`

ANTIMICROBIAL EVALUATION OF VOLATILE OILS OBTAINED FROM THE LEAVES OF *CITRUS SINENSIS* AND *EUPATORIUM CAPILLIFOLIUM*

BY

IZEVBUWA EFOSA FELIX

PHA1908533

SUPERVISED BY

DR GODFREY EDOKPIA UMHENIN

DEPARTMENT OF PHARMACEUTICAL MICROBIOLOGY AND BIOTECHNOLOGY,

FACULTY OF PHARMACY,

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PRESENTED TO THE DEPARTMENT OF PHARMACEUTICAL MICROBIOLOGY AND BIOTECHNOLOGY,

FACULTY OF PHARMACY,

UNIVERSITY OF BENIN

A RESEARCH WORK SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF THE DOCTOR OF PHARMACY DEGREE BY THE FACULTY OF PHARMACY UNIVERSITY OF BENIN

FEBRUARY, 2025.

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CERTIFICATION

This is to certify that this project research work was carried out by IZEVBUWA EFOSA FELIX, matriculation number: PHA1908533 of the department of Pharmacy, Faculty of Pharmacy, University of Benin, Benin city Edo state, Nigeria

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IZEVBUWA EFOSA FELIX DATE

(Student)

______________________ _________________________

DR. GODFREY EDOKPIA UMHENIN DATE

(Project supervisor)

_________________________ ___________________________

DR. (MRS) UPE FRANCISCA BABAIWA DATE

(Head of department)

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DEDICATION

This project work is dedicated to GOD Almighty for His infinite mercies, love, guidance and protection that saw me through the course of this programme and to my lovely parents MR. and MRS, FELIX IZEVBUWA, who through God's power helped in providing for me not just financially and morally but in all way round and my siblings Ogbemudia, Itohan, Oduwa and Omorotiomnwan for their support, prayers and patience. Not forgetting my Grandmother MRS. ESTHER EDO for her prayers and good wishes

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ACKNOWLEDGEMENT

Special thanks to GOD Almighty whose Mercy and Grace saw me through this project work. My profound gratitude goes to my loving parents Mr. and Mrs. Felix Izevbuwa for their wonderful support morally, spiritually and financially all through this journey. I am also grateful to my siblings, Ogbemudia, Itohan, Oduwa, Omoriotiomnwan. Also, to my friends Eghosa, Bright, Jackson, Obinna, Favour, Kelvin, Testimony and so many others for their support in diverse ways. I am thankful to my supervisor, Dr. Godfrey Edokpia Umhenin for his availability, support, discipline and academic advice. I also want to thank my HOD Dr. (Mrs) Upe Francisca Babaiwa

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Table of Contents

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LIST OF TABLES

`

LIST OF FIGURES

`

ABSTRACT

The increasing global challenge of antimicrobial resistance (AMR) underscores the urgent need for novel bioactive agents from natural sources. This study evaluated the antimicrobial potential and chemical composition of volatile oils obtained from *Citrus sinensis and Eupatorium capillifolium*, two plants widely used in traditional medicine. Fresh leaves of both species were subjected to hydro-distillation using clevenger apparatus, and the percentage yield was determined to be 0.3% for *Citrus sinensis* and 0.6% for *Eupatorium capillifolium*. Gas Chromatography (GC-MS) analysis revealed that Citrus *sinenesis* oil contain major components such as D-limonene (11%), linalool (7.94%), gamma-terpene (9.93), 2, 6-octadienal (7.03), while *Eupatorium capilifolium* revealed alpha-phellandrene (8.84%), 3-carene (8.67%), alpha-pinene (2.95%).

Antimicrobial activity was assessed using the cup-plate diffusion method against selected bacterial and fungal strains, including *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Klebsiella aerogenes, Candida albicans, and Aspergillus niger*. The volatile oil of *C. sinensis* demonstrated a high level of activity against the fungal species at all concentration (5 to 25%w/v), the zone of inhibition range from 12mm to 30mm for *Candida albicans* and 8mm to 20mm for *Aspergillus aerogenes*, and showed weak inhibitory activities against bacteria species with activities shown at the highest concentration of (25%w/v), with a zone of inhibition of 10mm for *P.aeruginosa*, 14mm for K.aerogenes, and 15mm for *B.subtilis*, with no inhibition against *S.aureus* and *E.coli*

E. capillifolium oil showed high level of inhibitory activity at all concentration (5 to 25%w/v) with range of 10mm to 20mm for *E. coli* and 11mm to 19mm for *P.aeruginosa*, with no activity seen against *K.aeregenes, S.aureus, B.subtilis, C.albicans* and *A.niger*.

The result obtained indicate that the volatile oil of each plants has pharmaceutical importance and can be utilize d in the treatment of some ailments.

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CHAPTER ONE

1.0 INTRODUCTION/LITRATURE REVIEW

Plants can secrete volatile oils sometimes referred to as essential oils or ethereal oil, from a plant’s glandular hairs, secretion ducts or oil cells all of which contribute to its distinct aroma. The food, cosmetic and fragrance industries all use these oils. Additionally, they have a wide range of pharmacological effects including analgesic, antimicrobial, carminative, expectorant, and antispasmodic effects. (Yash G Kulkarni, 2023). When they are fresh volatile oils are colorless liquids, some are solids that are either crystalline or amorphous. They are kept tightly in an amber-colored bottle in a cool dry place because they turn darker over time especially when exposed to direct sunlight. Water barely dissolves volatile oil but ether, alcohol and the majority of organic solvents readily dissolve it (Yash G Kulkarni., 2023). Volatile oils have a long history of use primarily in traditional medicine, cooking, religious ceremonies, and embalming (Bakkali et al, 2020). But according to recent studies the use of volatile oils has expanded across a number of industries as a multipurpose bioactive agent with promise in pharmacology and environmentally friendly packaging (De Oliveira et al.,2023; Wang et al., 2024). Because they have antiviral, antifungal and antibacterial properties, volatile oils are also used for antimicrobial purposes. These plants and their volatile oils must be investigated as new and alternative remedies due to the rapidly increasing antimicrobial agent resistance.

The complex chemical makeup of volatile oils determines their characteristics which change based on the plant species, extraction technique, storage conditions, and place of origin. (Rad Sharifi et al., 2022).

Terpenoids and terpenes: The most prevalent ingredients limonene, menthol, and thymol have antibacterial and anti-inflammatory properties (Kumar et al. 2019).

Phenylpropanoids like cinnamon, aldehyde and eugenol contribute to both antifungal and preservative properties *(Mahmoudi et al., 2020).*

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1.1 Problem of antimicrobial resistance

The efficacy of treatments for bacterial, viral, fungal and parasitic infections is threatened by antimicrobial resistance (AMR), a serious threat to global health. According to recent estimates the highest burden of bacterial antimicrobial resistance was found in sub-Saharan Africa and South Asia accounting for 1.27 million deaths and nearly 5 million deaths worldwide in 2019 *(Antimicrobial Resistance Collaborators 2022).*

Antimicrobial resistance has multiple contributing factors. The development and spread of resistant strains are accelerated by improper use of antibiotics in human and veterinary medicine, insufficient infection prevention and control (IPC), inadequate sanitation and environmental contamination (WHO 2024). The issue is made worse in many low- and middle-income nations by inadequate laboratory capacity and restricted access to diagnostics which leads to the use of broad-spectrum antibiotics and the postponement of targeted therapy (WHO 2025).

Antimicrobial resistance effects go beyond its effects on clinical outcomes. Hospital stays are prolonged, mortality rates are increased and healthcare expenses are increased by resistant infections. According to estimates if current trends continue there will be significant global losses with resource-constrained settings bearing a disproportionate amount of the impact (CDC 2022; WHO 2024).

Antimicrobial resistance is therefore a governance and biomedical problem. Global collaboration, fair funding, and a persistent political commitment are necessary to address it and maintain the effectiveness of antibiotics for coming generations.

1.2 Biological activity of essential oils in general

Essential oils (EOs) are volatile, aromatic extracts obtained from plants and composed of complex mixtures of terpenes, phenolics, and other phytochemicals (D. D. de Sousa et al., 2023). Recent research has shown that these compounds possess diverse biological activities, including antimicrobial, antioxidant, anti-inflammatory, and immunomodulatory properties (N. Mahboub et al., 2025). The specific activity of each

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essentials oils depends on its chemical composition, extraction process, and concentration (Liang-yung wei et al., 2022).

1.2.1 Antibacterial activity

Essential oils have been extensively documented to have antimicrobial properties especially against bacteria that are resistant to multiple drugs. Methicillin-resistant Staphylococcus aureus (MRSA) and other pathogens have been shown to be strongly inhibited by clove oil which is high in eugenol (Sathish Kumar et al., 2024). Similarly, oils extracted from garlic, eucalyptus and ginger also exhibit broad-spectrum antibacterial activity though their effectiveness varies depending on the type of bacteria and oil concentration (Sathish Kumar et al., 2024). Thymol, carvacrol and cinnamonaldehyde are among the essential oil components that was reviewed (Khwaza and Aderibigbe 2025). They observed that these compounds work by rupturing microbial membranes interfering with enzymes and obstructing quorum-sensing mechanisms.

1.2.2 Anti-inflammatory effects

It is becoming more widely acknowledged that essential oils can alter inflammatory pathways. Numerous plant-derived essential oils, with notable anti-inflammatory properties in vitro and in vivo were reported in a systematic review by (Qiang Zhoa et al., 2022). By inhibiting the NF-κB pathway (Nuclear Factor Kappa-light-chain-enhancer of activated B cells), Citrus peel oils have been shown to reduce pro-inflammatory cytokines like TNF-α and IL-6 (Jiyoon Yang et al., 2023). Additionally, by stimulating the NRF2 antioxidant response chamomile oil decreased inflammation mediated by macrophages (P. De Cicco et al., 2023).

1.2.3 Antioxidant activity

Because they contain flavonoids and phenolic compounds many essential oils have potent antioxidant qualities. For example, in DPPH and ABTS tests, citrus peel oils can efficiently scavenge free radicals, which makes them beneficial for lowering oxidative stress in biological and food systems *(Abid and Yahya 2023*). Rosemary essential oils, rosmarinic acid and carnosic acid content have been linked to both antioxidant and anti-inflammatory properties in vitro *(Khalil and Hassan 2024*).

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1.2.4 Other biological activities and challenges

Clove oil has shown promise as a natural food preservative and the essential oils also have wound-healing, analgesic, antiviral, antiparasitic and insecticidal qualities (Sathish Kumar et al.,2024). However, a number of issues still need to be resolved such as the potential for toxicity at high concentrations, lack of standardization, chemical variability among plant sources, and an incomplete understanding of mechanisms *(Qiang Zhoa et al., 2022)*.

1.3 Reported biological activities of *Citrus sinensis*

The sweet orange (*Citrus sinensis*) is the source of the essential oil. A natural product with significant commercial and medicinal value. Mostly made up of D-limonene which makes up more than 90% of the oil. This volatile mixture has a wide variety of biological activities that have been the focus of much recent scientific research (Hinda Hacib et al., 2023). Because of its strong antimicrobial, antioxidant, and psychoactive qualities, *Citrus sinensis* oil is becoming more and more recognized as a sustainable and natural substitute for synthetic agents in the food, cosmetic and pharmaceutical industries.

1.3.1 Antibacterial and antifungal efficacy

Among the most extensively documented biological activities of *Citrus sinensis*, the potent antimicrobial properties of the essential oil have been shown to be effective against a wide range of pathogenic and spoilage microorganisms such as bacteria and fungi (Akarca et al., 2021). studies have continuously proven that the oil, and mainly its foremost constituent D-limonene, possesses bactericidal and fungicidal (Delia Mirela et al., 2023).

Citrus sinensis oil regularly shows stronger inhibition to Gram-positive microorganism like *Staphylococcus aureus* as compared to Gram-negative microorganism which include *Escherichia coli and Pseudomonas aeruginosa* (Sado et al., 2022, Akarca et a.,l 2022).This difference is commonly attributed to the Gram-negative outer membrane, that's rich in lipopolysaccharides and provides a more hydrophilic barrier that restricts the passage of the oil's hydrophobic monoterpenes (Emily A. Lundstedt et al., 2020).

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1.3.2 Antioxidant properties

The high content of monoterpenes, particularly D-limonene, contributes substantially to the strong antioxidant activities of *Citrus sinensis* oil (Jiyoon Yang et al., 2025). Oxidation is a chemical process that produces free radicals, leading to cell harm, ageing, and numerous continual diseases. The essential oil has been extensively assessed through the use of numerous in vitro assays, together with the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, continually demonstrating a remarkable ability to scavenge unfastened radicals and decrease oxidative harm (Jiyoon Yang et al., 2025).

1.3.3 Neuroprotective and psychoactive agent

Aromatherapy with essential oils, including *Citrus sinensis* has been practiced since ancient times and remains popular for its holistic nature in health and symptoms relief (Pooja Agarwal et al., 2022).

Clinical and preclinical studies show that *citrus sinensis* oil and other oils can help reduce anxiety, depression, stress, and insomnia and may support cardiovascular and cognitive health (Pooja Agarwal et al., 2022).

The oils effects are linked to its volatile compounds (e.g., D-limonene), which can influence neurotransmitter systems and the limbic system, leading to anxiolytic and mood enhancing effects (Jieqiong Cui et al., 2022).

Essential oils are generally considered safe when used in controlled aromatherapy settings, with few or no side effects reported in most studies (Pooja Agarwal et al., 2022).

1.4 Reported biological activities of *Eupatorium capillifolium*

one of the most considerable and nicely-documented activity of *Eupatorium capillifolium* oil is its mighty insecticidal and repellent movement. This has drawn specific interest inside the development of natural alternatives to artificial insecticides for agricultural and public health functions (Tabanca et al., 2010).

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1.4.1 Adulticidal activity

The oil has demonstrated a linear dose-reaction in inflicting mortality in adult insects, significantly in opposition to the azalea lace bug (*Stephanitis pyrioides*). In some bioassays, the dog-fennel oil exhibited extra potency than the conventional insecticide malathion, suggesting its potential as a effective botanical insecticide (Tabanca et al., 2010)

1.4.2 Mosquito control

Research have shown that the essential oil possesses promising repellent activity in opposition to the yellow fever mosquito (*Aedes aegypti*) (Tabanca et al., 2010). Moreover, the essential oil and crude extracts of the plant's leaves have shown great larvicidal efficacy in opposition to the primary insect larvae of *Aedes aegypti* and other order vectors like *Culex quinquefasciatus* (Tabanca et al., 2010; Barrios et al., 2017).

1.4.3 Antifungal activities

The presence of phenolic compounds and terpenoids inside the essential oil and other extracts of Eupatorium species shows an ability for antifungal activity (Carrillo-Hormaza et al., 2015)..

Whilst the essential oil has shown weak antifungal activities towards certain plant pathogens, including *Colletotrichum acutatum, C. fragariae, and C. gloeosporioides* in precise bioassays (Tabanca et al., 2010), the wider genus of Eupatorium is extensively identified for its antifungal activity towards various human and plant fungi, often attributed to sesquiterpene lactones (Carrillo-Hormaza et al., 2015). The search for new antifungal drugs is particularly relevant given the rising hassle of microbial resistance.

1.4.4 Anti-inflammatory activities

Even though many research on the anti-inflammatory activities of the *Eupatorium capillifolium* essential oil itself are less distinguished in comparison to the insecticidal research, different species within the Eupatorium genus are historically and scientifically cited for such activities (Tabanca et al., 2010). The presence of compounds known to cause

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anti-inflammatory actions, consisting of terpenes, implying a potential that warrants further devoted research for anti-inflammatory and antinociceptive (pain-relieving) outcomes.

1.5 Chemical composition of *Citrus sinensis*

Citrus sinensis (sweet orange) is one of the world's economically and nutritionally significant fruit crops, valued for both is juice and its abundance of beneficial chemical constituents. Latest research, especially since 2020, highlights sweet orange as a major source of essential nutrients and diverse phytochemicals, with a particular focus on the high concentration of bioactive compounds in by- products like the peel and leaves (Ogo Ogo et al., 2024).

1.5.1Terpenoids

The characteristic aroma of the sweet orange is due to the essential oil mostly located within the flavedo (outer peel) (V. Ferrer et al., 2022). This oil is overwhelmingly composed of monoterpenes, with D-limonene being the major constituent, regularly accounting for over 90% of the essential oil (V. Ferrer et al., 2022).

1.5.2 Limonoids

Limonoids, together with limonin, are highly oxygenated triterpene derivatives responsible for the bitterness in Citrus fruit and juice, specifically upon processing. they're observed at some stage in the fruit, with big amounts in the seeds and peel, and are associated with anti-cancer properties. (Yu – Sheng Shi et al., 202).

1.5.3 Oxygenated compounds

Oxygenated compounds, which are often alcohols, aldehydes, esters, or ketones derived from terpenes, are found in low concentrations than that of hydrocarbons but make a contribution disproportionately to the feature candy orange taste and aroma.

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1.5.4 Aldehydes

Key aromatic compounds frequently diagnosed consist of decanal and octanal, which make a contribution to the sparkling and citrusy nature.

1.5.5 Alcohols

Brilliant oxygenated monoterpenes consist of linalool and α-terpineol. while their chances are low (often less than 1–2%), they are critical to the taste profile and are also diagnosed for his or her own biological sports.

The exact chemical composition of citrus sinensis is not static and is subject to variation based on the several factors like

- Geographic region
- Extraction method
- Maturity of fruit

1.6 Chemical composition of *Eupatorium capillifolium*

The essential oil of *Eupatorium capillifolium* (dog-fennel), a member of the Asteraceae family, has been investigated for its diverse chemical composition, which supports its traditional use and emerging applications in pest control. Analysis of the volatile oils, generally via gas chromatography-mass spectrometry (GC-MS), although the exact profile can differ considerably based on geographic location, plant component analyzed, and developmental stage. Current studies keep to focus on the presence of key compounds which are ordinarily answerable for the oil's organic activity Numerous researches have constantly identified a number of compounds as the predominant components of *Eupatorium capillifolium* volatile oil. A chemical analysis, whose records is often mentioned and remains relevant for understanding the chemical nature of certain species, stated that the oil from aerial components become overwhelmingly composed of oxygenated monoterpenes and monoterpene hydrocarbons. The three most abundant compounds identified were thymol, methyl ether, (additionally called methyl thymol), 2,5-dimethoxy-p-cymene, and the acyclic monoterpene hydrocarbon (myrcene) (Tabanca et

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al., 2010). those 3 components often constitute over 70% of the full oil composition, establishing them as key markers for its chemical nature.

1.6.1Thymol Methyl Ether

An oxygenated monoterpene, this compound is a by-product of the phenolic compound thymol, regarded for its robust aromatic and organic properties. Its high concentration (often exceeding 35%) shows it's far a main contributor to the oil's features, smell, and bioactivity.

1.6.2 2,5-Dimethoxy-p-cymene

This aromatic ether is an oxygenated compound, contributing to the general complexity and synergy of the oil's activity.

1.6.3 Myrcene

An unusual monoterpene hydrocarbon in essential oils, myrcene is vital, though not as effective as a main constituent, but additionally as an precursor inside the biosynthesis of different terpenes.

1.7 Biological significance

The detailed chemical profile shows direct insight into the bioactivity of the essential oil. The excessive text of oxygenated monoterpenes and associated fragrant compounds, which include thymol, methyl ether and a couple of,2,5-dimethoxy-p-cymene, is frequently associated with the essential oil's confirmed activity, specifically its ability as a herbal insecticide and repellent. Research has particularly evaluated the insecticidal activity of *Eupatorium capillifolium* oil towards pests just like the yellow fever mosquito, *Aedes aegypti*, and the azalea lace trojan horse, *Stephanitis pyrioides*, suggesting that the most important compounds are the main active components (Tabanca et al., 2010).

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1.8 Taxonomy classification

Citrus sinensis

Kingdom: Plantae

Class: Angiosperms

Class: Eudicots

Class: Rosids

Order: Sapindales

Genus: Citrus

Species: Citrus sinensis (L.) Osbeck

Eupatorium capillifolium

Kingdom: Plantae

Class: Tracheophytes

Class: Angiosperms

Class: Eudicots

Class: Asterids

Orders: Asterales

Genus: Eupatorium

Species: Eupatorium capillifolium LAM.

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1.9 Botanical description

1.9.1 *Citrus sinensis*

Citrus sinensis trees are the most commonly grown trees worldwide growing in warm temperate semitropical and tropical climates. The tree is now found in the pacific and warm regions of the world despite its Asian origins. Although it typically reaches 7 to 5 meters this evergreen flowering tree can grow up to 15 meters with its compact crown and primarily spiny branches (Tahir Khurshid et al., 2024). The leaves are smooth dark green and 3–5 millimeters wide. They alternately have oval or elliptical toothed blades. Stem-winged petioles connect them. The leaves high oil content gives them a powerful citrus scent (M. Dutt et al., 2022).

1.9.2 *Eupatorium capillifolium*

Eupatorium capillifolium commonly referred to as dog fennel is a herbaceous perennial that grows somewhat weedy in North America. It typically grows on erect slender stems that reach heights of 3-6 meters and spread to a width of 3 centimeters. Every plant produces several tall stiff woody stems that are often reddish and upright. These stems are covered in feathery finely divided leaves that resemble threads and are pinnately divided into segments. There is an unpleasant smell to crushed leaves. Large leafy end clusters that resemble pyramids are made up of numerous tiny heads with three to five flowers. Flowers are in full bloom from September to November.

1.10 Method of evaluation of volatile oil

The evaluation of volatile oils is crucial for quality control, authentication, and understanding their chemical and sensory properties. Modern analytical techniques have greatly enhanced the accuracy, speed, and reliability of volatile oil analysis.

1.10.1 Extraction and sample preparation

The first step in volatile oil evaluation is extraction from plant material. Common methods include steam distillation, solvent extraction, and hydrodistillation. These methods

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minimize sample loss and are suitable for both qualitative and quantitative analysis (Letseka et al., 2022).

1.10.2 Analytical techniques

Gas chromatography (GC) and Gas Chromatography- Mass Spectrometry (GC-MS)

Gas chromatography, often coupled with mass spectrometry (GC-MS), is the gold standard for the separation, identification, and quantification of volatile oil componenets. GC-MS provides high sensitivy and specificity, allowing for the detection of even trace components

Spectroscopic methods

Fourier-transform infrared (FTIR) spectroscopy, especially when combined with chemometric analysis, offers a rapid, non-destructive approach for assessing oil purity and authenticity. FTIR can oils of different botanical origins and detect adulteration (Agatonovic-Kustrin et al., 2020)

1.11 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 vapourize 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.

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As each compound leaves the GC column, it proceeds to the next stage: the mass spectrometer (MS). This unit work 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).

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1.12 Rationale of the study

Antimicrobial resistance is now one of the most critical threats to global public health. The World Health Organization (WHO) reported that AMR caused, directly an estimated 1.27 million deaths worldwide in 2019 alone, and in addition nearly 5 million deaths through co-infections. The rapid development of resistance to standard antimicrobial drugs has been accelerated directly by the misuse and overuse of antibiotics in humans, animals, and agriculture, which 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 by pass resistance mechanisms.

Essential oils from medicinal plants such as Citrus sinensis and Eupatorium capillifolium can present an exciting, naturally occurring alternative. Essential oils from these plants exhibit serious antimicrobial activity against many microbes, such as bacteria and fungi, including antibiotic resistant strains. Eupatorium capillifolium, essential oil also known as dog fennel have been shown to demonstrate insecticidal and weak fungal activity.

Similarly, the leaves of Citrus sinensis possess high D-limonene oil content that has demonstrated strong inhibitory properties against pathogenic organisms.

1.13 Aim of the study

To determine the chemical composition of *Citrus sinensis and Eupatorium capillifolium and to compare the antimicrobial activities to standard antimicrobial agents*

1.14 Specific objective

I. To extract the volatile oils of *Citrus sinensis and Eupatorium capillifolium by hydrodistillation process using Clevenger apparatus*
II. To determine the bioactive compound *of Citrus sinensis and Eupatorium capillifolium* using GC-MS analysis
III. To evaluate the antimicrobial activity of *Citrus sinensis and Eupatorium capillifolium* and compare with standard antimicrobial agents

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Chapter 2

2.0 Materials and methods

2.1 Materials

2.1.1 Plant materials

Eupatorium capillifolium and *Citrus sinensis* (leaves) was used as the plant material for this study.

2.1.2Glassware

Beakers, Conical flask, Measuring cylinders, Pasteur pipette, Petri dishes, Universal bottles, Micro-pipettes.

2.1.3 Equipment

Clevenger apparatus (Glassco, India), Weighing balance (H80, Mettler, Switzerland), Autoclave (Gallenkamp, UK), Bunsen burner, Hot air oven (Gallenkamp, UK), Incubator (Gallenkamp, UK), Refrigerator (500-Model, Haier Thermocool), Condenser, Heating mantel, Distillation flask, Storage bottle

2.1.4 Solvents for extraction

Distilled water- Used in hydrodistilation extraction of volatile oils

2.1.5 Culture Media for Antimicrobial Assay

Nutrient agar and broth (MicroMaster, Maharashtra India) - General purpose medium for bacterial growth.

Sabouraud dextrose agar (Fluka, Germany) - Selective medium for fungal growth.

Sabouraud dextrose broth (Fluka, Germany) -Liquid form of sabouraud dextrose agar designed for the cultivation of fungi.

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2.1.6 Antbacterial Agents (Positive Controls) and Anti-fungal Agents

Standard antibiotics (Analytical grade Ciprofloxacin)- Used as positive control and Antifungal agent (Ketoconazole) were used as reference antimicrobial agents.

2.1.7 Microorganisms for antimicrobial analysis

Gram positive bacterial (*Staphylococcus aurues, Bacillus subtilis*)

Gram negative bacterial *(Escherichia coli, Pseudomonas aeruginosa*, *Klebsiella aeregones*)

Fungal strain (*Candida albicans, Aspergilus niger*) were the microorganisms used in the study and they were gotten from the Department of Pharmaceutical Microbiology and biotechnology of Faculty of Pharmacy, Benin city, Edo state.

2.2 Method

2.2.1 Collection and identification of plant materials:

The fresh sample of the plant *Citrus sinensis* and *Eupatorium capillifolium* was collected inside the environs of Faculty of art, University of Benin, Edo state, Nigeria and University of Benin Teaching Hospital. The fresh sample of the plant *Citrus sinensis* and *Eupatorium capillifolium* was correctly identified by Professor Henry Akinnibosun, a plant taxonomist from the Department of Plant Biology and Biotechnology, Faculty of Life Science, University of Benin, Benin city, with the voucher number UBH-C441 and UBH-E465

2.2.2 Preparation of plant material

The fresh leaves of *Citrus sinensis* and *Eupatorium capillifolium* was washed in water and cut into pieces to make room for large surface area. The leaves were weighed using a weighing balance.

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2.2.3 Extraction of volatile oil

Fresh leaves of *Citrus sinensis* and *Eupatorium capillifolium* were subjected to hydrodistillation with Clevenger under optimal operational conditions with a temperature of 100 – 150 degrees Celsius (Olascuaga-*Castillo et al., 2024*). four hundred grams (400g) of fresh leaves of Citrus sinensis and Eupatorium capillifolium was mixed 800ml of distilled water. The distillation process was performed for 3 hours, and the obtained essential oil was collected

Percentage yield = (weight of extract in grams/initial weight of plant materials) * 100

2.2.4 GC-MS analysis

GC-MS analysis of the extract was performed using an Agilent 5977B GC/MSD system coupled with Agilent 8860 auto-sampler, a Gas Chromatography interfaced to a Mass Spectrometer (GC-MS) equipped with an Elite-5MS (5% diphenyl/95% dimethylpolysiloxane) fused a capillary column (30*0.25micrometeer ID *0.25micrometer df). For GC-MS detection, an electron ionization system was operated in electron impact mode with an ionization energy of 70eV. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1 ml/min, and an injection volume of 1micrometer was employed (a split ratio of 10:1).

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

2.2.5 Antimicrobial assays

Clinical isolates of five bacterial comprising three Gram negative (Escherichia *coli, Pseudomonas aeruginosa,* and *Klebsiella aerogenes*) and two Gram positive bacteria (Bacillus *subtilis,* and *Staphylococcus aureus*) were used for antibacterial assay, and two fungi (*Candida albicans* and *Aspergillus niger*) were used for antifungal assay. The

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organisms were obtained from the department of Pharmaceutical Microbiology and Biotechnology Laboratory, Faculty of Pharmacy, University of Benin, Benin city, Nigeria. The purity of the culture was confirmed by conventional, cultural, morphological and biochemical methods prior to use. The microbial culture was maintained in Nutrient agar and Sabouraud dextrose agar for bacteria and fungi respectively at 4 degrees Celsius.

2.2.6 Preparation of nutrient medium

Nutrient agar, Nutrient broth and Sabouraud dextrose agar were prepared in accordance with the manufacturer's guidelines and sterilized at 121 degrees Celsius for 15 minutes using an autoclave. All laboratory techniques and procedure were adhered strictly to standard microbiological practices.

2.2.7 Bacteria and Fungi Suspension Preparation

Sterile media, including Nutrient agar, Sabouraud dextrose agar, and broth were prepared following standard microbiological procedures. A sterile inoculating loop was used to extract bacteria and fungi samples from pure subcultures aseptically. Each colony was suspended in 4ml of water, and vortex mixing was performed to ensure even distribution. The turbidity of bacterial suspensions was standardized using 0.5 McFarland's solution

2.2.8 Inoculation of Microbial Isolates

Sterile swab stick was immersed in a 10ml suspension of the test microorganisms, including *Escherichia coli, Staphylococcus aureus, Pseudomonas aureginosa, Bacillus subtilis, Klebsiella aerogenes, Candida albicans and Aspergillus niger*. The swab was carefully rotated against the inner tube wall to remove excess fluid before being used for inoculation. The plates were rotated at 60 degrees each after each streaking motion to ensure uniform distribution of the inoculums across the agar surface.

2.2.9 Cup plate method

30ml of the media was prepared and sterilized at 121 degrees Celsius for 15 minutes. Microbial suspension was spread across the agar surface to create a uniform lawn of growth. Sterile cork borers (10 mm) were used to create wells in the solidified agar. The base was sealed to prevent leakage. A sterile pipette was used to add a fixed volume of the volatile oil gotten from *Citrus sinensis* and *Eupatorium capillifolium* into the wells at different concentration (5%-25%w/v). A control well containing only the solvent (Tween

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80) was included. Ciprofloxacin and Ketoconazole were placed in separate wells as reference antibiotics and antifungal agents (serving as positive control).

The plate was incubated at 35–37-degree Celsius for 24 hours for bacteria and at 25-28 degree Celsius for 48-72 hours for fungi. The inhibition zones (clear areas where microbial growth was prevented) were measured using a caliper or ruler, with MIC (minimum inhibitory concentration) also determined.

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Chapter 3

3.0 Result

3.1 Table 1: Percentage yield of volatile oils

Plant material	Average weight of plant materials (g)	Average weight of volatile oil obtained (g)	Average percentage yield (%)
Citrus sinensis	400	1.2	0.3
Eupatorium capillifolium	400	2.4	0.6

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3.2 Antimicrobial evaluation

Table 2: Antimicrobial activity of Eupatorium volatile oil

Organism	Diameter of zones of inhibition (mm)							
	Volatile oil extract (%w/v)						Cp (10ug/ml)	Ket (10ug/ml)
	5	10	15	20	25	50% Tween 80		
E. coli	10±0.02	13±0.01	15±0.01	17±0.03	20±0.02	0	32±0.15	ND
P. aeruginosa	11±0.01	13±0.03	14±0.02	16±0.01	19±0.01	0	30±0.23	ND
K. aerogenes	0	0	0	0	0	0	33±0.32	ND
S. aureus	0	0	0	0	0	0	40±0.23	ND
B. subtillis	0	0	0	0	0	0	34±0.13	ND
C. albicans	0	0	0	0	0	0	ND	34±0.54
A. niger	0	0	0	0	0	0	ND	33±0.35

Values expressed as mean±SEM, zero (0) indicates no activity, ND indicates Not determined, Ket indicates Ketoconazole, Cp indicates Ciprofloxacin, $P < 0.05$.

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Table 3: Antimicrobial activity of citrus sinensis volatile oil

Organism	Diameter of zones of inhibition (mm)							
	Volatile oil extract (%w/v)						Cp (10ug/ml)	Ket (10ug/ml)
	5	10	15	20	25	50% Tween 80		
E. coli	0	0	0	0	0	0	32±0.23	ND
P. aeruginosa	0	0	0	0	10±0.01	0	30±0.12	ND
K. aerogenes	0	0	0	0	14±0.03	0	33±0.31	ND
S. aureus	0	0	0	0	0	0	40±0.26	ND
B. subtillis	0	0	0	0	15±0.01	0	34±0.34	ND
C. albicans	12±0.02	15±0.04	20±0.01	25±0.03	30±0.02	0	ND	34±0.16
A. niger	8±0.03	10±0.02	12±0.03	15±0.05	20±0.03	0	ND	33±0.24

Values expressed as mean±SEM, zero (0) indicates no activity, ND indicates Not determined, Ket indicates Ketoconazole, Cp indicates Ciprofloxacin, P <0.05.

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3.3 GC-MS Analysis

Table 4: GC-MS analysis of *Citrus sinensis*

Peak	Compound name	Retention time	Area%
1	Gamma-Terpinene	5.050	9.93
2	Beta-Myrcene	5.307	2.22
3	D-Limonene	5.954	11.00
4	Linalool	7.167	7.94
5	2, 6-Octadienal	9.153	7.03
6	Caryophyllene	11.447	1.74

6 most significant compounds found from the GC-MS

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Table 5: GC-MS analysis of *Eupatorium capillifolium*

Peak	Compound name	Retention time	Area%
1	alpha-Pinene	4.403	2.95
2	3-Carene	5.084	8.67
3	alpha-Phellandrene	5.570	14.70
4	Alpha-Phellandrene	5.633	8.84
5	o-Cymene	5.879	5.83
6	Benzoic acid, 4-(1-methylethyl)	8.975	18.83
7	Benzene, 1,4-dimethoxy-2-methyl	11.367	5.12
8	2, 3, 5-Trimethylanizole	12.179	10.00

8 most significant compounds identified from the GC-MS

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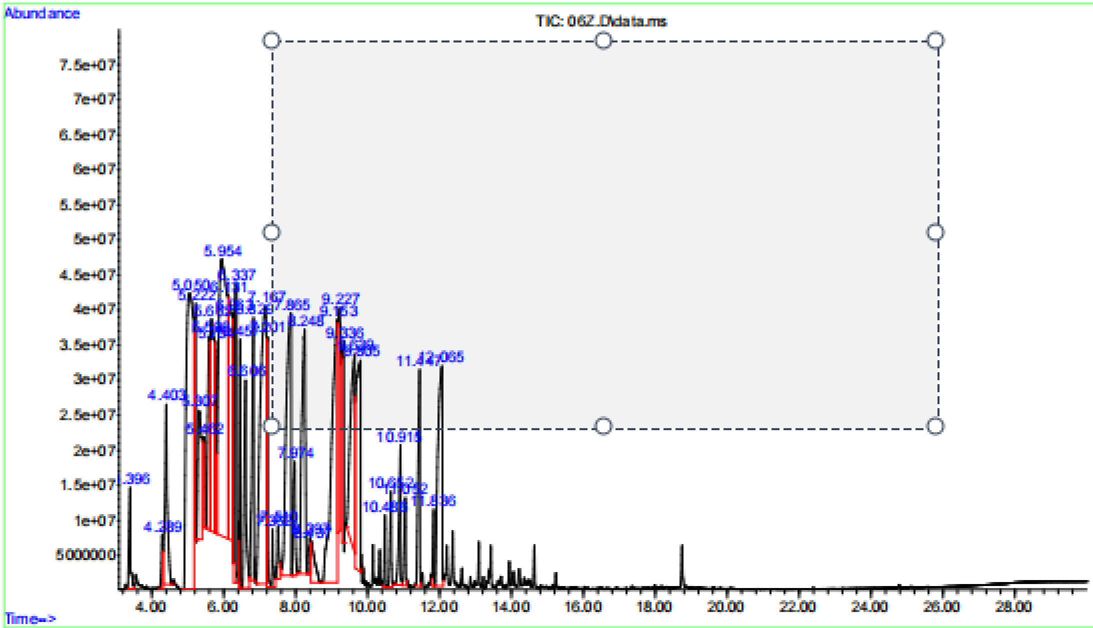

Figure 1: Total ion chromatogram (TIC) of *Citrus sinensis*

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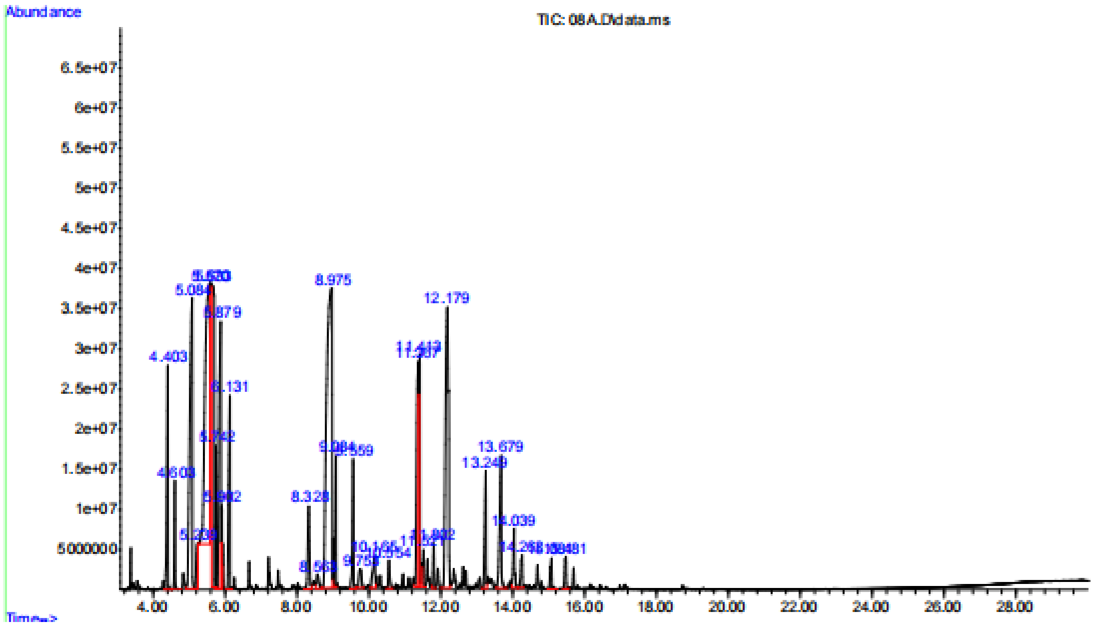

Figure 2: Total ion chromatogram (TIC) of *Eupatorium capillifolium*

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Chapter 4

Discussions

Medicinal plants have been the main source for the formation of herbal drugs in pharmaceutical area due to the prevalence of antimicrobial resistance. The presence of

Linalool, limonene, beta-myrcene, o-cymene, alpha phellandrene e.t.c.in various volatile oil is responsible for the antimicrobial activity of plants. So that *Citrus sinensis* and *Eupatorium capillifolium* possess remarkable antimicrobial, insecticidal and anti-inflammatory activities. The results obtained in this study revealed the antimicrobial efficacy of the volatile oil obtained from *Citrus sinensis* and *Eupatorium capillifolium.*

Numerous volatile compounds mostly monoterpenes and aromatic derivatives are found in *Eupatorium capillifolium* according to Gas Chromatography-Mass Spectrometry (GC-MS) analysis. Phytochemical studies have widely recognized the antimicrobial properties of these compounds especially the monoterpenes. The synergistic action of these several constituents is probably what gives the essential oil its overall antimicrobial potential (Al-(Kong et al., 2025).

According to the GC-MS data the essential oil of *Eupatorium capillifolium.* Eight primary compounds were highlighted, three of which make up more than half of the total area percentage (between 52.37%). Though the bioactive compound obtained from the GC-MS analysis differs from the one gotten in literature like myrcene, and thymol-methyl-ether (Tabanca et al., 2010)

Monoterpenes are known for their bioactivities and make up a sizable portion of the compounds that have been identified.

The terpenes groups most representative members are monoterpenes which can make up to 90% of the total oil concentration due to their extensive distribution (Amri et al. in 2023). monoterpenes are divided into regular and irregular varieties as well as iridoids. Monoterpene hydrocarbons contain a number of essential compounds and conventional monoterpenes are the most common (Bhattacharya et al. 2021ab). Alpha phellandrene is

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one of a pair of cyclic monoterpene and double-bond isomers which can be identified as phellandrene. Alpha phellandrene have antifungal activity by disruption of fungal cell membrane integrity *(Costa et al. 2021).*

Alpha-pinene also a monoterpene studies showed the antibacterial properties when applied to certain microorganisms. However, it was evidenced that its effectiveness is directly linked to its concentration and the interaction with certain bacterial strains having more activity against gram positive bacterial than gram negative (MFA Borges et al 2022)

Numerous studies have been done on 3-carene which demonstrated that this bicyclic monoterpene has antibacterial properties potentially targeting both Gram-positive and Gram-negative bacteria (Shu et al, 2019). Its suggested mechanism involves damaging membranes interfering with the structure of genomic DNA and interfering with metabolic enzymes (shu et al. 2019).

The oils composition is largely composed of aromatic compounds which are also known for their antimicrobial properties, such as o-cymene, 2, 3, 5-Trimethylanizole and Benzoic acid,4-(1-methylethyl)

The essential oil of *Citrus sinensis* (sweet orange) is analyzed using Gas Chromatography-Mass Spectrometry (GC-MS) which yields a chemical profile that directly influences its antimicrobial activity. The oils high concentration of particular monoterpene hydrocarbons is largely responsible for the activity, potency, and range.

D-limonene has a well-established therapeutic effect in literature showing antibacterial properties against many bacteria like *Streptococcus mutans*, *Streptococcus salivary, Streptococcus spp., Salmonella spp*., *Pseudomona spp.*, *Streptococcus sobrinus, Staphylococcus aureus, Candida albicans and oralis.*

Limonene has. become a subject of curiosity. its capacity to fight bacteria. has been compared to a wide range of studies. According to a study by limonene exhibited antibacterial properties, ability to combat both Gram-positive and Gram-negative bacteria including *Pseudomonas aeruginosa* and *Staphylococcus aureus respectively*. Accordingly, D-limonene was more sensitive to Gram-positive when compared to Gram-negative

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microorganism. The more intricate structure of *Pseudomonas aeruginosa* justifies this. An efflux system that can eliminate antibiotics substances that are found in the intracellular medium to the extracellular medium. (Adyelle Dantas Ribeiro et al. 2022)

A terpenic alcohol that belongs to the monoterpene family linalool (37-dimethyl-octa-16-diene-3-ol) has been identified as a significant volatile component in the essential oils of numerous plants (Xue Liu et al. 2020)

A natural chemical with a pleasing floral aroma linalool finds extensive use in the food pharmaceutical and cosmetics industries particularly in fragrances and perfumery (Xue Liu et al. 2020)

Previous research indicates that linalool has antibacterial, anti-cholesterol and anxiolytic properties. (Xue Liu et al. 2020)

For antibacterial activity it showed antimicrobial activity against *Campylobacter jejuni, Campylobacter coli and Pseudomonas aeruginosa* (Xue Liu et al. 2020)

The findings demonstrated that linalool exhibited strong antibacterial activity against *Pseudomonas aeruginosa*. Linalool minimum inhibitory concentration and minimum bactericidal concentration in relation to *Pseudomonas aeruginosa* were 431 μg/mL (0. 5 μL/mL) and 862 μg/mL (1. 0 μL/mL) respectively demonstrating that linalool exhibited the optimal bactericidal and bacterial inhibition activity against *Pseudominas. aeruginosa* (Xue Liu et al. 2020). Gamma terpinene, beta- myrcene, 2, 6 octadienal and caryophyllene also possess antimicrobial activity but in lesser amount compared to Limonene and Linalool.

Table 3 shows the antimicrobial activity of *Eupatorium capillifolium* volatile oil against five bacteria and 2 fungi species. The effectiveness is measured by the use of the zone of inhibition in millimeter (mm). A larger zone of inhibition indicates greater antimicrobial activity. The table also include positive control, ciprofloxacin and ketoconazole a known antibiotic and antifungal drug for comparison.

The volatile oil of *Eupatorium capillifolium* shows some level of antibacterial activity against two of the tested five bacteria. The extract effectiveness varies depending on the

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bacteria. It was most effective against *Pseudomonas aeruginosa and Escherichia coli*, which are both gram negative bacteria, with zone of inhibition of 11mm,13mm,15mm,17mm,20mm for *Pseudomonas aeruginosa an*d 10mm,13mm,15mm,17mm,20mm for *Escherichia coli* with different concentration from 5%w/v to 25%w/v. This activity against gram negative bacteria is due to the phytochemical agent present in the volatile oil alpha pinene and 3-carene which are known to have antibacterial properties (shu et al. 2019; MFA Borges et al 2022). It showed no activity against gam positive bacteria like staphylococcus aureus and Bacillus subtilis and also a gram-negative bacteria klebsiella aerogenes at all concentration. It also showed no zone of inhibition against fungal organism such as Candida albican and Aspergillus niger. This is in contrast to previous studies which showed Eupatorium capillifolium volatile oil to have a weak activity against some antifungal species (Tabanca et al., 2010). The positive control ciprofloxacin and ketoconazole indicated a larger zone of inhibition. This is expected as ciprofloxacin and ketoconazole are a purified and potent pharmaceutical antibiotic and antifungal drug. This type of research is a preliminary step in ethnobotanical studies, which explore the medicinal properties of plants used in traditional medicine. The result suggests that *Eupatorium capillifolium* has compound with potential antibacterial activity that need further investigation. Further research could involve isolating and identifying compounds responsible for this activity and testing their efficacy and safety in a more controlled manner

Table 3 also illustrated the minimum inhibitory concentration (MIC) of the volatile oil extract of *Eupatorium capillifolium* against the susceptible bacteria, *Pseudomonas aeruginosa and Escherichia coli*. The minimum inhibitory concentration is a fundamental parameter whose determination is the cornerstone of antimicrobial susceptibility testing.

Principle of minimum inhibitory concentration

The principle of the MIC test is based on the concept of serial dilution. The volatile oil extract is diluted to a series of decreasing concentration in a liquid growth medium, and a standardized amount of the test organism is added to the tube or well. After a fixed incubation period, the tubes are examined for turbidity (cloudiness), which signifies bacterial growth. The lowest concentration of the extract in which no visible growth is

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detected is defined as MIC. This value provides a quantitative measure of the antimicrobial potency as a lower MIC value indicates a more effective agent. The MIC test is a critical step in drug development and research for several reasons. It helps to quantify the efficacy of a new antimicrobial compound or extract, determine appropriate dosage for therapeutic use, compare the activity of different antimicrobial agents a specific pathogen, identify potential resistance in a bacterial strain.

Table 3 also present the minimum inhibitory concentration (MIC) of Eupatorium capillifolium volatile oil against the two susceptible Gram-negative bacteria *Pseudomonas aeruginosa and Escherichia coli.* The MIC is the lowest concentration of a substance that prevents the visible growth of a microorganism. A lower MIC value indicates a more potent antimicrobial agent. The result presented inhibition of growth at different concentration from 5 to 25%w/v for the two susceptible organisms,11mm,13mm,14mm,16mm,19mm for *Pseudomonas aeruginosa.* Therefore, the MIC for *pseudomonas aeruginosa* was seen at 11mm. This suggests a good potent drug with low concentration for the volatile oil extract.

For *Escherichia coli* the zone of inhibition was 10mm, 13mm, 15mm, 17mm, 20mm. Therefore, the MIC for *Escherichia coli* was seen at 10mm. This suggests a good potent drug with low concentration for the volatile oil extract

Table 4 shows antimicrobial activity of *Citrus sinensis* against five bacteria species and 2 fungi species, the effectiveness is measured by the use of the zone of inhibition in millimeter (mm). A larger zone of inhibition indicates greater antimicrobial activity. The table also include positive control, ciprofloxacin and ketoconazole a known antibiotic and antifungal drug for comparison.

The volatile oil extract shows some level of antimicrobial activity against three of the five bacteria and showed a high-level activity against the two fungi. This showed *Citrus sinensis* volatile oil have activity against both bacterial and fungi. It zones of inhibition against *pseudomonas aeruginosa, Klebsiella aerogenes* (gram negative) and *Bacillus subtilis* (gram positive), With zone of inhibition seen only at the highest concentration (25%w/v), with zone of inhibition of 10,14, 15. This signify the low sensitivity of Citrus sinensis against bacteria. According to previous studies the phytochemical characteristics

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of *Citrus sinensis* like Linalool and D-limonene are known to have antimicrobial activity against gram positive bacterial and gram-negative bacteria bacterial *((Xue Liu et al. 2020)*. But the test result showed otherwise as it was unable to inhibit staphylococcus aureus, Escherichia coli, and inhibited Pseudomonas aeruginosa, Bacillus subtilis and Klebsiella aerogenes at the highest concentration (25%w/v) with a value of 10, 15, 14.

The antifungal activity had high sensitivity at all tested concentrations, as the inhibitory zone diameter increases as the concentration increase from 5% to 25%w/v, with Candida albican having a zone of inhibition of 12, 15, 20, 25, 30 and Aspergillus niger having a zone of inhibition of 8,10, 12, 15, 20. As previous studies have shown the antifungal effect of Citrus sinensis due to the presence of D-limonene (Adyelle Dantas Ribeiro et al. 2022). The table also include a positive control, Ciprofloxacin and Ketoconazole which is a known antibiotic and antifungal drug for comparison, which have a higher zone of inhibition than the extract.

Table 4 also show the minimum inhibitory concentration of both the bacterial and the fungi species.

The MIC for Pseudomonas aeruginosa, Klebsiella aerogenes and Bacillus subtilis was 10mm,14mm, 15mm as the was the only concentration (25%w/v) that growth was not was. This suggests a good potent drug with low concentration for the volatile oil.

The MIC for Aspergillus niger and Candida albicans was 8mm and 12mm which is the lowest concentration that inhibited the growth of the fungal organism.

This result for both Eupatorium capillifolium and Citrus sinensis proves it can be used as an alternative medicine to orthodox drug or in combination in the treatment of different ailment like wound healing skin infection and different range of bacterial and fungal infection etc.

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Chapter 5

Conclusion

The result of this study confirms that *Eupatorium capillifolium* and *Citrus sinensis* contain important bioactive compounds and has antimicrobial properties. The GC-MS analysis showed the presence of various compound for both *Eupatorium capillifolium* and *Citrus sinensis* like alpha-Pinene, 3-Carene, alpha-Phellandrene, o-Cymene etc. which are present in *Eupatorium capillifolium*, While D-Limonene, Linalool, Beta-Myrcene, Gamma-Terpinene etc. are present in *Citrus sinensis*, which are known to have medicinal benefits.

The antimicrobial test showed that *Eupatorium capillifolium* volatile oil was effective against *Pseudomonas aeruginosa and Escherichia coli*. However, it was not active against Staphylococcus *aureus, Bacillus subtilis, Klebsiella aerogenes, Candida albicans, Aspergillus niger*. Which showed *Eupatorium capillifolium* has more activity against bacteria than fungi.

The antimicrobial test showed that *Citrus sinensis* volatile oil was effective against *Aspergillus aerogenes, Candida albicans* and showed low antimicrobial activity against *Pseudomonas aeruginosa, Klebsiella aerogenes and Bacillus subtillis*. which demonstrates that Citrus sinensis have a strong antimicrobial effect.

These finding support the ethnomedicinal use of *Eupatorium capillifolium and Citrus sinensis* in the treatment of various infection cause by bacterial and fungi.

Further research should focus on isolating these compounds and exploring their potential as new antimicrobial agents, especially with increasing antibiotic resistance worldwide.

REFERENCE

Abid, K.Y. & Yahya, M.Q. (2023) 'Antimicrobial and antioxidant activities of essential oils derived from some citrus peel', *MMSL*, 92(1), pp. 64–74. doi:10.31482/mmsl.2022.025.

Agatonovic-Kustrin, S., Ristivojevic, P.M., Gegechkori, V., Litvinova, T.M. & Morton, D.W. (2020) 'Essential Oil Quality and Purity Evaluation via FT-IR Spectroscopy and Pattern Recognition Techniques', *Applied Sciences*, 10(20), pp. 1–15.

Akarca, P. M., & Sevik, R. (2022) 'Biological Activities of *Citrus limon* L. and *Citrus sinensis* L. Peel Essential Oils', *Journal of Essential Oil Bearing Plants*.

Antimicrobial Resistance Collaborators (2022) 'Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis', *The Lancet*, 399(10325), pp. 629–655. doi:10.1016/S0140-6736(21)02724-0.

Antimicrobial Resistance Collaborators (2024) 'Global burden of bacterial antimicrobial resistance, 1990–2021', *The Lancet*. [Online]. Available at: **https://www.thelancet.com** (Accessed: 22 September 2025).

Barrios, D. E., Santos, R. P. D. L., Dela-Vega, (2017) 'Larvicidal of Efficacy of *Eupatorium capillifolium* (Dog-fennel) Crude Extract on *Aedes aegypti* and *Culex quinquefasciatus*', *J. Bio. & Env. Sci.*, 11(5), pp. 385–396.

Borges, M.F.A., Lacerda, R.S., Correia, J.P.A., Melo, T.R. and Ferreira, S.B. (2022) 'Potential Antibacterial Action of α-Pinene', *Medical Sciences Forum*, 12(1), p. 11. doi:10.3390/eca2022-12709.

Carrillo-Hormaza, L., Mora, C., Alvarez, R., Alzate, F., & Osorio, E. (2015) 'Chemical composition and antibacterial activity against *Enterobacter cloacae* of essential oils from Asteraceae species growing in the Páramos of Colombia', *Industrial Crops and Products*, 77, pp. 108–115.

`

Centers for Disease Control and Prevention (CDC) (2022) *Antimicrobial Resistance Threats Update 2022*. CDC. [Online]. Available at: **https://www.cdc.gov/antimicrobial-resistance/data-research/threats/update-2022.html** (Accessed: 22 September 2025).

De Cicco, P., Ercolano, G., Sirignano, C., Rubino, V., Rigano, D., Ianaro, A., & Formisano, C. (2023) 'Chamomile essential oils exert anti-inflammatory effects involving human and murine macrophages: Evidence to support a therapeutic action', *Journal of Ethnopharmacology*, 116391. doi:10.1016/j.jep.2023.116391.

De Sousa, D., Damasceno, R., Amorati, R., Elshabrawy, H., De Castro, R., Bezerra, D., Nunes, V., Gomes, R., & Lima, T. (2023) 'Essential Oils: Chemistry and Pharmacological Activities', *Biomolecules*, 13. doi:10.3390/biom13071144.

Dutt, M., Mahmoud, L., Nehela, Y., Grosser, J., & Killiny, N. (2022) 'The *Citrus sinensis* Tiller Angle Control 1 (CsTAC1) Gene Regulates Tree Architecture in Sweet Oranges by Modulating the Endogenous Hormone Content.', *Plant science : an international journal of experimental plant biology*, pp. 111401. doi:10.1016/j.plantsci.2022.111401.

Farrar, A.J., Farrar, F.C. (2020) 'Clinical Aromatherapy', *Nurs. Clin. N. Am.*, 55, pp. 489–504.

Hasan, Z., Al-Halbosiy, M., Al-Lihaibi, R., & Al-Nauimi, E. (2022) 'Short Communication: Antimicrobial of lemongrass (*Cymbopogon citratus* L.) volatile oil and cytotoxic effects against L20B and MCF-7cell lines', *Biodiversitas Journal of Biological Diversity*. doi:10.13057/biodiv/d231039.

Jang, S.-K., Kim, K.-J. & Park, M.-J. (2023) 'Anti-Inflammatory Effects of Essential Oils from the Peels of Citrus Cultivars', *Pharmaceutics*, 15(6), Article 1595. doi:10.3390/pharmaceutics15061595.

Khalil, D.Y. & Hassan, O.M. (2024) 'Anti-inflammatory and Antioxidant Activity of Rosemary Essential Oil', *Journal of Angiotherapy*, 8(4), pp. 1–6.

`

Khurshid, T., Creek, A., Sanderson, G., & Zhao, X. (2024) 'Tree Performance, Yield, and Fruit Quality of 'Valencia' Sweet Orange (*Citrus sinensis* L. Osbeck) Selections on New *Poncirus trifoliata* Rootstocks', *Horticulturae*. doi:10.3390/horticulturae10040393.

Letseka, T., Sepheka, N.J., Dubery, I. & George, M. (2022) 'Bioprospecting of Essential Oil-Bearing Plants: Rapid Screening of Volatile Organic Compounds Using Headspace Bubble-in-Drop Single-Drop Microextraction for Gas Chromatography Analysis', *Plants*, 11(19), pp. 1–15.

Liu, X., Cai, J., Chen, H., Zhong, Q., Hou, Y., Chen, W. and Chen, W. (2020) 'Antibacterial activity and mechanism of linalool against *Pseudomonas aeruginosa*', *Microbial Pathogenesis,* 141, p. 103980. doi:10.1016/j.micpath.2020.103980.

Mahboub, N., Cherfi, I., Laouini, S., Bouafia, A., Benaissa, A., Alia, K., Alharthi, F., Al-Essa, K., & Menaa, F. (2025) 'GC/MS and LC Composition Analysis of Essential Oil and Extracts From Wild Rosemary: Evaluation of Their Antioxidant, Antimicrobial, and Anti-Inflammatory Activities', *Biomedical Chromatography: BMC*, 39(5), e70084. doi:10.1002/bmc.70084.

Olascuaga-Castillo, K., Castillo-Medina, O., Villacorta-Zavaleta, M., Diaz-Ortega, J., Blanco-Olano, C., Altamirano-Sarmiento, D., & Valdiviezo-Campos, J. (2024) 'Extraction of essential oils by hydrodistillation of four aromatic species: Conditioning, extraction conditions, yield and chemical composition', *Scientia Agropecuaria*, 15(3), pp. 385-408.

Ribeiro, A.D., Cardoso, M.N.A., Freire, J.C.P., Figueiredo Júnior, E.C., Costa, M.M.A., Silva, P.G., Gomes, D.Q.C., de Brito, E.M.M. and Pereira, J.V. (2023) 'Antimicrobial activity of limonene: Integrative review', *Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas*, 22(5), pp. 581–593. doi:10.37360/blacpma.23.22.5.42.

Sado, M., Yusuf, Z., Desta, M., & Idris, M. (2022) 'Physicochemical Properties, Antioxidant and Antimicrobial Activities of Sweet Orange (*Citrus sinensis* L. OSBECK) Fruit Peel and Pulp Oil Extracts', *The Open Biotechnology Journal*.

Sathish Kumar, N. et al. (2024) ‘Biochemical, Antioxidant, and Antimicrobial Profiling of Essential Oils of Indian Origin for Culinary Applications’, *International Journal of Food Science*, 2024, Article 9326683. doi:10.1155/ijfo/9326683.

Shu, H., Chen, H., Wang, X., Hu, Y., Yun, Y., Zhong, Q., Chen, W. and Chen, W. (2019) 'Antimicrobial Activity and Proposed Action Mechanism of 3-Carene against *Brochothrix thermosphacta* and *Pseudomonas fluorescens*', *Molecules*, 24(18), p. 3246. doi:10.3390/molecules24183246.

Silva, A. M., Peixoto, C., Costa, R., et al. (2023) ‘Chemical Composition and Assessment of the Anti-Inflammatory, Antioxidant, Cytotoxic and Skin Enzyme Inhibitory Activities of *Citrus sinensis* (L.) Osbeck Essential Oil and Its Major Compound Limonene’, *Molecules*, 28(12), 4652.

Tabanca, N., Bernier, U. R., Tsikolia, M., Becnel, J. J., Sampson, B., Werle, C., ... & Wedge, D. E. (2010) 'Eupatorium capillifolium Essential Oil: Chemical Composition, Antifungal Activity, and Insecticidal Activity', *Natural Product Communications*, 5(9), pp. 1409–1415.

Wei, L., Yang, H., Li, H., Zhu, M., Mi, S., Lu, Q., Liu, M., & Zu, Y. (2022) ‘Comparison of chemical composition and activities of essential oils from fresh leaves of *Pelargonium graveolens* L'Herit. extracted by hydrodistillation and enzymatic pretreatment combined with a solvent-free microwave extraction method’, *Industrial Crops and Products*. doi:10.1016/j.indcrop.2022.115204.

World Health Organization (WHO) (2025) *Global Antimicrobial Resistance and Use Surveillance System (GLASS) — AMR surveillance and antimicrobial consumption report*. WHO. [Online]. Available at: **https://www.who.int/initiatives/glass** (Accessed: 22 September 2025).

Zhao, Q., Zhu, L., Wang, S., Gao, Y., & Jin, F. (2022) ‘Molecular mechanism of the anti-inflammatory effects of plant essential oils: A systematic review’, *Journal of Ethnopharmacology*, 11 5829. doi:10.1016/j.jep.2022.115829.