

**ANALYSIS OF ANTIMICROBIAL ACTIVITY OF THE VOLATILE OILS OF
Cymbopogon citratus AND *Ageratum conyzoides***



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BIOTECHNOLOGY,
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NOVEMBER, 2025

CERTIFICATION

This is to certify this is an original research work carried out by **MARLOW PHELM** in the Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmacy, University of Benin, in partial fulfillment of the requirements for the award of Doctor of Pharmacy (Pharm D) degree.

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DATE

DEDICATION

This project work is dedicated to God Almighty for the gift of life and strength throughout this project, and to my entire family and friends for standing by me through the tough times and the process.

ACKNOWLEDGEMENT

I express my deepest gratitude to God and to everyone who contributed to the success of this project. I appreciate the guidance and support of my supervisor, Dr Godfrey E. Umhenin, whose expertise and encouragement were invaluable. I also thank my project colleagues Efosa, Lesley, and, hephziba, my family, especially my parents, the HOD of pharmaceutical microbiology and biotechnology, Dr (Mrs.) Upe Francisca Babaiwa, and lecturers in the Faculty of Pharmacy for their unwavering support, encouragement, and academic mentorship. Your contributions have been instrumental in shaping me academically and personally.

ABSTRACT

Antimicrobial resistance is a major global health problem that requires exploration for new natural drug alternatives. This study examines the extraction process, chemical analysis, and assessment of the antimicrobial properties of the volatile oils from *Cymbopogon citratus* and *Ageratum conyzoides*. Whole volatile oils were extracted from the two plants by hydrodistillation. The chemical constituents of both are compared by gas chromatography-mass spectrometry (GC-MS). Five bacterial and two fungal strains were chosen for the antimicrobial studies. The cup-plate agar diffusion method was used to test microbial susceptibility to the volatile oils. The oil yield of the two plants was 1.4% for *C. citratus* and 0.0079% for *A. conyzoides*. GC-MS analysis indicated that citral is the main ingredient of *C. citratus*, while *A. conyzoides* was high in precocene. *C. citratus* oil exhibited potent broad-spectrum antibacterial activity against in vitro-tested pathogenic bacteria and fungi in a dose-dependent concentration. With a potent antifungal activity. On the other hand, the essential oil of *A. conyzoides* showed weak performance and hence inhibited only *P. aeruginosa* and *C. albicans* at the highest concentration tested (25% w/v) and exhibited a weak effect against the other clinical isolates used in the study.

C. citratus volatile oil demonstrated a very good antimicrobial activity and could serve as a good antimicrobial agent against bacterial and fungal infection.

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CHAPTER 1

1.0 INTRODUCTION

Essential oils are volatile, concentrated liquids that are extracted from different plant components and are usually recognized by their pleasant scent (Tingting Z et al., 2024). Their names are given based on the plant they are obtained from. Though they are usually recognized by their aroma, there also exist a variety of nonaromatic essential oils that have been identified. Essential oils are oily compounds and generally do not dissolve in water, known to have a variety of chemical and medical activities, and have been the culprit of the therapeutic activity of herbal plants, which have been used for centuries in human history, as well as being used for embalment in ancient Egypt, traditional Chinese medicine, and Indian Ayurvedic medicine (Tingting Z et al., 2024).

The biological activity of volatile oil has been extensively studied over the years, and its activity is irrefutable, ranging from antibacterial, antifungal, antiviral, larvicidal, antipyretic, antidiabetic, treatment of dyspepsia, etc., which has made essential oils an interesting aspect of study for treatment and management of disease conditions as well as for cosmetic purposes (Yamina Ben Miri, 2025).

Microorganisms (bacteria, fungi, and viruses) have been instrumental in the cause of disease worldwide; they have been responsible for the dangerous diseases that have plagued human existence, affected our children, and limited the ability of children to survive past the age of 5. Modern medicine, through evidence-based medical practices and substances, has in part eliminated this harsh reality through strong vaccination efforts and effective antibiotics, ensuring a majority of children now make it to adulthood with improved life expectancy (BMC Public Health, 2021).

The advent of antibiotics was a significant breakthrough for mankind, which drastically improved the quality of life of the world population. With the discovery of new antibiotics, we envisioned a time when microbial infection morbidity and fatality would be nothing more than a stain in our history as a species. The onset of resistance created a massive gap in this vision and made the need for multiple options for therapy much more relevant. Since the 2000s, the discovery of new novel antibiotics has been increasingly difficult, which is why the search is so much more important at this time in our lives (H. Brüssow, 2024).

Lemongrass, also known as *Cymbopogon citratus*, is a tropical plant from the grass family that's famous for its unique scent, similar to a lemon. It's known to contain essential oils and is used as a herbal remedy for indigestion, constipation, stomach aches, bloating, pain relief, and fever. It's believed to have antimicrobial properties and can reduce stress and anxiety (Sabita Dangol et al, 2023). This study explores the antimicrobial activity of lemongrass, asking: Does it actually have this activity? Are the essential oils responsible? How broad is the activity spectrum, and how does it compare to standard antimicrobial agents?

Billy goat weed, also known as *Ageratum conyzoides* L., is an annual aromatic herb that belongs to the Asteraceae family. Many countries, especially in tropical and subtropical areas, have a long history of using it in traditional medicine. In Hindi, it's commonly called Jangli pudina or appa grass, while in Portuguese, it's known as Mentrasto. In Spanish, it's called Mejorana (Rubal Chahal et al, 2021).

1.1 The Problem of Antimicrobial Resistance

The increase and widespread incidents of antimicrobial resistance (AMR) are a legitimate concern with increasing urgency. As more time goes by, there is currently a wide variety of antimicrobial drugs available for treatment, but with the increasing incidence of AMR, the

number of viable options for therapy has continued to shrink yearly. Though the promotion of proper antimicrobial stewardship is important, there is an obvious need for an increase in the current antimicrobial compounds.

According to a global modelling effort published in *The Lancet* (Murray et al., 2022), there are an estimated 4.95 million deaths associated with Bacterial AMR in 2019 alone. Of these deaths, 1.27 million were directly attributable to the resistant infection and not any underlying conditions in the patients. This is the reality of the burden of AMR, not only presented in the loss of lives but also in the cost of AMR on a global scale. There are estimated annual healthcare costs of \$4.6 billion for the treatment of AMR.

1.2 Reported Biological Activities of *Cymbopogon citratus*

Although lemongrass has a well-established antimicrobial profile, more recent studies have identified a wider range of pharmacological effects such as gastrointestinal, neurobehavioral, anti-inflammatory, antidiabetic, anticancer, and antioxidant effects (P. Upadhyay et al, 2021).

1.2.1 Antimicrobial Activity

The antimicrobial properties of *C. citratus* essential oil are effective against several clinically significant pathogens. With notable inhibitory effects against the skin and mucosal infections caused by bacteria *Staphylococcus aureus*, *Streptococcus mutans*, *Escherichia coli*, and *Candida albicans* (Carolina Chaves-Quirós et al. 2020). The oil's capacity to inhibit microbial growth was demonstrated by the study's use of agar diffusion assays, which showed distinct zones of inhibition. The traditional use of lemongrass oil to treat skin infections and its inclusion in cosmeceutical formulations are both supported by these findings. An additional study conducted by Hasan et al (2022) investigated the antibacterial properties of *C. citratus* volatile oil against the cause of acute gastrointestinal infections, *Shigella sonnei*. Strong inhibitory activity was demonstrated by the oil, Additionally, the study found that the oil functioned as a competitive inhibitor of the extracellular protease generated by *S. sonnei*,

indicating a dual mechanism of action that includes both enzymatic inhibition and microbial growth suppression. The therapeutic value of lemongrass oil is increased by this dual action, especially when it comes to infections that cause tissue damage due to proteases. The high citral content of citratus oil, which is a blend of the geranial and neral isomers, is primarily responsible for its properties. Cell death results from citrals' disruption of microbial cell membranes, interference with enzyme activity, and induction of oxidative stress. Furthermore, fatty acid esters may improve membrane permeability and make it easier for active substances to enter microbial cells. All of these processes work together to produce the broad-spectrum antimicrobial activity seen in different investigations.

1.2.2 Antioxidant and Anti-Inflammatory Properties

C. citratus is mostly recognized for its abundance of phenolic compounds and flavonoids, including luteolin, apigenin, kaempferol, and quercetin. Reactive oxygen species and oxidative stress, which are linked to aging and chronic illnesses, are neutralized by these substances. Oladeji & Associates. (2019) found that in DPPH and ABTS tests, lemongrass extracts demonstrated strong free radical scavenging activity, indicating that they may be useful in preventing oxidative damage and conditions linked to inflammation.

Apart from its anti-oxidant properties *C. citratus*, by inhibiting pro-inflammatory cytokines and enzymes like COX-2, has shown anti-inflammatory activity. Citral and geraniol, according to Mishra (2024), modulate inflammatory pathways, which makes lemongrass a promising treatment option for inflammatory bowel disease and arthritis.

1.2.3 Hypoglycemic and Antidiabetic Effects

Numerous investigations have examined *C. citratus* potential as an antidiabetic. Especially its capacity to control blood sugar levels. In animal models, the plant's aqueous extracts and essential oils have been demonstrated to lower fasting blood glucose and improve insulin sensitivity. Lemongrass may have a hypoglycemic effect by promoting the activity of

pancreatic β -cells and preventing the absorption of glucose from the intestines, according to Mishra (2024). These results lend credence to its long-standing application in the treatment of metabolic syndrome and diabetes.

1.2.4 Antimutagenic and Anticancer Activity

According to new information, because of its citral content, *C. citratus* has anticancer properties. In several cancer cell lines, including those from the breast, colon, and liver, citral has been demonstrated to cause apoptosis and stop proliferation. Lemongrass extracts demonstrated antimutagenic properties by shielding DNA from oxidative damage and decreasing tumor growth in experimental models according to (A. Kiełtyka-Dadasiewicz et al. 2024). Its potential as an adjuvant agent in cancer treatment is demonstrated by these findings.

1.2.5 Neurobehavior and Anxiety

The effects of *C. citratus* on the nervous system. Studies have also been conducted specifically on its function in mood and cognitive regulation. Because of its calming properties, essential oils are frequently used in aromatherapy. Lemongrass oil, when inhaled or taken orally, has been shown in studies to improve sleep quality and lessen anxiety-like behavior. Citrals' sedative and anxiolytic effects may be explained by their interactions with GABAergic receptors, according to Mishra (2024).

1.2.6 Digestive and Antidiarrheal Properties

C. citratus has been used historically to treat digestive disorders. In pharmacological studies, *citratus* has shown antispasmodic and antidiarrheal properties. Because its extracts decrease fluid secretion and inhibit intestinal motility, they are useful in treating gastrointestinal cramps and diarrhea. Lemongrass ethnomedicinal use was supported by (A. Kiełtyka-Dadasiewicz et al. 2024), confirming that it decreased castor oil-induced diarrhea in animal models.

C. Citratus has demonstrated potential in the treatment of parasitic infections, pneumonia, and malaria. Because of its bioactivity and volatility, its essential oil is also utilized in food and cosmetic products as a natural preservative and insect repellent. The plant's pharmacological diversity and therapeutic potential are highlighted by its adaptability in both conventional and alternative medicine.

1.3 Reported biological activities of *Ageratum conyzoides*

1.3.1 Antimicrobial Agent

Forty compounds were found in the volatile oil at a study in Nepal, with precocene I accounting for 61.7% and precocene II for 23.5% of the total composition (Satyal et al. 2018). While their antimicrobial potency varies according to chemotype and geographic origin these compounds are thought to play a role in the plant's pharmacological effects.

A variety of bacterial and fungal pathogens have been tested against *Ageratum conyzoides* essential oils. The effectiveness of oils derived from the plant's various parts against pathogens like *Bacillus subtilis*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and several *Candida* species has been a subject of significant research (Afolayan et al., 2021). Studies show that the essential oils exhibit considerable antimicrobial properties, with minimum inhibitory concentrations (MICs) often falling within a range that indicates sensitivity for most tested microbes. For instance, research by Djeussi et al. (2022) demonstrated that oils rich in precocene I and II were effective against a panel of pathogenic bacteria, corroborating earlier findings.

However, it is important to note that the efficacy can vary. The resistance of gram-negative bacteria like *Pseudomonas aeruginosa* has been observed, suggesting the oils may be more potent against gram-positive bacteria and certain yeasts.

Despite these encouraging results, other studies report limited antimicrobial activity. This disparity highlights how environmental factors, the maturity of the plant, and extraction techniques significantly influence the chemical makeup and bioactivity of the oils. Research has shown that variations in the relative concentrations of key compounds, such as precocene I and II, lead to different chemotypes of the plant. A study by Abdullahi et al. (2020) analyzed oil samples from different geographical regions and used hierarchical cluster analysis to reveal distinct chemotypes, which correlated with differing levels of antimicrobial efficacy. This variability helps explain why some studies report strong antimicrobial action while others find the activity to be modest.

1.3.2 Anti-inflammatory

It's one of the most researched activities of *A. Conyzoides* has an anti-inflammatory effect. Bioactive substances found in the plant that alter inflammatory pathways include coumarins, alkaloids, and flavonoids. Pro-inflammatory mediators like prostaglandins and cytokines are inhibited by these substances. Sivakrishnan and Kavitha (2017) claim that extracts of *A. Conyzoides* has shown strong anti-inflammatory properties in animal models, lowering tissue inflammation and edema. The plant also possesses analgesic qualities which have long been used to treat toothaches, headaches, and stomachaches. It is thought that both central and peripheral mechanisms involving the inhibition of pain receptors mediate the analgesic effect.

1.3.3 Antipyretic and antimalarial properties

A traditional treatment for fever and malaria symptoms is *A. conyzoides*. Its extracts have been found to have antispasmodic activity, which prevents *Plasmodium falciparum* from growing. In addition to its antimalarial action, it has an antipyretic effect that lowers fever and inflammation throughout the body. Laboratory studies have confirmed the leaves' effectiveness in reducing elevated body temperatures in experimental models and decoctions made from the

leaves are frequently used in folk medicine to treat feverish conditions (Sivakrishnan and Kavitha 2017).

1.3.4 Dermatology and Wound Healing Applications

Has the capacity to heal wounds *A. Conyzoides* has antiseptic, anti-inflammatory, and antioxidant qualities, which lend credence to this. The leaves' flavonoids and tannins encourage tissue repair and lessen microbial contamination. Crushed leaves are directly applied to wounds, burns, and ulcers in traditional medicine to hasten the healing process. Dr. Barbara Health (2024) emphasizes the application of *A. Conyzoides* is used in skin care with good results in treating fungal infections, rashes, and insect bites. The plant's dermatological advantages are attributed to its capacity to promote collagen synthesis and lessen oxidative stress.

1.3.5 Antioxidant activity

A key factor in the etiology of chronic illnesses is oxidative stress, in a number of tests *A. conyzoides* has demonstrated potent antioxidant activity. Because it contains flavonoids and phenolic compounds, it can scavenge free radicals and shield cellular constituents from oxidative damage. Rajput and colleagues. (2022) showed that the plants' methanolic extracts had a strong antioxidant capacity in the DPPH and FRAP tests, indicating that it may stop degenerative diseases and boost immunity.

1.3.6 Insecticidal and Mosquito Repellent Properties

In addition to its therapeutic benefits, *A. Conyzoides* has insect-repelling properties. The plant is called Billy goat weed because it releases a strong smell when crushed, resembling that of a male goat. Precocene I and II two volatile compounds that disrupt insect hormonal systems, are the source of this odor. According to Sivakrishnan and Kavitha (2017), the plant's essential oil has been utilized in integrated pest management strategies to control agricultural pests and repel mosquitoes.

1.3.7 Gastrointestinal and respiratory support

In traditional medicine, *A. conyzoides* has been used to treat bronchitis, asthma, and cough related to the respiratory system. Its expectorant qualities facilitate breathing by releasing mucus. Additionally, the leaves' decoctions are used to treat gastrointestinal disorders like dysentery, diarrhea, and colic. The plant's anti-inflammatory and spasmolytic properties, which relax smooth muscles and lessen intestinal inflammation, are probably what cause these effects (Dr. Health Barbara 2024).

1.4 Methods of Evaluation of Essential Oils

Physical tests can only evaluate the bulk oil, and thus chromatographic methods are necessary to decipher the intricate chemical composition of the oil. The most detailed and closest to being a "gold standard" method is Gas Chromatography (GC), which is usually combined with Mass Spectrometry (GC-MS). GC isolates the oil to its individual chemical components, and the mass spectrometer identifies and quantifies them according to their unique mass-to-charge ratio (Kaname Obara et al. 2024). This "chemical fingerprint" is what enables the exact determination of the main active components (for instance, menthol in peppermint oil) and, importantly, the identification of synthetic adulterants or the lack of naturally occurring compounds.

1.5 Botanical Description

1.5.1 *Cymbopogon citratus*

Lemongrass (globally known as *Cymbopogon citratus*) belongs to the Poaceae family. With around 780 genera and around 12,000 species, the Poaceae is the fifth-largest plant family, following the Asteraceae, Orchidaceae, Fabaceae, and Rubiaceae. the Poaceae family is well known as a source of cellulose, hemicellulose, and lignin, and is of economic and medical importance (Kamaruddin, Z. H. et al., 2021).

With several stiff stems and thin blade-like leaves that droop toward the tips, the grass grows in dense clumps. The blue-green leaves have a strong lemon scent when they are damaged, and they turn red in the autumn. Lemongrass rarely blooms in more northern latitudes, but when grown in the tropics, it produces large compound flowers on spikes. Lemon grass will grow for several years and can reach a height of 1.8 meters (6 feet), its economic lifespan is usually four years. Although a wild variety of lemongrass is unknown, it is most likely native to Malaysia or Sri Lanka and is also known as ginger grass or citronella grass. *Cymbopogon citratus* thrives in warm, humid tropical and subtropical regions with growth temperatures ranging from 10 to 33°C, with optimal growth at 25 to 30°C. They grow preferably on fertile well well-drained loam soil with a pH range of 5.0 to 8.4 and are propagated by dividing rhizome, cuttings, or seeds.



Fig 1. Image of *Cymbopogon citratus* taken at the Federal Government Girls College Road, Benin city, Nigeria.

1.5.2 *Ageratum conyzoides*

Outside of its native range, *Ageratum conyzoides* is naturalized in Asian temperate, Brazil, and all of Africa with the exception of arid regions. *Ageratum conyzoides* is widespread in disturbed sites and degraded areas, grows well in any garden soil and is frequently found close to habitation. Wetlands, coastal dunes, riparian zones (banks of watercourses), grasslands, cultivated land, and forests are all invaded. A significant weed of overgrazed pastures and plantation crops is *Conyzoides*. The annual herb *Ageratum conyzoides* is upright, branching, soft, and has fibrous shallow roots. It has a slight scent. It reaches a height of about 1 m. The leaves which are up to 7 and a half centimeters long and have an egg-shaped broad end at the base (ovate), are covered in fine white hairs. About 30 to 50 flowers are grouped in close terminal flower-heads and the flowers are purple, blue, pinkish, or white. They are less than 6 mm across. The fruits are tiny brown achenes with a single seed.

The Asteraceae plant family is unique in that its flowers are grouped in a head, a type of inflorescence that can draw pollinators for cross-pollination, which aids in rapid population growth. The fruit's unique organ, which functions as a parachute is another efficient mechanism for dispersing seeds over long distances. This weed invades easily because of its large seed-producing capacity which aids in rapid colonization. It produces many fruits (achene) with pappus ranging from 8000 to 100000 per plant. These are easily spread by animals, water, and the air. The seeds have a one-year survival period and are photoblastic (J. Hernandez et al. 2023).



Fig 2. Image of *Ageratum conyzoides* taken at the Federal Government Girls College Road, Benin city, Nigeria

1.5.3 Common Names

Cymbogonom citratus

India: Gavati Chaha, Gandhatrina, Sera.

Indonesia/Malaysia: Serai, Sereh

Philippines: Tanglad, Salai, Balioko

Thailand: Takhrai

Vietnam: Sả

China (Mandarin): Níng méng cǎo

Japan: Remon-gurasu

Korea: Remon-geuraseu

Nepal: Pirhe ghans

UK/General English: Lemongrass

France: Verveine des Indes

Germany: Zitronengras

Spain: Zacate de limón, Hierba de limón

Italy: Cimbopogone

African vernacular names west africa (igbo): Nri-ewu, (Yoruba): Imieshu yarnigbei.

Ageratum conyzoides

Brazil: Catinga de Bode, Erva de São João

Mexico: Mexican *Ageratum*

Caribbean: Rompesaraguelo

Central America: *Ageratum*, Billygoat Weed

Kenya: Gathenge

Uganda: Adwolo

Tanzania: Kimavi cha kuku

India: Visamustih (Sanskrit), Visadodi (Hindi), Uralgidda (Kannada), Pokasung (Odisha),
Bheda Jhar (Nepali), Khongjainapi (Manipuri)

Malaysia: Rumput tahi ayam

Philippines: Aru batu

1.5.4 Taxonomical Classification

Ageratum conyzoides

Kingdom: Plantae Subkingdom

Angiosperm Class: Eudicot

Order: Asteraceae

Family: Asteraceae

Genus: *Ageratum*

Species: *conyzoides*

Binomial name: *Ageratum conyzoides* Linn.

Cymbopogon Citratus

Kingdom: Plantae

Claude: Tracheophytes → Angiosperms → Monocots → Commelinids

Order: Poales

Family: Poaceae (Grass family)

Subfamily: Pancoideae

Genus: *Cymbopogon*

Species: *Cymbopogon citratus*

Binomial name: *Cymbopogon citratus* (DC.) Stapf

1.5.5 Botanical Description

Ageratum conyzoides

Ageratum conyzoides appears morphologically as an upright branching herb that can grow up to one meter tall. The leaves are ovate, opposite and have serrated edges, while the stem is soft, hairy, and frequently reddish. These leaves have glandular trichomes covering them which add to the distinctive scent of the plant. Tiny tubular flowers, usually white, blue, or purple, are grouped in dense corymbs to form the inflorescence. The fruit is a tiny achene with ribs that has a pappus to help spread by wind. Despite having high fecundity its seeds are photoblastic, meaning they need light to germinate and their viability rarely lasts longer than a year.

Roadsides fields and degraded areas are among the disturbed habitats where *Ageratum conyzoides* flourishes ecologically. It is considered an invasive species in many areas due to its capacity for quick colonization and fierce competition with native plants. It is also recognized to serve as a breeding ground for nematodes and crop diseases, endangering agricultural output.

Notwithstanding these difficulties, the plant has attracted a lot of scientific attention due to its adaptability and biochemical diversity. (Amarpreet Kaur et al. 2023).

1.5.6 *Cymbopogon citratus*

Cymbopogon citratus grows to a height of 1 to 5 meters and is distinguished by its tall tufted growth habit. The plant has long linear leaves that when crushed release a potent citrus aroma and it grows in dense clumps via a rhizomatous root system. The leaves have rough edges taper and are bluish-green. In cultivation flowering is uncommon and when it does occur the inflorescence consists of tiny spikelets that are characteristic of grasses (Yasmine F Madi et al. 2022). Lemongrass is a warm-climate plant that grows best in full sun and well-drained loamy soils. Since seed germination is unpredictable vegetative division is the main method of propagation.

1.6 Phytochemical Characteristics

Natural bioactive substances called phytochemicals are present in plants and help explain some of their therapeutic qualities. Because of its broad-spectrum biological activities and varied phytochemical composition, *Ageratum conyzoides* has attracted a lot of attention. Its chemical components have been the subject of numerous investigations, finding compounds with cytotoxic, antioxidant, antimicrobial, and anti-inflammatory qualities (P. Mucha et al, 2021).

1.7 Major Phytochemical Constituent

1.7.1 *Ageratum conyzoides*

Alkaloids.

The pharmacological effects of nitrogen-containing compounds are known as alkaloids. In its antimicrobial and analgesic qualities are attributed to *A. conyzoides*, which contain alkaloids like lycopsamine and echinatine (P. Mucha et al., 2021). Alkaloid content varies among plant parts according to quantitative analysis, with leaves having higher concentrations than flowers (Oyeyemi et al. in 2019).

Flavonoids.

Flavonoids are polyphenolic substances that have strong antioxidant properties. Flavonoids found in *A. conyzoides*, including luteolin, kaempferol, and quercetin, are what give it its anti-inflammatory and free radical-scavenging properties. The plant's highest flavonoid concentrations have been found in ethyl acetate extracts with values in leaves ranging from 5 to 80 mg QE/g (Kushwaha and Jain 2025).

Terpenoids.

An extensive class of organic compounds called terpenoids is made up of isoprene units. In Monoterpenes and sesquiterpenes, including precocene I and II have been identified. The plant is beneficial for cancer research and pest control because of these compounds' cytotoxic antifungal and insecticidal properties (P. Mucha et al. 2021).

Saponins.

Saponins are glycosides that have surfactant qualities and are well-known for their immunomodulatory and antimicrobial capabilities. The saponins found in *A. conyzoides*. Phytochemical screening has confirmed the presence of *conyzoides* with concentrations in leaves ranging from 0 to 13 mg/g (Oyeyemi et al. 2019).

Tannins.

Tannins are polyphenolic substances that give plants their antibacterial and astringent qualities. With recorded values of 3–86 mg TEA/g they are especially prevalent in leaf extracts (Kushwaha and Jain 2025).

Phenolic Compound

One of the main sources of antioxidant activity is phenol. Phenolic content overall in *A. conyzoides* has a strong potential for mitigating oxidative stress as evidenced by measurements of 8–46 mg TEA/g in leaves (Kushwaha and Jain 2025).

Chromene and Coumarin Compounds

Contains lesser-known but pharmacologically important compounds called coumarins and chromenes. Conyzoids. These compounds have further improved the plant's medicinal profile by exhibiting cytotoxic and antimicrobial qualities (Sharma & Sharma, 2021).

1.7.2 Cymbopogon Citratus

Lemongrass (*Cymbopogon citratus*), a member of the Poaceae family, finds widespread use in cosmetics, cooking, and traditional medicine. A diverse range of secondary metabolites, such as terpenoids, flavonoids, phenolic acids, alkaloids, and essential oils, is thought to contribute to its therapeutic potential. These substances make lemongrass an important tool in pharmacognosy and phytotherapy because they have antimicrobial, antioxidant, anti-inflammatory, and anxiolytic properties (Kiani et al. 2022).

Flavonoids

Lemongrass leaves and extracts contain flavonoids like luteolin kaempferol and quercetin. These substances have strong antioxidant properties and help lower inflammation and oxidative

stress. Chauhan (2024) noted that methanolic and ethanolic extracts had a notable flavonoid content that was associated with potent radical scavenging activity.

Phenolic Compounds

Among the many phenolic acids found in lemongrass are caffeic acid ferulic acid and chlorogenic acid. These substances support the antimicrobial and antioxidant properties of the substance. The extraction technique and solvent used affect the total phenolic content polar solvents produce higher concentrations (Kiani et al. in 2022.)

Saponins and Alkaloids

Extracts from lemongrass have been found to contain alkaloids and saponins albeit in smaller amounts. Saponins are recognized for their immunomodulatory and cholesterol lowering qualities while alkaloids may have sedative and analgesic effects (Shah et al., 2021).

Cardiac Glycosides and Tannins

Lemongrass contains tannins, which have antibacterial and astringent qualities. Cardiac glycosides may affect cardiovascular activity and should be further studied for potential therapeutic uses despite their trace levels (Chauhan et al, 2024).

1.8 GC-MS Analysis

This technology is a gold standard in analytical chemistry. It couples two very strong methods: one method for separating the compound and the other for identifying them, thus resulting in just one single, integrated system with extraordinary capabilities.

The introductory stage of the whole thing, Gas Chromatography (GC), is a sorting system of the highest order. The method is set in motion when a sample, either liquid or solid, is heated to vaporize it and the resulting gas is injected into the machine. This gaseous mixture is then

carried by an inert carrier gas (for example helium or nitrogen) into a long, narrow, spiral column which is also called the tube. The inside of this column is coated with a special material. The different compounds in the mixture, as they move through the column, attach themselves to the various parts of the coating to a different extent depending on their own chemical properties, such as boiling point and polarity. Consequently, there are some compounds which move very fast, while there are some which move very slowly, and this causes the separation of the mixture into its individual substances that leaves the column one at a time during a certain time interval.

As each compound leaves the GC column, it proceeds to the next stage: the mass spectrometer (MS). This unit works in identification. The pure compound, which is the one analyzed, is energetically hit by high-energy electrons inside the spectrometer. The strong blow is enough to forcibly remove electrons from the compounds molecules thus forming positively charged ions. At the same time, the molecules break into smaller, typical fragments, which are also charged. The bundle of ions-one being the intact parent ion and the other being the unique fragment pieces-are then speed up by electric field and separated by a mass analyzer according to each ions mass-to-charge (Hassan Khalifea et al., 2025).

The outcome of this operation is a graph called a mass spectrum, which indicates the relative abundance of each fragment at its particular mass. This spectrum is a very special chemical “fingerprint” for the exact compound. A match between this fingerprint and a huge digital library of standard spectra can be made by a computer within a very short time and with a very high degree of certainty the compound can be identified. The GC technique which gives the unique mass fingerprint of a compound are the two separate and reliable data points that the analysts get for confirmation. The effectiveness of GC-MS is due to this dual-system synergy which is capable of identifying substances even at extremely low levels (Hassan Khalifea et al., 2025).

The list of such a printout and trustworthy method is long and it affects numerous parts of human life. The use of GC-MS in forensics is mainly to identify illicit drugs, analyze arson accelerants, and detect trace evidence. Moreover, in environmental science, it helps in keeping water and air clean through the detection of pollutants such as pesticides and industrial byproducts. The food and beverage industry employ it for the purpose of safety, along with testing for contaminants and analyzing flavor and fragrance profiles, in addition, medicine and toxicology, it is a major instrument for drug testing, disease diagnosis via metabolic profiling, and pharmaceutical research. Briefly, GC-MS is a must have instrument that offers the precise and final chemical solutions demanded by contemporary science (Hassan Khalifea et al., 2025).

1.10 Rationale of the Study

1.10.1 Antimicrobial Activity Rationale

Antimicrobial resistance (AMR) is now the most critical threat to global public health. The World Health Organization (WHO) projects that AMR directly, an estimated 1.27 million deaths worldwide in 2019 alone, and in addition, nearly 5 million deaths through co-infection. The rapid development of resistance to standard antimicrobial drugs has been accelerated directly by the misuse and overuse of antimicrobials in humans, animals, and agriculture. This complicates the treatment of infectious diseases and threatens modern medical advances. AMR is responsible for limited effective treatment options, increasing health care costs, and risking food security, which demonstrates an unprecedented need for potentially alternative antimicrobial agents that bypass resistance mechanisms (Geneva Environment Network, 2024.)

1.11 Aims and Objectives of the Study:

To determine the chemical composition of *Cymbopogon citratus* and *Ageratum conyzoides* and compare the antimicrobial activity to standard antimicrobial agents

1.12 Specific Objectives

This study aims to achieve the following

- I. To extract volatile oils of *Ageratum conyzoides* and *Cymbopogon citratus*
- II. To investigate the chemical constituents present in the volatile oils of *Ageratum conyzoides* and *Cymbopogon citratus*.
- III. To evaluate the antimicrobial activities of the volatile oils of the leaves of *Ageratum conyzoides* and *Cymbopogon citratus* and compare them with standard antimicrobial agents

CHAPTER 2

2 MATERIALS AND METHODS

2.1 MATERIALS

2.1.1 Plant Materials

Ageratum conyzoides (whole plant, leaves, or aerial parts) and *Cymbopogon citratus* (whole plant, leaves, or aerial parts) were used as the plant materials for this study

2.1.2 Glassware

Beakers, conical flasks, petri dishes, measuring cylinder, round-bottom flask, micro pipette, universal bottles, and macerating jars.

2.1.3 Equipment

Weighing balance (H80, Mettler, Switzerland) refrigerator (500-Model, Haier Thermocool), Autoclave (Gallenkamp, UK), Microscope (Olympus) Bunsen burner, Aluminum foil paper, Wire gauze, Cotton swab, Marker, Clevenger apparatus (Glassco, India), Hot air oven (Gallenkamp, UK), Incubator (Gallenkamp, UK), Storage bottle.

2.1.4 Solvent for Extraction

Distilled water – Used for the hydro distillation extraction of volatile oil with the Clevenger apparatus

2.1.5 Culture Media for Antimicrobial Assay

Nutrient broth (MicroMaster, Maharashtra India)

Nutrient agar (MicroMaster, Maharashtra India)

Sabouraud dextrose agar (Fluka, Germany)

Sabouraud dextrose broth (Fluka, Germany)

2.1.6 Antimicrobial Agents Used

Standard antibiotic: Ciprofloxacin

Standard antifungal agent: Ketoconazole

Both were used as controls in this study

2.1.7 Microorganisms for Antimicrobial Assay

Gram-positive: *Staphylococcus aureus* and *Bacillus subtilis*

Gram-negative: *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella aerogenes*

Fungal: *Candida albicans* and *Aspergillus niger*

The microorganisms used in this study were all obtained from the department of Pharmaceutical Microbiology and Biotechnology in the University of Benin.

2.2 METHOD

2.2.1 Collection and identification of plant materials

Fresh samples of *Ageratum conyzoides* and *Cymbopogon citratus* were collected from Federal Government Girls' College Road and the Staff Quarters of the University of Benin respectively in Benin City, Edo State.

Both plants were validated and identified by Prof. Akinibosun from the Department of Plant Biology and Biotechnology (PBB) and herbarium numbers UBH-A344 and UBH-C451 for *Ageratum conyzoides* and *Cymbopogon citratus* were assigned respectively.

2.2.2 Preparation of the plant materials

The plants were washed properly under tap water to remove dirt from the leaves. After this, the leaves of the plants were sliced to increase the surface area of the plants for extraction. The flowers of *Ageratum conyzoides* were added to the sliced leaves and weighed before being transported into a large conical flask for extraction.

2.2.3 Extraction of volatile oils

Fresh leaves of *Ageratum conyzoides* and *Cymbopogon citratus* were subjected to hydro distillation using a Clevenger apparatus at a temperature of 100 - 150 °C. The Clevenger oil armrest was fixed with a condenser from which chilled water was dispersed to avert low volatiles from escaping. The oil acquired upon isolation from the drug was dried over anhydrous sodium sulphate, kept in sealed glass bottles, and stored in a fridge freezer (Olascuaga-Castillo et al., 2024).

2.2.4 Percentage yield of volatile oil

The weight of the oil after a single extraction was obtained by using a weighing balance. The process was repeated multiple times and the percentage yield of the plant was calculated by using the average weight of the leaves and the average weight of the oil using the following formula.

Percentage yield = (weight of volatile oil/Weight of plant material) x 100%

2.3 GC-MS Analysis

GC-MS analysis of the extract was performed using an Agilent 5977B GC/MSD system coupled with an Agilent 8860 auto-sampler, a Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with an Elite-5MS (5% diphenyl/95% dimethyl polysiloxane) fused a capillary column (30 × 0.25µm ID × 0.25 µm df).

For GC-MS detection, an electron ionization system was operated in electron impact mode with an ionization energy of 70 eV. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1 ml/min, and an injection volume of 1µl was employed (a split ratio of 10:1).

The injector temperature was maintained at 300 °C, and the ion-source temperature was 250 °C, and the oven temperature was programmed from 100 °C (isothermal for 0.5 min), with an increase of 20 °C/min to 280°C (2.5 min), Mass spectra were taken at 70 eV; a scanning interval of 0.5 s and fragments from 45 to 450 Da. The solvent delay was 0 to 3 min, and the total GC/MS running time was 21.33min.

2.4 Antimicrobial Assay

Clinical isolates of five bacteria comprising of two-gram positives (*Staphylococcus aureus* and *Bacillus subtilis*) and three gram negative (*Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella aerogenes*) were used for antibacterial assay and two fungal species were used for the antifungal assay. The organisms were obtained from the Department of Pharmaceutical Microbiology and Biotechnology Laboratory, Faculty of Pharmacy, University of Benin, Benin City, Nigeria. Standard conventional morphological approach and biochemical methods were used to confirm the purity of the culture. The bacterial cultures were maintained in nutrient agar, and the fungal culture in Sabouraud dextrose agar at 4°C

Preparation of inoculum

An overnight culture was used for the preparation of microbial suspension with a turbidity equivalent to that of 0.5 Mcfarland's standard

2.4.1 Preparation of Nutrient Medium

Nutrient agar and Sabouraud dextrose agar were prepared according to the manufacturer's guidelines and sterilized at 121°C for 15 minutes using an autoclave. All laboratory techniques and methods used were in accordance with the standard microbiological practice.

2.4.3 Inoculation of Bacterial Isolates

A sterile swab stick was immersed in a 4ml suspension of the test microorganisms. The swab was rotated around the inner tube wall to remove excess fluid before inoculation was carried

out. The plate was rotated 60 degrees after each streak and streaked again to ensure even distribution of the inoculum across the agar surface.

2.4.4 Preparation of Volatile Oil Solution

2g of the volatile oil was mixed with 2ml of 50% Tween 80 to create the solution used to create the volatile oil solution.

2.4.5 Agar well diffusion method

The media were prepared and sterilized at 121°C for 15 minutes. A total of 30ml Nutrient agar was seeded with bacterial culture and allowed to solidify and on each plate wells of 10mm in diameter were made. The open wells were filled with different concentrations of the extract ranging from 5% w/v to 25% w/v, and incubated at 37°C for 24 hrs. for antifungal assay, sabouraud agar was used in place of Nutrient agar and the medium incubated at 28°C for 2 days. All tests were carried out in triplicates. The inhibition zone diameter was measured and compared with ciprofloxacin and ketoconazole for antibacterial and antifungal assays respectively.

Statistical analysis: All the data were expressed as mean \pm SEM (Standard error of mean) and statistical significance was evaluated using the student t-test. P-value of <0.05 was considered statistically significant.

3.0 RESULTS

GC-MS Analysis

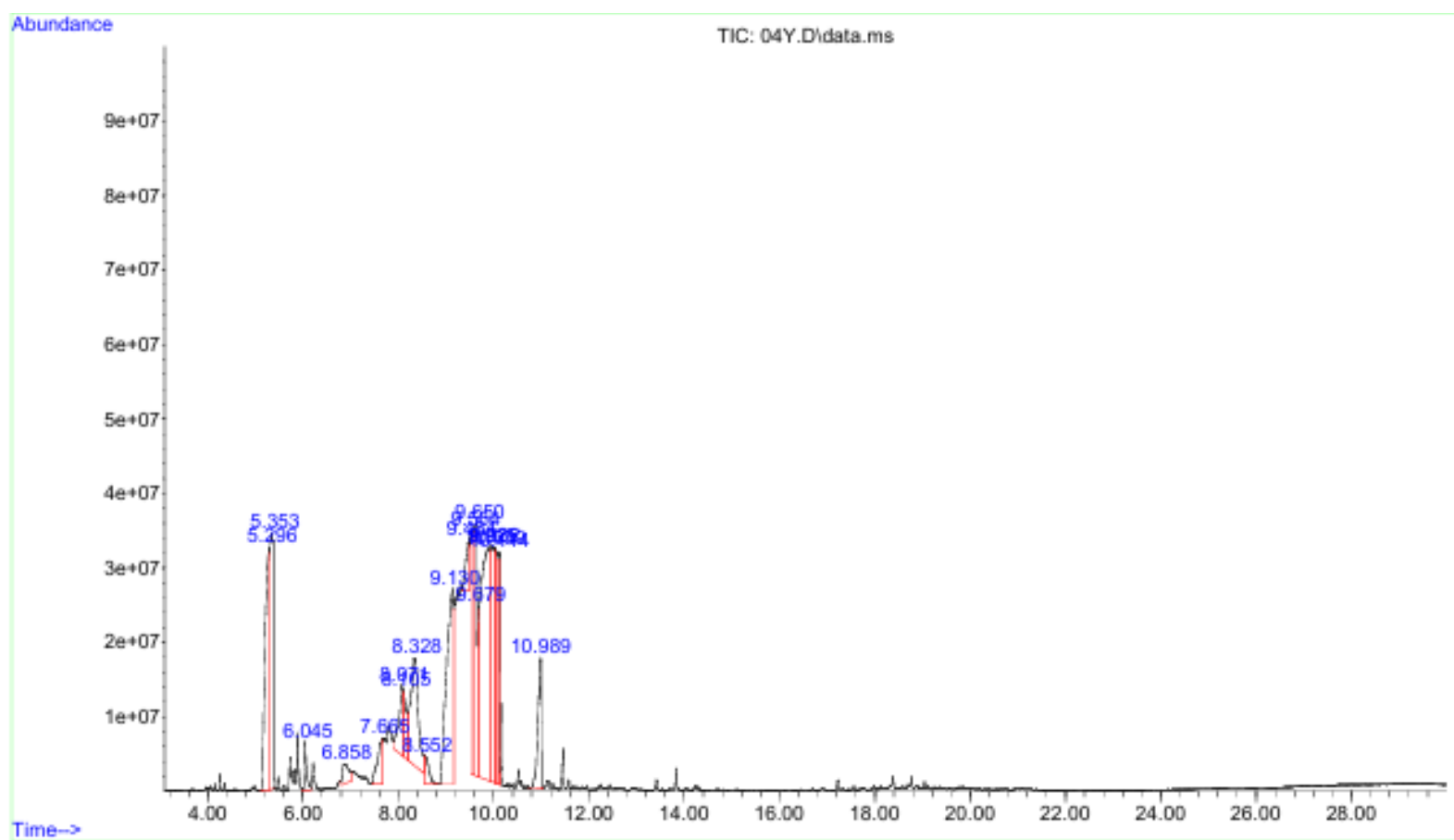


Fig 3. Total ion chromatogram (TIC) of *Cymbopogon citratus*

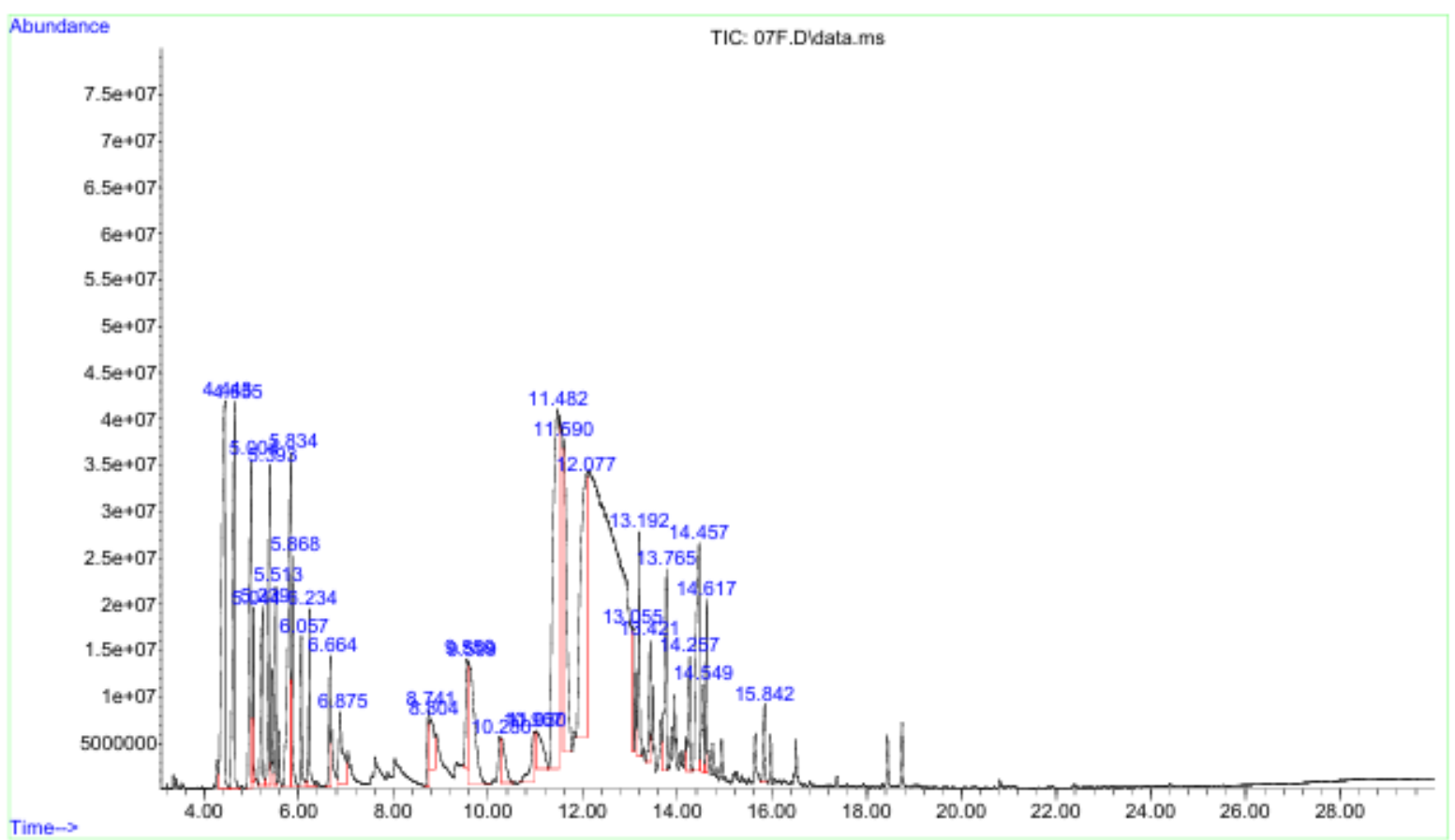


Fig 4. Total ion chromatogram (TIC) of *Ageratum conyzoides*

3.1 PERCENTAGE YIELD

Table 1. Percentage yield of volatile oils

Plant material	Average weight of plant material (g)	Average weight of volatile oil obtained (g)	Average percentage yield (%)
<i>Ageratum conyzoides</i>	400	0.0316	0.0079
<i>Cymbopogon citratus</i>	400	5.6	1.4

3.3 GC-MS Analysis Table

Table 2. GC-MS significant constituent of *Cymbopogon citratus*

Peak	Compound name	RT	Area%
1	beta-Myrcene	5.296	9.53
2	Isoneral	8.328	7.17
3	Estragole	8.552	1.09
4	Citral	9.925	20.07
5	Geranyl acetate	10.989	3.63
6	6-Octenal, 7-methyl-3- methylene-	7.665	2.03
7	Linalool	6.858	1.10

Table 3. GC-MS significant constituent of *Ageratum conyzoides*.

Peak	Compound name	RT	Area%
1	alpha-Pinene	4.443	8.34
2	Camphene	4.655	6.05
3	Bicyclo [3.1.0] hexane, 4-methylene-1-(1-methylethyl)	5.004	5.00
4	Cyclohexene, 1-methyl-4-(1-methylethylidene)	5.393	3.88
5	D-Limonene	5.834	6.25
6	Bornyl acetate	9.599	4.57
7	Caryophyllene	11.482	12.41
8	Precocene I	12.077	10.57
9	Precocene I	13.055	1.35
10	Caryophyllene oxide	13.765	2.41
11	2H-1-Benzopyran, 6,7-dimethoxy-2,2-dimethyl	14.457	4.58

Table 4. Antimicrobial activity of *Cymbopogon citratus*. IZD (inhibitory zone diameter)

organisms	Diameter of zone of inhibition (mm)							
	Volatile oil (%w/v)						Cp (10µg/ml)	Keto (10µg/ml)
	5	10	15	20	25	50%Twee n 80		
<i>Staphylococcus aureus</i>	0	0	0	12±0.02	17±0.01	0	40±0.65	ND
<i>Bacillus subtilis</i>	0	0	8±0.02	15±0.02	18±0.01	0	34±0.54	ND
<i>Escherichia coli</i>	7±0.02	8	9±0.01	11±0.01	13±0.05	0	32±0.72	ND
<i>Pseudomonas aeruginosa</i>	0	0	0	0	10±0.01	0	30±0.68	ND
<i>klebsiella aerogenes</i>	0	0	0	11±0.02	16±0.03	0	33±0.82	ND
<i>Candida albicans</i>	10±0.0 3	13	15±0.0 1	18±0.03	25±0.01	0	ND	34±0.47
<i>Aspergillus niger</i>	10±0.0 1	12	15±0.0 3	18±0.01	22±0.03	0	ND	33±0.64

Values expressed as mean±SEM, zero (0) indicates no activity, ND indicates Not determined,

Keto indicates Ketoconazole, Cp indicates Ciprofloxacin, P <0.05.

Table 5. Antimicrobial activity of *Ageratum conyzoides* IZD (inhibitory zone diameter)

organisms	Diameter of zone of inhibition (mm)							
	Volatile oil (%w/v)						Cp (10µg/ml)	Keto (10µg/ml)
	5	10	15	20	25	50% Tween 80		
<i>Staphylococcus aureus</i>	0	0	0	0	0	0	40±0.81	ND
<i>Bacillus subtilis</i>	0	0	0	0	0	0	34±0.43	ND
<i>Escherichia coli</i>	0	0	0	0	0	0	32±0.72	ND
<i>Pseudomonas aeruginosa</i>	0	0	0	0	11±0.01	0	30±0.26	ND
<i>klebsiella aerogenes</i>	0	0	0	0	0	0	33±0.71	ND
<i>Candida albicans</i>	0	0	0	0	15±0.02	0	ND	34±0.37
<i>Aspergillus niger</i>	0	0	0	0	0	0	ND	33±0.62

Values expressed as mean ± SEM, zero (0) indicates no activity, ND indicates Not

determined, Keto indicates Ketoconazole, Cp indicates Ciprofloxacin, P <0.05.

CHAPTER 4

4.1 DISCUSSION

The chemical and antimicrobial properties in the experiment were the main objective of the experiment, which ranged from the percentage yield and GC-MS identifying the different chemical constituents of the various plants. The analysis was carefully carried out to prevent any incident of contamination or unintended adulteration and a reduction in the accuracy of the methods. Both the *Ageratum conyzoides* and *Cymbopogon citratus* were weighed, and the percentage yield of the EO in the plants was found to be 0.079% and 1.4% respectively, which, on analysis and comparison to the available literature, seems to align with the standard reported percentage yields in literature from hydro distillation (Anggraeni et al, 2018).

The GC-MS analysis of *Ageratum conyzoides* and *Cymbopogon citratus* was performed and various compounds were identified for each plant, with 7 key components of significant area% identified in Tables 3 and 4

The largest compound present in the essential oil of *Cymbopogon citratus* is citral, an important compound found in many citrus-containing compounds. It is also responsible for the characteristic scent of the plant and the volatile oil, giving it its lemon essence, which is currently a strong identifier of the plant (Sabita Dangol et al, 2023).

Citral is a monoterpene aldehyde that is a major constituent of lemon grass at approximately 85%. It is a mixture of 2 isomers, namely Neral and geranial, and is also present in a variety of other plants like lemon, oranges, tomatoes, etc. It has been reported to have a number of biological activities, including antibacterial, antifungal, and anti-parasitic properties. Citral, in general, has been tested and verified as safe for use (Generally Recognized as Safe (GRAS)) and in the United States by the FDA. And has shown potential because of its antimicrobial activity for use as a preservative for different products, which would protect against spoilage.

The study on citral and its ability to inhibit the growth of not only bacterial but also fungal organisms is robust, and with its composition in the test plant, it suggests the plant essential oil would have some antimicrobial properties. Citral does seem to be present in literature in a similar composition to that of the GC-MS conducted. (Heriberto Torres-Moreno

Et al, 2023).

Myrcene is another monoterpene that is a major constituent of many plant species, including lemon grass (*Cymbopogon citratus*). It is commonly used as a food additive in flavouring used in food and beverage production. It was found in lemon grass in the GC-MS in two peaks and a cumulative area of 16.3% making it the second most abundant constituent of the lemon grass. Myrcene, over multiple studies have been reported to possess antioxidant, anti-inflammatory, and analgesic properties (Surendran et al, 2021).

Myrcene has been found to have antimicrobial activity against some bacterial species, like *Staphylococcus aureus* and *Escherichia coli*, where its action, in synergy with citral, was able to inhibit the growth of these bacterial species and would be a contributing factor to the activity of *Cymbopogon citratus* (Karolina Połec et al, 2020)

According to the results of the GC-MS examination, the essential oil (EO) of *Cymbopogon citratus* exhibited a chemical profile that was mainly composed of monoterpene aldehydes, and monocyclic compounds could account for the most potent and significant antimicrobial activities that were directly observed in the experiment. In particular, the major compounds that were determined to be the geometrical isomers of geranial and neral (citral), which, when combined, made up more than 75% of the entire oil composition. This discovery perfectly correlates with the chromatographic analyses of *C. citratus* EO from different parts of the world made recently by Cortes torres et al. (2023).

The antimicrobial activity evidenced in the research and tested against both Gram-positive and Gram-negative bacteria is evidence of a high concentration of citral. Citral, as an α , β -unsaturated aldehyde, is held responsible for the biocidal activity (Gao et al., 2020). The process includes the fast destabilization of the microbial cytoplasmic membrane and the subsequent destruction. This breakage results in the leakage of intercellular content, the AMF (proton motive force) as well as the total inhibition of highly significant metabolic pathways, resulting in cell death (Zedong Liao et al, 2023). Additional reports about citral's mechanism also point to the fact that it is multi-targeted, leading to lower ATP content and emission of reactive oxygen species (ROS) (Zedong Liao et al, 2023).

Moreover, the monoterpene myrcene and the alcohol geraniol (which make up 5.5% and 3.2%, respectively) are substances that may be promoting, along with the rest, the bioactivity of the plant. Initially, citral destroys the membrane, while these minor oxygenated compounds can help the EO to be more effective by opening the cell wall with higher permeability or targeting internal cell structures (Cortes-Torres et al., 2023) and (chaves-quiros et al 2020). Thus, aiding synergy is essential because the EO generally shows more considerable antimicrobial abilities than the separate isolation of the components, thus the so-called 'ensemble' action of the volatile matrix being confirmed.

In recent studies, GC-MS analysis has shown *Ageratum conyzoides* essential oil (EO) as being largely composed of chromene derivatives, mainly precocene I and precocene II, with a considerable contribution of sesquiterpenes like β -caryophyllene and caryophyllene oxide. The proportion of these substances might change depending on the time of the year, the part of the plant, and the method of extraction; however, precocene I and II are almost always the primary components. In most cases, they make up the oil for more than 50% (Priya M Karthigai et al, 2024).

The antimicrobial effect of *A. conyzoides* EO is mostly determined by its chemical components. Some studies have shown that the plant volatile oil has antibacterial and antifungal effects of a moderate to strong level (J. Kwembe et al, 2021, and Rubal Chahal et al., 2021). This statement is mostly explained by the three major components precocene I, precocene II, and β -caryophyllene (Quoc T., & Pham, L. 2020; J. Kwembe et al, 2021). The bacteria *Salmonella enteritidis* and fungi *Aspergillus ochraceus* and *Lasiodiplodia theobromae* have been inhibited, among others, by the use of the essential oil. The main mechanisms proposed are the removal of the microbial cell membrane and the interruption of metabolic pathways. Supported by both in vitro and in silico studies (K. Agarwal et al, 2022).

Seasonal and circadian changes can impact the amount and the components of the EO to a great extent, where in some cases, higher oil yields and elevated levels of the main bioactive compounds are mainly reported for the rainy season or certain hours of the day. Such fluctuation may also have an impact on the antimicrobial strength of the oil; therefore, it can be inferred that the best harvesting time is essential to obtaining the highest bioactivity (Priya M Karthigai et al. 2024, Rosário et al. 2024).

α -pinene found in *Ageratum conyzoides* has been shown to possess anti-inflammatory properties due to its ability to suppress inflammatory pathways, like the NF- κ B and MAPK. (K. Rahimi et al 2023) which results in a lower production of pro-inflammatory cytokines and enzymes in the body. It reduced inflammation in models of formalin-induced pain, brain ischemia, and neurodegenerative diseases, amongst others (K. Rahimi et al, 2023).

It has been found to possess antioxidant properties by enhancing the activity of oxidative enzymes (SOD, glutathione peroxidase, catalase), which in turn reduces levels of oxidative stress markers (MDA, NO), protecting against oxidative damage in the neural tissue (K. Rahimi et al, 2023).

It has been shown to exhibit neuroprotective effects, as well as showing potential for pain relief in animal studies. It also exhibits a moderate antimicrobial property, although its highest activity is as an anti-inflammatory agent (Khan-Mohammadi-Khorrami et al, 2022).

Though alpha pinene may not be solely responsible for the antimicrobial activity of *Ageratum conyzoides*, it perhaps plays a more synergistic role and is worth exploring more for its anti-inflammatory properties (A. Elbouzidi et al, 2024).

Bornyl acetate is present in a significant quantity in *Ageratum conyzoides* according to the results obtained from the GC-MS in Table 2. Having a cumulative percentage of 8.68 across all peaks. Bornyl acetate has been reported to possess anti-inflammatory properties by its action on NF-kB and MAPK, causing inhibition, which in turn downregulates the pro-inflammatory cytokines and has reduced inflammation in models of arthritis, lung injury, and in vitro macrophage activation (Zhenkun Zhao et al. 2023).

It also possesses immunomodulatory effects as well as analgesic effects, providing pain relief in animal models.

Bornyl acetate also has antimicrobial properties, showing potential as a natural antimicrobial agent. Hence, bornyl acetate would contribute to the antimicrobial activity of *Ageratum conyzoides* seen in this experiment.

The results showed strong antimicrobial properties, which is expected when compared to similar studies on its activity. From the five bacterial samples tested, it was very strong against *E. coli*, with an inhibition of growth at the smallest concentration tested of 5% w/v of the plant, with an inhibitory zone of 7mm, which is really impressive and promising at that concentration.

The other bacterial cultures showed growth at this concentration, showing the essential oil had no effect to kill or inhibit the growth of those organisms at that concentration. This high susceptibility of *E. coli* does not seem to be related to the bacteria being a gram-negative

bacterium, as other gram-negative bacteria like *Pseudomonas aeruginosa* and *Klebsiella aerogenes* did not show the same level of susceptibility.

It was interesting to see that the only other bacteria to be inhibited at 15%w/v was a gram-positive bacterium, which indicated no trend or pattern in the inhibition of growth of the bacteria, indicating a strong gram-negative and gram-positive inhibition of bacterial growth without exception or noticeable weakness to either at this point.

At 20%w/v, there was a strong inhibition of the growth of all of the bacterial species except *Pseudomonas aeruginosa*, which proved resistant to the action of the volatile oil at this concentration, the zone of inhibition of *E. coli* was below that of *B. subtilis*, which had an inhibitory zone of 15mm with *E. coli* now having the lowest IZD of all of the non-resistant organisms at this stage

At the highest concentration, all of the bacterial species were inhibited by lemon grass volatile oil. The result showed an interesting paradox with *E. coli*; the volatile oil was quick to show noticeable inhibition, but the quick onset was met with slow progression of the inhibition. Though at the initial lower concentration, there wasn't a noticeable difference in inhibition between gram-positive and gram-negative bacteria, the higher concentrations seem to paint a different picture, with a small but more noticeable inhibition of gram-positive when compared to gram-negative organisms.

At the end of the *B. subtilis* experienced more inhibition than any other bacterial species tested. The Gram-positive bacterial species both had similar IZD, experiencing a higher inhibition. *Cymbopogon citratus* was shown to have activity against both gram-positive and gram-negative organisms which is in accordance with the available literature on the antimicrobial activity of the essential oil.

From the result in table 3. The *Cymbopogon citratus* showed inhibition across both fungal species of *Aspergillus niger* and *Candida albicans* where there was a significant inhibition of growth of fungal species at the lowest concentration of 5% w/v of the volatile oil. This result is significant and showcases the higher activity of *Cymbopogon citratus* against fungal than against bacterial species from the results.

From the result, the volatile oils have a slightly higher inhibitory zone for *Candida albicans* than for *Aspergillus*, which shows a higher potency of antimicrobial action for *Candida albicans*.

The minimum inhibitory concentration of the volatile oil from the experiment was shown to vary by bacterial species, with 5% w/v for *E. coli*, 15% w/v for *B. subtilis*, and 20% w/v for *S. aureus* and *K. aerogenes*, and 25% w/v for *P. aeruginosa*.

The MIC for the fungal species was determined to be 5% w/v, which is the lowest concentration tested in the experiment carried out

For both bacterial and fungal species, the volatile oil when compared to standard antibiotic Ciprofloxacin and standard antifungal agent ketoconazole showed a lower potency than the standards which was expected but for the fungal agents the inhibitory diameter was close to that of the standard hence showing potential for use as an alternative antifungal agent for the treatment of infections or for use as a preservative for many preparations and production of food which use is currently continuously investigated.

There doesn't seem to be much difference in the result with geographical changes as the results seem to match that of the previous studies already established for *Cymbopogon citratus*

For the extracted volatile oil of *Ageratum conyzoides*, the results was interesting and differ significantly from the available literature as the result showed no activity at a concentration of 25% w/v of 2g of the oil for all but one of the bacterial species and showing weak activity for

E. coli at this highest concentration with a IZD of 11mm with no inhibition to staphylococcus as showed in available literature and other bacterial agent as shown in the available studies that has been previously conducted, this change in activity could potentially be explained by the absence of prococene II which has shown to be an important component known to be responsible for the antimicrobial activity of *Ageratum conyzoides* but was absent in the sample from the GC-MS results available in table 4.

For the fungal species, only *Candida albican* showed any level of susceptibility to the volatile oil, with no growth from the *Aspergillus* species. From Table 4. The volatile oil only inhibited the activity of *Candida albicans* at the highest concentration of 25%w/v of the oil, with an inhibitory diameter of 15mm, which shows it has limited activity against fungal agents and a lower spectrum of activity.

When compared to the standard anti-bacterial agent, ciprofloxacin, and the standard antifungal ketoconazole, the standard showed a superior activity and inhibition of bacterial and fungal growth, which indicates the potential for use as an alternative antibacterial and antifungal agent is very limited.

The minimum inhibitory concentration for this volatile oil can therefore only be determined for two organisms, one bacterial and one fungal organism, of which the MIC for *E. coli* and *C. albican* were both 25% w/v

The differences in the results obtained and other previous studies could stem from geographical and vegetative differences of the plant from region to region as similar plants show different composition based on location and timing of harvesting and extraction of the plant.

CHAPTER 5

5.1 CONCLUSION

The results of the experiment confirm the presence of antimicrobial activity in the volatile oils of *Cymbopogon citratus* and *Ageratum conyzoides* against different antibacterial agents. The percentage yield of *Cymbopogon citratus* and *Ageratum conyzoides* were 0.0079% and 1.4% respectively. The GC-MS carried out showed the presence of prococene I in *Ageratum conyzoides* as well as other constituents which are known to possess antimicrobial activity and are most likely responsible for the antibacterial and antifungal activity of *Ageratum conyzoides* observed in the experiment.

The GC-MS of *Cymbopogon citratus* showed high levels of citral, which is the chief compound responsible for its antimicrobial activity, as well as myrcene and other compounds that act synergistically with citral to increase its antimicrobial action.

The antibacterial test for *Ageratum conyzoides* showed moderate activity against only *Pseudomonas aeruginosa* among the five bacterial species. In contrast, *Cymbopogon citratus* performed better, showing moderate to strong activity against all five.

The *Ageratum conyzoides* showed moderate activity against *C. albicans* but no activity against *Aspergillus niger*, while the *Cymbopogon citratus* showed strong activity against both fungal agents.

These findings support the traditional uses and reported activity of both plants across multiple traditions for the treatment of infections of both gram-positive and gram-negative bacteria and for fungal species.

The geographical variation in the components of these plants, as well as their potential for use as an antimicrobial alternative to standard drugs, was shown, and the *Cymbopogon citratus*

seems to show higher levels of promise for this than the *Ageratum conyzoides*, from the results showing weak activity against five of the seven microbes tested.

Further research needs to be carried out to isolate these compounds and further examine their safety and efficacy as potential antimicrobial agents to combat the constantly growing incidence of resistance of many microorganisms to available antimicrobial medications (WHO, 2023).

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