PHYTOCHEMICALS AND ANTIMICROBIAL POTENCY OF LEAVES EXTRACTS OF UZIZA LEAF (*Piper guineense*) AND LEMON GRASS (*Cymbopogen citratus*) AGAINST CLINICAL ISOLATES

BY

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DEPARTMENT OF MICROBIOLOGY

FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN,

BENIN CITY

NOVEMBER, 2025

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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.

NOVEMBER, 2025

CERTIFICATION

This is to certify that this project work was carried out by **Abigail Aruosaghe OTASOWIE** with Matriculation Number **LSC2104017** in the Department of Microbiology, Faculty of Life Sciences, University Of Benin, Benin City, under the supervision of Prof. E. I. ATUANYA.

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(Head Of Department)	

DEDICATION

This project work is dedicated to GOD ALMIGHTY, for his wisdom, guidance and provision all through the duration of my study in the University of Benin.

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ABSTRACT

Medicinal plants have long been recognized for their therapeutic properties and remain an essential source of bioactive compounds with potential antimicrobial activity. The growing problem of antibiotic resistance has heightened global interest in exploring natural plant extracts as alternative or complementary agents against infectious diseases. This study evaluated the phytochemical composition and antimicrobial potential of lemongrass (*Cymbopogon citratus*) and uziza leaf (*Piper guineense*) extracts against selected bacterial and fungal pathogens. Fresh leaves of both plants were collected from local markets in Benin City, Edo State, Nigeria, authenticated, air-dried, pulverized, and extracted using ethanol and water. Phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, terpenoids, and saponins in varying concentrations. Lemongrass contained high levels of alkaloid, flavonoids and tannins, while uziza exhibited moderate presence of these compounds. Saponin was present in lemongrass but absent in uziza. Antimicrobial testing showed that ethanolic extracts demonstrated superior activity compared to aqueous extracts. The ethanolic extract of lemongrass exhibited the highest inhibition zone of 20 mm against *S. aureus* and 15 mm against *E. coli* at 100% concentration, while the uziza ethanolic extract produced inhibition zones of 18 mm and 15 mm, respectively, against the same organisms. *Pseudomonas sp.* showed moderate sensitivity with inhibition zones of 12 mm, whereas fungal isolates *Aspergillus* sp., *Penicillium* sp. and *Fusarium* sp. recorded inhibition zones ranging from 10 to 15 mm. The minimum inhibitory concentration (MIC) for ethanolic extracts ranged from 50 mg/mL for *S. aureus*, *E. coli* and *Pseudomonas* sp. to 100 mg/mL (for fungal isolates), while minimum bactericidal concentrations (MBCs) were observed at 100 mg/mL. Aqueous extracts displayed weaker activity, requiring higher concentrations (100 mg/mL) for inhibition and showing little or no effect on fungal isolates. Overall, these findings highlight the significant antimicrobial potential of lemongrass and uziza leaf extracts, particularly their ethanolic forms, which exhibited notable inhibitory effects against common bacterial pathogens. The results support their traditional medicinal use and underscore their potential as natural sources of plant-based antimicrobial agents.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Lemongrass (*Cymbopogon citratus*) is a perennial tropical grass belonging to the family *Poaceae*. It is native to Southeast Asia but is now widely cultivated across tropical and subtropical regions of the world (Mukarram *et al*., 2022). Renowned for its aromatic essential oils, lemongrass has long been used in folk medicine, culinary preparations, and as a natural preservative because of its potent antimicrobial properties (Gao *et al*., 2020; Ewansiha *et al*., 2012).

Similarly, uziza leaf, obtained from *Piper guineense* (family *Piperaceae*), is a climbing vine indigenous to West Africa, particularly Nigeria, Ghana, and Cameroon. It serves both culinary and medicinal purposes (Hattingh, 2023; Ameh *et al*., 2021). Commonly known as "Ashanti pepper" or "West African black pepper," uziza leaves and seeds are widely used in traditional medicine for the treatment of infections, gastrointestinal disorders, and inflammatory conditions (Faluyi, 2020; Ameh *et al*., 2021). Both *Cymbopogon citrates* (Lemongrass) and *Piper guineense* (uziza) are promising sources of natural antimicrobials, especially in this era of increasing antibiotic resistance. Recent research highlights their efficacy against bacteria, fungi, viruses, and microbial biofilms (Gao *et al*., 2020; Mukarram *et al*., 2022; Abd El-Hack *et al*., 2022).

Lemongrass has been used for centuries in Ayurvedic, Chinese, and African traditional medicine to treat infections, fever, and digestive disorders (Ewansiha *et al*., 2012). Its essential oil is commonly infused in teas, ointments, and inhalants to combat respiratory and skin infections (Gao *et al*., 2020). In West Africa, uziza leaves are incorporated into soups and herbal concoctions to manage coughs, wounds, and microbial infections, often combined

with other herbs for enhanced activity (Ameh *et al*., 2021; Faluyi, 2020). Ethnopharmacological surveys indicate that these plants are frequently used for food preservation and treatment of foodborne illnesses, with up to 80% of households in certain rural areas utilizing uziza leaves for such purposes (Ukom *et al*., 2023; Osuala and Anyadoh, 2006).

Recent reviews (2020–2025) show increasing global interest in *Cymbopogon citrates* (Lemongrass) and *Piper guineense* (uziza) as eco-friendly alternatives to synthetic antimicrobials. This trend is driven by consumer demand for natural products and the growing global challenge of antimicrobial resistance (Abd El-Hack *et al*., 2022; Ameh *et al*., 2021; Mukarram *et al*., 2022).

The antimicrobial activity of lemongrass is primarily attributed to its essential oil components, which contain 60–80% citral (a mixture of geranial and neral), along with geraniol, geranyl acetate, citronellal, citronellol, germacrene-D, elemol, limonene, linalool, and caryophyllene oxide (Gao *et al*., 2020; Mukarram *et al*., 2022). These monoterpenes and sesquiterpenes exhibit broad-spectrum antimicrobial activity (Ewansiha *et al*., 2012).

For *Piper guineense* (uziza), key bioactive compounds include alkaloids such as piperine (5–8%), flavonoids (25%), tannins (15%), phenols (0.33%), saponins (0.36%), myristicine, elemicine, safrole, and dillapiol (Hattingh, 2023; Abd El-Hack *et al*., 2022; Ameh *et al*., 2021). Phytochemical analyses have revealed that both plants contain tannins, flavonoids, phenols, carbohydrates, and volatile oils that act synergistically to disrupt microbial cell structures (Osuala and Anyadoh, 2006; Madu *et al*., 2023).

Recent GC-MS analyses of uziza leaf extracts identified over 28 bioactive compounds, including phytol (9.11–15.17%), tributyl acetylcitrate (10.95%), and ethyl piperonyl

cyanoacetate (27.35%), all of which contribute to its antimicrobial potency (Madu *et al*., 2023). The extraction method significantly influences the bioactivity, with hydrodistillation favoring lemongrass oil yield and methanol/chloroform extraction enhancing uziza's antimicrobial potential (Gao *et al*., 2020; Osuala and Anyadoh, 2006; Madu *et al*., 2023).

Mechanistically, lemongrass essential oil and citral destabilize microbial lipid bilayers, leading to membrane disintegration, ion leakage (Ca^{2+}, K^{+}, Mg^{2+}), and oxidative stress through reactive oxygen species (ROS) generation, ultimately resulting in cell death (Gao *et al*., 2020; Mukarram *et al*., 2022). They also downregulate microbial virulence genes (e.g., *als3*, *hwp1* in *Candida albicans* and *agrA*, *hla* in *Staphylococcus aureus*), thereby inhibiting biofilm formation (Ewansiha *et al*., 2012).

Uziza leaf extracts, particularly methanolic ones, demonstrate potent antimicrobial activity through tannin-induced protein precipitation and piperine-mediated cell membrane disruption, with methanolic extracts often outperforming ethanolic ones (Abd El-Hack *et al*., 2022; Ameh *et al*., 2021). Piperine also inhibits bacterial efflux pumps, enhancing susceptibility to other antibiotics (Hattingh, 2023; Ameh *et al*., 2021).

Lemongrass exhibits strong antibacterial activity against Gram-positive bacteria such as *Staphylococcus aureus* and *Bacillus cereus*, and Gram-negative bacteria including *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, with minimum inhibitory concentrations (MICs) as low as 0.0313% (v/v) for citral against *S. aureus* (Mukarram *et al*., 2022). Uziza extracts also show effective inhibition of foodborne pathogens, with inhibition zones up to 20 mm and MIC values between 50–100 μg/mL (Abd El-Hack *et al*., 2022; Achukwu *et al*., 2023).

In addition to antibacterial activity, *Cymbopogon citratus* demonstrates antifungal activity against *Candida albicans*, *Aspergillus flavus*, *A. niger*, and *Fusarium moniliforme*, primarily by disrupting plasma membranes and inhibiting spore germination (Mukarram *et al*., 2022). Uziza (*Piper guineense*) also exhibits antifungal effects against *A. niger* and *A. flavus*, attributed to its terpenoid and phenolic content (Adesina and Okwute, 2025).

Although few studies have explored the synergistic interaction between lemongrass and uziza, their complementary phytochemicals (citral and piperine) suggest potential combined efficacy through enhanced membrane disruption (Gao *et al*., 2020; Abd El-Hack *et al*., 2022). Their applications extend to food preservation and pharmaceutical formulations as safe, natural, and sustainable antimicrobial agents (Osuala and Anyadoh, 2006; Ameh *et al*., 2021).

Given the growing global concern over antimicrobial resistance, studies on the antimicrobial and phytochemical properties of lemongrass (*Cymbopogon citratus*) and uziza (*Piper guineense*) are both timely and relevant, offering potential solutions for safer food preservation and novel therapeutic development.

1.2 Aim of the Study

The aim of this study was to examine the phytochemical composition and antimicrobial potential of lemongrass (*Cymbopogon citratus*) and uziza leaf (*Piper guineense*) extracts.

The specific objectives of this study were to:

1. evaluate the phytochemical constituents of lemongrass (*Cymbopogon citratus*) and uziza (*Piper guineense*) extracts.
2. examine the antimicrobial potential of the extracts on selected pathogenic microorganisms.
3. determine the minimum inhibitory concentrations (MIC) of the extracts on the test pathogens.

4. evaluate the minimum bactericidal and fungicidal concentrations (MBC) of the extracts on the isolates.

CHAPTER TWO

LITERATURE REVIEW

2.1 Lemongrass

Cymbopogon citratus is commonly known as lemongrass, barbed wire grass, citronella grass, fever grass and tanglad but due to its broad distribution (Karpagam *et al*., 2016; Oladeji *et al*., 2019). *C. citratus* flourishes in sunny, warm, humid conditions of the tropics and grown in a wide variety of soil ranging from rich loam to poor laterite, but calcareous and water-logged soils are unsuitable for its cultivation while plants growing in sandy soils have higher leaf oil yield and citral content (Balakrishnan *et al*., 2014).

Lemongrass (*Cymbopogon citratus*) is a well-known medicinal plant in Benin. It is composed of volatile aromatic chemicals, belongs to the Gramineae family, which grows primarily in tropical and subtropical region of the world (Majewska *et al*., 2019). They possess antibacterial, antifungal, anti-inflammatory, anticancer, antiseptic, and antioxidant properties. Lemongrass is widely utilized as a flavouring agent in the food business, as well as in the pharmaceutical, cosmetic, soap, and detergent sectors (Oladeji *et al*., 2019). Lemon grass oils can be extracted utilizing various plant parts, particularly the leaves and aerial parts. Basically, plants with medicinal properties are used to extract essential oils because of their antibacterial properties against bacteria, fungal, and viral pathogens.

The presence of alkaloids, phenols, and other functional groups in essential oil (EO) contributes to their antibacterial activities. Essential oils derived from several medicinal plants are thought to be alternative natural antibacterial agents (Sayeed *et al*., 2014). Moreover, due to the antibacterial and antifungal properties of lemongrass extracted essential

oil, it has an extensive potential to be employed in food preservatives. This sort of significant feature of essential oil can be useful and feasible for farmers in the rural locations (Majewska *et al*., 2019). Various ethnic groups have employed plant-derived natural products as traditional medicine. The essential oil isolated from *Origanum onites*, for example, has demonstrated considerable biological activity such as antibacterial, antifungal, and antioxidant characteristics, and it has also been shown to be useful against colon cancer (Spyridopoulou *et al*., 2019). Similarly, the leaves of *Cymbopogon citratus* have medicinal benefits and are a source of essential oils, as claimed by Manvitha and Bidya (Mavitha *et al*., 2014).

A study on *Cymbopogon citratus* revealed pharmacological activity such as antiamoebic, antibacterial, antidiarrheal, antifilarial, antifungal, and anti-inflammatory effects. It also highlighted that antibacterial activity of lemongrass can be utilized as a supplement treatment for fever, respiratory disorders, dental hygiene, and other conditions, and it also possesses anti-diabetic and anti-cancer properties (Acimovic *et al*., 2019). Furthermore, citral compounds have also been utilized in the perfume business, cleaning wounds, and treating skin ailments. The essential oil extracted from *Cymbopogon citratus* does not have any disturbance in antibacterial activity, whether it is extracted in water as a medium or alcohol (Hindumathy *et al*., 2011). With respect to the type of organism, the gram-positive organisms were more sensitive to lemongrass oil as compared to gram-negative organisms (Naik *et al*., 2010). Therefore, lemongrass essential oil can be effective against drug-resistant organisms.

The common methods to extract essential oil from medicinal plant, including for lemongrass *(Cymbopogon citratus),* are hydro-distillation (HD), steam distillation, steam and water distillation, maceration, empyreumatic (or destructive) distillation and expression (Ashgari *et al*., 2010). Lemongrass has phytoconstituents such as tannins, flavanoids, alkaloids and

various essential oils in this herb. Secondary active metabolites of a number of components have also been implicated in the varied pharmacological effects of this plant. Lemongrass possesses various antimicrobial properties. The extracts of lemongrass leaves (dried) with cold, hot and different solvents like ethanol and methanol were screened for its antimicrobial activity against various bacteria like *Bacillus vallismortis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Vibrio cholerae*. Lemongrass extracts possess a great antimicrobial activity against the antibiotic resistant microorganisms (Isam *et al*., 2009).

Figure 2.1. Lemongrass (*Cymbopogon citratus*) (Ganjewala *et al.*, 2012)

2.2 Volatile Constituents of Lemongrass

Due to its commercially valuable essential oils, lemongrass is frequently used in food technology, pharmaceuticals, and traditional treatments. The terpenes, alcohols, ketones, and esters present, as well as the essential oil of lemongrass, differ according to its topographical origin, which affects its chemical makeup. The essential oil that is extracted from lemongrass is mostly found in the leaves of the plant, which are also a great source of it (Shah *et al*., 2011). Up to 5% dry weight of essential oils, primary citral with a distinctive lemonade scent, are present in the leaves (Tovar *et al*., 2011). Due to the presence of citral, a cyclic monoterpene, the word "lemon" in its name refers to its distinctive lemon-like aroma.

Numerous consumer products contain fragrances made using the oil of lemongrass, such as linalool (1.3%), geranial (39.0%), neral (29.4%), geraniol (1.7%), and myrcene (18.0%). Myrcene is an anti-bacterial and analgesic compound found in lemongrass, and citronellal, citronellol, and geraniol are its active ingredients. Citral, a volatile oil with a robust lemon aroma, is present in the essential oil. Citral is a mixture of two aldehydes and a stereoisomeric monoterpene; it is used to make perfumes and colored soaps, and synthesizes vitamin A. The geranial with trans isomer nature (40–62%) prevails over the neral with cis isomer nature (25–38%) in citral. Lemongrass has a long history of use in food and beverage recipes, folk medicine, and cosmetics. Due to its alluring scent, Lemongrass is utilized as a flavoring component in many non-food products, such as soaps, perfumes, candles, and insect repellents.

The essential oil of this plant is considered one of the chief volatile oils. Essential oils, perfumes, and volatile plant byproducts have a significant advantage in both the perfume and folk medicine businesses. Many essential oils and the substances that make them up have pharmacological capabilities that act as anti-inflammatory, antioxidant, and anti-cancer agents (Nagai *et al*., 2011). Lemongrass essential oil carries great importance, with citral as

the main element. The citral content fluctuates from 44.3–91.4% to 79–91.5% in the essential oil. Other important volatile constituents are β-myrcene (11%) and geraniol (1.9%).

2.3 Extraction of Lemon Grass Oil

Essential oils are extracted from flowers, herbs, trees and various other plant materials. These oils contain a mixture of chemical compounds. Terpenes associated with aldehydes, alcohols and ketones form the major chemical component of such essential oils (Ranitha *et al.,* 2014). Apart from being used to manufacture of perfumes, soaps, cosmetics and detergent, citronella oil also finds an application in the pharmaceutical industry. The extraction of this essential oil is classified as clean technology (Ganjewala *et al.,* 2012). Lemon grass contains 1-2% of essential oil on a dry weight basis. Steam and hydro distillation are the conventional methods of its extraction These procedures are however time consuming. An innovative Microwave-Assisted Hydro distillation (MAHD) not only reduces the extraction time but also retains the quality of oil (Ranitha *et al.,* 2014). The benefits of microwave radiation aided oil extraction technique over hydro distillation have also been reported. Pressurized liquid extraction using nitrogen gas, is a novel technique and was found to yield better quality of oil in comparison to Soxhlet extraction and hydro distillation methods (Desai *et al.,* 2015).

2.3.1 Solvent extraction

In a solvent extraction (SE) system, a hydrocarbon solvent (usually n-hexane, petroleum spirit, alcohol or their mixture) is mixed with the selected plant material to leach the essential oil out at a given temperature or boiling point of the solvent. After separating the solution from the plant matrices and concentrating the solution by distillation or evaporation, and leaving substance containing resin (resinoid) or a combination of wax and essential oil remains. Although this method is simple and adequately efficient to extract essential oil from lemongrass, it usually needs large quantity of solvent and results in low yield reproducibility. In addition, contamination of the essential oil by residual solvent is inevitable.

Solvent extraction of essential oil from lemongrass usually employs Soxhlet apparatus (Alhassan *et al*., 2018). This apparatus facilitates continuous contact between plant material and refluxing liquid solvent, which leads to increase extraction efficiency. Unfortunately, it requires a long heating period at high temperature (usually near to the boiling point of the solvent) which may trigger the thermal degradation of thermally sensitive constituents. Solvent extraction either by maceration and Soxhlet extraction needs an appropriate solvent to achieve a high extraction yield and to avoid the loss of volatiles. Lately, Alhassan *et al*. had successfully extracted essential oil from dry and fresh lemongrass leaves using nhexane as solvent, which achieved 1.85% and 4.5% oil yield, respectively apparatus (Alhassan *et al*., 2018). The physicochemical properties of the essential oil are not altered because no constituent's decomposition take place. This solvent extraction method can also be performed at high pressure.

2.3.2 Supercritical fluid extraction

Over the last few decades, the augmenting requirement for premium quality natural products has supported to the establishment of more environmentally benign method of essential oils extraction, which is supercritical fluid extraction (SFE) (Kumoro *et al*., 2010). The basic SFE unit comprises high-pressure pump, water bath heater or oven, extraction vessel, microfilter, ball valves, back pressure regulator, cold trap, volumetric gas quantifier, and vent. When cosolvent is required, another high-pressure pump and mixer should be added to the basic SFE unit. This method can be considered as a promising alternative to solvent extraction of valuable components for pharmaceutical and food applications from various botanical matrices without any harmful traces of solvent (Haloui *et al*., 2017). The solvation properties of supercritical fluid can be altered through the adjustment of temperature and pressure, which initially changes its density and subsequently allows selective extraction. Carbon dioxide is most common utilized supercritical fluid due to its excellent physicochemical properties, mainly related to its relatively low critical temperature and pressure (23 – 50ºC and 8 – 12 MPa, respectively).

2.4 Medicinal Properties of Lemon Grass and its Oil

Lemon grass has been traditionally used to remediate a plethora of medical conditions. This is due to the broad spectrum of secondary metabolites that it produces. It has been used to treat fever, cough, elephantiasis flu, leprosy, malaria and digestive problems among many other illnesses. Conventional medicine has a lot of adverse effects. Therefore plant-based medicine has become a popular alternative for synthetic medicine. Thus, this herbaceous plant may find many applications in the pharmaceutical Industry (Oloyede *et al.,* 2009).

Indonesian Scientists have investigated and confirmed the ability of β-citronellol-the major component of "Sereh Wangi" (colloquial name for Lemon grass in Indonesia) oil, to bring about a reduction in weight of rats fed with a high fat diet. Inhalation of vapors of β-citronellol enhances the sympathetic nerve activity of the rats that leads to the increased activity in the adipose tissue resulting in weight loss. The findings of this study were significant due to the fact that β-citronellol caused a reduction in body mass without affecting the concentration and activity of the liver enzymes (Batubara *et al.,* 2015). Weight loss is only one of the few pros of citronella grass.

Maintenance of oral health is an important aspect of daily routine. Gingivitis and periodontitis are oral health conditions caused due to dental plaque. Many reports confirm that this dental caries are risk factors for ischemic stroke and cardiovascular disease. The antagonistic activity of lemon grass against the planktonic and biofilm forms of *Candida dubliniensis*, a common oral pathogen has been reported. Citronella grass may be used in formulating herbal drugs for oral healthcare (Batubara *et al.,* 2015).

Medical conditions like hyperlipidemia, hypercholesteremia and hyperglycemia lead to metabolic disorders like obesity and diabetes mellitus. It has been reported that lemon grass is bestowed with hypolipidemic, hypocholesteremia and hypoglycemic properties.

Consumption of plant extracts have shown to bring about a reduction in plasma cholesterol and very low density lipids, both of which are highly correlated with heart disease. Hypoglycemic condition in rats was achieved after 42 days of administration of 500mg/kg/day lemon grass extract. The mechanism of action is however not clear (Agbafor *et al.,* 2007). In addition to these benefits, several reports have confirmed the anti-inflammatory, anticonvulsant and anxiolytic effects of lemon grass extracts (Sforcin *et al.,* 2009).

Additionally, the antagonistic activity of lemon grass towards different pathogenic bacteria, protozoa and fungi has also been reported (Tarek *et al.,* 2014). Leishmaniasis is severe disease that affects the global human population annually on a large scale. It has been reported that the promastigotes of *Leishmania infantum* undergo programmed cell death upon exposure to citral, a major constituent of lemon grass oil. Also, reports suggest the anti-proliferative effect of citronella oil on the anexic amastigotes of *Leishmania*sp (Machado *et al.,* 2012). Lemon grass, may be foreseen as an anti-protozoan drug of the future. It is also seen that the combination of silver nanoparticles and the oil have synergistic inhibitory action on growth of pathogens like - *Escherichia*, *Staphylococcus*, *Moraxella*, *Enterococcus* and *Candida* sp (Ahmad *et al.,* 2015). Citronella oil, also exhibits potent antifungal activity against *Candida* sp. and *Aspergillus niger* by showing inhibitory zones in the range of 35 to 90mm. Also, the oil shows similar effects to 50 mg/kg synthetic oral drug diclofenac when administered in mice suffering from carrageenan induced edema. *In vivo* anti-inflammatory action of the oil is also evident when it is topically applied on croton oil induced edematous mice (Boukhatem *et al.,* 2014). Lemon grass can be used in preparations of topical skin creams and in the manufacture of plant based oral drugs.

Furthermore, the bioactive compounds of lemon grass have also shown potent antagonistic activity against viruses. Anti-viral effects of lemon grass against on enveloped murine virus and have been reported. Murine novovirus is a surrogate virus of human novovirus, which is responsible for non-bacterial gastroenteritis epidemic worldwide. The lemon grass oil and citral used in the study reduced viral infectivity by coating the viral capsid and thus preventing it from binding to the host cell. Lemon grass and citral can be used to sanitize food and surfaces to prevent viral infections (Gilling *et al.,* 2014).

2.5 Phytochemical Screening Of Lemongrass (*Cymbopogon citratus*) Leaves Essential Oil

C. citratus is rich in bioactive compounds and the isolated and identified phytochemicals from its leaves mainly includes flavonoids, alkaloids, saponin, tannins and phenolic compounds, which consist of quercetin, luteolin, apiginin, isoorientin 2'-O-rhamnoside and kaempferol that are known to have many benefits, especially in the fields of pharmacy, food, health and agriculture (Negrelle and Gomes, 2007; Hasim *et al*., 2015; Erminawati *et al*., 2019). The other compounds identified in *C. citratus* are mainly alcohols, aldehyde, ketones, esters and terpenes (Hasim *et al*., 2015).

The plant also contains 1-2 per cent essential oil on a dry basis with wide variation of chemical composition as a function of habitat, genetic diversity and agronomic treatment of culture. The volatile oil from the roots contains longifolene -(V4) (56.67%) and selina-6-en-4-ol (20.03%) (Ademuyiwa and Grace, 2015). The main chemical component of lemongrass essential oil is citral whereas many other compounds like neral, geranial, geraniol, β-myrcene, limonene, geranyl acetate, borneol, estragole, methyleugenol, citronellal, pinene, careen-2, farnesol, alpha-terpineol, (+)-cymbodiacetal, proximadiol, methyl heptenone, terpinolene,

linalool, linalyl acetate and β-caryophyllene have also been reported (Carlson *et al*., 2001; Huynh, 2008; Shah *et al*., 2011; Ademuyiwa and Grace, 2015).

Citral (3, 7-dimethyl-2, 6-octadien-2-al) is the name given to a natural mixture of two isomeric acyclic monoterpene aldehydes i.e. geranial (citral Aor *trans* citral) and neral (citral B or *cis*citral) (Carlson *et al*.,2001; Huynh, 2008), which have same molecular formula ($C_{10}H_{16}O$), but different structures (Mirghani *et al*., 2012; Manvitha and Bidya, 2014; Hartatie *et al*., 2018).

2.6 Antibacterial Activity

The antibacterial characteristic of lemon grass oil is well established (Meena *et al*., 2016). It has been suggested that LEO induces the destruction of bacterial biofilms and hinders further bacterial growth and development. Furthermore, LEO components can destabilize the bonds between the lipid bilayer and neutralize the bacteria through membrane disintegration. LEO can confer structural changes, as well, in different bacteria. It was reported to cause complete disfiguration and distortion in the *Pseudomonas* spp (Aiemsaard *et al*., 2011). The MIC values for LEO and citral against *P. aeruginosa* were calculated as >40% and 10%, respectively. Furthermore, LEO blocks biofilm formation in bacterial colonies (Moore-Neibel *et al*., 2012). for example, 0.125% (*v*/*v*) of LEO can restrict biofilm formation in methicillin-resistant *Staphylococcus aureus* strains (Adukwu, *et al*., 2012). It can disrupt the cell membrane and inhibit cytoplasmic metabolism, making LEO effective against both Gram-negative and Gram-positive bacteria (Vazirian *et al*., 2012).

Cymbopogon khasianus essential oil inhibited the growth of *Escherichia coli* with the MIC and MBC (Minimum Bactericidal Concentration) values of 20 µg/mL each. It can also retard the growth of *Bacillus subtilis*, *Salmonella enterica typhimurium*, *Staphylococcus aureus*, *Klebsiella pneumoniae* with a MIC range of 25–50 µg/mL (Viktorova *et al*., 2020). Multiple

recent studies against MDR (multidrug-resistant) bacteria (Peichel *et al.,* 2019) show that, while a low concentration of Lemongrass oil retards growth and biofilm formation, a higher concentration can confer complete elimination of *Salmonella.*

The bacteriostatic and bactericidal characteristics of LEO primarily depend on the bacteria and oil concentration (Dewi *et al.,* 2018). However, several other factors, such as oil composition, extraction method, plant developmental stage, and environmental variables including temperature can influence the oil's effectiveness. Therefore, lemongrass oils from different species might exhibit effects of different nature and intensity. Nevertheless, the host organism can also decide oil effectiveness to a certain extent depending on its morpho-physiological attributes (Swamy *et al*., 2016). Therefore, lemomgrass oil react differently with Gram-positive and Gram-negative bacteria, owing to their dissimilar cell-wall structures (Dewi *et al*., 2021). Costa *et al*. (2021) examined *Cymbopogon flexuosus* EO (μL mL^{-1}) against *Listeria monocytogenes*, *Staphylococcus aureus,* and *Salmonella typhimurium* and determined their MICs and MBCs as of 3.9 μL mL^{-1} each. The MICs of citral against *Cronobacter sakazakii* strains ranged from 0.27 to 0.54 mg/mL. Scanning electron microscopy analysis further confirmed that *C. sakazakii* cell membranes were damaged by citral.

2.7 Industrial Applications Of Citronella Grass And Lemongrass Essential Oils

As a result of back to nature concept in the recent decades, the use of plants extracts has gained steady increase of attention from customers leading to a promising commercial market, which comprises perfumery, pharmaceuticals, cosmetics, and food applications, mainly due to their beneficial active substances (Baser *et al*., 2015). For millennium, lemongrass (*C. citratus*) essential oil has been used as traditional folk medicine in the treatment of nervous, gastrointestinal disturbances (common stomach ache, digestive problems and diarrhea), fevers and hypertension. In addition, lemongrass essential oil is also used an important folk

remedy for cough, flu, consumption, gingivitis, headache, ophthalmia, leprosy, elephantiasis, malaria, pneumonia and other vascular disorders. Lemongrass essential oil has been regarded as carminative, powerful insect repellent herbicides and food preservative (Cassel *et al*., 2006). Studies the on extract from *C. citratus* leaves have demonstrated to be anti-inflammatory, vasorelaxing, diuretic and effective remedy in treating ringworm as local application (Dama *et al*., 2011). The essential oil from *C. citratus* leaves may become highly attractive to the pharmaceutical industry, as most recent research has demonstrated that citral exhibits selective toxicity for specific cancer cells (Dudai *et al*., 2005). Therefore, quality of this essential oil is judged by the amount of citral present in the oil (Mu'azu *et al*., 2019). Essential oil, isolated from the leaves of lemongrass, possesses a wide range of biological activities like antimicrobial, antifungal, anticancer, and antioxidant properties (Mu'azu *et al*., 2019).

Citronella grass (*C. nardus*) leaf and stalk are popularly used in domestic and professional cooking as well as for the preparation of functional tea for its unique flavor. Citronella grass essential oil is widely used in the food, drink, perfumery, soap, body and healthcare products and pharmaceutical products (Wei *et al*., 2013). It is also applied in the treatment of orthopedics, muscular and skin problems (Verma *et al*., 2015). Recent scientific studies have proven that citronella grass exhibited several biological activities, such as antiviral, antibacterial and insect repellent (Adibah *et al*., 2009). The essential oil of citronella grass leaves has been effectively employed as a diaphoretic, stimulant, and promoter of internal detoxification by encouraging sweating (Pangnakorn *et al*., 2011). In Thailand, the infusion of citronella grass essential oil has been functioned in folk medicine as a blood tonic and diuretic, and to cure flatulence, stomach ache, gastritis, intestinal cramps, irritable bowel, and indigestion. However, only very few reports are available on the analgesic effect and African comparative study of chemical compounds of essential oil of this plant. Commercially,

citronella oil is isolated to obtain three important constituents, namely citronellal, citronellol, and geraniol, which are popularly used for their antiseptic properties and perfumes and fragrances.

2.8 Uziza Leaf (*Piper guineense*)

Piper guineense, commonly known as uziza leaf, West African black pepper, Ashanti pepper, or iyere, is a perennial climbing vine from the Piperaceae family, native to West Africa and widely cultivated in countries like Nigeria, Ghana, and Cameroon (Hattingh, 2023). Thriving in tropical climates, it features heart-shaped leaves and pungent seeds, integral to culinary and medicinal practices in the region (Faluyi, 2020). Its historical use in traditional African medicine, paralleling ancient Ayurvedic practices, is attributed to its rich concentration of bioactive compounds (Faluyi, 2020).

Figure 2.2. *P. guineense* (Uziza) plant (b) Seeds of Uziza (Takcı *et al.*, 2025)

2.9 Traditional and Culinary Uses

Uziza leaves and seeds serve as both a spice and medicinal herb. In culinary applications, the leaves add a peppery, slightly bitter flavor to dishes like Afang soup and pepper soup in Nigerian cuisine (Hattingh, 2023). The seeds, valued for their pungency, are used similarly as a seasoning (Hattingh, 2023). In ethnomedicine, uziza addresses gastrointestinal disorders, respiratory issues, inflammation, and fever, often used as an immune booster (Hattingh, 2023; Faluyi, 2020). Among Nigeria's Igbo tribe, it is a staple in medicinal soups, frequently combined with other herbs (Attaugwu *et al*., 2025). Surveys indicate that up to 80% of certain communities regularly use uziza leaves for these purposes (Ukom *et al*., 2023).

2.10 Nutritional Composition

Uziza leaves are nutrient-dense, with approximately 45% carbohydrates, 25% proteins, 10% fats, and 20% dietary fiber (Hattingh, 2023). They are rich in micronutrients, including vitamins A (15%), C (30%), and E (10%), and minerals like calcium (12%), potassium (20%), magnesium (8%), and iron (5%) (Hattingh, 2023). Uziza seeds show a proximate composition of 4.29–7.30% protein, 21.90–62.35% moisture, 6.93–8.05% fat, 1.75–7.61% ash, 13.77–38.34% crude fiber, and 3.39–6.83% carbohydrates, varying with processing methods like soaking and air-resting (Attaugwu *et al*., 2025). Seeds contain high levels of calcium (10.77 mg/L), iron (11.16 mg/L), molybdenum (3.07 mg/L), and selenium (0.64 mg/L), surpassing some spices (Evuen *et al*., 2022). Antioxidant vitamins in seeds include vitamin A (329.05–908.14 IU), C (64.92–95.38 mg/100 g), and E (3.7–7.5 mg/100 g), though processing can reduce vitamin C content (Attaugwu *et al*., 2025). Cooking methods impact retention; sautéing preserves more B-group vitamins and β-carotene in leaves compared to blanching, which causes up to 50% nutrient loss due to leaching (Ukom *et al*., 2023).

2.11 Phytochemical Constituents

Uziza's pharmacological potency stems from its bioactive compounds, including alkaloids (20% in leaves), flavonoids (25%), tannins (15%), phenolic acids (30%), and piperine (10%) (Hattingh, 2023). Seed analysis reveals flavonoids (2.73%), alkaloids (1.57%), phenols (0.33%), saponins (0.36%), phytate (0.66%), oxalate (0.05%), and tannins (0.22%) (Evuen *et al*., 2022). GC-MS profiling of leaf extracts identifies phytol (9.11–15.17%), tributyl acetylcitrate (10.95%), ethyl piperonyl cyanoacetate (27.35%), and piperine (5.36%), contributing to therapeutic effects (Sulaimon *et al*., 2020). These compounds exhibit antioxidant, anti-inflammatory, antimicrobial, and anticancer activities, with cooking reducing anti-nutritional factors like phytates and tannins (Ukom *et al*., 2023).

2.12 Pharmacological Properties and Health Benefits

Uziza's antioxidant properties, driven by high phenolic content (108–320 mg/100 g in seeds) and activities like DPPH scavenging (55.76–88.43%) and FRAP (66.54–116 mg/100 g), neutralize free radicals and reduce oxidative stress (Hattingh, 2023; Attaugwu *et al*., 2025). Piperine-driven anti-inflammatory effects inhibit pro-inflammatory cytokines, aiding conditions like arthritis and asthma (Hattingh, 2023; Faluyi, 2020). Its antimicrobial activity combats bacterial and fungal pathogens, validating traditional uses for infections (Hattingh, 2023; Faluyi, 2020). Antidiabetic effects are notable, with leaf extracts inhibiting α-amylase (IC50 = 0.33 µg/mL) and α-glucosidase (IC50 = 0.24 µg/mL), surpassing drugs like acarbose in some fractions (Sulaimon *et al*., 2020).

Cardiovascular benefits include hypolipidemic effects, reducing cholesterol and promoting weight gain in animal models (Faluyi, 2020; Ukom *et al*., 2023). Uziza supports respiratory health through expectorant properties and enhances digestive health by stimulating enzymes, alleviating indigestion and ulcers (Hattingh, 2023; Faluyi, 2020). Emerging evidence

suggests anticancer potential via piperine-induced apoptosis, fertility enhancement through increased testosterone and sperm count due to zinc and magnesium, memory improvement in degenerative models, and gut microbiome promotion for immunity (Hattingh, 2023; Faluyi, 2020). It also aids in pain relief, wrinkle reduction, oral health, and smoking cessation (Faluyi, 2020). Optimized processing, like 30-minute air-resting or sautéing, preserves antioxidants and nutrients, enhancing its role in addressing nutrient deficiencies and chronic diseases in Sub-Saharan Africa (Attaugwu *et al*., 2025; Evuen *et al*., 2022; Ukom *et al*., 2023).

2.12.1 Antioxidant Activity

Uziza leaf exhibits potent antioxidant properties, neutralizing free radicals and reducing oxidative stress through high levels of vitamins A (15%), C (30%), E (10%), and phenolic compounds (108–320 mg/100 g) (Hattingh, 2023; Faluyi, 2020). Mechanisms include inhibition of lipid peroxidation, enhancement of enzymes like superoxide dismutase and catalase, and scavenging reactive oxygen species (Faluyi, 2020; Asiamah *et al*., 2021). Studies show it prevents LDL oxidation and supports cellular protection against chronic diseases like cancer and neurodegeneration (Ameh *et al*., 2021; Faluyi, 2020). In lead-induced neurotoxicity models, uziza combined with honey restored glutathione peroxidase and reduced lipid peroxidation in rat brains by up to 40% (Ogueji *et al*., 2022). A 2023 study confirmed DPPH scavenging activity (55.76–88.43%) and FRAP (66.54–116 mg/100 g), underscoring its role in combating oxidative stress (Hattingh, 2023).

2.12.2 Antimicrobial and Antiparasitic Effects

Uziza leaf extracts demonstrate broad-spectrum antimicrobial activity against Gram-positive and Gram-negative bacteria (e.g., *Escherichia coli*, *Staphylococcus aureus*), fungi, and mycobacteria like *Mycobacterium tuberculosis* (Hattingh, 2023; Ameh *et al*., 2021; Faluyi, 2020). Time-kill assays reveal reductions in viable enteric pathogens like *Shigella*

dysenteriae and *Salmonella typhi* within 3–8 hours at MIC levels, with inhibition zones up to 20 mm (Achukwu *et al*., 2023). Antiparasitic potential includes antiplasmodial effects, with ethanol extracts reducing parasitemia by 62.69% in *Plasmodium berghei*-infected mice at 400 mg/kg (Dike-Ndudim *et al*., 2016). These properties support its use in treating infections and as a natural preservative in pharmaceutical formulations (Ameh *et al*., 2021; Achukwu *et al*., 2023).

2.12.3 Anti-inflammatory and Analgesic Properties

Uziza inhibits pro-inflammatory cytokines (e.g., TNF-α, IL-1β) and enzymes via piperine, alleviating inflammation in conditions like arthritis and asthma (Hattingh, 2023; Faluyi, 2020; Asiamah *et al*., 2021). In egg albumin-induced paw edema models, methanolic extracts normalized CRP, MDA, LDH, and GGT levels, achieving results comparable to diclofenac at 100 mg/kg (Ogueji *et al*., 2020). Analgesic effects reduce writhing by up to 59.07% in acetic acid-induced pain tests, suggesting central and peripheral mechanisms (Dike-Ndudim *et al*., 2016). These effects position uziza for anti-inflammatory drug development (Faluyi, 2020; Ogueji *et al*., 2020).

2.12.4 Neuroprotective and Antineurodegenerative Effects

In geriatric brain aging, uziza modulates NF-κB, reduces neuroinflammation, and enhances BDNF signaling to improve memory and synaptic plasticity (Asiamah *et al*., 2021). It inhibits AChE by 30–40%, prevents amyloid-β aggregation in Alzheimer's models, and protects dopaminergic neurons in Parkinson's, restoring neurotransmitters like dopamine (Asiamah *et al*., 2021). Against lead neurotoxicity, uziza ameliorates hippocampal damage, enhances social behaviors, and improves motor function in rats, with a 25% increase in open-field activity (Ogueji *et al*., 2022). These findings suggest potential in neuroprotective supplements (Asiamah *et al*., 2021; Ogueji *et al*., 2022).

2.12.5 Reproductive and Fertility Effects

Uziza enhances male fertility by boosting sperm motility, testicular steroidogenesis, and libido, acting as an aphrodisiac (Ameh *et al*., 2021; Ojiako and Ojiako, 2022). It ameliorates oxidative stress in metal-induced reproductive toxicity, improving testosterone and sperm parameters by up to 35% (Ojiako and Ojiako, 2022). For women, it supports postpartum recovery by promoting uterine contractions (Okwunodulu *et al*., 2023). However, chronic high doses may cause testicular atrophy in males and inhibit follicle maturation in females, necessitating moderation (Ameh *et al*., 2021). These properties indicate potential in fertility-enhancing formulations (Ojiako and Ojiako, 2022).

2.12.6 Renal Protective Effects

Ethanolic extracts protect against paraquat-induced kidney toxicity, reducing urea and creatinine levels by 20–30% and ameliorating histological damage like inflammation and necrosis in Wistar rats (Adolphus *et al*., 2024). This suggests preventive potential in oxidative stress-related renal disorders (Adolphus *et al*., 2024).

2.12.7 Other Properties

Anticancer potential involves piperine-induced apoptosis and proliferation inhibition in breast and colon cancer cells (Hattingh, 2023; Faluyi, 2020). Antidiabetic effects include inhibition of α-amylase (IC50 = 0.33 µg/mL) and α-glucosidase (IC50 = 0.24 µg/mL), surpassing acarbose (Sulaimon *et al*., 2020). Cardiovascular benefits include lipid metabolism improvement and cholesterol reduction, with a 15% decrease in LDL in animal models (Hattingh, 2023; Ukom *et al*., 2023). Uziza also aids in pain relief, wrinkle reduction, oral health, and smoking cessation (Faluyi, 2020).

2.13 Pharmaceutical Applications

Pharmaceutically, uziza's piperine enhances drug bioavailability, increasing curcumin absorption by up to 2000%, making it an adjuvant in formulations (Hattingh, 2023; Faluyi, 2020). Its antimicrobial properties position it as a natural preservative or antibiotic alternative (Ameh *et al*., 2021; Achukwu *et al*., 2023). Extracts are explored for neuroprotective supplements, anti-inflammatory drugs, and fertility enhancers (Asiamah *et al*., 2021; Ojiako and Ojiako, 2022). Low toxicity (LD50 >2000 mg/kg) supports its integration into nutraceuticals and ethnomedicines (Dike-Ndudim *et al*., 2016). Recent studies suggest potential in topical creams for skin infections and oral health products due to its antimicrobial and anti-inflammatory effects (Ameh *et al*., 2021; Faluyi, 2020).

2.14 Safety and Toxicology

Uziza is generally safe at moderate doses, with LD50 >2000 mg/kg, but high chronic intake may impair renal or reproductive function (Ameh *et al*., 2021; Dike-Ndudim *et al*., 2016). Temporary effects like appetite loss occur at high doses, emphasizing the need for controlled use (Dike-Ndudim *et al*., 2016). Acute toxicity studies show no significant adverse effects at therapeutic levels (Adolphus *et al*., 2024).

2.15 Antibacterial Activity

Uziza leaf extracts demonstrate potent activity against both Gram-positive and Gram-negative bacteria, including *Escherichia coli*, *Staphylococcus aureus*, *Shigella dysenteriae*, *Salmonella typhi*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* (Hattingh, 2023; Ameh *et al*., 2021; Achukwu *et al*., 2023). A 2023 study reported that ethanolic leaf extracts achieved inhibition zones of up to 20 mm against *S. aureus* and *E. coli* at minimum inhibitory concentrations (MICs) ranging from 50 to 100 µg/mL (Achukwu *et al*., 2023). Time-kill

assays showed significant reductions in viable cells of enteric pathogens like *S. dysenteriae* and *S. typhi* within 3–8 hours at MIC levels, indicating bactericidal effects (Achukwu *et al*., 2023). The mechanisms involve disruption of bacterial cell membranes, inhibition of efflux pumps, and interference with biofilm formation, particularly effective against multidrug-resistant strains (Ameh *et al*., 2021; Faluyi, 2020). These properties position uziza leaf as a potential alternative to synthetic antibiotics, especially in addressing antibiotic resistance.

2.16 Antifungal Activity

Uziza leaf exhibits significant antifungal activity against pathogens such as *Candida albicans*, *Aspergillus niger*, and *Aspergillus flavus* (Ameh *et al*., 2021; Dike-Ndudim *et al*., 2016). A 2025 study highlighted the efficacy of uziza leaf essential oils, with concentrations as low as 525 µL/L inhibiting conidia of *Aspergillus* species, attributed to terpenoids and phenolic compounds disrupting fungal cell walls and ergosterol synthesis (Adesina and Okwute, 2025). Ethanolic extracts showed MIC values of 25–50 µg/mL against *C. albicans*, comparable to standard antifungal agents like fluconazole (Dike-Ndudim *et al*., 2016). This antifungal potential supports its traditional use in treating skin infections and oral candidiasis, suggesting applications in topical antifungal formulations (Faluyi, 2020).

2.17 Mechanisms of Action

The antimicrobial mechanisms of uziza leaf involve multiple pathways. Piperine and phenolic compounds disrupt microbial cell membranes, increasing permeability and causing leakage of cellular contents (Hattingh, 2023; Achukwu *et al*., 2023). Flavonoids inhibit DNA gyrase and topoisomerase, essential for bacterial replication, while tannins precipitate proteins, impairing microbial metabolism (Ameh *et al*., 2021; Madu *et al*., 2023). Essential oils, including phytol and terpenoids, interfere with fungal ergosterol biosynthesis and bacterial efflux pumps, enhancing susceptibility to other antimicrobials (Adesina and Okwute, 2025; Dike-Ndudim *et*

al., 2016). These synergistic actions make uziza leaf effective against resistant pathogens, offering a multi-targeted approach to infection control.

CHAPTER THREE

MATERIALS AND METHOD

3.1 Sample preparation and Extraction

The leaf of lemon grass and uziza leaves were purchased at a local market in Benin city, Edo State, Nigeria. The leaves were air dried and pulverized with an electric blender and stored in an airtight container till further analysis. Five hundred grams of the sample were soaked in Alcohol and water (Ethanol and Aqueous extraction) after which it was filtered with Whatman No 1 filter paper and evaporated to obtain the extract (Abdul-Hammed *et al*., 2021).

3.2 Preparation and Sterilization of Culture Media

All culture media were prepared according to the manufacturer's instructions. Sterilization will be at 121ºC at 15psi for 15 min unless otherwise stated by manufacturer.

3.2.1 Nutrient agar

Twenty-eight grams (28 g) of nutrient agar were dissolved in 1000 ml of distilled water in a conical flask corked with cotton wool and foil paper and allowed to dissolve in 1000 ml of distilled water in a conical flask. The medium will be placed in an autoclave to sterilize it for 15 minutes at 121 °C. After sterilization, the flask was allowed to cool.

3.2.2 Mueller Hinton agar

Thirty -eight grams of mueller hinton agar were dissolved in 1000ml of distilled water and boil to completely dissolve agar. The autoclave was sterilized for 15 minutes at 15 psi (121^{o}C). It was then cool to 60^{o}C and before pouring into sterile Petri dishes.

3.3 Determination of phytochemicals Components

3.3.1 Total flavonoid:

Total flavonoid content (TFC) were determined with slight modification of Pandey *et al*. (2020). A small quantity of the sample (0.5 ml) was combined with 4 ml distilled water and 0.3 ml of 5% NaNO2 (Sodium Nitrite) solution. After 5 minutes, 0.3 ml of 10% $Alcl_3$ (Aluminium Chloride) was added and after 6 minutes 2 ml of 1M NaOH (Sodium Hydroxide) was added. After 30 minutes, the absorbance was measured at 510 nm against a blank solution. TFC was calculated using a quercetin calibration curve and represented as mg of Quercetin equivalents (QE/g) of extracts (Bouret *et al.,* 2015).

3.3.2 Total phenols:

Total phenol content were determined by Folin Ciocalteu method (Slinkard and Slingleton, 1997). Using distilled water, a known aliquot of samples was made up to 1.5 ml. After that, 0.5 ml Folin-Ciocalteu reagent was added and then 10 ml Sodium Carbonate, was added after

which the solution was incubated for 1 hour at 37 °C. The absorbance values were read at 750 nm. Total phenolic content was expressed as mg gallic acid equivalent (GAE)/g of extracts by using the calibration curve of gallic acid (Bouret *et al.*, 2015).

3.3.3 Tannins:

Tannin content were determined by using Folin Denis reagent (AOAC 2005). Known aliquot of sample was added to a volumetric flask containing 75 ml of water in a 100 ml volumetric flask. Later 5 ml of Folin Denis reagent and 10 Na2CO3 solution was added. After 30 minutes, the colour at 760 nm was measured against an experimental blank calibrated to 0 absorbency. Tannic acid percentage was determined by mg tannic acid from standard curve (Bouret *et al.*, 2015).

3.4 Confirmatory Test for Isolates

3.4.1 Gram staining:

This test was done to confirm the cell type of the bacteria to be used. Gram staining techniques was used for differentiation between Gram-positive and Gram-negative bacteria. Organisms that retain the primary stain are called Gram positive while those that do not retain the primary stain when decolourized are called Gram negative. The non-retention of the stain is due to the cell composition. The Gram stain procedure is as follows:

A smear of the bacteria isolate was made on grease free slide and heat fix by passing over flame. The smear was flooded with crystal violet which is the primary stain for 1min then washed with distilled water. Subsequently the slides were flooded with Lugol's iodine solution for 30sec and then washed off with distilled water. 95% alcohol was used for decolorization for 10sec and immediately washed off with distilled water. Finally, the smear

was counter stained with saffranin for 1min and washed off. The slides were allowed to air dry before observing under the microscope using an oil immersion objective lens of ×100 magnifications to view the slides.

3.5. Biochemical identification

Biochemical test was carried out so as to help in the identification of the bacteria isolates as phenotypic (cultural) characteristics is not sufficient. The various biochemical test carried out are shown below;

3.5.1 Oxidase test

This is mainly used to differentiate between *Pseudomonas* from other Gram-negative rods. Oxidase test was carried out to identify bacteria species that will produce cytochtome oxidase enzyme. *Staphylococcus aureus* and *Escherichia coli* which are Gram-positive and Gram-negative respectively were employed as control. A piece of filter paper using sterilized wire loop 2-3 drops of freshly prepared oxidase reagent (1% aqueous tetramethyl-3-phenyl nediamine dichloride) was added. A positive oxidase test is indicated by purple colouration within 10 seconds (Dhakad *et al.*, 2015).

3.5.2 Urease test.

This is used to test organisms that have the ability to produce the enzyme urease which catalyzes the breakdown of urea to produce ammonia. The test is usually used to differentiate organisms like *Proteus mirabilis* from other non-urease positive organism. A sterilized medium was dispensed into test tubes aseptically and the test bacteria isolated were inoculated into the medium and incubated at 37 ^{0}C for 24 hours. A change in colour from yellow to red-pink confirmed the presence of urease.

3.5.3 Indole production test

This test was used to determine which of the isolates has the ability to split indole from tryptophan present in peptone water. The test is usually used in differentiating Gram-negative Bacilli especially those of enterobacteriaceae. Five grams of commercially available peptone broth was dissolved in 1litre of distilled water. The medium was then sterilized by autoclaving at 121 degree centigrade for 15 minutes. The 4 ml of the medium was dispensed into sterile test tube and each of the bacterial isolates was inoculated into the peptone broth. The inoculated media was incubated at 37 ^{0}C for 24 hours after which few drops of KOVAC reagent was added. KOVAC reagents consist of 150 ml of amylalcohol, 10 g dimethylamino benzaldehyde and 150 ml of concentrated hydrochloric acid. Positive test was indicated by the red colouration that occurs immediately at the upper part of the test tube.

3.5.4 Citrate utilization test

This test is used to identify which of the isolate can utilize citrate as the sole source of carbon for metabolism. The medium used for this test is Simon`s citrate agar. In the preparation, 22 g of commercially available Simon's citrate agar was dissolved in a litre of distilled water and sterilized by autoclaving at 121 ^{0}C for 15 minutes. The medium is dispensed into test tubes and the test organism was inoculated by stabling the medium on the tubes using sterile straight inoculation wire containing culture. The tubes were incubated at 37 ^{0}C for about 24 hours. Positive result is indicated by a change in colour from green to bright blue colouration (Amin *et al.*, 2018).

3.5.5 Catalase test

This is a test to detect the presence or absence of catalase enzyme. The catalase enzyme catalyses the breakdowns of hydrogen peroxide to release free oxygen gas and the formation of water. A few drops of freshly prepared 3% hydrogen peroxide were added onto the bacterial isolates smeared on a slide. The production of gas bubble indicated catalase enzyme positive.

$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$

3.5.6 Sugar fermentation and production of gases using Triple sugar iron agar (TSI)

TSI was prepared following manufacturer's instruction and the prepared media was placed in a test tube and kept in a slant position for it to solidify. The slant and butt of the medium was inoculated with the test bacterium using a sterile loop and it was incubated for 18- 24 hours. The results were read on the basis of acid or alkaline production in the slant or butt region of the tube and gas production was confirmed by the presence of crack or air bubbles in the slant or but region. More so, production of hydrogen sulphide was confirmed by the blackening of the medium. A prepared laboratory chat was used for result interpretation in line with microbiological standard protocol as well as other biochemical tests carried out on the isolates to confirm or ascertain their identity.

3.6 Antimicrobial sensitivity of extract

3.6.1 Inoculation of plates

This was done by the modified method of Acar and Goldstein using flood- inoculation technique. Bacterial suspension having turbidity equivalent to 0.5 McFarland was freshly prepared and 2 ml of this was transferred onto the Muellar Hinton Agar plate and distributed gently over surface of medium with gentle rocking. The excess fluid was removed from the plate and the plate were kept in incubator at 37ºC for 30 minutesfor drying before application of discs.

3.6.2 Agar Well Dilution

This was carried out using the modified method of Bauer *et al* . (2012). Muellar Hinton Agar was prepared and after sterilization, cooled and poured into the petri dishes and allow to solidify. Upon solidification of the agar, sterile corn-bore of 6mm was used to make well into the agar plates, about 4mm deep. The bacteriocin in different concentrations was transferred aseptically into the hole and labeled accordingly, and the plate was flooded with bacteria isolates. The petri dishes were incubated at 37°c up to 24 hours after which the radius of the zone of growth of the isolates were measured using graduated millimeter (mm).

3.6.3 Determination of Minimum Inhibitory Concentration (MIC) and MBC using Broth Dilution Method

The Broth dilution method (Nagalakshmi *et al*., 2019) was used for the determination of Minimum Inhibitory Concentration (MIC) of the extract against bacteria from spoilt food. The extracts were diluted into various concentrations 25µg/ml, 50 µg/ml, 75 µg/ml and 100 µg/ml in a sterile Nutrient broth in test tubes. Using standard wire loop (Hi-media), a loopful of the bacterial culture was inoculated into test tubes containing various concentrations of oil extract in Nutrient broth. The tubes were incubated at 37 °C for 24 hours and thereafter observed for growth or turbidity.

3.6.4 Determination of Minimum Fungicidal Concentration

Small aliquots from wells from MIC test that show no visible growth are plated on fresh medium containing leaf extract and incubated. The MFC was the lowest concentration that showed either no growth or fewer than three colonies.

3.7 Data Analysis

The data were analysed using the SPSS package version 21.0. All data are mean of three replicates. The mean, range and standard deviation of each parameter was determined. The means were separated using Duncan's Multiple Range test (SPSS, 2010).

CHAPTER FOUR

RESULTS

Table 4.1 presents the results of the phytochemical analysis of lemongrass and uziza leaf extracts. The results reveal that both extracts contained important secondary metabolites such as alkaloids, flavonoids, terpenoids, tannins, and saponins in varying concentrations. Lemongrass showed a higher presence of alkaloids, flavonoids, and tannins (++), while uziza

exhibited a moderate presence (+) of these compounds. Saponin was present in lemongrass but absent in uziza.

Table 4.2 shows the antibacterial and antifungal activities of aqueous and ethanolic extracts of lemongrass at different concentrations (100%, 50%, 25%, and 12.5%). The ethanolic extract demonstrated stronger antimicrobial activity than the aqueous extract against all tested organisms. The highest inhibition zones were recorded against *Staphylococcus aureus* (20 mm) and *Escherichia coli* (15 mm) at 100% concentration of the ethanolic extract. Both extracts showed decreasing activity with dilution. Fungal isolates such as *Aspergillus* sp., *Penicillium* sp., and *Fusarium* sp. were also inhibited, although to a lesser degree compared to bacterial isolates. Ciprofloxacin and fluconazole (controls) exhibited the highest inhibition zones, confirming the relative effectiveness of the extracts.

Table 4.3 illustrates the inhibitory effects of uziza leaf extracts on selected bacterial and fungal isolates. The ethanolic extract exhibited greater antimicrobial activity than the aqueous extract, producing inhibition zones of 18 mm against *Staphylococcus aureus* and 15 mm against *E. coli* at 100% concentration. The aqueous extract demonstrated moderate activity, while lower concentrations (25% and 12.5%) produced little or no inhibition. Fungal isolates such as *Aspergillus* sp. and *Penicillium* sp. showed mild to no inhibition at all concentrations tested.

Table 4.4 presents the minimum inhibitory concentration (MIC) values of both ethanolic and aqueous extracts of lemongrass against bacterial and fungal isolates. The ethanolic extract showed lower MIC values (50 mg/mL) against *E. coli*, *Staphylococcus aureus*, and *Pseudomonas* sp., indicating higher potency. Fungal isolates such as *Aspergillus*, *Penicillium*, and *Fusarium* sp. required higher concentrations (100 mg/mL) for inhibition. Table 4.5 shows the MIC values of uziza extracts against bacterial and fungal isolates. The

ethanolic extract exhibited moderate inhibitory activity, with MIC values of 50 mg/mL against *Staphylococcus aureus* and 100 mg/mL against *E. coli* and *Pseudomonas* sp. The aqueous extract demonstrated lower activity, requiring higher concentrations (100 mg/mL) to inhibit growth. No inhibitory activity was recorded against fungal isolates *Aspergillus* sp. and *Fusarium* sp.

Table 4.6 displays the minimum bactericidal and fungicidal concentrations of lemongrass extracts. The ethanolic extract exhibited bactericidal activity at 100 mg/mL against *E. coli*, *Pseudomonas* sp., *Aspergillus* sp., and *Penicillium* sp. The aqueous extract demonstrated similar bactericidal effects against *E. coli* and *Pseudomonas* sp. but lacked activity against *Staphylococcus aureus* and the tested fungi.

Table 4.7 presents the MBC and MFC results of uziza leaf extracts. The ethanolic extract exhibited bactericidal activity at 100 mg/mL against *E. coli*, *Staphylococcus aureus*, and *Pseudomonas* sp., whereas the aqueous extract showed activity only against *E. coli*. No fungicidal activity was observed against *Aspergillus*, *Penicillium*, or *Fusarium* species.

Table 4.1: Phytochemical screening of lemongrass (*Cymbopogon citratus*) and uziza leaf (*Piper guineense*) Leaf extracts.

Phytochemicals	**Lemon leaf**	**Uziza leaf**
Alkaloid	++	+

Flavonoid	++	+
Terpenoids	+	+
Tanins	++	+
Saponin	+	-

Key: + = present

- = absent

Table 4.2: Antibiotic potential of aqueous and ethanolic extracts of lemongrass (*Cymbopogon citratus*) on Bacterial Isolates.

		Zones of Inhibitions (mm)				
Test organism	**Extract**	**100%**	**50%**	**25%**	**12.50%**	**CPX/FLU (control)**
Staphylococcus aureus	Ethanolic	20	16	0	0	28
	Aqueous	18	13	0	0	
Escherichia coli	Ethanolic	15	11	0	0	24
	Aqueous	14	11	0	0	
Pseudomonas sp	Ethanolic	12	10	0	0	
	Aqueous	12	9	0	0	17
Aspergillus sp	Ethanolic	10	7	0	0	
	Aqueous	8	0	0	0	20
Penicillium sp	Ethanolic	12	10	0	0	
	Aqueous	10	7	0	0	18
Fusarium sp	Ethanolic	15	10	0	0	
	Aqueous	8	0	0	0	20

Key: CPX = Ciprofloxacin, FLU=fluconazole

Table 4.3: Antibiotic potential of aqueous and ethanolic extracts of uziza leaf (*Piper guineense*) on Bacterial Isolates.

		Zones of Inhibitions (mm)				
Test organism	**Extract**	**100%**	**50%**	**25%**	**12.50%**	**CPX (control)**
Staphylococcus aureus	Ethanolic	18	10	0	0	30
	Aqueous	10	10	0	0	
Escherichia coli	Ethanolic	15	9	0	0	24
	Aqueous	11	10	0	0	
Pseudomonas sp	Ethanolic	12	10	0	0	
	Aqueous	12	9	0	0	17
Fusarium sp	Ethanolic	15	10	0	0	
	Aqueous	8	0	0	0	20
Aspergillus sp	Ethanolic	10	0	0	0	
	Aqueous	8	0	0	0	20
Penicillium sp	Ethanolic	10	0	0	0	
	Aqueous	7	0	0	0	18
Fusarium sp	Ethanolic	10	0	0	0	
	Aqueous	0	0	0	0	20

Key: CPX = Ciprofloxacin

Table 4.4: Minimum inhibitory concentration of lemongrass (*Cymbopogon citratus*) leaf extract

	ETHANOL (extract)	**AQUEOUS (extract)**
E.coli	50mg/ml	100mg/ml
Staphylococcus aureus	50mg/ml	100mg/ml
Pseudomonas sp	50mg/ml	100mg/ml
Aspergillus sp	100mg/ml	-
Penicillium sp	100mg/ml	-
Fusarium sp	100mg/ml	-

Table 4.5: Minimum inhibitory concentration of Uziza Leaf (*Piper guineense*)

	ETHANOL (extract)	**AQUEOUS (extract)**
E.coli	100mg/ml	100mg/ml
Staphylococcus aureus	50mg/ml	100mg/ml
Pseudomonas sp	100mg/ml	-
Aspergillus sp	-	-
Penicillium sp	100mg/ml	-
Fusarium sp	-	-

Table 4.6: Minimum bactericidal and fungicidal concentration of lemongrass (*Cymbopogon citratus*) leaf

	ETHANOL (extract)	**AQUEOUS (extract)**
E.coli	100mg/ml	100mg/ml
Staphylococcus aureus	-	-
Pseudomonas sp	100mg/ml	100mg/ml
Aspergillus sp	100mg/ml	-
Penicillium sp	100mg/ml	-
Fusarium sp	-	-

Table 4.7: Minimum bactericidal and fungicidal concentration of Uziza Leaf (*Piper guineense*)

	ETHANOL (extract)	**AQUEOUS (extract)**
E.coli	100mg/ml	100mg/ml
Staphylococcus aureus	100mg/ml	-
Pseudomonas sp	100mg/ml	-
Aspergillus sp	-	-
Penicillium sp	-	-
Fusarium sp	-	-

CHAPTER FIVE

DISCUSSION

The phytochemical screening of *Cymbopogon citratus* (lemongrass) and *Piper guineense* (uziza) leaves revealed the presence of alkaloids, flavonoids, terpenoids, tannins, and saponins in varying concentrations. Lemongrass showed higher intensity (++ for alkaloids, flavonoids, and tannins), while uziza exhibited moderate presence (+) of most constituents and lacked saponins. These findings align with earlier reports indicating that lemongrass contains abundant secondary metabolites—particularly citral, geraniol, and flavonoids—that contribute to its antimicrobial and antioxidant potential (Mukarram *et al*., 2022; Gao *et al*., 2020). Similarly, the phytochemical profile of uziza corroborates previous studies that identified alkaloids, flavonoids, phenolics, and piperine as its major bioactive components (Hattingh, 2023; Ameh *et al*., 2021).

The presence of alkaloids and tannins in both plants is noteworthy because these compounds are known to interfere with microbial enzyme activity and cell wall synthesis (Osuala and Anyadoh, 2006). Flavonoids act as antioxidants and disrupt microbial membranes by binding to proteins and lipids, while terpenoids and phenolic compounds are known to destabilize microbial cell membranes and interfere with biofilm formation (Ewansiha *et al*., 2012; Abd El-Hack *et al*., 2022). The absence of saponins in uziza suggests a difference in secondary metabolite expression between both plants, possibly due to species variation or extraction solvent polarity, which influences phytochemical solubility (Adesina and Okwute, 2025).

Both aqueous and ethanolic extracts of lemongrass demonstrated varying degrees of antibacterial and antifungal activity against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas sp.*, *Aspergillus sp.*, *Penicillium sp.*, and *Fusarium sp.*. The ethanolic extract exhibited the highest inhibition zones, with 20 mm against *S. aureus* and 15 mm against *E.*

*coli*at 100% concentration, while aqueous extracts showed moderate inhibition (14–18 mm). This finding aligns with prior studies that demonstrated ethanol's superior extraction efficiency for phenolic and terpenoid compounds, leading to stronger antimicrobial effects (Ewansiha *et al*., 2012; Mukarram *et al*., 2022).

The enhanced sensitivity of *S. aureus* to lemongrass extract agrees with earlier research by Gao *et al*. (2020), who reported that citral-rich lemongrass oil achieved up to 97% inhibition of *S. aureus* at concentrations as low as 0.0313% v/v. Similarly, Mukarram *et al*. (2022) found minimum inhibitory concentrations (MICs) between 20–50 µg/mL against *E. coli* and *Pseudomonas aeruginosa*. The present study recorded MIC values of 50 mg/mL (ethanolic) and 100 mg/mL (aqueous), consistent with the notion that crude extracts generally display lower potency than purified essential oils (Vazirian *et al*., 2012).

Fungal isolates (*Aspergillus*, *Penicillium*, and *Fusarium*) were moderately susceptible to the ethanolic extract, with inhibition zones ranging from 10–15 mm. This observation agrees with Adesina and Okwute (2025), who reported that lemongrass oil inhibited *Aspergillus flavus* and *Fusarium moniliforme* at MICs below 0.078% v/v. The antifungal mechanism is attributed to citral-induced membrane disruption and inhibition of ergosterol biosynthesis, leading to cell lysis (Mukarram *et al*., 2022).

Uziza (*Piper guineense*) extracts exhibited notable activity, particularly against *S. aureus* and *E. coli*, though less potent than lemongrass. Ethanolic extracts produced inhibition zones of 18 mm for *S. aureus* and 15 mm for *E. coli*, while aqueous extracts recorded lower values (10–11 mm). This observation corroborates the findings of Achukwu *et al*. (2023), who reported inhibition zones of up to 20 mm for uziza methanolic extracts against similar pathogens at concentrations between 50–100 µg/mL.

The moderate antimicrobial activity observed in this study supports the results of Hattingh (2023) and Ameh *et al*. (2021), who attributed uziza's bactericidal effects to piperine, flavonoids, and phenolic compounds that damage bacterial cell membranes and inhibit efflux pumps. The higher activity against *S. aureus* compared to *E. coli* can be explained by the thicker peptidoglycan layer in Gram-positive bacteria, which is more easily penetrated by hydrophobic phytochemicals like alkaloids and terpenoids (Faluyi, 2020).

However, uziza extracts were less effective against fungi, with little to no inhibition of *Aspergillus*, *Penicillium*, or *Fusarium* species. This result diverges slightly from Adesina and Okwute (2025), who observed significant antifungal activity of uziza essential oil at 525 µL/L. The discrepancy could be attributed to differences in extraction solvent and concentration, as essential oils typically contain higher levels of active volatile terpenes compared to crude ethanolic or aqueous extracts.

The MIC results revealed that ethanolic extracts of both plants were more effective than aqueous extracts. For lemongrass, MICs ranged from 50–100 mg/mL, while uziza extracts recorded 50 mg/mL for *S. aureus* and 100 mg/mL for other pathogens. These findings correspond with prior studies by Abd El-Hack *et al*. (2022), who reported MIC values of 50–100 µg/mL for uziza methanolic extracts and 25–75 µg/mL for lemongrass oils. The MBC and MFC values of both plants were also within the same range, suggesting that the extracts possess both bacteriostatic and bactericidal properties, depending on concentration.

The observed dose-dependent pattern, where antimicrobial activity decreased with dilution, mirrors the concentration-dependent inhibition reported by Gao *et al*. (2020) for lemongrass and Achukwu *et al*. (2023) for uziza. The relatively high MIC and MBC values recorded in this study could be linked to the crude nature of the extracts and potential interference of non-bioactive compounds (Mukarram *et al*., 2022).

When compared, lemongrass demonstrated stronger and broader antimicrobial efficacy than uziza, particularly against both Gram-positive and Gram-negative bacteria and fungi. This aligns with literature reports that lemongrass essential oil possesses one of the highest antimicrobial indices among tropical medicinal plants, primarily due to its high citral (neral and geranial) content (Gao *et al*., 2020; Mukarram *et al*., 2022). Conversely, uziza's moderate activity may be attributed to lower concentrations of volatile compounds and absence of saponins, which enhance permeability and synergy in antimicrobial mechanisms (Hattingh, 2023; Ameh *et al*., 2021).

Nevertheless, uziza's bioactive alkaloids and piperine make it particularly valuable for synergistic applications. Piperine has been shown to potentiate the antimicrobial activity of other compounds by inhibiting bacterial efflux pumps (Faluyi, 2020). Thus, combining lemongrass and uziza extracts could yield synergistic antimicrobial effects, an area that warrants further exploration.

While ciprofloxacin and fluconazole (controls) exhibited higher inhibition zones (17–30 mm), the plant extracts displayed substantial activity at 100% concentrations. This observation supports the growing evidence that plant-derived antimicrobials can serve as complementary or alternative agents to synthetic antibiotics, especially against resistant strains (Abd El-Hack *et al*., 2022). The lower potency compared to standard drugs is expected, given that plant extracts are complex mixtures rather than purified compounds (Gao *et al*., 2020).

The findings are particularly significant in the context of antimicrobial resistance (AMR), where natural products like lemongrass and uziza can offer multi-targeted mechanisms of action with minimal side effects (Hattingh, 2023; Mukarram *et al*., 2022). Their roles in disrupting biofilms and enhancing drug absorption (via piperine) further highlight their pharmaceutical potential (Ameh *et al*., 2021).

5.2. Conclusion

This study revealed that both lemongrass (*Cymbopogon citratus*) and uziza (*Piper guineense*) possess important phytochemical constituents such as alkaloids, flavonoids, tannins, saponins, and terpenoids, which contribute to their antimicrobial activity. The ethanolic extracts exhibited higher inhibitory effects against both bacterial and fungal isolates compared to the aqueous extracts, indicating that ethanol was a more efficient solvent for extracting active compounds. The observed antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas* spp., and some fungi supports the traditional use of these plants in the treatment of infections. The findings suggest that lemongrass and uziza could serve as potential sources of natural antimicrobial agents for pharmaceutical and food preservation purposes. Further studies are recommended to isolate and characterize the active compounds responsible for these effects.

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APPENDIX

NUTRIENT AGAR

Composition (g/L):

Peptone – 5.0

Beef extract – 3.0

Sodium chloride – 5.0

Agar – 15.0

Distilled water – 1000 ml

pH 7.4 ± 0.2

Preparation:

28 g of nutrient agar powder was dissolved in 1000 ml of distilled water, sterilized at 121 °C for 15 min, cooled, and poured into sterile Petri dishes.

POTATO DEXTROSE AGAR (PDA)

Composition (g/L):

Potato infusion – 200.0

Dextrose – 20.0

Agar – 15.0

Distilled water – 1000 ml

pH 5.6 ± 0.2

Preparation:

39 g of PDA was dissolved in 1000 ml distilled water, sterilized at 121 °C for 15 min, cooled, and dispensed aseptically.

MUELLER-HINTON AGAR (MHA)

Composition (g/L):

Beef infusion solids – 300.0

Casein hydrolysate – 17.5

Starch – 1.5

Agar – 17.0

Distilled water – 1000 ml

pH 7.4 ± 0.2

Preparation:

38 g of MHA was dissolved in 1000 ml distilled water, boiled to dissolve completely, sterilized at 121 °C for 15 min, cooled, and poured into sterile Petri dishes.

BIOCHEMICAL REAGENTS

Indole Medium

Peptone – 20.0 g

Sodium chloride – 5.0 g

Distilled water – 1000 ml

pH 7.4

Preparation: 25 g of indole medium dissolved in 1000 ml distilled water, autoclaved at 121 °C for 15 min, and dispensed aseptically.

Oxidase Reagent (Kovac's oxidase)

p-dimethyl-aminobenzaldehyde – 0.5 ml

Amyl alcohol – 15.0 ml

Concentrated HCl – 50 ml

Preparation: aldehyde dissolved in alcohol, acid added slowly. Stored refrigerated.

Catalase Test Reagent

3% Hydrogen peroxide solution

FUNGAL STAINING REAGENT (Lactophenol Cotton Blue)

Composition:

Lactic acid – 20 ml

Phenol crystals – 20 g

Glycerol – 40 ml

Cotton blue – 0.05 g

Distilled water – 20 ml

Preparation & Use:

Cotton blue dissolved in lactic acid and phenol, then mixed with glycerol and water. A drop was placed on a fungal culture smear, covered with a coverslip, and observed under the microscope.

FOLIN–CIOCALTEU REAGENT (for Total Phenols)

Composition:

Sodium tungstate – 10 g

Sodium molybdate – 2.5 g

Phosphoric acid – 5 ml

Hydrochloric acid (conc.) – 10 ml

Lithium sulfate – 15 g

Distilled water – up to 100 ml

Preparation & Use:

Reagent prepared and stored in a dark bottle. Used by mixing 0.5 ml with sample extract; in the presence of phenols, a blue–green colour develops, read at 750 nm.

FOLIN–DENIS REAGENT (for Tannins)

Composition:

Phosphomolybdic acid – 10 g

Phosphotungstic acid – 20 g

Distilled water – 100 ml

Preparation & Use:

Both acids dissolved in hot distilled water, cooled, and stored in amber bottles. Used by adding reagent to extract, followed by sodium carbonate; development of blue colour indicates tannins, absorbance read at 760 nm.