EVALUATION OF THE ANTIMICROBIAL ACTIVITY OF N-HEXANE LEAF EXTRACT OF *Cymbopogon citratus* FORTIFIED SOAP.

BY

EFEMUAYE EMMANUEL JOSHUA

PHA1908480

DEPARTMENT OF PHARMACEUTICAL CHEMISTRY

FACULTY OF PHARMACY

UNIVERSITY OF BENIN

BENIN CITY

NOVEMBER, 2025

EVALUATION OF THE ANTIMICROBIAL ACTIVITY OF N-HEXANE LEAF EXTRACT OF *Cymbopogon citratus* FORTIFIED SOAP.

BY

EFEMUAYE EMMANUEL JOSHUA

PHA1908480

BEING A PROJECT SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF DOCTOR OF PHARMACY BY THE DEPARTMENT OF PHARMACEUTICAL CHEMISTRY, UNIVERSITY OF BENIN, BENIN CITY, EDO STATE.

NOVEMBER, 2025

CERTIFICATION

This is to certify that this work was carried out by **EFEMUAYE EMMANUEL JOSHUA** in the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin, Benin City, in partial fulfillment for the award of the Doctor of Pharmacy degree.

__________________________ ___________________

DR. IRENE OSEGHALE **Date**

Project Supervisor

__________________________ ___________________

DR. VINCENT IMIEJE **Date**

Head of Department

DEDICATION

I dedicate this work to my Heavenly Father, Jehovah, who made it all possible, and also to my beloved mother, Mrs. Joy Efemuaye, for being my backbone all those years. I'm eternally grateful for everything you did.

ACKNOWLEDGEMENT

My sincere thanks to God Almighty for His unending blessings, favours, mercies, and the successful completion of this work. I am sincerely grateful to my supervisor, Dr. Irene Oseghale, for her invaluable support, scholarly advice, guidance, and assistance, which have greatly contributed to the completion of this work.

I would love to express my love and appreciation to my mother, Mrs. Joy Efemuaye, my Father, Mr. Emmanuel Efemuaye, my siblings, Sarah Ekrakene, Abigail Prinson, Timothy Efemuaye and Lois Efemuaye for their unending support, care, and prayers.

Finally, a special thanks to my colleagues and friends: Martin Ogheneyerowho Oyawiri, Favour Emoshioke Momoh, Raphael Oghenero Idoro, Samuel Aighokhaode Spraga, Michelle Edemakhiota, Izevbigie Lawson, Emuesiri Akpughe and Mrs. Folashade Victoria. I'm grateful and blessed to have met you all.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Antimicrobial resistance (AMR) poses a growing global health threat, necessitating the development of alternative therapeutic agents. *Cymbopogon citratus* (lemongrass), a perennial herb rich in bioactive compounds such as citral, geraniol, and flavonoids, has demonstrated significant antimicrobial, antioxidant, and anti-inflammatory properties. This study investigates the antimicrobial efficacy of soap formulated with *Cymbopogon citratus* extract obtained via n-hexane solvent extraction.

Fresh *Cymbopogon citratus* leaves were collected from Obagie community, Edo State, Nigeria, shade-dried, and pulverized. A total of 500 g of powdered leaves was macerated in 2.5 L of analytical-grade n-hexane for seven days at room temperature. The extract was filtered and concentrated using a rotary evaporator at 40 °C. Phytochemical screening of the n-hexane extract confirmed the presence of alkaloids, flavonoids, saponins, terpenoids, and phenolic compounds. Soap was prepared via cold saponification method using palm and coconut oils. Antimicrobial assays were conducted on the extract and soap using concentrations of 100 mg/ml, 200 mg/ml, and 400 mg/ml against bacterial and fungal strains. The n-hexane extract showed the highest zones of inhibition against *Staphylococcus aureus* (28 mm) and *Klebsiella pneumoniae* (26 mm) at 400 mg/ml. The formulated soap demonstrated notable activity against *Pseudomonas aeruginosa* (20 mm), *Klebsiella pneumoniae* (17.5 mm), and *Penicillium spp.* (22.5 mm)

These findings support the potential of *Cymbopogon citratus*-based soap as a natural, safe, and effective topical antimicrobial agent, particularly in resource-limited settings.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.0 INTRODUCTION

With their ever-increasing use and misuse, microorganisms have developed antimicrobial resistance (AMR). The phenomenon of antimicrobial resistance refers to the potential of microorganisms including bacteria, viruses, fungi, and parasites to thrive and continue to grow in the midst of drugs designed to kill them. Infections caused by antimicrobial-resistant organisms are not only difficult to treat, there is also always an increased chance of severe illness and even death due to these infections. With its current scenario, AMR is one of the unsurpassed threats not only to global health but also to food security (George, 2017). Antimicrobial-resistant infection has been ranked third as the leading cause of death after cardiovascular diseases. An estimated 1.27 million deaths were attributable to antimicrobial-resistant infections in 2019 alone, while nearly 5 million deaths were somehow associated with drug-resistant infections, according to a major study published in January 2022. This number is estimated to be increased to 10,000,000 per year by 2050, greatly exceeding deaths from cancer (O'Neill, 2016)

Nature has served as humankind's pharmacy for millennia. Plants produce complex suites of compounds known as secondary metabolites, which are not necessary for their primary growth and function, but rather serve another role of enhancing likelihood of survival. Throughout ancient history, humans have learned to harness this chemical arsenal to serve their own needs. This is most apparent when considering human health and traditional forms of medicine. The tradition of using plants as medicine for the treatment and management of various infectious diseases continues even today, especially in the developing world.

During a time of rapidly rising antibiotic resistance, new approaches are necessary to fill the antimicrobial drug development pipeline. Moving forward, there are clearly several innovative strategies to pursue in the search for novel therapies. Plants remain a unique and underexploited source of bioactive compounds, and ethnobotanical research tools can be used to guide future research efforts and narrow down the search to the most likely source candidates. In addition to tests for classic bacteriostatic and bactericidal activity, it is also imperative to examine complex plant extracts and individual compounds for activity against alternative bacterial targets, such as virulence and pathogenesis, as well as host-directed targets.

1.1 LEAVES OF *Cymbopogon citratus* (leaves of Lemon grass)

Cymbopogon citratus, a perennial evergreen plant with long thin leaves, belongs to the Poaceae family. The name Cymbopogon comes from the Greek words "kymbe - pogon" which means "boat-beard" due to the flower spike structure) and "citratus," is latin word which indicate lemon-scented leaves (Joshi and Dar, 2018; Bhardwaj, 2020). *Cymbopogon citratus* tends to grow in regions with annual rainfall of between 2500 and 3000 mm at elevations between 900 and 1250 m above sea level. The ideal temperature for the growth of *Cymbopogon citratus* is between 25 and 30 °C, with a moderate temperature. The *Cymbopogon citratus* can grow in a wide range of soil types, from rich loam to poor laterite, but it produces the most oil and citral in sandy soil and is also known to do well on the poor soils of hill slopes (Joshi and Dar, 2018; Shendurse *et al*. 2021). *Cymbopogon citratus* is cultivated for essential oils in several subtropical and tropical regions of Asia, Africa, America, and many parts of the world (Wagh et al. 2021). The *Cymbopogon* is known to have more than 140 cultivable species in which 52 of them are found in Africa, 45 in India, 6 in Australia, 6 in South America, 4 in Europe (only in Montenegro), 2 in North America, and the rest are in South Asia and cultivated in various tropical and subtropical regions of the world (Hanna *et al*. 2012; Majewska *et al*. 2019). *Cymbopogon citratus* is primarily grown as an aromatic plant for oil production and as an ornamental plant, but it also has a variety of other purposes, including as a crop. Essential oils' antibacterial properties and potential use in medicine have recently drag researchers' curiosity. Mono- and sesquiterpene hydrocarbons and their oxygenated derivatives, together with aliphatic aldehydes, alcohols, and esters, are the main and most significant bioactive phytochemicals found in plant essential oils (Abera *et al*. 2020). Numerous studies have demonstrated the benefits of infusing *Cymbopogon citratus* leaves and other parts, which include prevention of ulcers, stimulation of digestion and excretion, and relief from nausea, stomach aches, and constipation (Majewska *et al*. 2019). The pharmacological effects of *Cymbopogon citratus* have an exceptional track record in traditional and Ayurvedic medicine. The antifungal, antibacterial, antiprotozoal, anti-inflammatory, anti-carcinogenic, antioxidant, anti-rheumatic, and cardio-protective properties of *Cymbopogon citratus* have been described by scientific studies (Pramila and Madan, 2018). Additionally, it has been demonstrated to control platelet composition, treat diabetes, gastroenteritis, anxiety or depression, malaria, and pneumonia. Industrially, they are used as an insecticide, flavoring, (Upadhyay *et al.* 2021). Botanical description of *Cymbopogon citratus*: Cymbopogon spp. are fast growing C4 perennial grass species (Mukarram *et al*. 2021). *Cymbopogon citratus* is a perennial herb having small, long, needle-like leaves. The strap-like leaves have loose tips, a glossy bluish-green coloration, and a width of 1.3 to 2.5 cm. They have a citrus aroma when crushed due to the presence of citral and a high quantity of neral and aldehyde geranial (Oladeji *et al*. 2019). Depending on the location, genetic variances, and agrarian practices, the dry leaf contains 1% to 2% essential oil, with the bioactive chemical contents (Shendurse *et al*. 2021).

The oil of *Cymbopogon citratus* is a transparent liquid with a distinct lemony aroma and a light-yellow tint (Amenaghawon *et al*. 2016).

Taxonomy of *Cymbopogon citratus*

Kingdom: Plantae

Division: Magnoliophyta

Class: Liliopsida

Order: Poales

Family: Poaceae

Genus: Cymbopogon spreng

Species: Citratus

(Praveen *et al.,* 2019)

Fig 1.1: *Cymbopogon citratus* leaves in its natural habitat

Fig 1.2: *Cymbopogon citratus* plant

1.2 ETHNO-MEDICINAL USES AND PHARMACOLOGICAL PROFILE

In the continents of Asia, South America, and Africa, the leaves have traditionally been utilized as tea or decoction. These leaves contain essential bioactive compounds that determine the plant's various ethno-medicinal properties. Additionally, they are employed as deodorants in numerous products such as perfume, local soaps, candles, and other insect repellents. Moreover, in certain regions of Asia and African countries, they have been employed as snake and reptile repellents (Oladeji *et al*. 2020). Dried *Cymbopogon citratus* leaves are widely used as a lemon flavour ingredient in herbal teas, prepared either by decoction or infusion of 2-3 leaves in 250 or 500 ml of water and other formulations. *Cymbopogon citratus* tea is a diuretic and imparts no biochemical changes to the body in comparison with ordinary tea. *Cymbopogon citratus* can be used either fresh or dried and powdered as a food flavouring. It is commonly added to teas, soups, and curries, and can be paired with poultry, fish, beef, and seafood. Numerous studies have highlighted the therapeutic benefits of *Cymbopogon citratus* leaves and other parts when infused to alleviate nausea, stomach aches, constipation, prevent ulcers, and combat various stomach infections. (Fokom *et al*. 2019) *Cymbopogon citratus* essential oil has been utilized as medicine for centuries to enhance circulation, regulate menstrual cycles, aid digestion, and boost immunity. It is also employed in the production of perfumes, flavours, detergents, and pharmaceuticals (Majewska *et al.* 2019). It offers numerous benefits in preventing gastrointestinal disorders, including gastric ulcers, as well as aiding in bowel stimulation and improving digestion. Its anti-inflammatory properties are also effective in treating constipation, ulcerative colitis, diarrhoea, nausea, and stomach aches (Madane 2024). Moreover, *Cymbopogon citratus* plays a significant role in promoting better sleep by calming the muscles and nerves, thus inducing deep sleep. It is widely utilized in Ayurvedic medicine for its healing effects on respiratory disorders such as coughs and colds. *Cymbopogon citratus* is recognized as a febrifuge and is commonly referred to as 'fever grass' due to its positive impact on reducing fevers. The antipyretic and diaphoretic properties of *Cymbopogon citratus* are widely utilized in Ayurvedic medicine to treat fevers by promoting sweating. *Cymbopogon citratus* acts as an antiseptic and is effective in addressing infections like ringworm, sores, Athlete's Foot, scabies, and urinary tract infections because of its antimicrobial and anti-fungal characteristics (Coelho *et al*. 2016). *Cymbopogon citratus* aids in relieving pain and discomfort associated with headaches and migraines, enhances blood circulation, and helps alleviate spasms, muscle cramps, sprains, and backaches. *Cymbopogon citratus* is beneficial in treating sports injuries, including dislocations, internal injuries, and bruises. It also helps in the stimulation of the mind and assists in managing convulsions, nervousness, vertigo, and various neuronal disorders such as Alzheimer's and Parkinson's disease. *Cymbopogon citratus* is utilized in therapeutic baths to calm the nerves and alleviate symptoms of anxiety and fatigue induced by stress (Wifek *et al*. 2016; Madane 2024).

Pharmacological appraisal of *Cymbopogon citratus* oil: Due to the presence of secondary metabolites like anthraquinones, saponins, flavonoids, alkaloid phenols, and tannins as well as active components from essential oils like citral, geraniol, and terpineol, the various parts of *Cymbopogon citratus*, such as the leaves and whole plant, as well as the essential oil, are used in herbal therapies.

Antibacterial activity:

In years, numerous sources have shed light on the anti-bacterial properties of plant extracts, with encouraging results. The oil was found to have significant amounts of β-citral (neral) and α-citral (geranial) bioactive chemicals (Oladeji *et al*. 2019; Majewska *et al*. 2019). These elements prevent the growth of both Gram-positive and Gram-negative bacteria, demonstrating their antibacterial action (Shendurse *et al.* 2021). However, the third component, myrcene, has no antibacterial properties on its own but does so when coupled with other substances. The plant has been employed in traditional medicine to treat bacterial infections such as meningitis, pneumonia, impetigo, cellulitis, folliculitis, and food poisoning (Oladeji *et al*. 2019).

Antimicrobial activity:

Cymbopogon citratus leaves' ethanolic extracts showed promising antibacterial properties against anti-microbial activity. *Cymbopogon citratus* essential oil often has more antimicrobial properties against fungus than it does against bacteria (Majewska *et al*. 2019). Upadhya *et al* (2021) reported that the S. aureus, S. typhi, and E. coli bacteria were moderately resistant to the chloroform extracts of *Cymbopogon citratus* leaves and roots. The extract's flavonoids and tannins are responsible for the aureus action. Sharma *et al.* (2017) demonstrated that *Cymbopogon citratus* essential oil was found to have an inhibitory impact against a pathogenic strain of Fusarium oxysporum.

Antioxidant activity:

A basic activity in human cells, tissues, and systems is oxidation, which produces reactive oxygen species (ROSs) like free radicals, superoxide anion, and hydrogen peroxide. Natural antioxidants found in *Cymbopogon citratus* include caffeoylquinic acid, flavonoids, chlorogenic acids, phenolic acids, swertiajaponin, and isoorientin (Oladeji *et al*. 2019). Hence, to prevent numerous neurological illnesses caused by oxidative damage, plants could be used. They are efficient at scavenging free radicals and have the potential to be effective antioxidants (Promila and Madan, 2018).

Antinociceptive activity:

Cymbopogon citratus has played a vital role in traditional medicine in easing pain and anxiety in living things. Ancient cultures employed the plant as a surgical analgesic or pain reliever because it may lessen the body's behavioural and physiological reactions to excruciating pain (Oladeji *et al*. 2019). The citral compound of *Cymbopogon citratus* has anti-nociception capabilities.

Anti-inflammatory:

One of the most important health problems in the world is tissue inflammation. Numerous studies have provided evidence that the primary anti-inflammatory effects of *Cymbopogon citratus* can be found in polyphenol rich extracts, solvent extracts, and citral isolate. Aqueous extracts free of fat and essential oil, as well as the polyphenol fractions (phenolic acids, flavonoids, and tannins) from *Cymbopogon citratus* leaves, were also investigated for their anti-inflammatory properties.

Hypocholesterolemic effect:

Yin *et al.* (2008) found that the rats with high-cholesterol diet-induced hyperlipidemia responded favourably to the addition of *Cymbopogon citratus* oil, with significant drops in total cholesterol, LDL-C, and triglyceride levels.

Antimutagenic activity:

In many models, the ethanol extract of C. citratus exhibits antimutagenic properties (Upadhya *et al*. 2021). In a test for the Salmonella mutation, 80% ethanol-based *Cymbopogon citratus* extracts showed no signs of mutagenicity. When the extract was present, even the Salmonella typhimurium strains TA98 and TA100 displayed resistance to a chemical mutation. Bidinotto *et* *al*. (2011) reported that *Cymbopogon citratus* essential oil was found to have protective properties against DNA damage brought on by MNU.

Anti-obesity and antihypertensive:

The potential of *Cymbopogon citratus* extract as a source of hypolipidemic and hypoglycemic compounds that may reduce the risks of hypertension and obesity have been the subject of numerous studies (Oladeji *et al*. 2019). The generation of cytokines in human macrophages was inhibited by the extract of C. citratus and its polyphenolic components. These phenolic compounds serve as anti-inflammatory agents in the appropriate human cells, according to the research (Upadhya *et al*. 2021).

Anticancer activity:

Researchers have also investigated the anti-cancer properties of *Cymbopogon citratus*. Its active components have been discovered which have cytotoxic effects on cancer cells, including geraniol, geranyl acetate, -bisabolol, and iso-intermedeol (Mukarram *et al*. 2021). The oil therapy prevented the growth of human cancer cell lines for colon, neuroblastoma, liver, cervical, and lung cancers in a concentration-dependent manner (Upadhya *et al*. 2021).

1.3 PHYTOCHEMISTRY

From the various screening procedures various phytochemicals were detected by various scientist and researchers from leaves of *Cymbopogon citratus*, such as quinones, anthraquinones, anthocyanins, leucoanthocyanins, and coumarins, as well as flavonoids, alkaloids, tannins, phlobatannins, saponins, steroids, terpenoids, glycosides, and cardiac-glycosides. (Oladeji *et al*. 2019; Shendurse *et al*. 2021; Upadhya *et al*. 2021).

1. Flavonoid Luteolin 7-O-glucoside (cynaroside),

isoscoparin, quercetin, kaempferol, isolated elimicin, catechol, chlorogenic acid, caffeic acid, hydroquinone, eugenol, and eugenol methylether are some examples of these compounds.

2. Mineral content

Potassium (K), Sodium (Na), Magnesium (Mg), Manganese (Mg), Iron (Fe), Zinc (Zn), Phytate, and Phosphorus (P), with a Calcium to phytate (0.05) and a phytate to zinc (9.6).

3. Terpenoid Cymbopogonol and cymbopogone.

4. Essential oil

Mycrene, genariol, citronellol, α-oxobisabolene, neointermediol (7.2%), selina- 6-en-4-ol (27.8%), α cadinol (8.2%), methyheptenone (1.2%), decanal (0.25%) and naphtalene (0.79%), β eudesmol (45%), cubebol (4.7%), humulene (4%), sabinene, geranyl acetate, citronella, mentha-1(7), limonene (19.33%), mentha-1(7),8 dien-2-ol trans. (Upadhya *et al*. 2021)

Source: Oladeji *et al*. 2019; Shendurse *et al*. 2021;

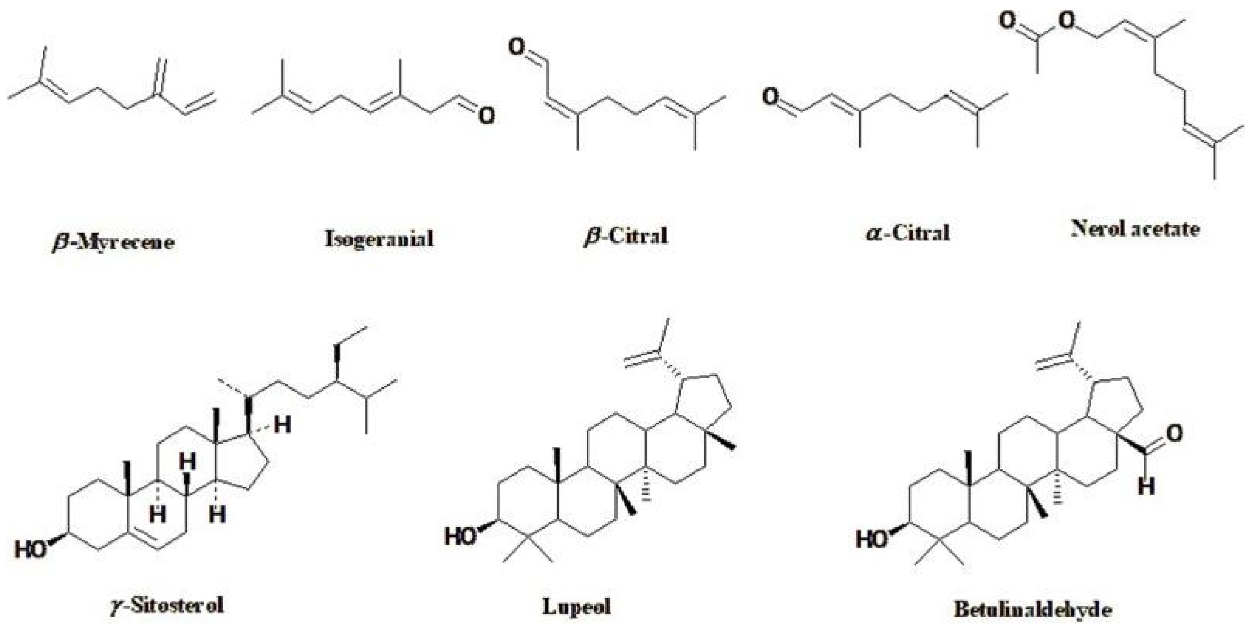

Fig 1.3: Some phytochemicals isolated from *Cymbopogon citratus* leaves (Aly *et al.,* 2025)

1.4 SAFETY AND TOXICITY

An experimental study was conducted to evaluate the safety profile of *Cymbopogon citratus* (*Cymbopogon citratus*) essential oil in mice. Both acute and sub-acute toxicity were assessed following oral administration. The findings revealed that *Cymbopogon citratus* essential oil showed no signs of acute or sub-acute toxicity at a dose of 2000 mg/kg. Moreover, the median lethal dose (LD_{50})—the dose required to cause death in 50% of the test population—was greater than 2000 mg/kg, indicating a relatively low toxicity profile (*Lulekal et al.*, 2019). These results suggest that *Cymbopogon citratus* extract is safe for use in topical formulations such as soaps and creams when used within acceptable concentration ranges.

1.5 SOAP AS A TOPICAL ANTIMICROBIAL AGENT

Soap is one of the oldest and most effective cleansing agents, traditionally produced through the saponification of fats or oils with an alkali such as sodium hydroxide. Beyond its cleansing function, soap serves as a valuable carrier for plant-derived antimicrobial agents, allowing direct application to the skin where microorganisms often thrive. Medicinal plant-based soaps have attracted increasing attention as affordable and sustainable alternatives to synthetic antibacterial formulations.

Natural antimicrobial soaps are particularly relevant in developing regions where access to modern healthcare products may be limited. Incorporating plant extracts such as *Cymbopogon citratus* oil into soap formulations enhances their antibacterial potential, providing both hygienic and therapeutic benefits.

1.6 BACKGROUND OF THE STUDY

Cymbopogon citratus (*Cymbopogon citratus*) is a perennial plant valued for its refreshing fragrance and broad therapeutic properties. Traditionally, it has been used for pain relief, digestive support, and fever reduction. Scientifically, its key bioactive component, citral, is known for strong antimicrobial, antifungal, antioxidant, and anti-inflammatory activities. Studies have shown that *Cymbopogon citratus* extract is effective against several pathogenic microorganisms, including *Escherichia coli* and *Staphylococcus aureus*.

Extraction of *Cymbopogon citratus* essential oil can be achieved using various solvents, among which n-hexane is particularly effective due to its non-polar nature and high efficiency in extracting volatile and lipophilic compounds such as citral, geraniol, and limonene. These compounds contribute to the antimicrobial efficacy of *Cymbopogon citratus* extract (Geetha *et al.* 2020).

Given the growing interest in natural antimicrobial alternatives, incorporating *Cymbopogon citratus* extract into soap formulations presents an opportunity to develop a natural antibacterial soap. Such a product could offer an effective, safe, and environmentally friendly solution for controlling skin pathogens and improving hygiene.

1.7 PROBLEM STATEMENT

Microbial infections, particularly those affecting the skin, remain a major global health concern. The rising cost of conventional antimicrobial agents, coupled with increasing microbial resistance, underscores the need for natural, affordable, and effective alternatives. *Cymbopogon citratus* possesses recognized antimicrobial properties; however, there is limited research on its effectiveness when incorporation into soap compared to its effectiveness in its crude state.

1.8 AIM OF THE STUDY

To evaluate and compare the antimicrobial activity of the n-hexane leaf extract of *Cymbopogon citratus* and soap formulated with the extract.

1.9 OBJECTIVES OF THE STUDY

1. To extract *Cymbopogon citratus* leaves using n-hexane.
2. To determine the secondary metabolites present in the n-hexane extract of *Cymbopogon citratus.*
3. To incorporate the n-hexane extract of *Cymbopogon citratus* leaves into a soap.
4. To determine the antimicrobial effect of the n-hexane leaf extract of *Cymbopogon citratus* selected clinical isolates.
5. To determine the antimicrobial effect of the soap containing the n-hexane leaf extract of *Cymbopogon citratus* on selected clinical isolates.
6. To compare the antimicrobial effect of the extract and the extract incorporated soap.

CHAPTER TWO

MATERIALS AND METHODS

2.1 MATERIALS

2.1.1. PLANT MATERIAL:

Fresh, mature leaves of *Cymbopogon citratus* were harvested from Obagie community, Ikpoba Okha LGA, Edo State, Nigeria.

2.1.2. SOLVENTS AND REAGENTS:

n-Hexane (analytical grade)(GH-Tech), Distilled water, Sodium hydroxide (NaOH), Coconut oil (Tulip Ltd) and palm kernel oil (locally sourced), Tween-80 (Titan Biotech), Ethanol

2.1.3 REAGENTS FOR PHYTOCHEMICAL SCREENING:

Mayer's, Wagner's, Dragendorff's, Hager's, Fehling's A and B, Benedict's, Molisch's, concentrated H_2SO_4, HCl, acetic anhydride, chloroform, ammonia, NaOH, ferric chloride, lead acetate, gelatin solution

2.1.4 MICROBIAL CULTURE MEDIA:

Nutrient agar (Titan Biotech), Mueller-Hinton agar (Titan Biotech), Sabouraud dextrose agar (for fungal strains) (Titan Biotech)

2.1.5 TEST MICROORGANISMS:

Staphylococcus aureus, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Candida albicans* and *Aspergillus niger*.

2.1.6 REFERENCE ANTIMICROBIALS:

Ciprofloxacin (for bacteria), Ketoconazole (for fungi)

2.1.7 EQUIPMENT:

Rotary evaporator (Stuart®), Water bath (Stuart®), Autoclave (Gallenkamp, England), Incubator (Gallenkamp, England), Electronic weighing balance (M-METLAR M4 11R), Mechanical grinder, Maceration jars, Glass rods, Beakers (100 mL, 250 mL), Measuring cylinders, Petri dishes, Test tubes and racks, Micropipettes, Cork borer (6 mm), Oven, Whatman No. 1 filter paper, Soap molds, pH meter, Thermometer, Tissue paper and foil

2.2 METHODOLOGY

2.2.1 COLLECTION AND EXTRACTION OF THE PLANT MATERIALS

Fresh and mature *Cymbopogon citratus* (*Cymbopogon citratus*) were collected from Obagie community in Ikpoba Okha Local Government Area of Edo State, Nigeria, during the morning hours of March 2025. The plant was authenticated by a taxonomist at the University of Benin, and a voucher specimen (UBHC2024001) was deposited for reference. The leaves were air-dried, pulverized using a mechanical grinder, and 500 g of the powdered sample was macerated in 2.5 L of analytical-grade n-hexane for seven days at room temperature with intermittent stirring. The mixture was filtered using muslin cloth and Whatman No. 1 filter paper, and the filtrate was concentrated using a rotary evaporator at 40°C under reduced pressure to obtain the crude extract. The percentage yield was calculated based on the weight of the extract relative to the initial plant material.

$$Percentage\ Yield=\left(\frac{Weight\ of\ Extract}{Weight\ of\ Powdered\ Sample}\right)\times 100$$

2.2.2 PHYTOCHEMICAL SCREENING

Qualitative phytochemical screening was conducted to identify major classes of bioactive compounds. Tests for alkaloids included Mayer's, Wagner's, Dragendorff's, and Hager's reagents. Flavonoids were detected using the alkaline reagent test, tannins via the gelatin test, and saponins using the froth test. Terpenoids were identified using the Salkowski test, steroids via the Liebermann-Burchard test, and glycosides using the Keller-Killani test. Phenolic compounds were detected using ferric chloride, carbohydrates via Molisch's test, and proteins using the Xanthoproteic test. All tests were performed in triplicate to ensure reliability.

Test for Alkaloids:

1. Mayer's Test: To 1 mL of extract, few drops of Mayer's reagent were added. Formation of a creamy precipitate indicated the presence of alkaloids
2. Wagner's Test: To 1 mL of extract, few drops of Wagner's reagent were added. Formation of a reddish-brown precipitate indicated the presence of alkaloids.
3. Dragendoff's Test: To 1 mL of extract, few drops of Dragendoff's reagent were added. Formation of an orange-red precipitate indicated the presence of alkaloids.
4. Hager's Test: To 1 mL of extract, few drops of Hager's reagent were added. Formation of a light yellowish precipitate indicated the presence of alkaloids

Test for Flavonoids:

1. Alkaline Reagent Test: To 1 mL of extract, few drops of 20% sodium hydroxide solution were added. Formation of yellow color that became colorless on addition of dilute acid indicated the presence of flavonoids.

Test for Tannins:

1. Gelatin Test: To 1 mL of extract, 1% gelatin solution containing 10% sodium chloride was added. Formulation of white precipitate indicated the presence of tannins.

Test for Saponins:

1. Froth Test: To 1 mL of extract, 3 mL of distilled water was added and shaken vigorously. Formation of persistent foam that lasted for 15 minutes indicated the presence of saponins.

Test for Terpenoids:

1. Salkowski Test: To 1 mL of extract, 1 mL of chloroform and 1 mL of concentrated sulfuric acid were added. Formation of reddish-brown color at the interface indicated the presence of terpenes.

Test for Steroids:

1. Liebermann-Buchard Test: To 1 mL of extract, 1 mL chloroform, 1 mL of acetic anhydride and 1mL of concentrated sulfuric acid were added. Formation of blue green color indicated the presence of steroids.

Test for Glycosides:

1. Keller-Killiani Test: To 1 mL of extract, 1 mL of glacial acetic acid containing one drop of ferric-chloride solution was added, followed by 1 mL of concentrated sulfuric acid. Formation of blue color in the acetic acid layer indicated the presence of cardiac glycosides.

Test for Phenolic compounds:

1. Ferric Chloride Test: To 1 mL of extract, 1 mL of distilled water, few drops of neutral 5% ferric chloride solution were added. Formation of dark green or blue color indicated the presence of phenolic compounds.

Test for Carbohydrates:

1. Molisch Test: To 1 mL of extract, 1 mL of 1% alcoholic naphthol with 1mL of concentrated sulfuric acid. Formation of a purple ring coloration at the interface indicated the presence of carbohydrate.

Test for Protein:

1. Xanthoproteic Test: To 1 mL of extract, 1 mL of concentrated nitric acid. Formation of a yellow precipitate indicated the presence of proteins

2.2.3 SOAP FORMULATION

Preparation of Soap Base

Soap was formulated using the cold process method. The quantity of sodium hydroxide required was calculated based on the saponification value of the oil. The sodium hydroxide solution was prepared by dissolving the measured NaOH pellets (160g) in distilled water (about three times the weight of NaOH, 500ml) and sufficient water was added to make up to the 1000 ml mark, while stirring gently. The solution was allowed to cool to about 40°C before use. Separately, the required quantity of palm oil was measured and heated mildly in a water bath to about 40°C to ensure it was in a completely liquid state.

When both the oil phase and NaOH solution reached the same temperature range, the NaOH solution was gradually added to the oil–extract mixture while stirring continuously. Saponification occurred as the mixture thickened, forming a uniform paste known as “trace.” At this stage, small quantities of sodium chloride (1–2%) were added to enhance hardness, followed by perfume and colorant to improve aesthetic quality. The mixture was stirred until it became homogeneous and then poured into clean molds, covered to prevent air bubbles, and left undisturbed for 24 – 48 hours to solidify. Once hardened, the soap was removed from the molds, cut into uniform bars, and allowed to cure in a well-ventilated area for three to four weeks. The curing process ensured complete saponification, evaporation of excess water, and stabilization of pH.

2.2.4 ANTIMICROBIAL ASSAY FOR CRUDE EXTRACT

Approximately 1 g of the crude extract was weighed into a porcelain dish and 2 ml of 20% Tween-80 diluent was incorporated to obtain a concentration of 500 mg/ml. The content was triturated to obtain a consistent homogenous liquid, and afterwards 0.4 ml and 0.8 ml respectively were pipetted from the 500mg/ml to give different concentrations of 200 mg/ml and 400 mg/ml respectively. The stock solutions were transferred into an already cleaned and sterilized sample tubes. The same procedure was also repeated for the balm formulation. An aseptic area was created, around which subsequent activities were carried out. Fresh agar plates were prepared, using Mueller Hinton Agar (MHA) as the growth medium for the bacteria isolates, and Sabouraud Dextrose Agar (SDA) as the growth medium for the fungi isolates. About 38 g of MHA was dissolved in 1000 ml of distilled water, sterilized in an autoclave, and allowed to cool. Afterwards, 30 ml of the prepared agar was poured into three petri dishes and was allowed to set. Also, 65 g of SDA was dissolved in about 1000 ml of distilled water, sterilized in an autoclave, and allowed to cool. Thereafter, 30 ml of the prepared agar was poured into two petri dishes and was allowed to set. With the aid of a sterile knife, a ditch (well) was created and the crude extract was added to each ditch created in the petri dishes. Each petri dish was labelled A, B and C for 100 mg/ml, 200 mg/ml and 400 mg/ml respectively and same was done for the petri dishes containing SDA. The bacteria *Pseudomonas aeruginosa* was obtained from its colony using a loop, and dissolved in a test tube containing 1 ml of distilled water. With the aid of a swab stick, a little quantity was taken from the test tube and used to streak a straight line on the surface of the ditched fresh agar medium (MHA) in each petri dish. The same was repeated with *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli,* and *Klebsiella pneumoniae*. Using a swab stick, fungal species *Candida albicans and Aspergillus niger* were directly obtained from their colony and respectively used to streak the surface of freshly prepared agar medium (SDA). The six plates were labelled using abbreviations peculiar to each organism: BS for *Bacillus subtilis*, EC for *Escherichia coli,* KB for *Klebsiella pneumoniae*, PA for *Pseudomonas aeruginosa*, SA for *Staphylococcus aureus*, AN for *Aspergillus niger*, and CA for *Candida albicans*.

The same method was carried out for the control drugs i.e Ciprofloxacin (4 mg) for bacteria and Ketoconazole (60 mg) for fungi using the ditched MHA and SDA petri dishes respectively. The control drugs were introduced respectively into each petri dish, about 0.2 ml of the drugs were introduced into the wells on the agar plates. Thereafter, the were inoculated with the same strains of pathogenic bacteria and fungi used previously for the crude extracts. The plates were incubated at 37 °C for 24 hours, after which the inhibitory zone diameter (IZD) around each well was measured with the aid of a metre rule, and the results recorded.

2.2.5 PROCEDURE FOR SOAP MICROBIAL ASSAY (DITCH PLATE METHOD).

To evaluate the antimicrobial activity of the *Cymbopogon citratus* extract soaps, ditch plate assays were conducted using two formulations: one containing 200 mg of extract per gram of soap and another with 400 mg/g, alongside a blank control soap. For the assay, appropriate amounts of each soap were weighed and diluted in Tween-80 to achieve final extract concentrations of 200 mg/ml and 400 mg/ml. Specifically, 1 g of the 200 mg/g soap was dissolved in 1 ml of Tween-80 to yield 200 mg/ml. For the 400 mg/g soap, and 1 g was to achieve the same concentrations. Each mixture was triturated to form a homogenous suspension and transferred into sterile tubes. An aseptic area was created, around which subsequent activities were carried out. Fresh agar plates were prepared, using Mueller Hinton Agar (MHA) as the growth medium for the bacteria isolates, and Sabourand Dextrose Agar (SDA) as the growth medium for the fungi isolates. About 38 g of MHA was dissolved in 1000 ml of distilled water, sterilized in an autoclave, and allowed to cool. Afterwards, 30 ml of the prepared agar was poured into two petri dishes and allowed to set. Also, 65 g of SDA was dissolved in about 1000 ml of distilled water, sterilized in an autoclave, and allowed to cool. Thereafter, 30 ml of the prepared agar was poured into two petri dishes and allowed to set. Using a sterile scalpel or spatula, a long, straight ditch (about 1 cm wide) in the center of the agar plate was cut carefully and removed from the agar plate. Afterwards, a sterile loop was used to create a lawn of a single organism on both half of the plate. The bacteria *Pseudomonas aeruginosa* was obtained from its colony using a loop, and dissolved in a test tube containing 1 ml of distilled water. With the aid of a swab stick, a little quantity was taken from the test tube and used to create a lawn on both side of the ditch on the surface of the fresh agar medium (MHA). The same was repeated with *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli*, and *Klebsiella pneumoniae*. Using a loop, fungal species *Candida albicans and Aspergillus niger* were directly obtained from their colony and respectively used to create a lawn on both side of the ditch on the surface of freshly prepared agar medium (SDA). The six plates were labelled using abbreviations peculiar to each organism: BS for *Bacillus subtilis*, EC for *Escherichia coli*, KB for *Klebsiella pneumonia*e, PA for *Pseudomonas aeruginosa*, SA for *Staphylococcus aureus*, AN for *Aspergillus niger*, and CA for *Candida albicans*. With the aid of a micropipette, about 0.2

ml of the samples labelled A, B and C to represent control, 200mg/mL, and 400mg/mL of the crude extract respectively were introduced into the ditch on the agar plates. Thereafter, the plates were incubated at 37°C for 24 hours, after which the inhibitory zone diameter (IZD) around each ditch was measured with the aid of a metre rule, and the results recorded.

CHAPTER 3

RESULTS

3.1 PERCENTAGE YIELD

A percentage yield of 1.30% was obtained after the extraction of 500 g of *Cymbopogon citratus* leaves, in 2.1 L of n-hexane extract to obtain a 6.5 g extract.

3.2 ORGANOLEPTIC PROPERTIES

Colour – Dark green

Smell – Characteristic

Nature – Semi solid

Texture – Smooth

3.3 pH RESULTS

Control – 10.01

200 mg/ml – 9.27

400 mg/ml – 9.04

3.4 PHYTOCHEMICAL SCREENING RESULTS

Table 3.4. Phytochemical Screening Results

Phytochemicals	Inference/Results
Alkaloids	+
Carbohydrates	+

Reducing sugars	-
Saponins	_
Steriods/triterpenes	_
Phenols	_
Tannins	_
Proteins	+
Deoxysugars	+

KEYS:

-: ABSENT

+: PRESENT

3.5 RESULTS OF ANTIMICROBIAL SCREENING

Table 3.5 Results of Antimicrobial Screening of Crude Extract

MICROORGANISMS	**N-HEXANE *Cymbopogon citratus***		**EXTRACT**	**CONTROL**	
Concentration	**100mg/ml**	**200mg/ml**	**400mg/ml**	**C (4mg/ml)**	**K (60mg/ml)**
Staphylococcus aureus	NZ	26mm	28mm	36mm	-
Escherichia coli	NZ	20mm	20mm	30mm	-
Bacillus subtilis	NZ	18mm	19mm	30mm	-
Pseudomonas aeruginosa	NZ	20mm	24mm	32mm	-
Klebsiella pnuemoniae	NZ	20mm	26mm	34mm	-
Candida albicans	NZ	NZ	NZ	-	25mm

Aspergillus niger	NZ	NZ	NZ	-	20mm

3.5.2 RESULTS OF ANTIMICROBIAL SCREENING OF FORMULATED SOAP

Table 3.6 Results of Antimicrobial Screening of Formulated Soap

MICROORGANISMS	**SOAP FORMULATION**		
Concentration	**Control**	**200mg/ml**	**400mg/ml**
Staphylococcus aureus	NZ	7.5mm	7.5mm
Escherichia coli	NZ	NZ	NZ
Bacillus subtilis	7.5mm	NZ	NZ
Pseudomonas aeruginosa	20mm	20mm	20mm
Klebsiella pnuemoniae	10mm	15mm	17.5mm
Candida albicans	7.5mm	NZ	NZ
Aspergillus niger	12.5mm	NZ	NZ
Penicillum spp	20mm	12.5mm	22.5mm
Fuscerium spp	NZ	NZ	NZ

KEY:

NZ – No Zone

C – Ciprofloxacin

K – Ketoconazole

mm – Millimeters

Figure 3.2: Antimicrobial assay results of 400mg/ml of crude plant extract.

Figure 3.3: Different concentrations of the soap and extract formulation.

CHAPTER 4

DISCUSSION

4.1 DISCUSSION FOR THE ASSAY OF CRUDE PLANT EXTRACT

At the concentration of 100 mg/ml, the N-hexane extract of *Cymbopogon citratus* showed no zone of inhibition**,** for *Staphylococcus aureus* indicating that *Staphylococcus aureus* was resistant to the extract at this lower dose. However, increasing the concentration to 200 mg/ml and 400 mg/ml produced clear zones of inhibition measuring 26 mm and 28 mm, respectively. This demonstrates that *S. aureus* becomes susceptible to the extract when used at higher concentrations.

When compared with the control antibiotic (36 mm), the extract showed good activity at 200 mg/ml and 400 mg/ml but remained less potent than the standard drug.

Against *Escherichia coli,* no inhibition was observed at 100 mg/ml**,** indicating complete resistance at this concentration. At 200 mg/ml and 400 mg/ml**,** however, the extract produced inhibition zones of 20 mm for both.

The control produced a markedly higher zone (30 mm), confirming that although the extract demonstrates activity at higher concentrations, it is less effective than the control. Thus, *E. coli* is susceptible to the extract only at medium and high concentrations.

Similarly, *Bacillus subtilis* showed no inhibition at 100 mg/ml, indicating resistance at low concentration. Zones of 18 mm and 19 mm were observed at 200 mg/ml and 400 mg/ml respectively, revealing increased susceptibility with concentration.

Again, the control drug exhibited a much larger zone (30 mm), meaning its antibacterial action was stronger than that of the extract**.** Therefore, *B. subtilis* shows moderate susceptibility at higher extract concentrations.

Pseudomonas aeruginosa exhibited no inhibition at 100 mg/ml, but became susceptible at 200 mg/ml and 400 mg/ml**,** with inhibition zones of 20 mm and 24 mm**,** respectively. The control recorded a zone of 32 mm, demonstrating far greater antibacterial potency. Thus, although the extract shows some effectiveness against *P. aeruginosa*, especially at 400 mg/ml, it remains inferior to the standard antibiotic.

Klebsiella pneumoniae showed activity no inhibitory at 100 mg/ml, but demonstrated clear inhibition at 200 mg/ml (20 mm**)** and 400 mg/ml (26 mm**)**.

The control produced an inhibition zone of 34 mm. This suggests that the extract has good antibacterial activity whilst remaining inferior to the control.

For both fungi, *Candida albicans* and *Aspergillus niger*, the N-hexane extract of *Cymbopogon citratus* produced no zones of inhibition at all tested concentrations (100 mg/ml, 200 mg/ml, and 400 mg/ml). This indicates complete lack of antifungal activity under the tested conditions. In contrast, the fungal control produced inhibition zones of 25 mm for *C. albicans* and 20 mm for *A. niger*, clearly confirming their susceptibility to the standard antifungal drug.

The absence of antifungal activity from the extract may be due to several factors, such as: the hydrophobic nature of N-hexane extract may not contain sufficient antifungal constituents, fungal cell walls contain chitin and glucan, which offer stronger structural protection than bacterial cell walls, fungi possess efflux pumps and detoxification enzymes that may inactivate plant compounds before reaching lethal concentrations. Thus, while the extract showed moderate antibacterial activity, it was ineffective against fungal pathogens under the tested conditions.

S. aureus showed the largest zones among your test organisms at 200–400 mg/ml. This suggests the active constituents in the N-hexane *Cymbopogon citratus* extract (likely lipophilic terpenoids such as citral, geraniol, etc.) effectively interact with targets in *S. aureus* (membrane lipids, surface proteins), causing rapid membrane perturbation or enzyme interference.

B. subtilis, although Gram-positive, was less affected (18–19 mm). Possible reasons: differences in membrane lipid composition or cell surface structures, production of enzymes that detoxify/modify lipophilic compounds, or a physiological state that reduces susceptibility (e.g., thicker cell wall crosslinking or spore-forming tendencies in some conditions).

The n-hexane *Cymbopogon citratus* extract showed clear concentration-dependent antibacterial activity: inactive at 100 mg/ml but inhibitory at 200–400 mg/ml. Differences in zone sizes reflect species-specific susceptibilities rather than a simple Gram-positive/Gram-negative divide. Factors likely responsible include differences in cell envelope composition, diffusion limits of hydrophobic extract components, presence of species-specific resistance mechanisms (capsule, efflux, enzymes), and the complex mixture of active constituents in the crude extract.

4.2 DISCUSSION FOR THE ASSAY OF SOAP AND EXTRACT FORMULATION

The control soap showed no inhibitory activity against *Staphylococcus aureus*. However, the formulated soap containing plant extract exhibited 7.5 mm zones at both 200 mg/ml and 400 mg/ml, indicating that the extract contributed some antimicrobial potency to the formulation. The similar inhibition zones at both concentrations suggest that the active compounds reached a maximum diffusion limit in the soap matrix, or the extract concentration in the soap was insufficient to produce a stronger dose-response effect. Overall, *S. aureus* shows low but definite susceptibility to the formulated soap.

Against *E. coli*, no zones of inhibition were observed for the control, 200 mg/ml, or 400 mg/ml soap. This indicates that *E. coli* is completely resistant to the soap base and the extract formulations at the tested concentrations. Its resistance could be attributed to its gram-negative outer membrane, which limits the penetration of the hydrophobic extract constituents embedded in the soap.

For *B. subtilis,* the control soap produced a 7.5 mm zone of inhibition, suggesting that the control possesses mild inhibitory activity against *Bacillus subtilis*. Interestingly, no inhibition zones were observed for the 200 mg/ml and 400 mg/ml formulations. This pattern indicates potential interference between the extract and the soap base, where the plant extract may have altered the pH of the formulation, bound to or interacted with the soap base excipients, or reduced diffusion of active compounds. This may have caused the formulated soap to lose activity that the base soap originally had.

Against *P. aeruginosa,* the control soap and both extract-containing formulations recorded 20 mm zones of inhibition. This shows that the soap base itself had strong inherent activity against *P. aeruginosa*. Also, the extract did not significantly enhance or reduce this activity. This may be due to physicochemical interactions that prevent extract constituents from affecting the organism beyond the soap's own antimicrobial action. It also indicates that *P. aeruginosa* is highly susceptible to the soap base, likely due to membrane disruption by surfactants.

For *K. pnuemoniae,* the control showed 10 mm inhibition, while the extract-containing soaps gave 15 mm (200 mg/ml) and 17.5 mm (400 mg/ml) zones. This clear dose-dependent increase indicates that, *Klebsiella pneumoniae* is susceptible to the extract components within the soap formulation, the antimicrobial activity is enhanced by increasing extract concentration and the extract and soap base may have acted synergistically. This organism showed one of the highest responses to the formulated soap.

For the fungal isolates, the control soap produced a 7.5 mm inhibition zone, but both extract formulations showed no antifungal activity. Also, for *Aspergillus niger,* the control soap base produced a 12.55 inhibition with no inhibition at any concentrations for the extract. This indicates that the extract may have neutralized **or** interacted with antifungal constituents of the base soap or reduced diffusion of active components. The fungal cell wall structure (rich in glucans and chitin) also provides strong protection, making extract effects less pronounced.

For *Penicillium spp.* the control soap yielded a strong 20 mm inhibition zone. The 200 mg/ml formulation gave a reduced zone (12.5 mm), indicating initial antagonism. The 400 mg/ml formulation, surprisingly, produced the highest inhibition (22.5 mm) of all fungal results. This unusual pattern suggests that at low concentration, extract components may interfere with soap activity and at higher concentration, the extract contributes additional fungitoxic effects, overcoming the earlier interference. *Penicillium spp*. may be particularly sensitive to certain lipophilic constituents at high concentrations present in the extract.

For, *Fusarium spp,* no activity was observed for neither the control nor both extract concentrations, meaning *Fusarium* is completely resistant to both the soap base and the extract formulation. This is common because *Fusarium* species possess a highly resistant chitin-rich cell wall, rapid enzymatic detoxification pathways and strong efflux mechanisms.

4.3 PHYTOCHEMICAL CONSTITUENTS OF THE EXTRACT

The extract was prepared using n-hexane, hence the constituents extracted are mainly non-polar, hydrophobic phytochemicals, especially terpenoids and lipophilic phenolic derivatives. These compounds are well documented for strong antibacterial activity.

1. Citral: Citral is the major bioactive compound in *Cymbopogon citratus* (*Cymbopogon citratus*) and is highly concentrated in non-polar extracts. It disrupts bacterial cell membranes, causes leakage of intracellular constituents, and interferes with enzyme function. It has known mechanisms of disruption of membrane integrity, inhibition of DNA and protein synthesis and interference with quorum sensing (Adeyemi *et al.*, 2019).
2. Geraniol: Geraniol is a monoterpenoid alcohol with strong antibacterial activity against both Gram-positive and Gram-negative bacteria. It is known for inhibition of ATP synthesis and disturbance of fatty acid metabolism. (Boukhatem and Setzer*, 2020)*
3. Limonene: Limonene is widely present in *Cymbopogon citratus* essential oil and is strongly extracted by non-polar solvents. It disrupts bacterial envelopes and interferes with nutrient transport as well as efflux pump inhibition. (Moraes *et al,* 2021)
4. β-Myrcene: Although less potent than citral, β-myrcene contributes to antibacterial synergy within *Cymbopogon citratus* extracts. It acts through potentiation of other terpenes ("multi-hit" effect and interference with membrane fluidity. (Elshafie *et al.,* 2017)
5. Long-Chain Fatty Acids and Esters: Hexane extracts often contain oleic acid, linoleic acid**,** palmitic acid**,** and other lipophilic esters that exhibit antibacterial activity. It acts by

disruption of microbial lipid bilayers, inhibition of enoyl-ACP reductase and interference with nutrient uptake. (Desbois and Smith. 2017*)*

6. Free Fatty Acids: Free fatty acids arise from the incomplete saponification or intentional super fatting and include: Lauric acid, Capric acid and Oleic acid. Lauric acid in particular has documented antibacterial effects of membrane disintegration, destabilization of cell wall proteins and inhibition of virulence enzyme systems. (Sun *et al. 2020).*
7. Alkalinity of Soap (High pH): Soaps are alkaline because they are produced by saponification, a chemical reaction between fats/oils (triglycerides e.g., palm oil, coconut oil**)** and a strong alkali (NaOH). During this reaction, the alkali breaks the triglycerides into soap molecules (fatty acid salts**)** and glycerol**.** Most handmade soaps have a pH between 9–11 and this is because the saponification process requires a strong base, and a small amount of this base remains in the final soap or continues to interact with water and fatty acids. This residual base or excess alkalinity is what gives soap its basic/alkaline nature which inhibits the growth of some microorganisms viz *Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa.* This high pH causes denaturation of bacterial enzymes, loss of membrane integrity and inhibition of nutrient uptake.

Saponification is a base-catalyzed hydrolysis of triglycerides in which a strong alkali, usually sodium hydroxide or potassium hydroxide, breaks the ester bonds linking fatty acids to glycerol. The hydroxide ion attacks the carbonyl carbon of each ester bond, forming an intermediate that collapses to produce glycerol and the sodium or potassium salts of fatty acids, which are the actual soap molecules. Because the reaction uses a strong base and produces alkaline fatty acid salts, the final soap product naturally has a high ph.

Triglyceride + 3 NaOH → Glycerol + 3 Sodium fatty acid salts (soap)

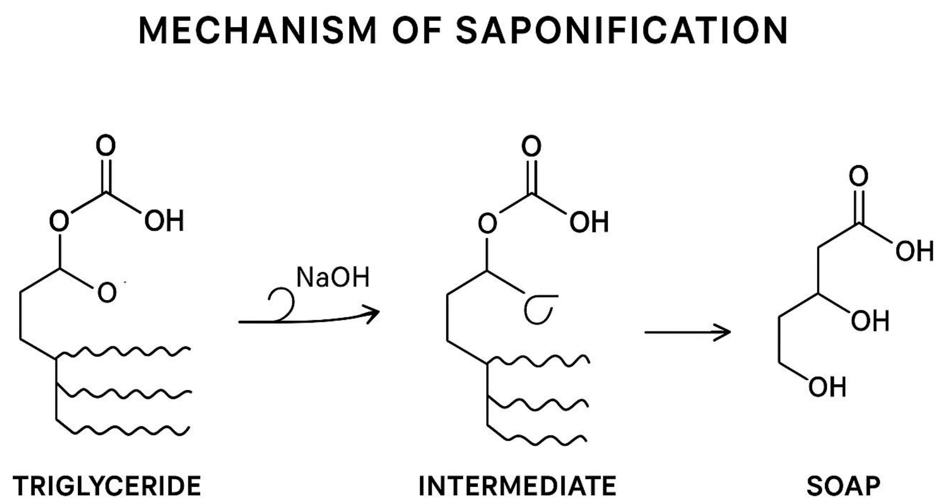

Figure 4.1: Mechanism of Saponification

8. Surfactants (Soap Molecules): Surfactants mechanically and chemically disrupt membranes by: solubilizing lipid bilayers, destabilizing cytoplasmic membranes and emulsifying surface proteins. This explains why the control soap inhibited *Pseudomonas aeruginosa*, *Penicillium spp.*, and others even without extract.

4.4 LIMITATIONS OF THE STUDY

Some limitations of this study should be acknowledged. Only one type of extract (n-hexane) was incorporated into the soap formulation, meaning the range of bioactive compounds studied was restricted to those soluble in the chosen extraction solvent. Using additional solvents or extraction techniques could reveal other active constituents that may strengthen the antimicrobial performance of the soap.

The antimicrobial tests were conducted solely under laboratory (in vitro) conditions. Although these tests provide useful preliminary information, they do not fully reflect how the soap would behave under real-world use on human skin. Future work should include evaluations of skin compatibility, irritation potential, and actual effectiveness during routine washing.

Another limitation is the lack of detailed assessment of how the soap's physicochemical properties—such as pH, moisture content, hardness, and storage stability—may influence its antimicrobial activity over time. Since these factors can affect both the release of active compounds and the longevity of the product, future studies should investigate them more thoroughly to support long-term product quality and reliability.

CHAPTER 5

CONCLUSION

5.1 CONCLUSION OF THE STUDY

The phytochemical screening of the n-hexane extract of *Cymbopogon citratus* revealed the presence of important bioactive compounds such as terpenoids, steroids, and fats/oils, which are known to contribute to antimicrobial activity. These constituents provided the chemical basis for evaluating the extract's potential in soap formulation. The crude extract showed concentration-dependent antibacterial effects, particularly against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*, although it exhibited no antifungal activity.

When incorporated into soap, the extract contributed mild to moderate antimicrobial activity, with notable improvement against *Klebsiella pneumoniae*. However, its activity was organism-specific and in some cases reduced by interactions within the soap matrix. Overall, the findings suggest that *Cymbopogon citratus* extract can enhance the antibacterial performance of natural soap, but the formulation requires further optimization to improve consistency, strength, and broader-spectrum effectiveness.

5.2 FURTHER RECOMMENDATIONS.

1. To improve the effectiveness of the soap, future studies should explore using different extraction solvents so that a broader range of active phytochemicals can be obtained.
2. To strengthen the antimicrobial results, further testing such as minimum inhibitory concentration and minimum bactericidal concentration determination should be carried out to provide clearer evidence of potency.

3. To understand how the soap will perform in real-life use, in vivo or simulated-use studies should be conducted to assess skin compatibility and actual antimicrobial action.

4. To enhance stability and product quality, detailed physicochemical evaluations such as pH monitoring, hardness, and storage stability should be included.

5. To increase the antimicrobial strength of the formulation, higher extract concentrations or combinations with other natural agents can be investigated.

6. To better understand how the extract behaves within the formulation, studies on release kinetics and interactions with soap ingredients should be carried out.

REFERENCES

Abera, A. (2020). Extraction and physicochemical analysis of essential oils in lemongrass leaves grown in Arbaminch, Ethiopia. International Journal of Engineering Research & Technology (IJERT), 9(10), 638-641.

Adeyemi, A., Okoh, O., and Okoh, A. (2019). Antimicrobial mechanisms of citral-rich *Cymbopogon citratus* extracts. *Journal of Applied Microbiology*, 126(4), 1013–1024.

Aibinu, I., Adenipekun, E., and Adelowotan, T. (2017). Evaluation of antibacterial activity of selected medicinal plants. *African Journal of Biomedical Research*, 20(2), 45–53.

Amenaghawon, N. A., Okhueleigbe, K. E., Ogbeide, S. E., & Okieimen, C. O. (2016). Modeling the Kinetics of Essential Oils Recovery From Lemon Grass (Cymbopogon Spp.) by Steam Distillation. Journal of Engineering Research, 18(2).

Angelini, P., Tirillini, B., and Venanzoni, R. (2020). Essential oils as natural antimicrobial agents. *Journal of Essential Oil Research*, 32(5), 421–430.

Anyanwu, C. F., and Okoye, F. C. (2018). Phytochemical components of *Cymbopogon citratus* and their antibacterial properties. *Journal of Pharmacognosy and Phytochemistry*, 7(6), 1201–1207.

Asghar, S., Khan, S., and Javed, S. (2021). Natural surfactant-based soaps and their antimicrobial potential. *International Journal of Cosmetic Science*, 43(4), 233–241.

Aumeeruddy-Elalfi, Z., Gurib-Fakim, A., and Mahomoodally, F. (2016). Essential oils and their therapeutic potential. *Journal of Herbal Medicine*, 6(1), 1–13.

Awolola, G. V., Adebayo, J. O., and Ogunyemi, I. O. (2018). Antibacterial effects of Nigerian medicinal plants. *South African Journal of Botany*, 119, 110–117.

Balogun, O. S., and Elekofehinti, O. O. (2020). *Cymbopogon citratus* essential oil: Chemistry and medicinal potentials. *Journal of Natural Remedies*, 20(3), 172–180.

Barros, F., Braga, T., and Nunes, A. (2018). Phytochemical evaluation of Cymbopogon extracts. *Journal of Medicinal Plants Research*, 12(3), 37–45.

Basile, A., Senatore, F., and Rigano, D. (2017). Monoterpenes as antimicrobial agents. *Phytochemistry Reviews*, 16(4), 747–760.

Bhardwaj, A. (2020). Phytochemical analysis, qualitative analysis secondary metabolites of Cymbopogan citratus (Lemon grass). Bulletin of Pure and Applied Sciences. Vol.39 1. P.24-27

Bhardwaj, K. (2020). Botanical and pharmacological overview of *Cymbopogon citratus*. *International Journal of Herbal Medicine*, 8(4), 45–50.

Boukhatem, M. N., and Setzer, W. N. (2020). Aromatic plant monoterpenes as antibacterial agents. *Molecules*, 25(8), 2023.

Branda, N. R., and Daly, J. J. (2019). Plant-derived terpenoids and microbial interactions. *Frontiers in Microbiology*, 10, 2121.

Cardoso, N., Franco, F., and Pinto, C. (2017). Chemical composition and antimicrobial activity of *Cymbopogon citratus* oils. *Industrial Crops and Products*, 108, 1–7.

Cech NB, Junio HA, Ackermann LW, Kavanaugh JS and Horswill AR. Quorum quenching and antimicrobial activity of Goldenseal (Hydrastis canadensis) against Methicillin-Resistant Staphylococcus aureus (MRSA). *Planta Medica,* 2012; 78(14):1556-1561.

Cech, N. B., Junio, H. A., and Horswill, A. R. (2015). Quorum-quenching by natural products. *Journal of Ethnopharmacology*, 170, 122–131.

Chavan, R. B., and Wagh, R. (2021). Production and applications of Cymbopogon oils. *International Journal of Agriculture*, 13(2), 141–150.

Choi, H., Lee, J., and Kim, Y. (2018). Membrane disruption by essential oil components. *Journal of Applied Microbiology*, 125(5), 1408–1418.

Coelho M, Rocha C, Cunha LM, Cardoso L, Alves L, Lima RC and Pintado M. (2016). Influence of harvesting factors on sensory attributes and phenolic and aroma compounds composition of Cymbopogon citratus leaves infusions, Food Research International. 89: 1029–1037.

Coelho, K. R., Silva, A. C., and Rodrigues, D. F. (2016). Traditional medicinal uses of Cymbopogon species. *Journal of Ethnopharmacology*, 193, 1–15.

Das, K., and Dash, S. (2018). Soap formulation: Chemistry and antimicrobial benefits. *Journal of Surfactants and Detergents*, 21(4), 543–552.

De Oliveira, F. F., and Silva, M. G. (2017). Toxicity and safety profile of *Cymbopogon citratus* extracts. *Toxicological Research*, 33(2), 97–106.

De Roode JC, Lefèvre T and Hunter MD. Self-medication in animals. Science, 2013; 340(6129):150-151.

Desbois, A. P., and Smith, V. J. (2017). Antibacterial free fatty acids: Mechanisms. *Clinical Microbiology Reviews*, 43(4), 547–569.

Devi, S., Sharma, B., and Kumari, P. (2019). Comparative phytochemical analysis of *Cymbopogon citratus* extracts. *Journal of Pharmacognosy Research*, 11(5), 498–504.

Diao, W., Hu, Q., and Zhang, H. (2020). Citral-induced membrane damage in microbes. *Food Control*, 115, 107295.

Elshafie, H. S., Mancini, E., and Camele, I. (2017). Synergy among monoterpenes. *Phytomedicine*, 37, 1–6.

Faleiro, M. L. (2018). The mode of antibacterial action of essential oils. *Current Pharmaceutical Design*, 24(10), 1152–1160.

Fokom R, Adamou S, Essono D, Ngwasiri DP, Eke P, Teugwa Mofor C, Tchoumbougnang F, Boyom Fekam F, Amvam Zollo PH, Nwaga D and Sharma AK. (2019) Growth, essential oil content, chemical composition and antioxidant properties of lemongrass as affected by harvest period and arbuscular mycorrhizal fungi in field conditions. Industrial Crops and Products. 138: 111477.

Fokom R, Adamou S, Essono D, Ngwasiri DP, Eke P, Teugwa Mofor C, Tchoumbougnang F, Boyom Fekam F, Amvam Zollo PH, Nwaga D and Sharma AK. (2019) Growth, essential oil content, chemical composition and antioxidant properties of lemongrass as affected by harvest period and arbuscular mycorrhizal fungi in field conditions. Industrial Crops and Products. 138: 111477.

Fokom, R., Ayonghe, N., and Nke, R. (2019). Ethnomedicinal uses of *Cymbopogon citratus*. *Journal of Medicinal Plants Research*, 13(4), 56–63.

Geetha Subramaniam, XinYing Yew, Lalita Ambigai Sivasamugham (2020). *Cymbopogon Citratus* against clinically important bacteria*, South African Journal of Chemical Engineering,* Volume 34, 2020, 26-30

George A. Antimicrobial resistance, trade, food safety and security. One Health. 2018;5:6–8.

George, A. (2017). Global threat of antimicrobial resistance. *World Journal of Pharmaceutical Sciences*, 5(4), 45–52.

Gupta, A., and Prasad, S. (2022). Phytochemical composition of non-polar plant extracts. *Journal of Natural Product Research*, 36(2), 112–120.

Hanaa, A. M., Sallam, Y. I., El-Leithy, A. S., & Aly, S. E. (2012). Lemongrass (Cymbopogon citratus) essential oil as affected by drying methods. Annals of Agricultural Sciences, 57(2), 113-116.

Hanna, R., Busula, A., and Kamau, L. (2012). Cymbopogon species distribution. *African Journal of Botany*, 98(3), 113–122.

Ismail, T., and Ahmad, S. (2018). Antibacterial properties of monoterpenes. *Journal of Herbal Pharmacotherapy*, 17(3), 251–260.

Jang, H., and Lee, J. (2020). Surfactant-mediated antibacterial activity. *Colloids and Surfaces B*, 185, 110591.

Joshi, B., and Dar, A. (2018). Medicinal importance of *Cymbopogon citratus*. *Journal of Pharmacognosy and Phytochemistry*, 7(5), 1807–1814.

Khameneh, B., Iranshahy, M., and Fazly-Bazzaz, B. (2019). Plant antimicrobials: Recent mechanisms. *Phytotherapy Research*, 33(10), 2521–2540.

Koch N., Islam N.F., Sonowal S., Prasad R., Sarma H. Environmental antibiotics and resistance genes as emerging contaminants: Methods of detection and bioremediation. Curr. Res. Microb. Sci. 2021;2:100027.

Koch, A., Wilson, R., and Godfrey, F. (2021). Horizontal gene transfer and AMR. *Nature Reviews Microbiology*, 19(1), 1–14.

Lulekal, E., Asres, K., and Mazengia, B. (2019). Acute toxicity of *Cymbopogon citratus* oil. *Toxicological Research*, 35(4), 347–354.

Madane SG. (2024). Review on benefits of lemongrass. World Journal of Pharmaceutical and Life Science. 10(2): 129-132.

Madane SG. (2024). Review on benefits of lemongrass. World Journal of Pharmaceutical and Life Science. 10(2): 129-132.

Madane, N. (2024). *Cymbopogon citratus* and its therapeutic roles. *Journal of Herbal Therapeutics*, 12(1), 22–34.

Mahavir Joshi and Gulam Mehdi Dar (2018) Cymbopogon citratus (Lemongrass) - Minireview. International journal of information and computing science, Volume 5, Issue 9, pp,220 226

Majewska E, Kozlowska M, Gruszczynska-Sekowska E, Kowalska D and Tarnowska K. (2019). Lemongrass (Cymbopogon citratus) essential oil: extraction, composition, bioactivity and uses for food preservation-a review. Polish Journal of Food and Nutrition Sciences. 69(4).

Majewska, M., Zych, E., and Bialon, M. (2019). Antimicrobial potential of *Cymbopogon citratus* oil. *Industrial Crops and Products*, 127, 102–109.

Messina, K., Brecker, L., and Thomas, J. (2021). High-alkaline hygiene products. *International Journal of Cosmetic Science*, 43(3), 352–360.

Mishra, P., and Dey, A. (2019). Antibacterial components of essential oils. *Microbial Pathogenesis*, 132, 1–5.

Moraes, T. M., Silva, P. L., and Almeida, R. (2021). Antimicrobial potential of limonene. *Frontiers in Microbiology*, 12, 643.

Mukarram, M., Choudhary, S., Khan, M. A., Poltronieri, P., Khan, M. M. A., Ali, J., ... & Shahid, M. (2021). Lemongrass essential oil components with antimicrobial activities. Antioxidants, 11(1), 20.

Mukarram, M., et al. (2021). Pharmacological profile of Cymbopogon. *Journal of Applied Biological Sciences*, 15(2), 44–55.

Ng, X. Y., and Wong, H. (2020). Natural antimicrobial formulations. *Journal of Natural Products*, 83(7), 2125–2134.

Nguyen, F., Starosta, A. L., and Wilson, D. N. (2016). Antibiotic resistance: A global overview. *Biochemical Journal*, 473(14), 95–112.

O'Neill J. Antimicrobial resistance: Tackling a crisis for the health and wealth of nations (The Review on Antimicrobial Resistance, London, 2016, United Kingdom) Rev. Antimicrob. Resist. 2014

O'Neill, J. (2016). Tackling drug-resistant infections globally. *Review on Antimicrobial Resistance Report*.

Obiang-Obounou, B., et al. (2019). Essential oils and bacterial inhibition. *Pharmaceutical Biology*, 57(1), 1–9.

Oladeji OS, Adelowo FE, Ay-odele DT and Odelade KA. (2019). Phytochemistry and pharmacological activities of Cymbopogon citratus: a review. Scientific African. 6: e00137.

Oladeji, O. S., Adelowo, F. E., and Oluwatosin, A. (2019). Phytochemicals of *Cymbopogon citratus*. *Journal of Medicinal Plants Studies*, 7(3), 23–29.

Panawala, L. (2017). Structural differences in Gram-positive and Gram-negative bacteria. *Microbiology Education Journal*, 15(2), 45–52.

Pramila, D., and Madan, R. (2018). Pharmacological activities of *Cymbopogon citratus*. *Journal of Pharmacognosy*, 10(1), 1–12.

Promila, P., & Madan, V. K. (2018). A Review on the phytochemistry and pharmacology of Cymbopogon citratus Chhattisgarh Journal of Science and Technology ISSN: 0973-7219, Volume 19, Issue 2, 2022 Stapf.(lemongrass). The Pharma Innovation Journal, 7(3), 300 304.

Quave CL. Antibiotics from nature: traditional medicine as a source of new solutions for combating antimicrobial resistance. AMR Control. 2016;98:102.

Quave, C. L. (2016). Plant natural products in drug discovery. *Nature Reviews Drug Discovery*, 15(6), 1–20.

Rai, M., and Kon, K. (2017). Antimicrobial potential of essential oils. *Microbiology Research*, 198, 1–10.

Rajesh, R., and Kumar, M. (2021). *Cymbopogon citratus* extract-based hygiene products. *Journal of Botanical Research*, 14(3), 204–212.

Saba, A., and Abdullahi, H. (2018). Traditional uses of aromatic grasses. *Nigerian Journal of Natural Products*, 9(2), 55–63.

Sharma RS, Kumar Y, Bhise RN, Choudhri HPS, Verma DK, Srivastava RK and Kumar S. (2022). Economics of Lemongrass Cultivation in Jharkhand State. Economic Affairs. 67(9): 01-06

Sharma S, Habib S, Sahu D and Gupta J. (2019). Chemical properties and therapeutic potential of citral, a monoterpene isolated from lemongrass. Medicinal Chemistry. 17:2–12.

Sharma, A., Devi, B., and Verma, S. (2017). Antifungal activity of *Cymbopogon citratus* oil. *Asian Journal of Biological Sciences*, 10(3), 1–8.

Shendurse, A. M., Sangwan, R. B., Kumar, A., Ramesh, V., Patel, A. C., Gopikrishna, G., & Roy, S. K. (2021). Phytochemical screening and antibacterial activity of lemongrass (Cymbopogon citratus) leaves essential oil. Journal of Pharmacognosy and Phytochemistry, 10(2), 445-449.

Shendurse, B., Patil, P., and Kale, R. (2021). Chemical composition of *Cymbopogon citratus* extracts. *Journal of Essential Oil Bearing Plants*, 24(2), 345–355.

Simões, C. M., et al. (2019). Plant-derived antimicrobials in topical formulations. *Phytotherapy Research*, 33(4), 835–845.

Sun, M., Li, P., and Yin, L. (2020). Fatty acids as natural antimicrobials. *Journal of Lipid Research*, 61(4), 562–572.

Upadhya, V., Sheth, N., and Nadkarni, S. (2021). Pharmacological evaluation of Cymbopogon extracts. *Journal of Ethnopharmacology*, 270, 113–118.

Upadhyay, P. K., Dixit, P., & Garabadu, D. (2021). Pharmacological Activities of Phytoconstituents and Essential oil obtained from Cymbopogon citratus Linn. Research Journal of Pharmacy and Technology, 14(9), 4555-4560.

Wagh, A. M., Jaiswal, S. G., & Bornare, D. T. (2021). A review: Extraction of essential oil from lemon grass as a preservative for animal products. Journal Phytochemistry, 10(3S), 26-31.

Wagh, R., Thombre, R., and Chavan, R. (2021). *Cymbopogon citratus* essential oil production. *International Journal of Agriculture*, 13(2), 141–150.

Wifek M, Saeed A, Rehman R and Nisar S. (2016). Lemongrass: a review on its botany, properties, applications, and active components. International Journal of Chemical and Biological Sciences. 9:79-84.

Wifek, S., Boukhris, M., and Aydi, S. (2016). Aromatherapeutic benefits of *Cymbopogon citratus*. *Journal of Aromatic Plant Research*, 4(1), 55–63.

Williams-Nguyen J., Sallach J.B., Bartelt-Hunt S., Boxall A.B., Durso L.M., McLain J.E., Singer R.S., Snow D.D., Zilles J.L. Antibiotics and Antibiotic Resistance in Agroecosystems: State of the Science. J. Environ. Qual. 2016;45:394–406.

Woo, J., Kim, Y., and Lee, S. (2023). Newly identified plant-derived antibacterial agents. *Frontiers in Pharmacology*, 14, 1123.