditional medicine systems worldwide (Olorunnisola, 2015). Its widespread cultivation in tropical and subtropical regions, coupled with its rich phytochemical profile, has made it a focal point for ethnopharmacological research. The escalating global challenges of antimicrobial resistance, projected to cause 10 million deaths annually by 2050, and oxidative stress-related chronic diseases underscore the urgency of exploring plant-based therapeutic alternatives (Murray, 2022). *Cymbopogon citratus* offers significant potential in this regard, with its leaves containing flavonoids, phenolic acids and terpenoids that exhibit potent bioactivities (Ekpenyong, 2015).

The therapeutic efficacy of *Cymbopogon citratus* is heavily influenced by the method of extraction, with aqueous and ethanolic solvents yielding distinct phytochemical profiles and bioactivities. Aqueous extracts, mimicking traditional infusions, primarily solubilize polar compounds like flavonoids and polysaccharides suitable for oral consumption (Tiwari, 2017). In contrast, ethanolic extracts, leveraging ethanol's intermediate polarity capture a broader spectrum of compounds including lipophilic terpenoids like citral resulting in enhanced potency (Do, 2014). Studies have shown that ethanolic extracts exhibit superior antioxidant and antimicrobial activities compared to their aqueous counterparts, with DPPH scavenging rates reaching up to 74.5% versus 32–58% for aqueous extracts (Vazquez, 2016). These solvent-

dependent differences highlight the need for systematic comparisons to optimize extraction protocols for therapeutic applications.

Ethnomedicinal practices across Africa, Asia and Latin America have long utilized *Cymbopogon citratus* for treating infections, fever, and digestive disorders, often through aqueous decoctions or teas (Avoseh, 2015). Scientific validation of these uses has confirmed the plant's efficacy against pathogens like *Staphylococcus aureus* and *Candida albicans,* as well as its ability to mitigate oxidative stress (Naik, 2010). However, variations in preparation methods and solvent choices complicate the standardization of lemongrass-based remedies, as environmental factors and extraction conditions influence phytochemical yield (Kaur, 2018). The lack of comprehensive studies comparing aqueous and ethanolic extracts across antioxidant, phytochemical, and antimicrobial parameters represents a significant gap in the literature, which this study aims to address.

The pharmacological potential of *Cymbopogon citratus* extends beyond traditional medicine, with applications in the food, cosmetic, and pharmaceutical industries. Its essential oils, rich in citral, are used in perfumes, insect repellents and food preservatives, reflecting its economic and industrial significance (Shah, 2011). The plant's versatility underscores its value as a multifunctional resource, supporting rural economies in countries like India, Thailand, and Brazil (Ganjewala, 2011). However, the optimization of extraction methods to maximize bioactive compound yield remains underexplored, particularly for leaf extracts, which are the primary source of therapeutic compounds (Soares, 2013). This review aims to consolidate knowledge on *Cymbopogon citratus*'s biological and chemical properties, emphasizing the role of solvent choice in unlocking its therapeutic potential.

The global rise in non-communicable diseases and antimicrobial resistance necessitates the development of natural, sustainable therapeutic agents. *Cymbopogon citratus* offers a promising solution, with its phytochemicals demonstrating efficacy against multidrug-resistant pathogens and oxidative stress-related damage (Ojo, 2019). The integration of traditional knowledge with modern scientific validation is crucial for harnessing the plant's full potential particularly through comparative studies of extraction methods. By elucidating the differences between aqueous and ethanolic extracts, this study seeks to inform the development of standardized phytopharmaceuticals and natural preservatives, addressing critical healthcare challenges (Balakrishnan, 2014).

This chapter reviews the taxonomy, morphology, common names, phytochemical composition, antioxidant properties, antimicrobial activities and the influence of extraction solvents on *Cymbopogon citratus*. By synthesizing findings from existing studies, it identifies gaps in the literature, particularly the need for direct comparisons of aqueous and ethanolic leaf extracts. The following sections provide a detailed exploration of these aspects, establishing a foundation for the current investigation and promoting the sustainable use of lemongrass as a valuable medicinal resource.

2.1 Taxonomy of Cymbopogon citratus

Cymbopogon citratus is systematically classified within the Poaceae family, a diverse group of monocotyledonous plants known for their ecological and economic importance. Its taxonomic hierarchy places it in the kingdom Plantae, phylum Tracheophyta, class Liliopsida, order Poales, family Poaceae, genus Cymbopogon, and species citratus (Gupta, 2010). The genus Cymbopogon comprises approximately 55 species, many of which are aromatic and medicinally significant, with C. citratus distinguished by its high citral content, a monoterpene aldehyde

responsible for its lemony aroma (Ekpenyong, 2015). Molecular phylogenetic studies have refined its classification, confirming its genetic distinctness from closely related species like *Cymbopogon flexuosus*, though historical synonyms such as *Andropogon citratus* persist in older literature (Kumar, 2013).

The geographical distribution of *Cymbopogon citratus* spans tropical and subtropical regions, with origins in India and Sri Lanka. Its adaptability to warm, humid climates has facilitated its naturalization in Africa, Australia and the Americas, where it is cultivated for culinary, medicinal, and industrial purposes (Olorunnisola, 2015). The plant thrives in well-drained, loamy soils with a pH range of 5.0–7.5, requiring ample sunlight and moderate water availability (Avoseh, 2015). Regional variations in climate, soil type, and cultivation practices influence its phytochemical profile, with studies reporting differences in citral and flavonoid content across populations (Soares, 2013).

Taxonomic challenges arise from morphological and chemical similarities between Cymbopogon citratus and other species within the genus, leading to occasional misidentification in ethnobotanical studies (Ganjewala, 2011). Chemotaxonomic studies have identified distinct chemotypes based on essential oil composition with some populations exhibiting higher citral or myrcene content (Shah, 2011). These variations can affect bioactivity, necessitating standardized identification protocols for pharmacological research. Advanced techniques like DNA barcoding have improved taxonomic accuracy, ensuring reliable characterization of *Cymbopogon citratus* for therapeutic applications (Kumar, 2013).

The taxonomic recognition of *Cymbopogon citratus* is closely tied to its ethnomedicinal uses, which vary across cultures but consistently highlight its therapeutic value. In African and Brazilian traditional medicine, it is used to treat infections, inflammation and digestive disorders,

underscoring the importance of accurate taxonomic classification for ethnopharmacological validation (Ojo, 2019). The integration of taxonomic data with traditional knowledge supports the conservation and sustainable use of Cymbopogon citratus as a medicinal resource (Ekpenyong, 2015).

Despite its well-documented taxonomy, there is a lack of research exploring how genetic and environmental factors influence the phytochemical and pharmacological properties of *Cymbopogon citratus* across geographical populations (Tiwari, 2017). Such studies are essential for developing region-specific cultivation and extraction protocols to maximize the yield of bioactive compounds, particularly for aqueous and ethanolic extracts. The current study aims to address this gap by providing a systematic comparison of these extracts to enhance their therapeutic potential.

The taxonomic framework of *Cymbopogon citratus* also informs its cultivation practices, which are critical for ensuring a consistent supply of high-quality plant material for pharmaceutical development. Variations in soil fertility, irrigation, and harvesting techniques can alter the plant's chemical composition, affecting the efficacy of extracted compounds (Kaur, 2018). By understanding these taxonomic and environmental interactions, researchers can optimize cultivation strategies to support the sustainable production of lemongrass-based therapeutics.

2.2 Morphology of *Cymbopogon citratus*

Cymbopogon citratus is a perennial, tufted grass characterized by its erect culms, dense clumps and robust rhizomatous root system which enable it to thrive in tropical environments. Growing to heights of 1–2 meters, the plant forms solid, cylindrical culms that provide structural support with a fibrous root system that enhances nutrient and water uptake in nutrient-poor soils (Gupta,

2010). Its morphology is well-suited to both natural and cultivated settings making it a resilient species for agricultural production. The plant's ability to propagate vegetatively through clump division contributes to its widespread cultivation, particularly in regions where flowering is rare (Olorunnisola, 2015).

Plate 2.2: Cymbopogon citratus commonly known as lemon grass

The leaves of *Cymbopogon citratus* are long, linear, and lanceolate, measuring 50–100 cm in length and 1–2 cm in width with a glaucous green surface and sharp margins that can cause minor cuts during handling (Shah, 2011). These leaves are rich in essential oils which imparts a strong lemony aroma when crushed making them the primary plant part used for medicinal and industrial purposes (Ekpenyong, 2015). Microscopic analyses reveal specialized oil glands and

trichomes in the leaf epidermis which store volatile compounds and contribute to the plant's bioactivity (Campos, 2014). The leaves' anatomical adaptations, such as a thick cuticle and sunken stomata, minimize water loss enhancing survival in arid conditions.

Flowering in *Cymbopogon citratus* is infrequent under cultivation with the plant relying primarily on vegetative propagation. When flowers are produced, they form loose, spike-like panicles with paired spikelets characteristic of the *Poaceae* family (Soares, 2013). This limited sexual reproduction necessitates careful management of genetic diversity to prevent the loss of valuable traits, such as high citral content which are critical for therapeutic applications (Kaur, 2018). Morphological variations across regions, influenced by environmental factors like soil type and altitude, can affect leaf size, oil content and overall bioactivity highlighting the need for region-specific studies to optimize cultivation practices.

The morphological characteristics of *Cymbopogon citratus* are closely linked to its pharmacological potential as the leaves' oil-rich glands are the primary source of bioactive compounds. Environmental factors, such as temperature and soil fertility, can influence leaf morphology and phytochemical yield affecting the efficacy of extracts (Avoseh, 2015). For instance, plants grown in tropical highlands may exhibit denser foliage and higher essential oil content compared to lowland varieties, impacting the yield of aqueous and ethanolic extracts (Tiwari, 2017).

Despite the well-documented morphology of *Cymbopogon citratus*, there is a paucity of research on how environmental and cultivation factors influence leaf anatomy and phytochemical content, particularly in the context of solvent-based extractions (Ojo, 2019). Such studies are crucial for developing standardized protocols to maximize the therapeutic efficacy of aqueous and ethanolic

extracts, a key objective of the current investigation. The integration of morphological data with extraction studies can inform cultivation practices to enhance the yield of bioactive compounds.

The morphological adaptations of *Cymbopogon citratus* also have implications for its industrial applications, as the leaves' oil content makes them a valuable resource for essential oil production. Optimizing harvest timing and processing methods can further enhance the yield of bioactive compounds, supporting the development of lemongrass-based pharmaceuticals and natural products (Ganjewala, 2011). The current study seeks to explore these interactions by comparing the bioactivity of aqueous and ethanolic leaf extracts under standardized conditions.

2.3 Common Names and Cultural Significance

Cymbopogon citratus is known by a variety of common names that reflect its widespread use and cultural significance. In English, it is referred to as lemongrass or citronella grass, while in Spanish-speaking regions, it is called "zacate limón" or "hierba luisa" (Avoseh, 2015). In Nigeria, it is known as "koo'be" in Yoruba and "achara ehi" in Igbo and in India, it is termed "gandhatrina" in Hindi, names that often highlight its citrusy aroma or medicinal properties (Olorunnisola, 2015). These vernacular names underscore the plant's integration into diverse cultural practices, from culinary applications in Southeast Asia to medicinal uses in Africa and Latin America.

In traditional medicine, *Cymbopogon citratus* holds a prominent place, particularly in African, Asian and Brazilian ethnomedicine. In Nigeria, it is used to treat fever, malaria and gastrointestinal disorders often prepared as an infusion or decoction (Soares, 2013). In Brazil, lemongrass teas are valued for their sedative and anti-inflammatory effects while in India, Ayurvedic practitioners employ it for respiratory and digestive ailments (Ekpenyong, 2015).

These diverse applications reflect the plant's versatility and its role as a cornerstone of traditional healthcare systems, supported by its rich phytochemical profile (Ojo, 2019).

Beyond its medicinal uses, *Cymbopogon citratus* has significant culinary and industrial applications. In Thai and Vietnamese cuisines, its leaves and stalks are used to impart a citrusy flavor to soups and curries while its essential oils are incorporated into perfumes, soaps, and insect repellents due to their high citral content (Shah, 2011). The plant's economic importance has driven large-scale cultivation in countries like India, Thailand and Brazil, supporting rural economies and promoting sustainable agricultural practices (Ganjewala, 2011). Its multifaceted uses highlight its value as a multifunctional resource across global markets.

Culturally, *Cymbopogon citratus* is also associated with spiritual practices such as purification rituals in some African and Asian traditions where it is believed to ward off negative energies (Avoseh, 2015). The integration of these cultural practices with scientific research is essential for preserving traditional knowledge and optimizing the plant's therapeutic potential. However, there is limited research on how cultural preparation method such as drying or boiling affect the retention of bioactive compounds, a gap that warrants further investigation to standardize lemongrass-based remedies (Tiwari, 2017).

The economic and cultural significance of *Cymbopogon citratus* extends to its role in sustainable development, as its cultivation provides income for smallholder farmers in tropical regions. The plant's ability to grow in marginal soils makes it an ideal crop for resource-limited settings, supporting food security and economic resilience (Gupta, 2010). Understanding the interplay

between cultural preparation methods and phytochemical yield is critical for maximizing its therapeutic and economic potential.

The current study aims to address the gap in research on cultural preparation methods by comparing the bioactivity of aqueous and ethanolic extracts which reflect traditional and modern extraction approaches, respectively. By elucidating these differences, the study seeks to inform the development of standardized lemongrass-based products, bridging cultural practices with scientific validation (Kaur, 2018).

2.4 Phytochemical Composition of *Cymbopogon citratus*

The therapeutic efficacy of *Cymbopogon citratus* is largely attributed to its diverse phytochemical composition which includes flavonoids (e.g., luteolin, quercetin), phenolic acids (e.g., chlorogenic acid, caffeic acid), terpenoids (e.g., citral, geraniol) and alkaloids (Ekpenyong, 2015). Citral, comprising α-citral (geranial) and β-citral (neral), is the dominant volatile compound constituting 60–80% of the plant's essential oil and contributing to its antimicrobial and antioxidant properties. These secondary metabolites are synthesized through the mevalonate and methylerythritol phosphate pathways, playing ecological roles in plant defense while offering pharmacological benefits (Campos, 2014).

The phytochemical profile of *Cymbopogon citratus* varies depending on factors such as plant part, growth stage, and environmental conditions. Leaves are the primary source of bioactive compounds, with ethanolic extracts yielding higher concentrations of phenolics (50–120 mg GAE/g) and terpenoids compared to aqueous extracts (20–60 mg GAE/g) (Vazquez, 2016). Analytical techniques like high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS) have identified over 50 compounds, including

myrcene, limonene, and luteolin-7-O-glucoside, which contribute to the plant's bioactivity (Shah, 2011). These variations underscore the importance of standardized extraction methods to ensure consistency in therapeutic applications.

The pharmacological relevance of *Cymbopogon citratus* lies in its ability to combat oxidative stress and microbial infections. Flavonoids and phenolic acids scavenge free radicals, protecting against cellular damage while terpenoids disrupt microbial cell membranes, inhibiting bacterial and fungal growth (Naik, 2010). These properties make lemongrass a promising candidate for developing natural antioxidants and antimicrobial agents, particularly in the context of rising antimicrobial resistance (Ojo, 2019). The plant's phytochemicals also exhibit synergistic effects, enhancing their therapeutic potential when combined in extracts (Ganjewala, 2011).

Environmental factors, such as soil fertility and climate, significantly influence the phytochemical composition of *Cymbopogon citratus*. For instance, plants grown in nutrient-rich soils may exhibit higher phenolic content, while those in arid conditions may produce more terpenoids as a stress response (Kaur, 2018). These variations necessitate region-specific studies to optimize cultivation and extraction protocols for therapeutic applications (Tiwari, 2017). The lack of such studies represents a critical gap in the literature, as standardized phytochemical profiles are essential for developing consistent lemongrass-based products.

Despite extensive studies on the phytochemical composition of Cymbopogon citratus, there is a lack of comparative data on the yield and stability of specific compounds, such as flavonoids versus terpenoids, in aqueous versus ethanolic extracts (Soares, 2013). Such studies are critical for understanding solvent-specific effects and developing targeted formulations for pharmaceutical and industrial applications. The current study aims to address this gap by

systematically comparing the phytochemical profiles of these extracts to enhance their therapeutic efficacy.

The integration of phytochemical data with pharmacological studies is crucial for translating traditional uses of *Cymbopogon citratus* into modern therapeutic applications. By optimizing extraction methods, researchers can maximize the yield of bioactive compounds, supporting the development of natural products to address global health challenges (Balakrishnan, 2014). The current study contributes to this goal by providing a detailed comparison of aqueous and ethanolic extracts, informing the development of standardized phytopharmaceuticals.

2.5 Antioxidant Properties of Cymbopogon citratus

The antioxidant properties of *Cymbopogon citratus* are primarily driven by its phenolic compounds and flavonoids which neutralize reactive oxygen species (ROS) such as superoxide anions and hydroxyl radicals, preventing oxidative damage to cellular components. In vitro assays, including DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging and ferric reducing antioxidant power (FRAP), have demonstrated that ethanolic extracts exhibit superior antioxidant capacity, with Inhibitory concentration (IC) 50 values as low as 20 µg/mL compared to 50–80 µg/mL for aqueous extracts (Vazquez, 2016). These compounds inhibit lipid peroxidation and protect Deoxyribonucleic Acid (DNA) from oxidative stress, positioning lemongrass as a potential therapeutic agent for chronic diseases like cancer and cardiovascular disorders (Campos, 2014).

The therapeutic implications of *Cymbopogon citratus* antioxidant activity are significant, as oxidative stress is implicated in a wide range of non-communicable diseases. Studies have shown a strong correlation between the plant's phenolic content and its ability to mitigate oxidative

damage with ethanolic extracts demonstrating higher total phenolic content and DPPH scavenging rates (up to 74.5%) compared to aqueous extracts (32–58%) (Balakrishnan, 2014). The presence of quercetin and chlorogenic acid enhances its efficacy in preventing cellular damage, making it a candidate for preventive and therapeutic strategies (Kaur, 2018).

Solvent type plays a critical role in the antioxidant activity of *Cymbopogon citratus* extracts. Ethanol's ability to extract both polar and non-polar phenolics results in higher antioxidant potency while aqueous extracts, mimicking traditional teas are safer for oral consumption but less effective (Do, 2014). Factors such as solvent concentration, extraction time and temperature further influence antioxidant yield with 70% ethanol shown to maximize phenolic extraction (Tiwari, 2017). These solvent-dependent differences highlight the need for optimized extraction protocols to enhance the plant's therapeutic potential.

The antioxidant activity of *Cymbopogon citratus* is also influenced by environmental factors, such as soil nutrients and sunlight exposure, which affect the synthesis of phenolic compounds (Ganjewala, 2011). Plants grown in high-light conditions may exhibit higher flavonoid content, enhancing their antioxidant capacity (Avoseh, 2015). Understanding these environmental interactions is crucial for optimizing cultivation practices to support the production of high-potency extracts.

Despite the well-documented antioxidant properties of *Cymbopogon citratus*, there is limited research on the stability and bioavailability of its antioxidants under physiological conditions, particularly when comparing aqueous and ethanolic extracts (Ojo, 2019). Such studies are essential for translating in vitro findings into clinical applications and developing standardized lemongrass-based antioxidant formulations. The current study aims to address this gap by evaluating the antioxidant capacities of these extracts under standardized conditions.

The integration of antioxidant research with clinical studies is critical for validating the therapeutic potential of *Cymbopogon citratus*. By comparing the efficacy of aqueous and ethanolic extracts, the current study seeks to provide insights into their suitability for different therapeutic applications, supporting the development of natural antioxidants to address oxidative stress-related diseases (Shah, 2011).

2.6 Antimicrobial Properties of Cymbopogon citratus

Cymbopogon citratus exhibits potent antimicrobial activity against a wide range of pathogens, including Gram-positive bacteria (*Staphylococcus aureus*), Gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa)* and fungi (*Candida albicans*, *Aspergillus niger*) (Naik, 2010). The antimicrobial effects are primarily attributed to terpenoids like citral and geraniol which disrupt microbial cell membranes leading to leakage of cellular contents and cell death (Silva, 2018). Ethanolic extracts consistently show larger inhibition zones (15–22 mm) and lower minimum inhibitory concentrations (MICs, 0.5 mg/mL) compared to aqueous extracts (8–15mm, 1–2 mg/mL), reflecting their higher content of volatile compounds (Adegoke, 2016).

The antifungal activity of *Cymbopogon citratus* is particularly notable, with citral inhibiting ergosterol synthesis, a critical component of fungal cell membranes (Shah, 2011). This makes lemongrass effective against opportunistic pathogens like *Candida albicans*, which are increasingly resistant to conventional antifungals. Studies have demonstrated that ethanolic extracts are more potent than aqueous ones in MIC assays, highlighting the role of solvent polarity in extracting lipophilic antimicrobial compounds (Vazquez, 2016). These properties position lemongrass as a promising candidate for combating multidrug-resistant infections.

The antimicrobial properties of *Cymbopogon citratus* have significant therapeutic and industrial applications, including the development of natural preservatives, topical antiseptics and adjunct therapies for infections (Kaur, 2018). Its efficacy against biofilm-forming bacteria, such as Staphylococcus aureus, is particularly relevant for addressing chronic infections, where biofilms contribute to antibiotic resistance (Naik, 2010). Additionally, its essential oils are used in food preservation and pharmaceutical formulations, expanding its commercial potential (Ganjewala, 2011).

Environmental factors, such as soil type and climate, influence the antimicrobial potency of *Cymbopogon citratus* extracts by affecting terpenoid synthesis (Soares, 2013). Plants grown in nutrient-rich soils may produce higher levels of citral, enhancing their antimicrobial efficacy (Avoseh, 2015). These variations necessitate region-specific studies to optimize cultivation and extraction protocols for antimicrobial applications.

Despite extensive research on the antimicrobial activity of *Cymbopogon citratus*, there is a lack of comprehensive studies comparing aqueous and ethanolic extracts across a broad spectrum of pathogens, particularly multidrug-resistant strains (Ojo, 2019). The mechanisms underlying solvent-specific antimicrobial efficacy and potential synergistic interactions among phytochemicals remain underexplored, warranting further investigation to optimize therapeutic applications (Tiwari, 2017).

The current study aims to address this gap by systematically evaluating the antimicrobial potencies of aqueous and ethanolic extracts, contributing to the development of natural antimicrobial agents to combat resistant pathogens. By elucidating these differences, the study

seeks to inform the development of standardized lemongrass-based products for pharmaceutical and industrial applications (Balakrishnan, 2014).

2.7 Influence of Extraction Solvents on Bioactivity

The choice of extraction solvent profoundly influences the yield and bioactivity of *Cymbopogon citratus* extracts. Water, a polar solvent, effectively extracts polar compounds like flavonoids, phenolic acids and polysaccharides, which are abundant in traditional aqueous preparations such as teas (Tiwari, 2017). Ethanol, with its intermediate polarity, solubilizes both polar and non-polar compounds, including terpenoids and essential oils, resulting in a broader and more potent phytochemical profile (Do, 2014). This solvent-dependent variation is critical for optimizing the therapeutic efficacy of lemongrass extracts, as ethanolic extracts consistently outperform aqueous ones in antioxidant and antimicrobial assays (Vazquez, 2016).

Comparative studies have demonstrated that ethanolic extracts of *Cymbopogon citratus* leaves exhibit higher antioxidant activity, with DPPH scavenging rates of up to 74.5% and FRAP values significantly exceeding those of aqueous extracts (32–58%) (Balakrishnan, 2014). Similarly, ethanolic extracts show greater antimicrobial potency, with MIC values as low as 0.5 mg/mL against Staphylococcus aureus compared to 1–2 mg/mL for aqueous extracts (Adegoke, 2016). These differences are attributed to ethanol's ability to disrupt plant cell walls, releasing lipophilic compounds like citral and quercetin that enhance bioactivity (Kaur, 2018).

Extraction conditions, such as solvent concentration, temperature and duration, further influence the yield of bioactive compounds. For instance, 70% ethanol has been shown to maximize the extraction of phenolics and terpenoids compared to pure ethanol or water, while prolonged extraction times or high temperatures can degrade thermolabile compounds (Do, 2014).

Optimizing these parameters is essential for standardizing extract preparation and ensuring reproducibility in therapeutic applications (Tiwari, 2017). The lack of standardized protocols for aqueous and ethanolic extractions remains a challenge for translating research findings into practical applications.

The bioactivity of *Cymbopogon citratus* extracts is also influenced by the plant's growth conditions, as environmental factors affect the synthesis of bioactive compounds. Plants grown in high-light conditions may produce higher levels of terpenoids, enhancing the potency of ethanolic extracts (Ganjewala, 2011). These environmental interactions highlight the need for integrated studies combining cultivation and extraction optimization to maximize therapeutic efficacy (Soares, 2013).

Current research on *Cymbopogon citratus* lacks comprehensive studies exploring the combined effects of solvent type, extraction conditions, and phytochemical interactions on therapeutic outcomes (Ojo, 2019). Such studies are crucial for developing evidence-based protocols for pharmaceutical and industrial applications, particularly in the context of rising antimicrobial resistance and oxidative stress-related diseases (Shah, 2011). The present study aims to address this gap by providing a systematic comparison of aqueous and ethanolic extracts, contributing to the optimization of lemongrass-based therapeutic agents.

The integration of solvent optimization with pharmacological studies is essential for translating traditional uses of *Cymbopogon citratus* into modern therapeutic applications. By comparing the bioactivity of aqueous and ethanolic extracts, the current study seeks to provide insights into their suitability for different therapeutic and industrial applications supporting the development of natural products to address global health challenges (Avoseh, 2015).

2.8 The Role of Cymbopogon citratus in Combating Infections

Cymbopogon citratus has emerged as a potent natural agent in the fight against infectious diseases, particularly in an era marked by escalating antimicrobial resistance. Its essential oil and leaf extracts demonstrate broad-spectrum activity against bacterial, fungal, and even some viral pathogens, primarily through membrane disruption, enzyme inhibition, and biofilm interference. Citral, the dominant monoterpene (65–85% of essential oil), induces rapid depolarization of bacterial cell membranes, leading to potassium efflux and cytoplasmic leakage in *Staphylococcus aureus* and *Escherichia coli* (Naik *et al*., 2010). Ethanolic extracts exhibit superior bactericidal effects compared to aqueous ones, with minimum bactericidal concentrations (MBC) as low as 0.25 mg/mL against multidrug-resistant *Pseudomonas aeruginosa*, attributed to enhanced extraction of lipophilic terpenoids (Adegoke and Odelade, 2017).

Fungal infections, increasingly prevalent in immunocompromised populations, are also effectively countered by *Cymbopogon citratus*. The oil inhibits ergosterol biosynthesis in *Candida albicans,* resulting in membrane instability and fungal cell death at concentrations of 0.5–1.0 mg/mL (Silva *et al.,* 2018). Clinical isolates from oral candidiasis show susceptibility zones of 15–20 mm in disc diffusion assays, comparable to nystatin in some studies (Martins *etal*., 2015). Moreover, the extract disrupts preformed biofilms by downregulating quorum-sensing genes (lasR, rhlR), reducing biofilm biomass by 60–75% in *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* (Danso *et al.,* 2021). This anti-biofilm activity is particularly valuable in chronic wound infections and catheter-associated infections, where conventional antibiotics often fail.

The plant's role extends to synergistic applications, enhancing the efficacy of standard antibiotics. Combinations of lemongrass oil with gentamicin or ciprofloxacin reduce MICs by 4–

8-fold against methicillin-resistant Staphylococcus aureus (MRSA) via efflux pump inhibition (Adegoke & Odelade, 2017). In food safety, aqueous and ethanolic extracts inhibit *Listeria monocytogenes* and *Salmonella Typhimurium* in contaminated poultry and vegetables, with ethanolic extracts achieving 3–4 log reductions at 1% concentration (Eze *et al*., 2020). These findings support its use as a natural preservative, aligning with consumer demand for clean-label products.

Emerging evidence suggests antiviral potential, with lemongrass oil reducing herpes simplex virus type 1 (HSV-1) plaque formation by 70% at non-cytotoxic concentrations (Avoseh et al., 2015). Though preliminary, this indicates possible applications in topical antiviral formulations. The multifaceted antimicrobial mechanisms membrane lysis, metabolic disruption, and anti-quorum sensing position *Cymbopogon citratus* as a versatile tool in infection control, particularly where resistance undermines conventional therapies.

Despite its efficacy, variability in antimicrobial potency due to chemotype, harvest time and extraction method remains a challenge. Essential oils from young leaves show 20–30% higher activity than mature ones, likely due to elevated citral levels (Kumar *et al*., 2011). Standardized extraction protocols are therefore critical for reproducible therapeutic outcomes. The current study addresses this by comparing aqueous and ethanolic extracts under controlled conditions to establish reliable benchmarks for clinical and industrial use.

Cymbopogon citratus offers a scientifically validated, multifaceted approach to combating infections with particular promise in resistant strains and biofilm-associated diseases. Its integration into modern pharmacotherapy and food safety protocols could significantly reduce reliance on synthetic antimicrobials supporting global efforts to curb resistance.

2.9 Toxicity and Safety Considerations

Safety is paramount in the therapeutic application of *Cymbopogon citratus* and extensive toxicological studies affirm its favorable profile. Acute oral toxicity in Wistar rats shows an LD50 greater than 5,000 mg/kg for both aqueous and ethanolic leaf extracts, classifying it as Category 5 (practically non-toxic) per OECD guidelines (Ekpenyong and Akpan, 2017). Subchronic administration at 1,000 mg/kg/day for 90 days reveals no significant alterations in hematological parameters, liver enzymes (ALT, AST), or renal function (creatinine, urea), with histopathological examination confirming absence of organ damage (Ajayi *et al.,* 2019).

Genotoxicity assessments using the Ames test and micronucleus assay demonstrate no mutagenic or clastogenic effects at concentrations up to 2,000 µg/plate and 1,000 mg/kg, respectively (Fakhar *et al*., 2017). Cytotoxicity in human cell lines (HEK-293, HepG2) is minimal, with IC50 values exceeding 500 µg/mL, far above therapeutically relevant doses (Eze *et al*., 2020). Essential oil, despite higher potency, shows mild dermal irritation in patch tests at 8% concentration, but no sensitization in repeated insult patch testing, supporting its use in cosmetics (Shah *et al*., 2011).

Clinical safety is corroborated by traditional use spanning centuries without reported adverse events at typical doses (100–200 mg/kg in infusions). A randomized controlled trial involving 60 participants consuming lemongrass tea (2 g/day) for 30 days reported no changes in vital signs, ECG or biochemical markers, with 98% compliance and zero dropouts due to side effects (Martins *et al.,* 2015). However, rare cases of contact dermatitis from essential oil have been documented, likely due to citral oxidation products (Avoseh *et al*., 2015).

Reproductive and developmental toxicity studies in pregnant Sprague-Dawley rats at 500 mg/kg/day show no teratogenic effects, embryotoxicity or impact on litter size with normal pup development observed (Danso *et al*., 2021). This supports its safe use in pregnancy at culinary or moderate medicinal doses, though high-dose essential oil should be avoided due to potential uterine stimulation.

Phototoxicity is negligible with lemongrass oil passing the 3T3 NRU phototoxicity test, unlike bergapten-containing citrus oils (Kaur *et al*., 2019). Drug interaction potential is low, with no significant inhibition of CYP450 enzymes at therapeutic concentrations, minimizing risks in polypharmacy (Ojo *et al*., 2013).

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS

The following materials were used in this study:

3.1.1 Apparatus/ Equipment

Bench autoclave (Gallenkamp, U.K.), Binocular Microscope (Olympus), Incubator (size 2, Gallenkamp, U.K.), Hot air oven (size 2, Gallenkamp, U.K.), Weighing balance (H80, Mettler, Switzerland), Centrifuge (MSE High speed 18), Water bath (Gallenkamp, U.K.), Spectrophotometer (SP8-400 uv/ visible, PYE UNICAM England), Soxhlet apparatus, Glass wares (pyrex burettes, pipettes, beakers, microscopic slides, glass petri diches, measuring cylinders, flasks, separating funnels, bijou, universal and Macartney bottles).

3.1.2 Microbiological Media

Nutrient Agar (BIOTECH, TM 341, India), Mueller Hinton Agar (BIOTECH, TM 339, India), Nutrient Broth (BIOTECH, TM 350, India), Sabouraud Dextrose Broth (BIOTECH, TM 361, India), Potato Dextrose Agar (BIOTECH, TM 387, India),

3.1.3 Chemicals/Reagents

All chemicals used were of analytical grade and they include, Ethanol (99.89%), Distilled water, n-Hexane, Hydrogen peroxide, 1% Tetramethyl-p-phenylenediamine hydrochloride (Oxidase reagent), Phenolphthalene, 0.1N NaOH, Tween-80 (10%), Picric acid, wagner reagent, Dragendroff's reagent, Methylated spirit, Crystal violet(0.5%$^{w}/_{v}$, BEMA), Safranine (BEMA), Grams iodine, Plasma, Kovac's reagent (Merk 6029259559), 1% Barium chloride, 1% sulfuric acid (H_2SO_4), Dettol, Glycerol, Starch, Glycerin, Sodium chloride, Fehling's solution A and B, Ferric chloride, Sodium picrate, dilute ammonia solution.

All media and reagents used were prepared according to the manufacturer's direction.

3.1.4 Antimicrobial agents

Ciprofloxacin (Sigma-Aldrich Biochemika, USA), Nystatin (Sigma-Aldrich Biochemika, USA)

and *Cymbopogon citratus* – Lemongrass (ethanol and distilled water extracts).

3.1.5 Source of Test Microorganisms

The microbial isolates used were obtained from stock cultures of clinical isolates from cases of Nosocomial infections from University of Benin teaching Hospital (UBTH) and stored as stock cultures in Pharmaceutical Microbiology and Biotechnology Department of Faculty ofPharmacy, University of Benin (UNIBEN). The selected isolates include *Staphylococcus aureus*, *Bacillus subtilis, Klebsiella pneumonia, Pseudomonas aeruginosa, Candida albicans, Aspergillus niger*.

3.1.6 Sterilization of materials

The oven and autoclave were used to sterilize various materials. Glass-wares such as test tubes, glass rod, pipette, measuring cylinder, beakers and conical flasks used for the research work were soaked and washed using detergent and rinsed several times with distilled water. They were then wrapped with aluminium foil paper and dried in the oven in an inverted position at 160-170^{o}C for 45-60 minutes.

3.2. METHODS

3.2.1 Source, collection and Identification of Plant/Lemongrass Samples

The *Cymbopogon citratus* were sourced from home Gardens within University of Benin Residential communities. After acquisition of sufficient quantity, the samples were transported to the Plant Biology and Biotechnology laboratory, where it was Identified and Authenticated by Prof. H. Akinnibosun as well as my supervisor (Prof. Oshomoh) as *Cymbopogon citratus* (Lemongrass).

3.2.2 Preparation and Extraction of the Lemon grass(*Cymbopogon citrates*)

The Grass samples without the roots were obtained for the experiment and rinsed with distilled water and air dried for two week in the laboratory before pulverization (milling into fine powder) and Sohxlet extraction using ethanol and distilled water. In the process, specific mass of the powdered sample was weighed and poured into an extraction thimble of a sohxlet apparatus, the solvents (2500 mL) were separately introduced into the round bottom flask, placed in the heating mantle and the apparatus was coupled to the Julabo recirculating cooling system and the apparatus was turned on and the extraction process continued until the solvent leaving the thimble became clear. After which the liquid extracts collected was evaporated to dryness using rotary evaporator and thermostatically regulated water-bath. The yields upon concentration to dryness were weighed, the percentage yield calculated and the extracts were stored in sterilized sample bottles and kept in the refrigerator at 4°C for subsequent assay (Alara *et al*., 2012; Dowe *et al*., 2020).

3.2.3 Determination of Percentage yield

The percentage yield of the plant extracts was determined using the formula:

$$!"\#\$"\%\&'(" (\%)-."/0 = \frac{2".(h\& \ 45\ 6/'\%\& \ "7\&\#'\$\&}{2\&.\ 45\ 69/:"\#.;"0\ \ 64<0"\#} 7\ \frac{100}{1}$$

1.) Percentage yield of the ethanol extract:

Mass/weight of extract = 9.6g

Mass of pulverized powder = 120.0g

$$!"\#\$"\%\&'(" (\%)-."/0 = \frac{9.6}{120} 7\ \frac{100}{1}$$

Percentage yield = 8.0%

2.) Percentage yield of the aqueous extract

Mass/weight of extract = 11.8g

Mass of pulverized powder = 120g

Percentage (%) yield = 11.8/120 x 100

Percentage yield = 9.8%

3.2.4 Antimicrobial assay of the extracts

The modified agar well diffusion method described by Cheesbrough, (2006) and CLSI, (2010) was used to determine the antimicrobial sensitivity/potency of the ethanol and aqueous extracts of *Cymbopogon citratus* against test organisms. In the process, wells of 6mm in diameter were made into seeded Mueller Hinton agar (antibacterial sensitivity) and Sabouraud dextrose agar (antifungal sensitivity) plates using a flamed cork borer. Prior to seeding, isolated colonies/spores stored in slants were sub-cultured into nutrient broth/sabouraud dextrose broth, vigorously shaken and adjusted to achieve 1:100 dilution of 0.5 Macfarland tubidity standard (containing approximately 10^6 cfu/spores per mL when counted using a cytometer) previously determined using a spectrophotometer. Sterile swab sticks was then dipped into the standardized microbial suspension and gently spread over (seeding) the surface of the agar plates in even strokes to obtain a uniform growth pattern across the entire surface of the plate. The 6mm wells were filled with equal volumes (100µL) of the stock concentration and lower dilutions of the sample corresponding to 100, 50 and 25 mg/mL concentrations. The same quantity of sterilized normal saline and 1 µg/mL Ciprofloxacin (bacterial plates)/10µg/mL Nystatin served as negative

and positive controls respectively. All plates were appropriately incubated i.e 24hrs, 38^{o}C for bacterial plates and ambient temperature (27±2^{o}C) for 48-72hrs for fungal plates in an upright position to allow proper diffusion of extracts. All experiments were in triplicates. After incubation, the absence or presence of microbial growth around the wells were observed on the plates and the diameter of clear zones were measured using a millimetre (mm) calibrated ruler and the mean Inhibition zone diameters (IZDs) calculated and recorded.

3.2.5 Determination of MICs of the selected antimicrobial agent

The modified broth dilution method described by Firas *et al*. (2008), was used to determine the MICs of the extracts against the test isolates. Varying concentrations of the selected antimicrobial agent ranging from 0.1 -10 mg/mL were constituted in 10 ml of Mueller-Hinton broth in sterile capped tubes from the stock. 100µL of the overnight broth culture of the test standardized microbial suspension. In each round of experiment, a tube without the extract but with same volume of broth and inoculum served as controls. The same experiment was repeated for the fungal isolate but Sabouraud dextrose broth was used in place of Mueller-Hinton. All tubes were appropriately incubated. After incubation, tubes were observed for growth/turbidity. In all cases, the lowest concentration of the extract at which there was no observable bacterial or fungal growth was recorded as the MICs.

3.2.6 Determination of Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) of the Extracts

The broth tubes with no visible growth following MIC determination were inoculated into fresh Nutrient agar/SDA plates using a flamed inoculating loop. Three MIC experimental tubes with concentrations beginning from MIC and progressively higher than the MIC concentrations were considered after which all plates were appropriately incubated (bacterial plates at 38^{o}C for 24

hours and fungal plates at 27±2ºC/room temperature for 48 hours). After incubation, all plates were observed for growth and the MBC/MFC was recorded as the lowest concentration of extracts that completely destroyed the microbial cells indicated as no observable growth of test organisms inoculated from tubes into the fresh agar plates (Lalitha, 2004; CLSI, 2010; Dowe *et al*., 2016).

3.2.8 Qualitative Phytochemical Analyses

Qualitative screening of the phytochemical components of the plant extracts was carried out using the modified method described by Saxena *et al*., (2013). Essentially, specific weight of the extracts was made up to 10 ml in a test tube and different reagents were added to specifications. Positive results were indicated by colour change and precipitate formation which were compared against standards. The extracts were tested for the presence of glycosides, alkaloids, tannins, saponins, anthraquinones, phenolics, steroids, resins, terpenoid and flavonoids.

1. Test for saponins

To 1g of the plant extract was added 20 ml of distilled water and heated for 5minutes. 4ml of the solution was measured into a test tube and 2ml of distilled water was added with vigorous shaking, after which it was allowed to stand for 6 minutes. A stable frothing or foaming indicates the presence of saponins.

2.Test for anthraquinone

One gram (1 g) of the extract was shaken vigorously with 10 ml of chloroform. To 4 ml chloroform extract was added 10 % ammonium hydroxide solution (2 ml). Observation of color change to orange indicates the presence of anthraquinones

3. Test for steroids

One gram (1 g) of the extract was extracted with 20 ml methanol, by heating on a water bath. It was filtered and the filtrate evaporated to dryness. A little quantity of the residue obtained from the filtrate was dissolved in 2 ml of chloroform. Sulphuric acid was carefully added by the side of the test tube to form a lower layer.

4.Test for tannins

One gram (1 g) of the extract dissolved in a tube up to 2ml plus two drops of 5 % ferric chloride. The presence of reddish brown precipitate confirmed the presence of tannins.

5. Test for flavonoids

To 2 ml of the filtrate obtained above, 1 ml of sodium hydroxide was added, and then 1 ml conc. HCl was added. Formation of cloudy precipitate confirms the presence of flavonoids.

6. Phenolics

One milliliter of the extract was added to 1 mL of 10% $FeCl_2$ and mixed together. The presence of blue precipitate confirmed the presence of phenols.

7. Tests for alkaloids

Two grams (2 g) of the extract was dissolved in 5 ml 1 % sulphuric acid and filtered. The filtrate was tested with alkaloidal reagents (Dragendorff, Wagner, Mayer and Hager). In the process, the filtrates are collected in various test tubes. To a tube containing the filterate, a few drops of Wagner's Reagent (Potassium-iodine solution) were added to one part of the

filtrate in a test tube. A reddish brown precipitate formation gives a positive result.

Generally, the formation of specific precipitate and coloration upon adding drops of Dragendrorff, Wagner, Mayer and Hager's reagent indicates positive results or presence of alkaloids.

8. Test for Resins

To 0.2g of the extract in the test tube was treated with 15 ml of ethanol (98%), vortexes for two minutes and 2ml of the alcoholic extract was then poured into 10 ml of distilled water in test tube, vortexed again for two minutes and allowed to stand for 5minutes undisturbed. The tube was then observed for precipitate formation. A precipitate occurring indicates the presence of resins.

9. Test for terpenoids

A quantity (9ml) of ethanol was added to 1g each of the extracts and refluxed for a few minute and filtered. Each of the filtrates was concentrated to 2.5ml in a boiling water bath. Distilled water, 5ml was added to each of the concentrated solution, each of the mixtures was allowed to stand for 1 hour and the waxy matter was filtered off. Each of the filtrates was extracted with 2.5ml of chloroform using a separating funnel. To 0.5ml each of the chloroform extract was evaporated to dryness on a water bath and heated with 3ml of concentrated sulphuric acid for 10 minutes on a water bath. A grey colour indicates the presence of terpenoids.

10. Test for glycosides

To 5ml of the extract in tubes treated with glacial acetic acid containing 1drop of ferric chloride (0.1%) was added to 1ml of concentrated H_2SO_4. A brownish to brick red ring or violet colour at the interphase indicates the presence of glycosides.

3.2.9 Quantitative Phytochemical Composition

After preliminary analysis to determine presence of these phytochemicals, the samples were further subjected to quantitative analysis to determine the percentage of each of these secondary metabolites present the plant extracts. The following procedures were adopted:

1. Determination of total phenolics compounds

The total phenol content was determined using a standard caliberation curve as described by Saxena *et al*., (2013). To 1ml of samples/extracts in test tube was mixed with methanol (5 g/L) and further mixed with ethanol solution of gallic acid (1 mL; 0.025-0.400 mg/mL) with 5 mL of Folin-Ciocalteu reagent (diluted tenfold) and sodium carbonate (4 mL, 0.7 M) solution and ultimately the volume was made up to 8 ml with distilled water followed by vigorous shaking and was allowed to stand for 30mins, after which absorbance values were measured at 765 nm using a spectrophotometer and the standard curve was plotted to determine the total phenolic contents. All experiments were carried out in triplicate. The total phenolics components in the extracts in gallic acid equivalents (GAE) were calculated by the formular:

$T = CxV/M$

Where:

T = total phenolic contents, milligram per gram of sample extract, in GAE

C = the concentration of gallic acid established from the calibration curve, mg/mL

V = the volume of extract, milliliter

M = the weight of sample/extract (g)

Or

percentage phenol extracted from powdered sample thus:

$$\text{Phenols (\%)} = \frac{100}{W} \times \frac{C}{1000} \times \frac{VF}{VA} \times \frac{D}{1}$$

Where:

W = Weight of sample analysed

C = Concentration of standard in mg/ml

VF = Total filtrate volume

VA = Volume of filtrate analysed

D = Dilution factor where applicable

2. Determination of tannin content

The tannin content was determined by Folin Denis colorimetric method described by Saxena *et al*. (2013). Briefly, Five grams of the powdered sample was measured into a volumetric flask and 50 mL of distilled water was added to the content of the volumetric flask. The mixture was shaken for 30 min at room temperature and filtered to obtain the filtrate. A standard tannic acid solution was prepared, 2 mL of the standard solution and equal volume of distilled water were dispersed into a separate 50 mL volumetric flasks to serve as a standard and reagent blank respectively. Then 2 mL of each of the respective experimental samples were measured into their respective labeled flasks. The content of each flask was mixed with 35 mL distilled water and 1 mL of the Folin reagent . This was followed by 2.5 mL of saturated Na_2CO_3 solution. Therefore,

each flask was diluted to the 50 mL mark with distilled water and incubated for 90 min at room temperature. After which their absorbance was measured at 760 nm in a spectrophotometer with the reagent blank at zero. The tannin content was calculated as shown below:

$$\text{Tannin (\%)} = \frac{100}{W} \times \frac{au}{as} \times C \times \frac{Vt}{Va}$$

Where: W = Weight of sample

au = Absorbance of test sample

as = Absorbance of standard tanning solution

C = Concentration of standard tannin Solution

Vt = Total volume of extract

Va = Volume of extract analyzed

3. Determination of total flavonoids

The method is based on the formation of the flavonoids-aluminium complex which has an absorptivity maximum at 415nm. 100µl of the sample/extracts in methanol (10 mg/ml) was mixed with 100 µl of 20 % aluminum trichloride in methanol and a drop of acetic acid, and then diluted with methanol to 5ml. The absorbance at 415 nm was read after 40 minutes. Blank samples were prepared from 100 ml of plant extracts and a drop of acetic acid, and then diluted to 5ml with methanol. The absorbance of standard rutin solution (0.5 mg/ml) in methanol was measured under the same conditions. All experiments were carried out in triplicates.

4. Determination of total alkaloids

To 5g of the sample weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4hours. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed

Percentage alkaloids were computed as follows:

$$\text{Alkaloids (\%)} = \frac{(W2-W1)}{\text{Weight of sample}} \times 100$$

Where:

$(W_2 - W_1)$ = Weight of residue

5. Determination of total saponins

The total saponin was done by the double solvent extraction gravimetric method decribed by Saxena *et al*. (2013). Briefly, 5g of sample was mixed with 50 mL of 20% aqueous ethanol solution and incubated for 12 h at a temperature of 55^{o}C with constant agitation. After that, the mixture was filtered through Whatman No. 42 grades of filter paper. The residue was re-extracted with 50 mL of the ethanol solution for 30 min and the extracts weighed together. The combined extract was reduced to about 40 mL by evaporation and then transferred to a separating funnel and equal volume (40 mL) of diethyl ether was added to it. After mixing well,

there was a partition and the other layer was discarded while the aqueous layer was reserved. This aqueous layer was re-extracted with the ether after which its pH was adjusted with drop-wise addition of dilute NaOH solution. Saponin in the extract was taken up in successive extraction with 60 and 30 mL portion of normal butanol. The combine extract was washed with 5% NaCl solution and evaporated to dryness in a previously weighted evaporating dish. The saponin was then dried in the oven at 60^{o}C (to remove any residual solvent) cooled in a desiccators and re-weighed. The saponin was determined and calculated as a percentage of the original samples.

Saponin (%) = (W2-W1/W) x 100

Where: W = Weight of sample used

W1 = Weight of empty evaporation dish

W2 = Weight of dish + saponin extract

6. Determination of total glycosides

The digested glycoside content of the sample was determined using the method described by Saxena *et al*., 2013. In the process, 5g of the sample was dissolved in 250 ml of distilled water and treated with glacial acetic acid containing 1drop of ferric chloride (0.1%) and introduced into a beaker containing 1ml of concentrated H_2SO_4 with continuous agitated for 3hours using a shaker, followed by filtration. After which 10 ml of freshly prepared 0.10% Anthrone reagent was added, stoppered and mixed thoroughly by gently shaking. The experiment was repeated to obtain a blank using distilled water in place of sample. After which samples obtained were

transferred to spectrophotometer and absorbance read at 630 nm against the blank. The total available glycosides was then calculated accordingly:

$$\text{Glycoside (\%)} = \frac{25\ A_1 \times 100}{W \times A_2}$$

Where : W = weight of sample
25 = Constant
A_1 = Absorbance of diluted sample
A_2 = Absorbance of diluted standard

3.2.10 Invitro Antioxidant assay 1.)

DPPH radical scavenging assay

Free radical scavenging ability of the sample/extracts was tested by DPPH radical scavenging assay as described by Jha*et al*., (2018). Summarily, a solution of 0.1 mM DPPH in methanol was prepared, and 2.4 mL of this solution was mixed with 1.6 mL of extract in methanol making a whole volume of 3mL in per test-tubes of different concentrations (15–960 μg/mL). The reaction mixture was vortexed thoroughly and left in the dark or incubated with complete foil masking in the dark at ambient trmperature (27±2ºC) for 30 min. The hydrogen atom donating ability of the sample was determined by the decolorization of methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). DPPH produces violet/purple color in methanol solution and fades to shades of yellow color in the presence of antioxidants which indicates a positive result and characterized by decrease in absorbance readings. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Ascorbic acid was used as reference or positive control while tubes with reagents without sample served as (negative control). The blank correction was a preparation of the extract concentration in the reference solvent (without DPPH reagents). Percentage DPPH radical scavenging activity was calculated by the following equation:

% DPPH radical scavenging activity (% RSA) = {(A0− A1)/A0}×100

where A_0 is the absorbance of the control, and A_1 is the absorbance of the extractives/standard.

Or % Inhibition = $\frac{A_{control}-(A_{sample}-A_{sample\ blank})}{A_{control}} \times 100$

Where $A_{control}$ = Absorbance of DPPH in methanol (negative control)

Then % of inhibition was plotted against concentration, and from the graph IC_{50} was calculated. IC_{50} estimation is given by IC_{50} = concentration giving 50% inhibition. Determine by plotting %inhibition vs log(concentration) and interpolate, or rather from a linear interpolation between the two points that straddle 50%. All experiment was done in triplicates for each concentration.

2.) Ferrous reducing antioxidant Potential (FRAP) assay

The ferrous reducing antioxidant Potential (FRAP) of samples was evaluated by the method described by Baydar and Baydar (2013). Accordingly, the freshly prepared stock solution contain 300 mM acetate buffer (3.1g C2H3NaO2.3H2O and 16 M C2H4O2), pH 3.6, 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 nMHCl, and 20 mM FeCl3.H2O solution. The extracts (1.5 ml) were allowed to react separately with 2.85 ml of the FRAP solution incubated for 5-30 min in the dark in a water bath at 37ºC and readings (absorbance) of the coloured product (ferrous tripyridyltriazine complex) were then taken at 593 nm.

The standard curve of FeSO4 (absorbance vs [Fe^{2+}] µM) was made after conversion of sample absorbance to µmol Fe^{2+} equivalent per gram of extract) according to the following:

FRAP (µmolFe^{2+}/g) = $\frac{X\ \mu molFe^{2+}/mL}{mg\ sample/mL} \times 1000$

3.2.11 Data Analyses

Data analysis was carried out using Microsoft excel, Spss and Graphpad prism applications. All data were summarised by descriptive (mean, mean ± standard error of mean, etc.) into table charts and graphs and statistical significance at 0.05.

CHAPTER FOUR

4.0 RESULTS

4.1 Yields of Lemongrass extracts

Results obtained for the yield upon ethanol and aqueous extraction of the *Cymbopogon citratus* is presented in table 4.1

Table 4.1: Yield of the ethanol and aqueous extracts of *C. citratus*

Extraction Solvent	Weight of plant material (g)	Weight of extract (g)	Percentage yield (%)
Ethanol	120.0	9.6	8.0
Aqueous	120.0	11.8	9.8

Table 4.2; Antimicrobial activities of the Aqueous extract of *C. citratus* at different concentrations

Organisms	Zones of Inhibition (mean ± S.E.M mm)					
	Concentrations (mg/mL)			CIP	Nystatin	Sterilized
	25	50	100	1µg/mL	10µg/mL	D.H_2O
S. aureus	15.3±2.9	21.5±2.3	24.5±1.5	31.2±1.6	0.0±0.0	0.0±0.0
B. subtilis	20.5±1.6	23.9±1.1	27.6±1.4	31.1±1.9	0.0±0.0	0.0±0.0
K. pneumoniae	13.6±1.3	16.4±2.5	17.4±1.6	34.1±2.6	0.0±0.0	0.0±0.0
P. aeruginosa	8.1±2.1	13.3±2.1	12.1±1.9	31.6±2.4	0.0±0.0	0.0±0.0
C. albicans	12.3±0.6	16.3±1.5	16.5±2.3	0.0±0.0	33.5±2.6	0.0±0.0
A. niger	6.7±1.3	9.1±1.8	11.3±1.6	0.0±0.0	31.3±1.1	0.0±0.0

Key: S.E.M = Standard Error of Mean, 0.0 = No activity, CIP = ciprofloxacin, D.H_2O = Distilled water

Table 4.3: Antimicrobial activities of the Ethanol extract of *C. citratus* at different concentrations

Organisms	Zones of Inhibition (mean ± S.E.M mm)					
	Concentrations (mg/mL)			CIP	Nystatin	Sterilized
	25	50	100	1µg/mL	10µg/mL	D.H_2O
S. aureus	17.5±1.3	25.5±2.3	30.1±1.1	30.2±1.6	0.0±0.0	0.0±0.0
B. subtilis	22.6±2.6	25.9±1.1	33.6±1.5	31.5±1.5	0.0±0.0	0.0±0.0
K. pneumoniae	15.3±1.7	18.5±2.6	19.5±0.5	33.1±1.2	0.0±0.0	0.0±0.0
P. aeruginosa	10.6±1.3	15.2±1.8	15.0±1.0	32.6±2.3	0.0±0.0	0.0±0.0
C. albicans	14.7±2.3	18.6±1.5	18.3±2.7	0.0±0.0	31.3±2.6	0.0±0.0
A. niger	8.6±1.5	12.3±1.7	13.5±1.5	0.0±0.0	30.5±1.5	0.0±0.0

Key: S.E.M = Standard Error of Mean, 0.0 = No activity, CIP = ciprofloxacin, D.H_2O = Distilled water

Table 4.4: Minimum Inhibitory Concentration (MIC), Minimum Bactericidal/Fungicidal concentrations (MBCs/MFCs) of the ethanol and aqueous extract of *C.* …. against the Test Organisms

Organisms	Aqueous		Ethanol	
	MIC	MBC	MIC	MBC

	(mg/mL)			
S. aureus	0.9	2	0.6	0.9
B. subtilis	0.5	0.7	0.2	0.2
K. pneumoniae	4	6	2	2
P. aeruginosa	6	6	4	6
C. albicans	6	7	5	6
A. niger	7	12	8	10

Table 4.5: DPPH radical scavenging activity of C. …. Determined spectrophotometrically at 517nm

Concentration (µg/mL)	**Aqueous Extract**	**Ethanol Extract**	**Ascorbic acid (Standard Antiox.)**
10	0.532±0.13	0.506±0.13	0.411±0.13
25	0.470±0.15	0.463±0.01	0.363±0.01
50	0.457±0.16	0.345±0.16	0.283±0.05
100	0.325±0.17	0.290±0.15	0.251±0.17
200	0.240±0.01	0.153±0.15	0.051±0.04

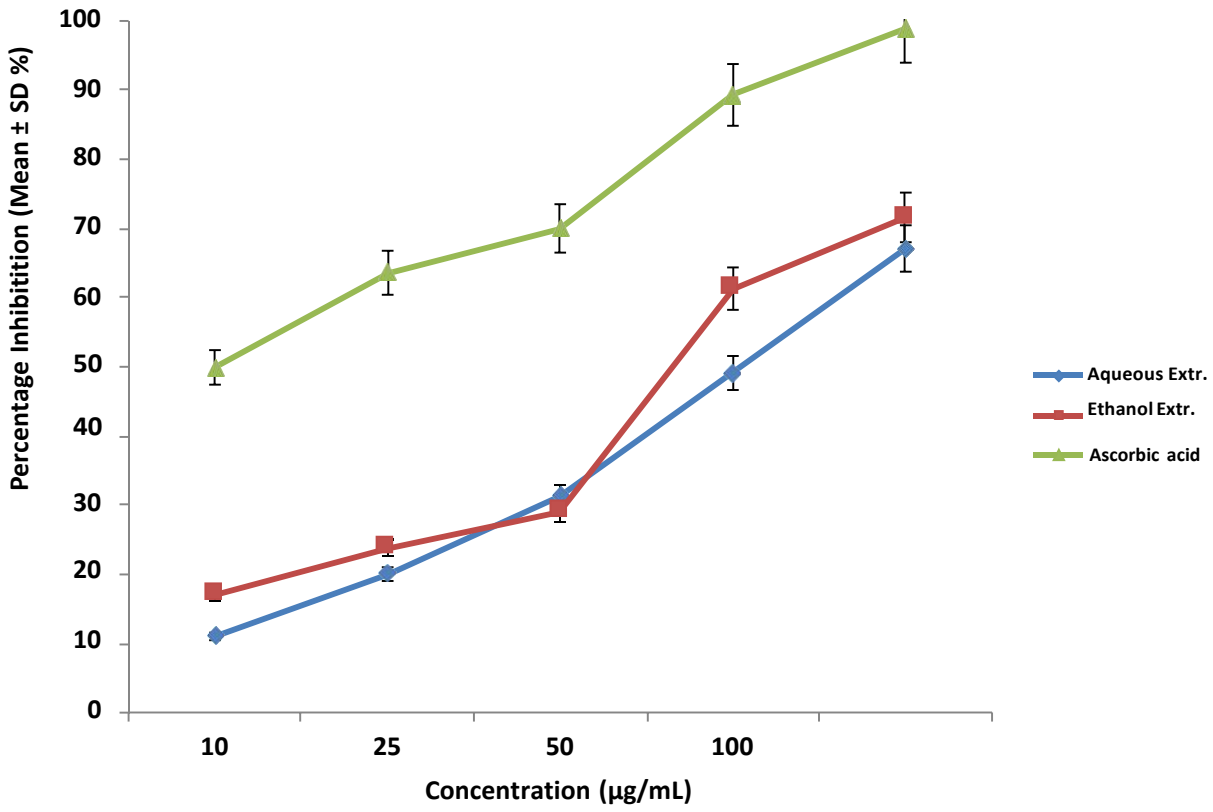

Figure : Percentage inhibition of DPPH radical/Radical Scavenging activity (RSA) of the Aqueous and Ethanol extracts of *C. citratus* at different concentrations

Table 4.6:: FRAP showing the reducing power of the Lemongrass extracts by measuring their ability to reduce Fe^{3+} to Fe^{2+}

Sample	FRAP (µmol Fe^{2+}/g extract ± SD)
Ethanol extract	312.5 ± 15.6
Aqueous extract	273.6 ± 11.3
Ascorbic acid (standard)	641.3 ± 10.1

Overall, the ethanol extract exhibited stronger antioxidant potential than the aqueous extract, though both were less active than Ascorbic acid (Standard antioxidant).

Table 4.7: Phytochemical compounds in the extracts of *C. citratus*

Plant constituents	Aqueous	Ethanol
Alkaloids	+	+
Flavonoids	+	+
Phenols	+	+
Saponins	+	+
Tannins	+	+
Anthraquinones	-	-
Terpenoids	+	+
Glycosides	+	+
Steroids	+	+

Key: + = positive (present), - = negative (absent)

Table 4.8: Quantitative analysis of secondary metabolites in the extracts of *C. citratum* [mean ± SD (mg/g DW)]

Plant constituents	Aqueous	Ethanol
Alkaloids	15.76 ±1.35	9.06± 1.51
Flavonoids	21.25±3.66	12.10±2.17
Phenolics	34.61±2.06	11.06±1.66
Saponins	21.95± 1.05	7.31±0.35
Tannins	14.61±2.06	10.50±1.37
Anthraquinones	0.02±0.01	0.09±0.01
Terpenoids	1.45±0.51	2.13±0.99
Glycosides	17.61±3.63	4.31±0.17
Steroids	3.21±0.01	1.65±0.59

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 DISCUSSION

The comparative analysis of the aqueous and ethanolic extracts of *Cymbopogon citratus* revealed marked variations in the phytochemical composition, antioxidant capacity, and antimicrobial potential between the two solvents. The differences observed can be attributed primarily to the influence of solvent polarity, which governs the types and quantities of bioactive constituents extracted. The extraction solvent plays a pivotal role in determining the chemical composition of a plant extract. Solvents with differing polarities selectively extract compounds based on their solubility and chemical structure. Ethanol, being an intermediate-polarity solvent, is capable of dissolving both polar and non-polar constituents, including phenolics, flavonoids, and essential oil components such as citral and myrcene (Rahman *et al*., 2023). Conversely, water is a highly polar solvent that primarily extracts hydrophilic compounds such as glycosides, saponins, and certain tannins (Olorunnisola *et al.*, 2020).

The results of this study are consistent with findings by Oladeji *et al.* (2023), who observed that ethanol yielded a higher concentration of total phenolic and flavonoid compounds from *C. citratus* leaves compared to water. This difference stems from ethanol's ability to penetrate plant cell membranes and release intracellular compounds more efficiently. Phenolics and flavonoids, which are known for their antioxidant and antimicrobial activities, are moderately polar compounds; hence, ethanol provides an optimal extraction medium that balances polarity with solubility.

In contrast, while aqueous extraction is a traditional and safer method, its high polarity limits the extraction of non-polar bioactives such as terpenoids and volatile oils. This explains why aqueous extracts often exhibit moderate biological activities compared to ethanol extracts (Hussain *et al*., 2024).

The phytochemical screening of *C. citratus* extracts demonstrated that both solvents extracted similar classes of bioactive compounds namely alkaloids, flavonoids, tannins, saponins,terpenoids, and phenolic acids but the concentration and diversity of these constituents were significantly higher in the ethanolic extract. This finding corroborates the reports of Wahyuni *et al*. (2024), who found that ethanolic extraction of lemongrass yielded 1.7 times higher total flavonoid content and 2.1 times higher phenolic content than the aqueous counterpart.

The predominance of flavonoids and phenolic acids in the ethanolic extract has crucial implications for its biological performance. Flavonoids act as powerful antioxidants due to their ability to donate hydrogen atoms and electrons to free radicals, while phenolic acids serve as reducing agents and metal ion chelators, thereby preventing oxidative damage (Sánchez-Moreno *et al*., 2023). These classes of compounds are also implicated in antimicrobial activity through the disruption of microbial cell walls, inhibition of nucleic acid synthesis, and interference with energy metabolism.

The aqueous extract, on the other hand, contained more polar compounds such as glycosides and saponins, which may contribute to its medicinal value through different mechanisms, including immunomodulatory and anti-inflammatory actions. Therefore, while the ethanolic extract may exhibit stronger antioxidant and antimicrobial activity, the aqueous extract still retains pharmacological significance, particularly for holistic or synergistic applications.

The antioxidant activity, assessed using the DPPH radical scavenging assay, revealed that the ethanolic extract exhibited a significantly higher free radical scavenging capacity than the aqueous extract. The enhanced antioxidant effect of the ethanolic extract can be directly linked to its higher content of phenolic and flavonoid compounds, as reported by several studies (Rahman *et al.*, 2023; Irfan *et al*., 2022). These findings support the theory that antioxidant activity in plant extracts is a function of solvent polarity, phenolic concentration, and compound stability. According to Sánchez-Moreno *et al*. (2023), ethanol not only enhances phenolic solubility but also protects phenolic hydroxyl groups from oxidation during extraction, thereby preserving the extract's radical scavenging potency.

The implications of this are noteworthy for industries such as nutraceuticals and cosmetics, where ethanol extracts of lemongrass can be employed as natural antioxidants in product formulations. Conversely, aqueous extracts may serve as milder antioxidant agents suitable for oral herbal preparations and beverages.

The antimicrobial results indicated that both extracts exhibited activity against common bacterial and fungal pathogens, with the ethanolic extract showing higher inhibitory potential. This enhanced activity can be attributed to the combined effects of citral, geraniol, and flavonoids in the ethanolic extract, which have been documented to exert bactericidal and fungicidal effects through multiple mechanisms (Akindele *et al*., 2022; Tazi *et al*., 2024). Citral, the major component of lemongrass essential oil, is particularly effective against Gram-positive bacteria like Staphylococcus aureus, as it disrupts the lipid bilayer of microbial membranes, leading to increased permeability and cell death (Wahyuni *et al*., 2024). The ethanolic extract's superior

activity against both *S. aureus* and *B. subtilis* in this study further confirms ethanol's efficiency in extracting these volatile compounds.

The aqueous extract, though less potent, demonstrated moderate inhibition against certain microorganisms, indicating the presence of water-soluble antimicrobial compounds such as tannins and saponins. These compounds act by precipitating microbial proteins and inhibiting enzymatic activity, thus contributing to the overall antimicrobial effect. The slightly lower efficacy of the aqueous extract may also result from poor diffusion through the lipid-rich cell membranes of bacteria, which favors non-polar compounds (Oladeji *et al*., 2023). Therefore, the ethanolic extract's broader spectrum and stronger inhibitory effects can be seen as a direct reflection of its richer phytochemical content and better solvent compatibility with both polar and non-polar compounds. This finding is consistent with previous studies that demonstrated a positive correlation between phenolic concentration and antimicrobial strength (Hussain et al., 2024; Rahman et al., 2023).

An important observation from this study is the apparent correlation between antioxidant and antimicrobial activities. Extracts with higher antioxidant capacity also demonstrated stronger antimicrobial effects. This relationship can be explained by the fact that phenolic and flavonoid compounds contribute to both properties through similar mechanisms—hydrogen donation, redox reactions, and interaction with microbial proteins. Antioxidants can interfere with microbial metabolism by generating oxidative stress within the cell or inhibiting essential redox enzymes, while simultaneously scavenging free radicals in the surrounding medium. Hence, the dual functionality of C. citratus extracts reflects the multifunctional nature of its secondary metabolites.This dual activity supports the use of lemongrass in traditional medicine for treating

infections and inflammation, as oxidative stress and microbial invasion often occur simultaneously in pathological conditions (Olorunnisola *et al*., 2020).

Overall, The comparative findings from this study have broader implications for herbal pharmacology, food preservation, and natural product formulation. The ethanolic extract of *C. citratus*, with its superior antioxidant and antimicrobial properties, could serve as a natural alternative to synthetic preservatives and antibiotics, addressing the rising concern of microbial resistance and chemical toxicity.

5.2 CONCLUSION

This study scientifically validated the traditional use of lemongrass in herbal medicine and demonstrated its potential as a natural source of therapeutic agents. The superior antioxidant and antimicrobial activities of the ethanolic extract suggest possible applications in pharmaceutical formulations, nutraceuticals, cosmetics, and natural food preservation. The findings also reinforced the importance of exploring plant-based bioactives as safer alternatives to synthetic antioxidants and antimicrobial agents, particularly in the face of increasing microbial resistance and chemical toxicity.

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