CHROMATOGRAPHIC ANALYSIS, ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF AERIAL PART OF ETHANOLIC FRACTION OF *Emilia praetermissa Milne Redhead (Asteraceae)*

BY

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COURSE CODE: PPJ 601

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CERTIFICATION

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DEDICATION

This work is dedicated to the memory of my late father, Mr. Monday Azekhuemhen for his encouragements and sacrifice and also to my mother and my brother for their ongoing support and care throughout this project.

ACKNOWLEDGEMENT

I give thanks to God Almighty for His preservation and guidance throughout my undergraduate journey.

I am profoundly grateful to Dr. Emmanuel E. Odion for his expert supervision, patient guidance, insightful feedback, and constant encouragement during this project. My appreciation also goes to the staff of the Department of Pharmaceutical Chemistry; Dr. Vincent Imieje (Head of Department), Dr. Uyi Ogbeide, and the departmental non-academic staff for granting laboratory access and providing essential technical support. I also thank the Faculty of Pharmacy for the broad teaching, mentorship, and practical experience that enriched my training.

Special thanks to Dr. Tony Aiwonegbe (Department of Chemistry, Faculty of Physical Sciences) for his invaluable assistance, and to Mr. Wilfred (Department of Pharmaceutical Microbiology) for his guidance with the antibacterial activity assays. I appreciate the Chemical Engineering Laboratory at the University of Ilorin for performing and analyzing the GC-MS spectra, and the University of Benin Teaching Hospital for supplying the bacterial isolates.

I am deeply indebted to my parents, Mrs. Gift Azekhuemhen and the late Mr. Monday Azekhuemhen, for their unwavering love and support. To my siblings, Emmanuel Azekhuemhen and Festus Azekhuemhen, thank you for your encouragement and advice throughout my PharmD programme. Finally, I extend my gratitude to my friend, the Time Masters, and to all others who helped me in various ways. God bless you all.

TABLE OF CONTENT

Title Page……..……..……..... I
Certification ii
Dedication iii
Acknowledgement iv
Table Of Content v
List Of Tables viii
List Of Figures ix
Abstract x
Chapter One 1
1.1 Introduction 1
1.2 Description 5
1.3 Botanical Classification Of *Emilia Praetermissa* 6
1.4 Ethno-Medicinal Use 7
1.5 Pharmacological Effect 7
1.6 Phytochemicals 8
1.6.1 Alkaloids 9
1.6.2 Phenolics 11
1.6.3 Flavonoids 12
1.6.4 Tannins 14
1.6.5 Saponin 15
1.7 Literature Review 17
1.7.1 Justication For The Study 20
1.7.2 Aims 21
1.7.3 Objectives 22
Chapter Two 23
Materials And Methods 23
2.1 Materials, Appararus And Reagents 23
2.2 Plant Collection And Identification 23
2.3 Extraction Process 24
2.4 Fractionation Process 24
2.5 Chromatographic Process 25

2.5.1 Sample Preparation For High Performance Liquid Chromatography 25
2.5.2 High-Performance Liquid Chromatography Procedure. 25
2.5.3 Sample Preparation For Gas Chromatography-Mass Spectrometry 26
2.5.4 Gas Chromatography Mass Spectrometry Procedure 26
2.6 In-Vitro Antioxidant Activity Of *Emilia Praetermissa* 27
2.6.1 DPPH Antioxidant Assay 28
2.6.2 FRAP Antioxidant Assay 29
2.6.3 ABTS Antioxidant Assay 29
2.6.4 TAC Assay 30
2.7 Antibacterial Activity Of *Emilia Praetermissa* 30
2.7.1 Specimen Collection 30
2.7.2 Preparation Of Test Organism 30
2.7.3 Antibacterial Susceptibility Tests And Determination Of Inhibitory Zone Diameter (Izd) 30
2.7.4 Determination Of Minimum Inhibitory Concentration (Mic) 31
2.7.5 Determination Of Minimum Bactericidal Concentration (Mbc) 32
2.7.6 Determination Of The Mode Of Action 33
2.8 Statistical Analysis: 33
Chapter Three 34
Result 34
3.1 High Performance Liquid Chromatography 34
3.2 Gas Chromatography-Mass Spectrometry 38
3.3 Antibacterial Activity 43
3.3.1 Minimum Inhibitory Concentration 44
3.3.2 Minimum Bactericidal Concentration 45
3.3.3 Mode Of Antibacterial Activity 46
3.4 Antioxidant Result 47
3.4.1 Dpph Antioxidant Result 47
3.4.2 Frap Antioxidant Result 48
3.4.3 Abts Antioxidant Result 49
3.4.4 Tac Antioxidant Result 50
Chapter Four 51
4.1 Discussion 51

Chapter Five 57
5.1 Conclusion 57
References 58
Appendix A 67
Appendix B 69

LIST OF TABLES

Table 1 Compounds identified through HPLC analysis

Table 2 Compounds identified through GC-MS analysis

Table 3 Minimum inhibitory concentration (MIC) results for Ethanolic fraction of *Emilia praetermissa* against clinical bacterial isolates.

Table 4 Minimum bactericidal concentration (MBC) results for Ethanolic fraction of *Emilia praetermissa.*

Table 5: Mode of Antibacterial activity of *Emilia praetermissa*

LIST OF FIGURES

Fig 1 Structure of Ammodendrine

Fig 2 Structure of Hydroxylupanine

Fig 3 Structure of Flavanone

Fig 4 Structure of Narigenin

Fig 5 Structure of Flavone

Fig 6 HPLC chromatogram of the ethanolic fraction of aerial part of *Emilia praetermissa* showing detected peaks and retention times for identified phytoconstituents.

Fig 7 Structures of compounds identified through HPLC

Fig 8 GC-MS chromatogram of the ethanolic fraction of aerial part of *Emilia praetermissa* showing retention times and relative peak areas of volatile and semi-volatile constituents.

Fig 9 Structures of compounds identified through GC-MS analysis

Fig 10 Antibacterial susceptibility of *Emilia praetermissa* ethanolic fraction of *Emilia praetermissa* (inhibition zone diameters in mm).

Fig 11 Comparative DPPH % Inhibition at 200–1000 µg/mL of Plant Extract and Ascorbic acid

Fig 12 Comparative FRAP % Inhibition at 200–1000 µg/mL of Plant Extract and Ascorbic acid

Fig 13 Comparative ABTS % Inhibition at 200–1000 µg/mL of Plant Extract and Ascorbic acid

Fig 14 Comparative TAC % Inhibition at 200–1000 µg/mL of Plant Extract and Ascorbic acid

Fig B1 Antibacterial activity of plant extract against *Escherichia coli*

Fig B2 IZD comparison between Ciprofloxacin and extract against *Staphylococcus aureus*

ABSTRACT

Introduction: *Emilia praetermissa* is used traditionally for treatment of infections, wound healing, and gastrointestinal complaints. Despite ethnomedicinal use, systematic chemical characterisation and laboratory evaluation of the ethanolic aerial fraction remain limited. This study profiles its phytochemicals, antibacterial and antioxidant activities.

Method: The aerial parts were extracted with 70% ethanol from which the ethanolic fraction were obtained and analyzed by HPLC and GC-MS to identify non-volatile and volatile constituents. Antibacterial activity against six clinical isolates (*Escherichia coli, Staphylococcus aureus, Enterobacter cloacae, Bacillus subtilis, Pseudomonas aeruginosa, Bacillus cereus)* was assessed by agar well diffusion method, Minimum Inhibitory Concentration (MIC) (agar dilution) and Minimum Bactericidal Concentration (MBC) determinations were carried out. Antioxidant capacity was measured using 1,1-diphenyl-2-picrylhydrazyl (DPPH), Ferric reducing antioxidant power (FRAP), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and Total Antioxidant Capacity (TAC) assays; ascorbic acid served as the standard.

Result: HPLC detected varying non-volatile constituents of which flavonoids (notably Flavanol, epicathecin, narigenin) were present with Cumulative Flavonoid concentration of 66.083 µg/mL (21.15%) while GC-MS identified 27 volatile/semi-volatile compounds dominated by fatty acid methyl esters and terpenoids. The extract produced moderate inhibition zones (15–18 mm) against the six clinical isolates. MIC testing showed complete growth inhibition at 40 mg/mL for all organisms; at 20 mg/mL only *S. aureus, P. aeruginosa and B. cereus* remained inhibited. MBC at 40 mg/mL was bactericidal for *S. aureus, E. cloacae, P. aeruginosa* and *B. cereus*. *Staphylococcus aureus, Enterobacter cloacae, Pseudomonas aeruginosa and Bacillus cereus* all have MBC/MIC ratios ≤ 4 and are therefore classified as bactericidal. In antioxidant assays the extract demonstrated strong radical scavenging (high DPPH and ABTS at increasing concentrations), modest FRAP activity, and a concentration dependent increase in TAC.

Conclusion: The ethanolic aerial fraction of *Emilia praetermissa* contains a complex mixture of alkaloids, phenolics, flavonoids, saponins and lipidic constituents that together confer measurable antibacterial and antioxidant activities in vitro.

CHAPTER ONE

1.1 INTRODUCTION

Plants generally are vast in nature, and they occur in almost every part of the world, where they are involved in the provision of basic needs of man, such as shelter, food, aesthetics and even medicine. (Coyago-Cruz *et al.,* 2023)

Medicinal plants remain fundamental to global healthcare, with an estimated 40 % of health practices relying on traditional medicine and roughly 85 % of those remedies being plant based; interest in these plants has grown since the mid 20th century because their defensive phytochemicals offer antibacterial, anticancer, anti-inflammatory, and other therapeutic activities, and because plant-derived treatments can be affordable and often have fewer side effects (Niazi and Monib, 2024).

Medicinal plants supply raw materials for therapeutic, aromatic, and culinary uses across industries from cosmetics to pharmaceuticals, and their use reflects centuries of indigenous knowledge documented in historical sources (Heinrich *et al.,* 1998; Heinrich *et al.,* 2017). The pharmacological relevance of many species derives from secondary metabolites that exhibit antitumor, antiviral, anti-inflammatory, and antibiotic effects, making plants a productive source for drug discovery (Alonso-Castro *et al.,* 2011; Sharma *et al.,* 2017; Wang *et al.,* 2019). It is estimated that about 25 % of modern bioactive drugs are plant derived, and that roughly 80 % of the world's population uses herbal remedies for primary healthcare (García de Alba *et al.,* 2012; Kew *et al.,* 2016).

Despite their promise, many medicinal plants lack complete scientific validation of chemical composition and toxicity, creating a need for rigorous pharmacological studies and standardized

extraction procedures (Alonso Castro *et al.,* 2018). Advances in green extraction technologies provide more sustainable methods for isolating bioactive phytochemicals and reduce reliance on hazardous solvents (Rodríguez *et al.,* 2015; Dai *et al.,* 2013).

The growing threat of antibacterial resistance increases the urgency of discovering novel antibacterial scaffolds and refining screening methodologies; contemporary reviews summarize traditional and advanced assays for evaluating antibacterial activity and discuss their relative strengths and limitations (Hossain, 2024). Factors that influence antibacterial efficacy include plant part, extraction solvent, and geographic origin; surveys report that leaves account for the largest share of tests, methanol and ethanol are the most effective solvents in many studies, and regions such as Cameroon, Indonesia, and South Africa are frequent sources of potent plant extracts (Zouine *et al.,* 2024). Specific phytochemicals illustrate diverse antibacterial mechanisms: for example, curcumin alters bacterial membrane fluidity and inhibits biofilm formation, while certain flavonoids interfere with DNA gyrase and protein synthesis (Cushnie *et al.,* 2005)

Reviews of antibacterial screening show that only a small fraction of global plant diversity has been systematically evaluated for antibacterial properties, though many studies report promising minimum inhibitory concentrations (MICs) for selected species. These reviews identify consistent patterns. Leaves are the most commonly tested plant part and often give the highest rates of activity, while polar organic solvents such as methanol and ethanol are frequently most effective because they extract a broad range of secondary metabolites (Zouine *et al.,* 2024). Mechanistic studies indicate that plant compounds act against bacteria through diverse routes, including membrane disruption, inhibition of protein and nucleic acid synthesis, alteration of membrane permeability, and inhibition of key metabolic enzymes (Zouine *et al.,* 2024).

Furthermore, medicinal plants are rich sources of natural antioxidants, which help neutralize reactive oxygen species (ROS) and prevent oxidative stress a key contributor to aging and chronic diseases such as cancer, diabetes, and cardiovascular disorders (Sayyed *et al.,* 2023). These plant antioxidants operate through several mechanisms, including radical scavenging, metal ion chelation, and inhibition of lipid peroxidation (Nwozo *et al.,* 2023).

Phytochemicals such as flavonoids, phenolic acids, tannins, and alkaloids are commonly implicated in antioxidant activity. These compounds protect cellular components by donating electrons or hydrogen atoms to stabilize free radicals, thereby preventing damage to DNA, proteins, and lipids (Sharma and Rajpal, 2023). Flavonoids such as quercetin and kaempferol provide clear examples of molecules with strong radical scavenging activity and are widely studied for their therapeutic potential (Jan *et al.*,2022)

Antioxidants are molecules that delay or prevent the oxidation of biomolecules by neutralizing reactive oxygen species (ROS) and other free radicals produced during normal metabolism or introduced from the environment. Oxidative stress arises when ROS generation exceeds endogenous antioxidant capacity, leading to damage to lipids, proteins, and DNA and contributing to aging, cardiovascular disease, inflammatory disorders, neurodegeneration, and impaired wound healing (Halliwell *et al.,* 2015). In plants, polyphenols (including flavonoids, phenolic acids, and tannins), alkaloids, saponins, and terpenoids act as natural antioxidants; these phytochemicals both protect the plant and, when consumed or applied, can confer antioxidant-mediated pharmacological effects such as tissue protection, anti-inflammatory actions, and improved wound repair (Hajam *et al.,* 2023)

Plants synthesize antioxidants as part of their defense against environmental stressors, and these compounds can be harnessed for human health (Halliwell & Gutteridge, 2015). Antioxidants act through several mechanisms which includes; free radical scavenging by donating electrons or hydrogen atoms to neutralize ROS, metal ion chelation by binding transition metals such as Fe^{2+} and Cu^{2+} that catalyze ROS formation, modulation of endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) and inhibition of lipid peroxidation, thereby preventing oxidative degradation of cell membranes (Hajam *et al.,* 2023).

Plants naturally produce ROS as byproducts of metabolism and in response to environmental stressors such as drought, UV radiation, and pathogen attack. While low levels of ROS function in cellular signaling, excessive accumulation leads to oxidative stress and damage to lipids, proteins, and DNA (Dumanović *et al.,* 2020). To counteract this, plants deploy a complex antioxidant defense system comprising enzymatic antioxidants (for example, SOD, catalase, and peroxidases) and non-enzymatic compounds such as flavonoids, phenolic acids, tannins, alkaloids, and vitamins (Mehla *et al.,* 2017).

Antioxidant activity in plants is therefore a key indicator of their pharmacological potential. Many medicinal plants used in traditional medicine owe their efficacy to antioxidant-rich phytochemicals that protect against oxidative damage, inflammation, and degenerative diseases in humans (Prenzler *et al.,* 2021).

One of the many examples of plants with medicinal value includes the genus *Emilia* (Family: Asteraceae, Subfamily: Asteroideae). The genus is made up of about 128 accepted species, among which is *Emilia praetermissa* (Milne Redhead).

Emilia praetermissa is an annual herbaceous plant that is found in the tropical and subtropical parts of Africa and Asia. *Emilia praetermissa* is native to West Africa, particularly Sierra Leone and Nigeria. It has also been identified in other western and central African countries such as the Democratic Republic of Congo, Cameroon and Cote D'Ivoire. *Emilia praetermissa* is also native to southeast China, particularly Taiwan *(*Chung *et al.,* 2009; Graveson, 2016; POWO, 2020). *Emilia praetermissa* is commonly known as tasselflower, pale tasselflower, wild lettuce, koyagipo, kipo, huang hua zi bei cao (China), odundun (Yoruba), banochi (Hausa), and nti-ene see (Igbo).

1.2 DESCRIPTION

Emilia praetermissa is an annual herbaceous plant that grows from about 60cm to 140cm with an erect stem that is simple or branched from the base with internodal space that ranges from 0.6 to 9cm. The texture of the stem can also be glabrous or smooth (Flora of China Editorial Committee, 2020; Plantiary, 2024). The leaves of *Emilia praetermissa* are simple and broadly ovate, measuring 4.0–6.0 cm in length and 4.5–6.0 cm in width. Both adaxial and abaxial surfaces are bright green and glabrous, exhibiting pinnate venation. The leaf apex ranges from obtuse to shortly acuminate, the margin is distinctly dentate, and the base is subcordate to rounded. The leaves are petiolate with a petiole length of 1.5–3.0 cm. The leaves of *Emilia praetermissa* are arranged alternately along the stem with an internodal length of 0.6 – 9 cm (Flora of China Editorial Committee, 2020). The inflorescence of *Emilia praetermissa* comprises solitary, terminal capitula borne on slender peduncles measuring approximately 1–3 cm in length. Each capitulum is cylindrical and discoid and attains a diameter of 1–1.5 cm at maturity (Chung *et al.,* 2009). The involucre is narrow and tubular, measuring 8–12 mm in length, and consists of 9–12 narrowly lanceolate phyllaries, each phyllary 8–11 mm long and pilose on the abaxial surface ((Flora of China Editorial Committee, 2020; Chung *et al.,* 2009).

The capitula are composed exclusively of tubular florets, with corolla tubes approximately 5 - 8 mm in length and limb lobes around 2 - 3 mm. Corolla colouration varies from creamy-white to pale yellow or pale orange, often displaying purple or orange tinges at the apex of the lobes (Chung *et al.*, 2009; Rojas-Sandoval, 2020). The anthers are syngenesious (fused into a tube), dark purple in colour, and typically exerted beyond the corolla mouth. Styles are bifid, terminating in capitate stigmatic branches positioned just above the anther tube to enhance pollen presentation. The fruit is a dry, indehiscent achene, oblong in shape, approximately 3 mm in length, distinctly ribbed, and densely pubescent (Flora of China Editorial Committee, 2020; Plantiary, 2024). Flowering occurs throughout the year in tropical regions, with peak blooming observed during the rainy season May to September (Rojas-Sandoval, 2020; World Flora Online, 2025). The floral structure and exposed reproductive organs attract a range of entomophilous pollinators, particularly bees and butterflies, consistent with the pollination syndrome typical of discoid heads within the Asteraceae family (Flora of China Editorial Committee, 2020; Rojas-Sandoval, 2020).

1.3 Botanical Classification of *Emilia praetermissa*

Domain	Eukaryota
Kingdom	Plantae
Phylum	Spermatophyta
Subphylum	Angiospermae
Class	Dicotyledonae
Order	Asterales
Family	Asteraceae

Genus	Emilia
Species	E. praetermissa

1.4 ETHNO-MEDICINAL USE

Extracts of *Emilia praetermissa* leaves (prepared as methanol, hot-water, cold-water, or ethanol extracts) are used widely in traditional medicine and applied as antibacterial remedies for bacterial and fungal infections (Afolayan *et al.,* 2017; Ikezu, 2023). The leaves are also crushed and used externally as a topical treatment for wounds (Afolayan *et al.,* 2017). Ethanolic leaf preparations are traditionally taken for cardiovascular support and are reported to lower blood pressure, reduce hyperlipidaemia, and exert anticoagulant effects (Ebhohon *et al.,* 2025). Aqueous decoctions are commonly used to relieve gastrointestinal complaints such as stomachache and ulcers (Ndji *et al.,* 2016; Ikezu, 2023). In some regions the leaves are eaten as a leafy vegetable to support general health (Ikezu, 2023; Useful Tropical Plants, 2024). Traditional healers also use *Emilia praetermissa* to treat colic in newborns and infants (Nwaefulu *et al.,*2016).

1.5 PHARMACOLOGICAL EFFECT

The pharmacological activities of *Emilia praetermissa* reported in ethnomedicine have been corroborated by experimental studies. Antibacterial activity against pathogens such as Staphylococcus aureus and Streptococcus pneumoniae as well as the traditional use for respiratory infections (sinusitis, otitis media, sore throat) have been demonstrated experimentally (Afolayan *et al.,* 2017). Aqueous extracts produced antihyperlipidemic effects in animal models, confirming traditional claims about lipid-lowering properties (Smith *et al.,* 2021). Decoction studies have shown mucosal protective effects consistent with reported anti-ulcer activity (Otto *et al.,* 2021). Ethanol-based preparations used for blood-pressure control is supported by recent evaluations of

antihypertensive activity (Ebhohon *et al.,* 2025). Anti-inflammatory and analgesic uses (for example in arthritis) have experimental backing, and traditional anticonvulsant applications—especially in paediatric folk practice have likewise been tested in preclinical models (Nwaefulu *et al.,* 2016). Multiple investigations confirm strong antioxidant and free-radical scavenging activity with potential relevance to cardiovascular protection and wound healing (Ndji *et al.,* 2016; Odion *et al.,* 2024; Bosson *et al.,* 2025). Finally, hepatoprotective effects of methanolic extracts reducing biochemical markers of liver injury after toxins such as paracetamol or CCl4 have been reported and experimentally validated (Nzewi *et al.,* 2016).

1.6 PHYTOCHEMICALS

Phytochemicals are naturally occurring chemical compounds found in plants, and they play important roles in protecting such plants from various threats like pests, diseases and environmental stress (Dai and Mumper, 2010; Mehla *et al.,* 2017). Although these phytochemicals are not essential in humans, as they are not required for basic survival or to protect deficiencies, they offer various health and medicinal benefits to humans, such as antioxidant properties, immune system support, reducing the risk of chronic disease and much more (Halliwell and Gutteridge, 2015; Sun *et al.,* 2023). Examples of common and extensively researched phytochemicals include glycosides, resins, tannins, carotenoids, flavonoids, polyphenolics, steroids, saponins and alkaloids (Dai and Mumper, 2010; Timilsena *et al.,* 2023)

Emilia praetermissa also contains some important phytochemicals that are believed to be implicated in the ethnomedicinal use as well as the pharmacological use of the plant (Ikezu, 2023; Odion *et al.,* 2024). *Emilia praetermissa* has been subjected to extensive research by various research teams to identify the phytochemicals present in the plant, and a wide variety of the phytochemicals have been identified and even quantified. Such compounds include cardiac

glycoside, tannins, flavones, flavonones, anthocyanidins, proanthocyanidins, cyanogenic glycosides, sapogenin, hydroxylupanin, ammodendrine, phytate and narigenin (Ikezu *et al.,* 2023). The presence of these phytochemicals has been evaluated and validated in both the traditional use and pharmacological use of *Emilia praetermissa.*

1.6.1 ALKALOIDS

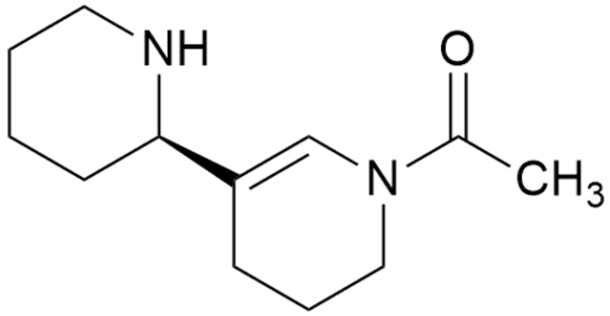
Alkaloids generally are a diverse class of organic compounds containing at least one nitrogen atom typically present in a heterocyclic ring. The nitrogen atom present often imparts alkali-like properties (Gutiérrez-Grijalva *et al.,* 2020; Leclerc and Fournier, 2024). Alkaloids are primarily found in plants but can also be found in fungi, bacteria and some animal species (Gutiérrez-Grijalva *et al.,* 2020). Alkaloids possess diverse and important physiological effects in humans and animals.

There are various classes of heterocyclic alkaloids, and they include piperidine alkaloids, indole alkaloids, pyrrolidine alkaloids, tropane alkaloids, quinolizidine alkaloids, and purine alkaloids (Kaur *et al.,* 2015). Some important alkaloids found in *Emilia praetermissa* include:

1.6.1.1 Piperidine Alkaloid:

Piperidine alkaloids are a subset of nitrogen containing natural products derived from the six-membered heterocycle piperidine. The core structure consists of an sp^3-hybridized ring containing one nitrogen atom and five carbon atoms. This structure is often bonded to various substituents (e.g., acyl, alkyl, or aromatic groups) giving rise to simple monomeric compounds. Piperidine alkaloids possess diverse structures and pharmacological activities highlighting their importance in the field of medicine, pharmacology and chemistry (Szõke *et al.,*2013). Some few examples of Piperidine alkaloid include; Coniine; which acts as a nicotinic receptor antagonist inducing neuromuscular blockade (Rischer, 2017), Lobeline; which modulates dopamine transporters in the

brain and nicotinic receptors (Shah *et al.,* 2024)**.** Another example of piperidine alkaloid is Piperine. It enhances gastric mucosal defense and exhibits analgesic properties by interacting with opioid and GABAergic pathways (Rashed, 2021). Other examples include Matrine, Aloperine and Solenopsin. An example of a piperidine alkaloid found in *Emilia praetermissa* is ammodendrine.



1-[5-[(2r)-piperidin-2-yl]-3,4-dihydro-2h-pyridin-1-yl] ethanone
(Ammodendrine)

Fig 1

Ammodendrine is a piperidine alkaloid predominantly isolated from the seeds of Lupinus formosus (a lupine species) and also present in *Emilia praetermissa* (Panter *et al.,* 1998; Lee *et al.,* 2008). It belongs to the N-acylpiperidine subclass and is biosynthetically derived from lysine via a piperidine intermediate. Ammodendrine occurs as a racemic mixture of D- and L-enantiomers. Ammodendrine is known for its teratogenicity in livestock, causing malformations in pregnant animals and it has limited pharmacological use (Panter *et al.,* 1998; Lee *et al.,* 2008).

1.6.1.2 Quinolizidine Alkaloid:

Quinolizidine alkaloids are a class of plant-derived nitrogenous compounds sharing the bicyclic quinolizidine (octahydro-2H-quinolizine) structure (Cely-Veloza *et al.,* 2023). They arise biosynthetically from lysine via decarboxylation to cadaverine, oxidative cyclisation to Δ^1-piperideine, and aldol-type coupling of two such units. A few examples of quinolizidine alkaloids include Sparteine-type alkaloids and Lupinine-type alkaloids, which inhibit the growth of Gram-

positive and Gram-negative bacteria. Matrine and other phenanthroquinolizidines induce apoptosis and inhibit proliferation in various human cancer cell lines (Cely-Veloza *et al.,* 2023; Yang *et al.,* 2020). These compounds modulate mitochondrial pathways and caspase activation, positioning them as promising anticancer leads. (Yang *et al.*, 2020)

An example of a quinolizidine alkaloid present in *Emilia praetermissa* is hydroxylupanine (Ikezu, 2023).

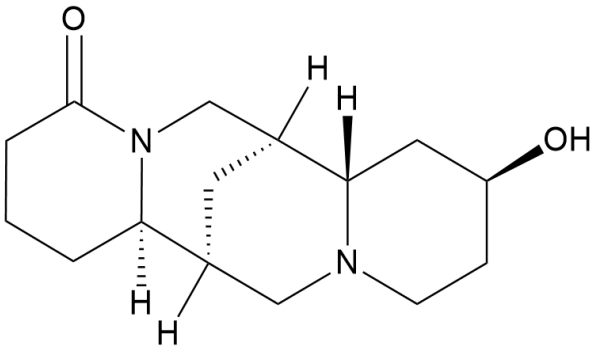


Fig 2

(1S,2R,9S,10S,12S)-12-hydroxy-7,15-diazatetracyclo[7.7.1.02,7.010,15]heptadecan-6-one (Hydroxylupanine)

Hydroxylupanine is a characteristic piperidine-type alkaloid predominantly isolated from the seeds of sweet lupins (Lupinus spp.), where it contributes to the plant's defensive chemistry. Hydroxylupanine (often referred to as 13-hydroxylupanine) is a hydroxylated derivative of lupanine that features a tetracyclic quinolizidine ring system with a hydroxyl group at the 13-position. Hydroxylupanine have been evaluated to show significant antibacterial activities (Erdemoglu *et al.,* 2007)

1.6.2 PHENOLICS

Phenolic compounds, or phenolics, are plant secondary metabolites containing one or more phenol units—an aromatic ring bonded to at least one hydroxyl (Singla *et al.,* 2019; Al Mamari, 2020). They range from simple molecules (e.g., phenol, hydroxybenzoic acids) to complex polyphenol

molecules (e.g., tannins, lignans) and are classified by their number of phenolic rings and linkage types into simple phenolics (monophenols and phenolic acids) and polyphenols (flavonoids, stilbenes, lignans, and tannins) (Singla *et al.,* 2019; Tijjani *et al.,* 2020). Phenolics are ubiquitous across the plant kingdom, accumulating in fruits, vegetables, cereals, and beverages such as tea and wine. (Dai *et al.,* 2010). In plants, they defend against ultraviolet radiation, pathogens, and herbivores, and contribute to colour, flavour, and astringency. By donating hydrogen atoms or electrons, phenolics scavenge reactive oxygen species (ROS), protecting cellular components from oxidative damage (Hajam *et al.,* 2023). They also chelate metal ions involved in radical formation and modulate enzymes related to oxidant production, reinforcing plant stress tolerance. Dietary phenolics exhibit potent antioxidant, anti-inflammatory, antibacterial, and anticancer activities in vitro and in vivo (Anantharaju *et al.,* 2016; Laganà *et al.,* 2019; Afnan *et al.,* 2022). Phenolic compounds that have been previously identified to be present in *Emilia praetermissa* include:

1.6.3 FLAVONOIDS

Flavonoids are one of the largest subclasses of polyphenolic compounds, defined by a 15-carbon skeleton (C6–C3–C6) synthesised via the shikimate and phenylpropanoid pathways (Rehan, 2021; Zhuang *et al.,* 2023). Flavonoids are subdivided into six classes based on the oxidation state and substitution pattern of the central pyran ring, and they include flavonols (e.g., quercetin, kaempferol), flavones (e.g., apigenin, luteolin), flavanones (e.g., hesperidin, naringenin), flavanols (e.g., catechin, epicatechin), anthocyanins (e.g., cyanidin, delphinidin), and isoflavones (e.g., genistein, daidzein) (Hrazdina, 2000; Zhuang *et al.,* 2023). Each subgroup has distinct properties and is found in various plant sources.

In plants, flavonoids protect against UV radiation (Takeda *et al.*, 1994, Del Valle *et al.*, 2020), contribute to flower and fruit colouration for pollinator attraction, and exhibit antibacterial properties to combat pathogens (Saini *et al.*, 2017). In humans, flavonoids provide dietary antioxidants that scavenge free radicals, supporting cardiovascular, neuroprotective, and anti-ageing effects when consumed through fruits, vegetables, tea, and cocoa (Rees *et al.*, 2018; Caraffa, 2021). They act as anti-inflammatory and anticancer agents studied in both preclinical and clinical settings for modulation of signalling pathways involved in cell proliferation and apoptosis (Ullah *et al.*, 2020; Pyo *et al.*, 2024) and phytoestrogenic compounds (especially isoflavones) used to alleviate menopausal symptoms and support bone health in nutraceutical formulations (Welch and Hardcastle, 2014; Sivakumar *et al.*, 2024). and also act as lead structures in drug discovery programmes targeting diabetes, metabolic syndrome, and neurodegenerative disorders due to their ability to modulate enzymes and receptors. Flavonoids that have been identified in *Emilia praetermissa* include Flavones, Flavanone, Anthocyanidins, Narigenin and Proanthocyanidin.

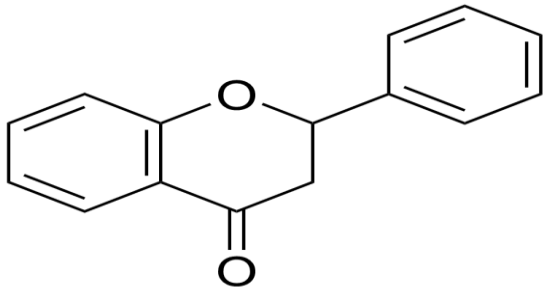


Fig 3 2-phenylchroman-4-one
(Flavanone)

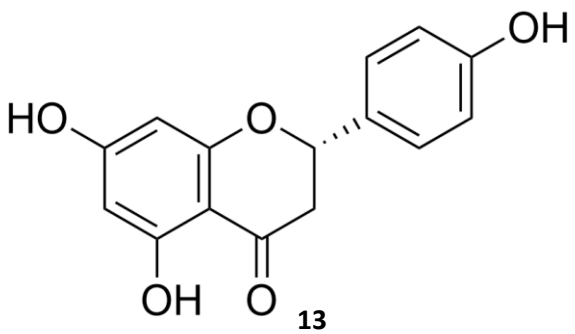
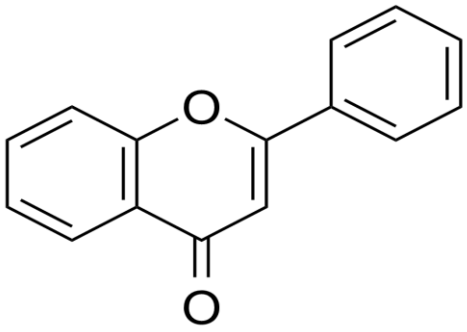


Fig 4

(2S)-5,7-dihydroxy-2-(4-hydroxyphenyl)-2,3-dihydrochromen-4-one
(Narigenin)

Fig 5



2-phenyl-4*H*-chromen-4-one
(Flavone)

1.6.4 TANNINS

Tannins are a fascinating group of naturally occurring compounds found in many plants, especially in their bark, leaves, seeds, and fruits (Jaiswal *et al.,* 2018). They are best known for their astringent taste, ability to precipitate protein and their wide range of biological (Cosme *et al.,* 2025) and industrial applications.

Pharmacologically, tannins exhibit antioxidant, antibacterial, antiviral, and anti-inflammatory properties, making them valuable in treating burns, diarrhoea, and infections. Their presence in foods and beverages like tea, wine, and fruit juices contributes not only to flavour and mouthfeel but also to potential health benefits, including cardioprotective and anticancer effects (Cosme *et al.,* 2025).

Despite their benefits, excessive tannin intake may interfere with nutrient absorption and cause gastrointestinal discomfort (Jaiswal *et al.,* 2018). Industrially, tannins are used in leather tanning,

ink production, and clarification of beverages due to their protein-binding and coagulating properties (Aires, 2020).

Tannins are polyphenolic compounds, meaning they have multiple phenol units that allow them to bind to proteins and other organic molecules (Okuda and Ito, 2011; Jaiswal *et al.,* 2018). Tannins are classified into two classes, namely, hydrolysable tannins, which break down in water to form simpler substances like gallic acid, and condensed tannins (proanthocyanidins), which are more complex and resistant to hydrolysis. In plants, tannins provide defensive functions by protecting against herbivores and protecting plants from bacterial infections (Jaiswal *et al.,* 2018; Iqbal & Poór, 2024). Tannins are found in oak bark, tea leaves, grapes, persimmons, pomegranates, and even chocolate (Okuda and Ito, 2011; Aires, 2020). In research studies, tannins have also been identified to be present in ethanolic leaf extract of *Emilia praetermissa (*Ikezu, 2023*).*

1.6.5 SAPONIN

Saponins are a class of naturally occurring plant glycosides known for their ability to produce soap-like foams when mixed with water (Cheok *et al.,* 2014; Timilsena *et al.,* 2023). The name "saponin" is derived from the Latin word *sapo,* meaning soap, which reflects their amphiphilic nature meaning having both hydrophilic and hydrophobic components (Cheok *et al.,* 2014). Chemically, saponins consist of a non-sugar aglycone (either a triterpenoid or steroid) linked to one or more sugar chains, such as hexoses or uronic acids, which contribute to their solubility and biological activity (Tamura *et al.,* 2012; Timilsena *et al.,* 2023).

These compounds are widely distributed across the plant kingdom. They occur in legumes like soybeans and chickpeas, grains such as oats and quinoa, and vegetables including garlic and spinach (Kareem *et al.,* 2022; Timilsena *et al.,*2023). Medicinal plants such as ginseng (*Panax*

spp.), licorice (*Glycyrrhiza glabra*), and soapwort (*Saponaria officinalis*) are also rich in saponin (Timilsena *et al.,* 2023). In quinoa, for instance, saponins are concentrated on the seed surface, contributing to its bitter taste and foaming behavior when rinsed (Cheok *et al.,* 2014). Saponins have attracted considerable attention due to their broad spectrum of biological activities. They are known to lower cholesterol levels by binding to bile acids and cholesterol in the gastrointestinal tract, reducing absorption and promoting excretion. Additionally, saponins exhibit antioxidant and anti-inflammatory properties, helping to neutralize free radicals and modulate immune responses. Their antibacterial and antiviral effects have made them promising candidates for pharmaceutical applications, and some saponins have demonstrated cytotoxicity against cancer cells, suggesting potential roles in cancer prevention and therapy (Kareem *et al.,* 2022; Timilsena *et al.,* 2023).

Industrially, saponins are valued for their surface-active properties. In the food sector, they are used as natural emulsifiers and foaming agents. In cosmetics, they serve as gentle cleansers and lathering agents in products like shampoos and facial washes. In pharmaceuticals, saponins are employed as vaccine adjuvants to enhance immune responses. Their ability to form complexes with drugs also makes them attractive for use in drug delivery systems (Cheok *et al.,* 2014; Timilsena *et al.,* 2023).

Despite their many benefits, saponins are not without limitations. In high concentrations, they can exhibit antinutritional effects by binding to essential minerals such as iron and zinc, thereby reducing their bioavailability (Samtiya *et al.,* 2020). Excessive intake may also cause gastrointestinal discomfort, including nausea and bloating. Moreover, some saponins possess haemolytic activity, meaning they can disrupt red blood cells, although this effect is typically not observed at dietary levels (Hassan *et al.,* 2009; Timilsena *et al.,* 2023).

Saponins in *Emilia praetermissa* contribute to its antibacterial activity, as demonstrated in studies where ethanolic and chloroform extracts showed zones of inhibition against *Escherichia coli* and *Staphylococcus aureus (*Ikezu, 2023). This suggests that the saponins may play a role in disrupting bacterial cell membranes or interfering with bacterial metabolism. Additionally, saponins are known for their antioxidant and anti-inflammatory properties, which may support the plant's traditional use in managing gastrointestinal and cardiovascular conditions.

Although specific structural details of the saponins in *Emilia praetermissa* have not yet been fully characterised, their presence aligns with the plant's observed therapeutic effects, including antibacterial, antihypertensive, and possibly immunomodulatory actions. For example, aqueous extracts of the plant have been shown to significantly reduce blood pressure and improve lipid profiles in salt-induced hypertensive rats, further supporting the pharmacological relevance of its phytochemical constituents (Ebhohon *et al.,* 2025).

1.7 LITERATURE REVIEW

Emilia praetermissa (Asteraceae) is a tropical African herb traditionally used to treat wounds, diarrhoea, respiratory complaints, and ear infections, and recent experimental studies provide preliminary laboratory support for some of these uses (Afolayan *et al.,* 2017; Ikezu, 2023). The species is recorded in West African floras and is commonly collected in regions where traditional healers use related Emilia species, which has prompted several pharmacological and phytochemical investigations (Odion *et al.,* 2024; Odetola *et al.,* 2012).

Phytochemical screenings of *Emilia praetermissa* consistently report the presence of alkaloids, flavonoids, saponins, tannins, and phenolic compounds, and these metabolite classes are proposed

to underlie the species' reported biological activities and justify further fractionation and compound isolation (Hanson, 2003; Edeoga *et al.,* 2005; Ikezu, 2023; Odion *et al.,* 2024). In vitro bioassays have shown cytotoxic potential in crude extracts of *Emilia praetermissa*, indicating bioactive that merit bioassay-guided isolation, structural elucidation, and comparative testing against nonmalignant cells to determine selectivity (Odion *et al.,* 2024).

In vivo studies using stress-induced ulcer models in Wistar rats report that *Emilia praetermissa* leaf extracts alter haematological indices and plasma biochemical markers, suggesting possible therapeutic effects on ulcer pathology while highlighting the need for systematic dose-finding and safety evaluations to establish therapeutic windows (Odetola *et al.,* 2012). Antibacterial investigations using agar diffusion and MIC assays have demonstrated activity of crude extracts and dichloromethane fractions against Gram-positive and Gram-negative bacteria commonly implicated in human infections, including Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa; fungal tests have included Aspergillus flavus and Candida albicans (Afolayan *et al.,* 2017). Reported MICs for active crude extracts ranged from 3.125 to 12.5 mg/ml, indicating in vitro potency for unrefined preparations but not directly translatable therapeutic dosing without pharmacokinetic and toxicity data (Afolayan *et al.,* 2017).

Preliminary antifungal screening of ethanol leaf extracts also indicates inhibitory effects against common fungal pathogens, supporting the need for standardized extraction methods, precise MIC determinations, and mechanistic studies to assess the therapeutic potential of *Emilia praetermissa*-derived antifungal agents (Nwankwo *et al.,* 2025). Ethnobotanical evidence linking Emilia species to gastrointestinal and topical wound treatments provides an ethnopharmacological

rationale for the laboratory research on ulcers and antibacterials (Odetola *et al.*, 2012; Odion *et al.*, 2024).

Emilia praetermissa has drawn interest from ethnobotanical and pharmacological investigators because its traditional uses imply the presence of bioactive compounds with protective properties (Odion *et al.,* 2024). Phytochemical analyses of the non-volatile extracts have revealed high concentrations of phenolic compounds and flavonoids in *Emilia praetermissa*, groups of molecules commonly linked to radical-scavenging and metal-chelating functions (Odion *et al.,* 2024).

Studies that profiled the plant's essential oils report a complex array of terpenoids and other volatile constituents, and comparative work indicates that both the chemical makeup of the oils and their measured antioxidant activity change with the extraction technique employed (Bosson *et al.,* 2025). When tested by standard in vitro methods such as DPPH and ABTS assays, both essential oil fractions and crude polar extracts of *Emilia praetermissa* show measurable radical-scavenging effects, though the observed potency varies according to extraction method, solvent polarity, and the specific chemical composition of each fraction (Bosson *et al.,* 2025; Odion *et al.*, 2024).

In vivo experiments using the leaf ethanol extract documented gastroprotective effects in rodent models accompanied by improved antioxidant markers; animals treated with the extract exhibited elevated endogenous antioxidant defenses and lowered biochemical indicators of oxidative damage (Ndji *et al.,* 2016). Phytochemical screening in that same study attributed the biological activity to phenolic and flavonoid constituents, offering a credible mechanistic explanation involving radical neutralization and metal chelation (Ndji *et al.,* 2016).

Comparative assays highlight different activity profiles between essential oils and polar extracts, reflecting the distinct chemistries of volatile terpenoids versus non-volatile phenolics and their differing abundances and modes of action across fractions (Bosson *et al.,* 2025). Mechanistically, the antioxidant effects reported for *Emilia praetermissa* are compatible with several actions: direct scavenging of free radicals by phenolics and flavonoids, chelation of transition metal ions by polyphenolic structures, modulation of endogenous antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT), and prevention of lipid peroxidation in cellular membranes (Odion *et al.,* 2024; Ndji *et al.,* 2016). The in vivo findings that suggest modulation of cellular antioxidant systems bolster the idea that crude extracts act through biological pathways as well as through direct chemical scavenging (Ndji *et al.,* 2016).

Nevertheless, gaps remain in the literature: there are few studies isolating and structurally characterizing the specific antioxidant molecules in *Emilia praetermissa,* dose–response relationships are not well defined, and lack of standardized extraction protocols hampers comparison between studies (Bosson *et al.,* 2025; Odion *et al.,* 2024). Limited toxicological data and scarce information on the bioavailability and metabolism of the active constituents further restrict the ability to evaluate therapeutic potential and safety before any clinical consideration (Ndji *et al.,* 2016; Odion *et al.,* 2024).

1.7.1 JUSTICATION FOR THE STUDY

1. Unverified traditional use: *Emilia praetermissa* is used widely for treatment of infections, wounds healing, gastrointestinal problems, management of hypertension and convulsions, yet controlled chemical and bioactivity data for the ethanolic aerial fraction are limited, making scientific validation necessary.

2. Incomplete chromatographic characterization: Existing reports note several phytochemicals in the species, but a systematic HPLC and GC-MS profile of the ethanolic aerial fraction is lacking, preventing reliable identification of which specific constituents drive biological effects.
3. Urgency from antibacterial resistance: The global rise in antibacterial resistance increases the need for new antibacterial scaffolds; locally available medicinal plants such as *Emilia praetermissa* represent accessible sources of novel bioactive compounds that could address resistant infections.
4. Mechanistic linkage between compounds and effects: Correlating quantified phytochemicals with measured antibacterial and antioxidant activities will clarify which classes of compounds (for example flavonoids, alkaloids, saponins) are responsible for observed traditional benefits.
5. Prioritization for further research: The combined phytochemical and bioactivity profile will allow evidence-based prioritization of *Emilia praetermissa* for advanced pharmacological, toxicological, and formulation research, maximizing efficient use of limited research resources.
6. Measuring antioxidant capacity and linking it to identified phenolic and flavonoid constituents provides mechanistic support for wound healing, gastroprotective, and cardioprotective activities reported in ethnomedicine.

1.7.2 AIMS

The aim of the study is to characterize the chemical composition and evaluate the antibacterial and antioxidant activities of the ethanolic aerial fraction of *Emilia praetermissa*, with the goal of

linking phytochemical constituents to observed bioactivities and assessing the extract's potential for further drug discovery and therapeutic development.

1.7.3 OBJECTIVES

1. To prepare and fractionate the ethanolic extract of the aerial parts of *Emilia praetermissa* using standard extraction and column fractionation techniques.
2. To identify and quantify phytoconstituents in the ethanolic fraction by High Performance Liquid Chromatography (HPLC).
3. To profile volatile and semi-volatile constituents of the ethanolic fraction using Gas Chromatography–Mass Spectrometry (GC-MS).
4. To determine in vitro antibacterial activity of the ethanolic fraction against selected clinical bacterial isolates using agar well diffusion and to measure inhibitory zone diameters.
5. To establish the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the ethanolic fraction for each tested organism.
6. To quantify and characterise the antioxidant capacity of the ethanolic fraction of the aerial part of *Emilia praetermissa* using appropriate in vitro assays and to relate antioxidant activity to chromatographically identified phytoconstituents.

CHAPTER TWO

MATERIALS AND METHODS

2.1 MATERIALS, APPARARUS AND REAGENTS

The Equipment and solvents used for this study encompassed various components. They include; 10 mm cork borer, 20 % Tween 80, absolute ethanol, acetone, beakers, Bunsen burner, cotton wool, dichloromethane, disinfectant (Purit, soap, detergent), a disinfectant jar, distilled water, an electric milling machine, foil paper, forceps, a freezer, gas cylinder, glass column, glass funnel, glass stirrer, hot air oven, incubator, an inoculating loop, lighter, maceration jar, measuring cylinder, micropipette, mortar, n-hexane, paper tape, pestle, Petri dish, plant extract, portable autoclave, porcelain dish, anhydrous pyridine, Potassium hydroxide, retort stand, rotatory evaporator, sample holder, separatory funnel, silica gel (mesh size 60–120), standard ciprofloxacin, sterile Mueller-Hinton nutrient agar, stock solutions of Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus. A swab stick, test tubes, a test tube holder, test tube rack, transparent measuring ruler, tripod stand, universal bottles, a water bath, and analytical weighing balance.

2.2 Plant Collection and Identification

The aerial part of the plant was collected from EDPA Ishior, in Ovia North East Local Government, Benin city, Edo state, Nigeria (Latitude: 6° 20′ 1.32″ N, Longitude: 5° 36′ 0.53″ E) in September 2024 and was taken for identification and authentication at the Department of Plant Biology and Biotechnology, University of Benin. It was identified by Professor Emmanuel Aigbokah as *Emilia praetermissa* with the herbarium number UBH-E407 and the plant was deposited in the herbarium for future reference.

2.3 Extraction Process

The harvested aerial part of *Emilia praetermissa* was placed under shade to dry for two weeks. It was pulverized to powder using a clean electric grinding machine and stored in a sterile air-tight container until required. 600 g of powdered plant was macerated with 2 × 2.5 L Ethanol 70 % (v/v) for a period of 3 days with initial agitation for every 30 min for 2 h at room temperature. The filtrate was decanted and filtered with a size 1 filter paper. The filtrate was then concentrated to dryness in a hot water bath at 60 ˚C after which the extract was stored in the refrigerator at a temperature of 4 ˚C for preservation until use.

2.4 Fractionation Process

The extract (20 g) was weighed with an analytical weighing balance and then transferred into a clean mortar. An equivalent weight of dry silica powder (60-120 mesh size) was added to the mortar, and the mixture was triturated thoroughly until a homogenous, fine mixture was attained.

A glass fractionating column was washed thoroughly and allowed to dry, and a small plug of cotton wool was gently inserted at the base of the column. The dry packing procedure began by carefully pouring silica powder (mesh size 60-120) into the column in small portions using a clean glass funnel. After each addition, the column was lightly tapped to settle the particles evenly, eliminate air pockets and achieve a flat, even surface.

A small amount of cotton wool was placed on top of the packed silica bed to act as a buffer layer. After which the previously triturated silica-extract mixture was introduced into the column in small portions using a small glass funnel with intermittent tapping to settle the particles, remove air pockets and achieve a flat, even surface.

For elution, the solvents were applied sequentially in order of increasing polarity. n-Hexane, Chloroform and Ethanol. Using a side wall pouring technique with a small glass funnel to prevent disruption of the packed bed the solvents were poured into the column and allowed to run down through the column. The stopcock at the base of the column was opened to allow the eluents to flow through and be collected in properly labelled receiving beakers which had been pre-rinsed with their respective solvents to prevent contamination,

After collection, the fractions were left to air dry under safe and controlled condition for further analysis. This setup allowed for a systematic separation of phytochemical constituents based on polarity.

2.5 Chromatographic process

2.5.1 Sample Preparation for High Performance Liquid Chromatography

0.2 g of Ethanolic fraction of *Emilia praetermissa* was weighed and transferred in a test tube and 15 mL ethanol and 10 mL of 50 %(w/v) potassium hydroxide was added. The test tube was allowed to react in a water bath at 60 $^{\circ}$C for 3h. After the reaction time, the reaction product contained in the test tube was transferred to a separatory funnel. The tube was washed successfully with 20 mL of ethanol, 10 mL of cold water, 10 mL of hot water and 3 mL of hexane, which was all transferred to the funnel. These extracts were combined and washed three times with 10 mL of 10 %v/v ethanol aqueous solution. the ethanol solvent was evaporated. The sample was solubilized in 1000 µL of pyridine of which 200 µL was transferred to a vial for analysis.

2.5.2 High-Performance Liquid Chromatography Procedure.

High-performance liquid chromatography (HPLC) analysis was performed using Shimadzu LC-10AD dual binary pumps, a Shimadzu CTO-10AS column oven, and a Shimadzu Prominence

SPD-20A UV/Vis detector. The analysis was performed using a C-12 reverse phase column (Phenomenex, Gemini 5 µ thick, 200 mm length × 4.8 mm internal diameter). The mobile phase consisted of acetic acid-acidified deionised water (pH 2.8) as solvent A and acetonitrile as solvent B at a flow rate of 0.8 mL/min.The column was equilibrated with 5 % solvent B for 20 min after each injection of samples. The column temperature was set to 38 °C, and the injection volume was 20 µL. The wavelengths were set to 280 nm. Standard compounds were used to qualify and quantify compounds from the ethanolic extract of the aerial part of *Emilia praetermissa* following comparison of the retention times and peak areas with pure standard compounds utilising the method of external standards to construct a calibration curve (Odion *et al.,* 2025).

2.5.3 Sample Preparation for Gas Chromatography-Mass Spectrometry

1g of Ethanolic fraction of *Emilia praetermissa* was weighed and transferred in a test tube and 25mL of ethanol was added. The test tube was allowed to react in a hotplate at 60 °C for 90mins. After the reaction time, the reaction product contained in the test tube was transferred to a separatory funnel. The tube was washed successfully with 20 mL of ethanol, 10 mL of cold water, 10 mL of hot water and 3 mL of hexane, which was all transferred to the funnel. These extracts were combined and washed three times with 10 mL of 10 %(v/v) ethanol aqueous solution. The solution as dried with anhydrous sodium sulfate and the solvent was evaporated. The sample was solubilized in 1000 µL of pyridine of which 1µL was transferred to a vial for analysis.

2.5.4 Gas Chromatography Mass Spectrometry Procedure

The gas chromatography–mass spectrometry (GC-MS) analysis was conducted using an Agilent 7890A gas chromatograph coupled with an Agilent 5975C inert mass selective detector equipped with a triple-axis detector. The system was operated and monitored using Agilent's MS Solution

software, which facilitated both instrument control and data acquisition. Sample injection was performed automatically using a 10 µL syringe.

Chromatographic separation was achieved using a capillary column (Agilent 19091-433HP-5Ms) coated with 5 % phenyl methyl siloxane as the stationary phase. The column measured 30 metres in length, with an internal diameter of 0.25 µm and a film thickness of 250 µm. Helium was used as the carrier gas, flowing at a rate defined by the instrument's parameters. A 1 µL sample of Ethanolic extract of *Emilia praetermissa* was injected in split mode with a split ratio of 1:50, and the injector temperature was maintained at 300°C. The oven temperature programme began at 35°C and was held for 5 minutes to allow initial separation. It was then ramped to 150 °C at a rate of 40 °C per minute, followed by a rapid increase to 250 °C at 200 °C per minute. The final temperature was held for 5 minutes, resulting in a total run time of 47.5 minutes.

Mass spectrometric detection was carried out using electron ionisation (EI) mode. The ion source was maintained at 250 °C, while the interface temperature was set to 300 °C. The system operated at a pressure of 16.2 psia, with an out time of 1.8 minutes. Compound identification was performed by comparing the acquired mass spectra with reference spectra from the NIST II library, ensuring accurate and reliable identification of analytes. (Odion *et al.*, 2025).

2.6 In-vitro Antioxidant Activity of *Emilia praetermissa*

In vitro antioxidant activity was carried out on the ethanolic fraction of *Emilia praetermissa* using four different assays which include DPPH, FRAP, ABTS and TAC

2.6.1 DPPH Antioxidant assay

To evaluate the free radical scavenging capacity of the extract against 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals, a slightly modified version of the method described by Brand-Williams et al. (1995) was employed. In this procedure, 0.5 mL of a 0.3 mM DPPH solution prepared in methanol was mixed with 2 mL of the extract at varying concentrations ranging from 200 to 1000 μg/mL. The resulting mixtures were placed in reaction tubes, which were then gently shaken and incubated for 15 minutes at room temperature in the absence of light to prevent photodegradation.

Following incubation, the absorbance of each sample was measured at a wavelength of 517 nm using a spectrophotometer. All experiments were conducted in triplicate to ensure reproducibility and accuracy. Ascorbic acid, prepared at the same concentration range as the test extracts, served as the standard control. Additionally, a blank sample was prepared by combining 0.5 mL of the DPPH solution with 2 mL of methanol, and it was subjected to the same treatment as the test samples.

The percentage of DPPH radical scavenging activity was calculated using the formula:

$$\text{DPPH radical scavenging activity (\%)} = \left(\frac{A_{\circ} - A_{t}}{A_{\circ}}\right) \times 100\%$$

where $A_{\circ}$ represents the absorbance of the blank (DPPH solution with methanol) and A_t denotes the absorbance of the sample containing either the extract or the standard.

2.6.2 FRAP Antioxidant assay

The Ferric reducing antioxidant power (FRAP) assay was performed following a modified Benzie and Strain (1996) method. Fresh FRAP reagent was prepared by mixing acetate buffer (300 mM, pH 3.6), 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mM HCl, and 20 mM ferric chloride ($FeCl_3 \cdot 6H_2O$) in the specified proportions to yield the working solution. Aliquots of the extract (1 mL) at concentrations of 200 to 1000 µg/mL were each combined with 1.5 mL of the freshly prepared FRAP reagent and incubated at 37 °C for 30 minutes. After incubation, the increase in absorbance was read at 593 nm. A calibration curve was constructed using $FeSO_4$ standards and ascorbic acid was included as a positive control. FRAP values for the extracts were calculated by interpolation from the $FeSO_4$ standard curve and expressed as mM Fe per gram of extract.

2.6.3 ABTS Antioxidant assay

The ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) Antioxidant assay was performed following the method of Pellegrini *et al.,* (1999). To 0.5 mL of each extract concentration of 200 to 1000 µg/mL, 0.3 mL of ABTS radical cation ($ABTS^{\bullet+}$) solution and 1.7 mL of phosphate buffer (pH 7.4) was added. The mixture was incubated at room temperature for 10 minutes, after which the absorbance was measured at 734 nm. Percent inhibition was calculated as described below.

$$\text{ABTS radical scavenging activity (\%)} = \left(\frac{A_{\circ} - A_{t}}{A_{\circ}}\right) \times 100\%$$

where $A_{\circ}$ represents the absorbance of the blank (ABTS solution with methanol) and A_t denotes the absorbance of the sample containing either the extract or the standard.

2.6.4 TAC assay

Total antioxidant activity was determined using the phosphomolybdenum assay (Prieto *et al.,* 1999). The assay relies on the reduction of molybdenum(VI) to molybdenum(V) by antioxidants in the sample, producing a green phosphomolybdenum(V) complex under acidic conditions. To perform the assay, 3.0 mL of each extract concentration (200 to 1000μg/mL) was mixed with 1.0 mL of molybdate reagent. The reaction mixtures were incubated at 95 °C for 90 minutes, then allowed to cool to room temperature for 20–30 minutes. Absorbance was measured at 695 nm, and ascorbic acid was used as the standard.

2.7 Antibacterial Activity of *Emilia praetermissa*

2.7.1 Specimen Collection

Bacteria used in this study were selected bacterial isolates obtained from the University of Benin Teaching Hospital, Benin City, Edo State, Nigeria. They are Staphylococcus *aureus, Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, Enterobacter cloacae* and *Bacillus cereus.*

2.7.2 Preparation of Test Organism

All test bacterial isolates were maintained in 20 % glycerol broth and frozen. Prior to use, test microorganisms were sub-cultured from stock into sterile nutrient agar plates and were incubated overnight at 37 °C. After incubation, bacterial colonies from the overnight plates of each microorganism were suspended in sterile broth for 12 h and adjusted to 0.5 McFarland standard to give an inoculum size of approximately 10 CFU/ml for each microorganism.

2.7.3 Antibacterial Susceptibility Tests and Determination of Inhibitory Zone Diameter (IZD)

An antibacterial susceptibility test was performed to evaluate the antibacterial activity of the ethanolic fraction of *Emilia praetermissa* using the agar well diffusion method (Perez *et al.,* 1990).

Six different bacterial isolates were selected, and each was assigned to a separate sterile Petri dish containing 30 mL of Mueller-Hinton agar. After the agar solidified, the plates were dried in a hot air oven at 40°C for approximately 5 minutes to remove surface moisture. A standardized bacterial suspension of each bacterial isolate containing approximately 10^7 colony-forming units per milliliter (CFU/mL) was prepared, and a sterile inoculating wire loop was used to streak each agar plate with its corresponding bacterial isolate. The wire loop was sterilized by flaming after each use to prevent cross-contamination. Two wells were bored into each agar plate using a sterile 10mm cork borer, and the base of the well was sealed with 0.02 mL Mueller-Hinton agar. A concentration of 200 mg/mL Ethanolic fraction of *Emilia praetermissa* was prepared, and 0.2 mL of the stock solution was introduced into one of the wells created in each petri dish for each isolate. A solution of 1.6 mg/mL of standard ciprofloxacin was also prepared to be used as the control for the analysis, and 0.2 mL of the standard solution was introduced into the second well created in each petri dish. The plates were incubated at 37 °C for 18–24 hours, and the growth of the organism in each petri dish was observed to assess its resistance or susceptibility to 200 mg/mL of the Ethanolic fraction of *Emilia praetermissa* for all bacterial isolates. The zones of inhibition around the wells were measured in millimeters to determine the antibacterial efficacy of the extract against each bacterial strain.

2.7.4 Determination of minimum inhibitory concentration (MIC)

The agar dilution method of Afoyan and Meyer (1997) was used in this study for the determination of the Minimum Inhibitory Concentration (MIC) of the ethanolic fraction of *Emilia praetermissa*. Four sterile petri dishes were used and labelled 5 mg, 10 mg, 20 mg and 40 mg. A twofold serial dilution of the test ethanolic fraction was prepared using 20% Tween 80 as the diluent to give concentrations of 50 mg, 100 mg, 200 mg and 400 mg. 1 mL of the prepared 50mg of the Ethanolic

fraction of *Emilia praetermissa* was introduced into a universal bottle containing 9 mL of Mueller-Hinton molten agar, and the mixture was swayed gently to ensure homogeneity. The mixture was poured into the petri dish labelled 5 mg. The agar was allowed to solidify, and the plates were dried in a hot air oven at 40 °C for approximately 5 minutes to remove surface moisture. The same procedure was repeated for 100 mg, 200 mg and 400mg of the test fraction. Using a marker, the base of each Petri dish was divided into six sections, and these sections in each plate were labelled to represent each bacterial isolate used for the analysis. From the standardized bacterial suspension of each bacterial isolate containing approximately 10^7 colony-forming units per milliliter (CFU/mL) prepared, a sterile inoculating wire loop was used to streak each section on each agar plate with its corresponding bacterial isolate. The wire loop was sterilized by flaming after each use to prevent cross-contamination. The plates were incubated at 37 °C for 18–24 hours. After the incubation, the plates were visually examined for growths on the inoculated spots, and the lowest concentration of the Ethanolic fraction of *Emilia praetermissa* that inhibits visible growth was considered as the Minimum Inhibitory Concentration (MIC).

2.7.5 Determination of Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentration was determined using the agar plate method. It was determined from the agar dilution of the MIC tests by sub-culturing into freshly prepared agar plates that did not contain any test extract. The dilution plates were then incubated at 37 °C for 18-24 hours. After incubation, the plates were visually examined for growths in the inoculated spots. The lowest concentration of the extract that showed no growth was considered as the Minimum Bactericidal Concentration.

2.7.6 Determination of the Mode of action

The bacteriostatic or bactericidal nature of the ethanolic aerial fraction was determined by calculating the MBC/MIC ratio from MIC and MBC values obtained. The MBC/MIC ratio was calculated by dividing the MBC (mg/mL) by the MIC (mg/mL) for bacteria whose MBC and MIC values were observed.

Values of MBC/MIC ≤ 4 were interpreted as bactericidal and MBC/MIC > 4 were interpreted as bacteriostatic.

2.8 Statistical Analysis:

A paired t-test was conducted using Microsoft Excel to compare the antibacterial activity (zone of inhibition) of 40 mg/mL ethanolic fraction of *Emilia praetermissa* against 1.6 mg/mL ciprofloxacin. This test was used to determine whether the observed differences in antibacterial efficacy were statistically significant.

CHAPTER THREE

RESULT

3.1 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Phytochemical analysis of the extract was carried out using chromatographic separation, with retention time, peak area, and calculated concentrations recorded for each identified compound. A total of 20 bioactive constituents were detected, spanning diverse phytochemical classes including alkaloids, flavonoids, glycosides, phenolics, steroids, and other secondary metabolites. Retention times ranged from 0.210 min (*Lunamarin*) to 44.170 min (*Ephedrine*), with concentrations varying between 3.8894 µg/mL (*Resveratrol*) and 19.2702 µg/mL (*Phytate*). Notably, high concentrations were observed for *phytate* (19.27 µg/mL), *cyanogenic glycoside* (17.16 µg/mL), and *ephedrine* (13.57 µg/mL), while compounds such as *resveratrol* and *flavonones* were present in lower amounts.

The diversity and relative abundance of these compounds suggest that the extract contains multiple bioactive agents that may contribute synergistically to its observed biological activities.

Table 1 Compounds identified through HPLC analysis

S/N	Compounds	Retention time (min)	Area (m^2)	Concentration (µg/mL)
1	Lunamarin	0.210	561.7208	7.9454
2	Naringin	2.390	3787.2370	9.7281
3	Cardiac Glycoside	4.120	12468.6878	6.0096
4	Flavan-3-ol	6.016	6439.0392	10.5922
5	Anthocyanin	7.470	18505.8704	10.9133
6	Ribalinidine	9.160	8168.2068	10.9403
7	Naringenin	12.970	19738.1656	5.9452
8	Sparteine	15.460	5983.7040	6.6912
9	Rutin	17.963	4717.6830	7.0437
10	Cyanogenic Glycoside	20.313	10773.6850	17.1582
11	Flavonones	22.730	12044.9656	4.1093
12	Steroids	25.650	9214.8346	12.9901
13	Kaempferol	27.536	9877.4402	7.9901
14	Epicatechin	29.860	11128.3451	8.2202
15	Phytate	32.993	5158.3293	19.2702
16	Flavone	34.600	14257.1034	5.6400
17	Oxalate	36.876	5611.0061	11.0497
18	Resveratrol	39.200	6421.6611	3.8894
19	Sapogenin	42.276	9871.3256	5.7676
20	Ephedrine	44.170	3468.1816	13.5688

HPLC chromatogram of the ethanolic aerial fraction of *Emilia praetermissa* showing resolved peaks with retention times and relative peak areas used for compound identification and quantification. Peaks labelled correspond to key nonvolatile phytoconstituents detected by comparison with external standards (examples: ephedrine, phytate, naringenin, proanthocyanidins, sapogenin, tannins and selected alkaloids).

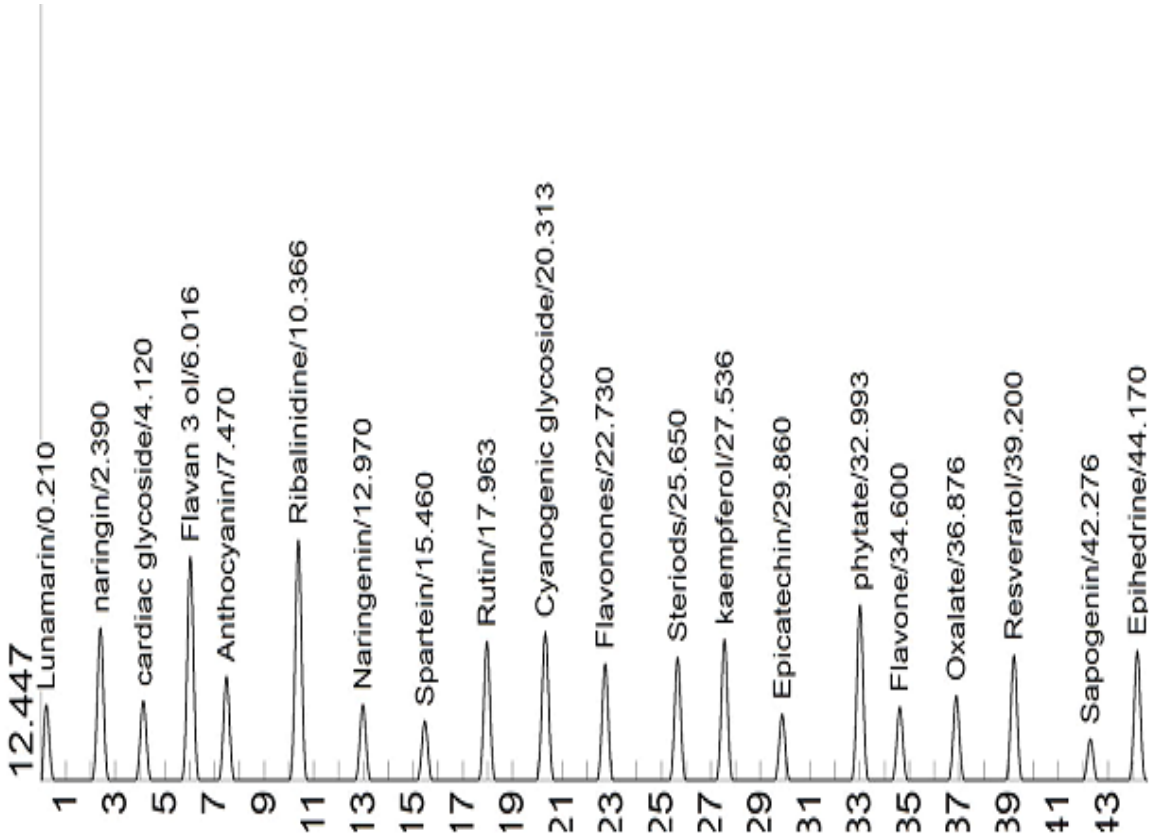
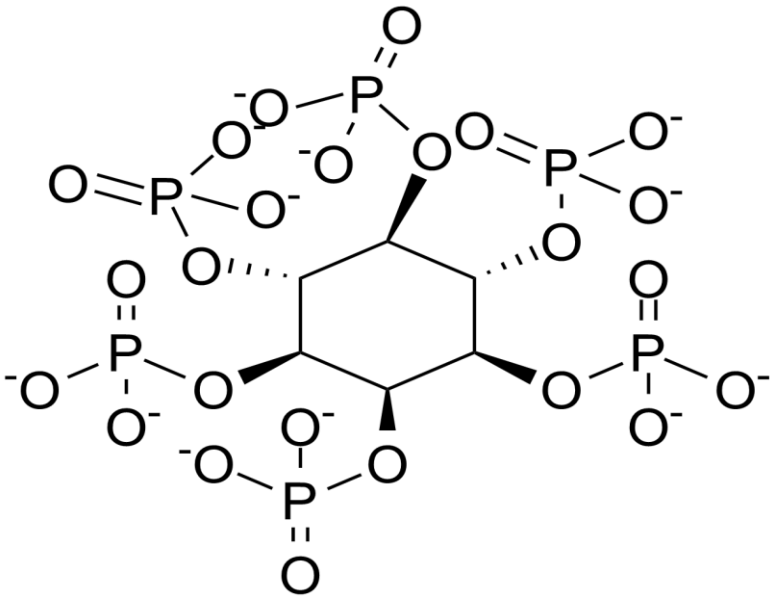
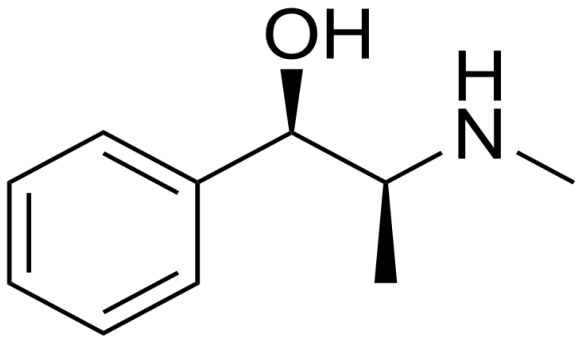


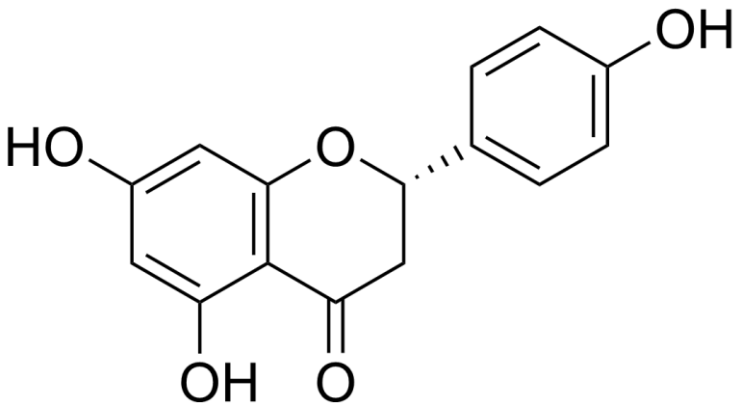
Fig 6: HPLC chromatogram of the ethanolic fraction of aerial part of *Emilia praetermissa* showing detected peaks and retention times for identified phytoconstituents.



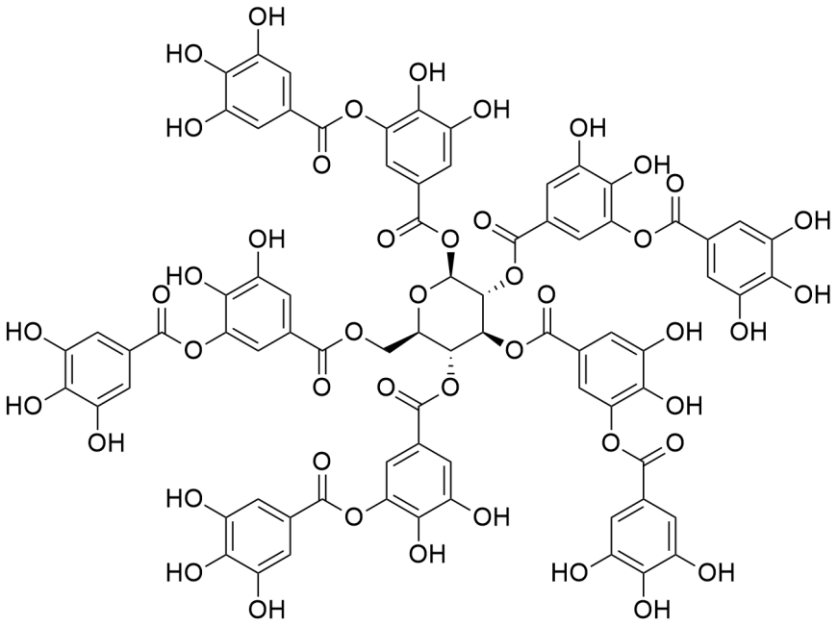
Phytate



Ephedrine



Narigenin



Tannin

Fig 7 Structures of compounds identified through HPLC

3.2 GAS CHROMATOGRAPHY-MASS SPECTROMETRY

The Gas Chromatography–Mass Spectrometry (GC-MS) analysis revealed a diverse profile of 27 bioactive compounds present in the sample, spanning a wide range of chemical classes, including alkanes, esters, alcohols, phenols, fatty acids, and terpenoids. These compounds were identified based on their retention times (RT), molecular weights, and area percentages, which reflect their relative abundance. Major compounds present include 11-Octadecenoic *acid methyl ester* (27.07%), *octanoic acid, 1-methyltridecyl ester* (17.88%), *n-Hexadecanoic acid methyl ester* (8.80%), and *2,4-Di-tert-butylphenol (4.4%).* The presence of fatty acid esters, terpenoids, and phenolic compounds suggests that the sample may possess antioxidant, anti-inflammatory, antibacterial, and skin-protective properties. These findings support its potential use in pharmacological and ethnomedicinal applications.

Table 2 GC–MS profile of volatile and semi-volatile constituents in the ethanolic aerial fraction of *Emilia praetermissa.*

S/N	RT (min)	Compound Name	Area (%)	Molecular Formula	MW (g/mol)
1	8.225	2,3,5-trimethyl carbonic acid	0.94	$C_5H_{10}O_2$	102.13
2	9.152	Cyclododecane	1.32	$C_{12}H_{24}$	168.32
3	9.622	2,4-Di-tert-butylphenol	4.4	$C_{14}H_{22}O$	206.32
4	10.314	Hexadecane	0.98	$C_{16}H_{34}$	226.44
5	11.064	Anthracene, 1,2,3,4,5,6,7,8-octahydro-(octahydroanthracene)	0.98	$C_{14}H_{18}$	186.29
6	11.161	Patchouli alcohol (patchoulol)	1.75	$C_{15}H_{26}O$	222.37
7	11.275	Cyclododecane	1.72	$C_{12}H_{24}$	168.32
8	11.481	Oxirane, tetradecyl-	1.73	$C_{16}H_{32}O$	240.43
9	12.54	Trichloroacetic acid, undec-10-enyl ester	0.95	$C_{13}H_{21}Cl_3O_2$	315.65
10	12.591	Longifolenaldehyde	1.44	$C_{15}H_{24}O$	220.35
11	12.935	1,2-Benzenedicarboxylic acid, diundecyl ester	1.17	$C_{30}H_{50}O_4$	498.71
12	13.186	(3S,3aS,6R,7R,9aS)-1,1,7-Trimethyl-decahydro-3a,7-methanocyclopenta[8]annulene-3,6-diol	0.87	$C_{14}H_{26}O_2$	230.35
13	13.318	Hexadecanoic acid, methyl ester (methyl palmitate)	2.77	$C_{17}H_{34}O_2$	270.45
14	13.427	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	1.43	$C_{19}H_{28}O_3$	320.43
15	13.73	n-Hexadecanoic acid (palmitic acid)	8.8	$C_{16}H_{32}O_2$	256.42

16	13.867	Hexadecanoic acid, ethyl ester (ethyl palmitate)	1	$C_{18}H_{36}O_2$	284.48
17	14.611	Pyridine, 2-[(3-chlorobicyclo[2.2.1]hept-2-yl)thio]-, 1-oxide	0.99	$C_{12}H_{16}ClNOS$	257.78
18	14.731	11-Octadecenoic acid, methyl ester	27.07	$C_{19}H_{36}O_2$	296.48
19	14.771	6-Octadecenoic acid, methyl ester	2.49	$C_{19}H_{36}O_2$	296.48
20	14.915	Methyl stearate	3.26	$C_{19}H_{38}O_2$	298.51
21	15.286	Octanoic acid, 1-methyltridecyl ester	17.88	$C_{21}H_{42}O_2$	334.55
22	16.334	Nonacos-1-ene	1.11	$C_{29}H_{58}$	406.78
23	16.557	Methyl 18-methylnonadecanoate	3.24	$C_{21}H_{42}O_2$	334.55
24	16.648	Squalene	2.71	$C_{30}H_{50}$	410.7
25	18.176	1-Methyl-4-phenyl-5-thioxo-1,2,4-triazolidin-3-one	0.88	$C_{11}H_{10}N_3OS$	232.29
26	19.012	Docosanoic acid, methyl ester (methyl behenate)	3.96	$C_{23}H_{46}O_2$	354.61
27	19.423	Bis(2-ethylhexyl) phthalate (DEHP)	4.15	$C_{24}H_{38}O_4$	390.56

GC–MS chromatogram of the ethanolic aerial fraction of Emilia praetermissa showing retention times and relative peak areas of volatile and semi-volatile constituents. Major GC–MS peaks (for example methyl esters of C16–C18 fatty acids, octanoic acid esters, 2,4-di-tert-butylphenol and squalene) are indicated and were identified by mass spectral matching to the NIST library; the chromatogram supports the GC–MS compound list and structural depictions in the results.

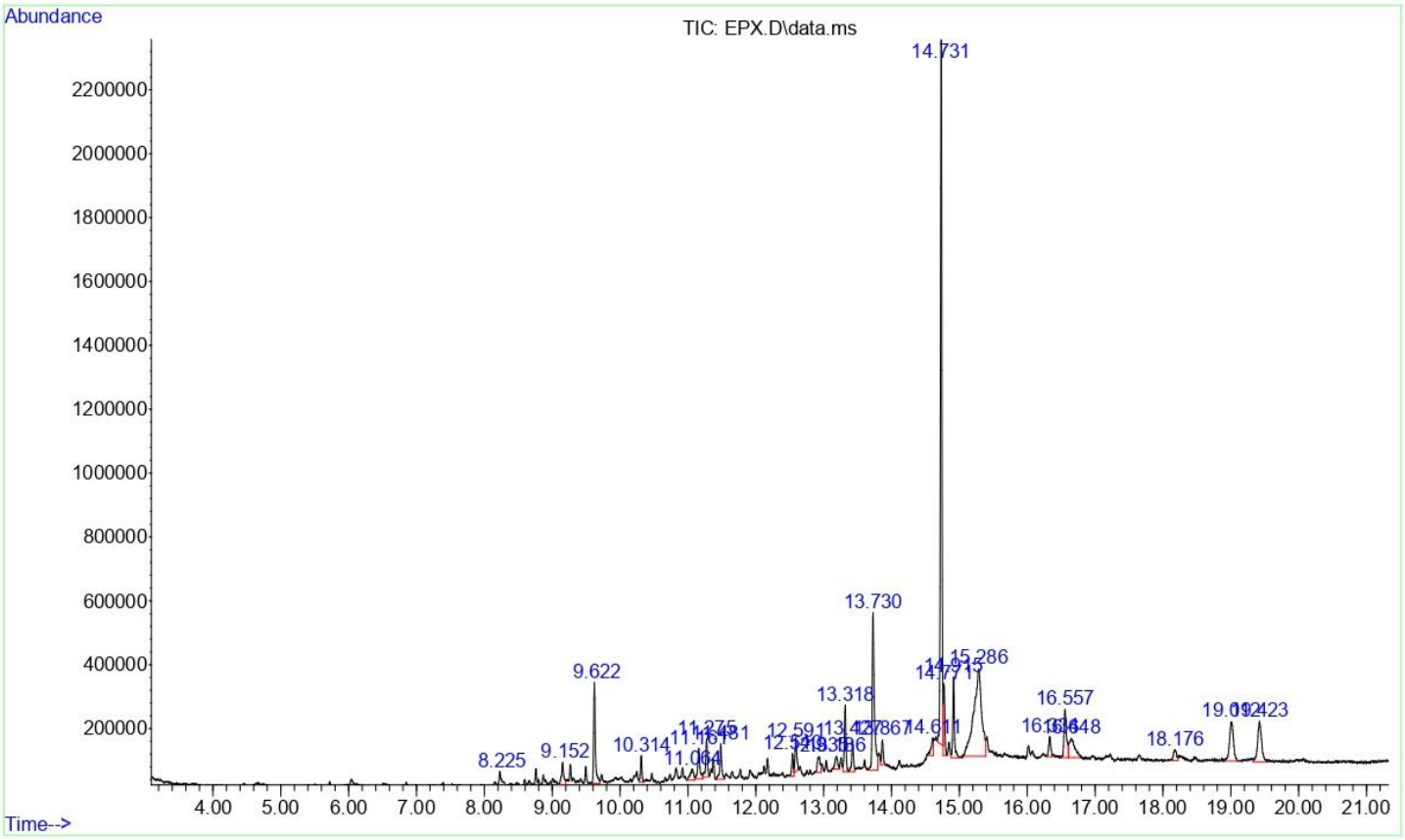
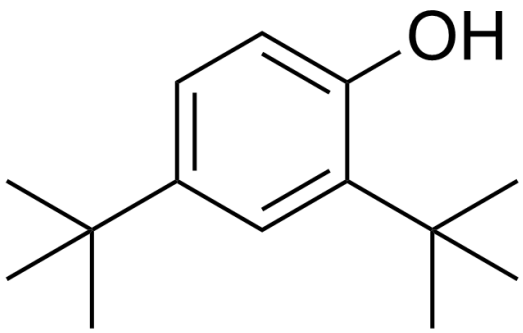
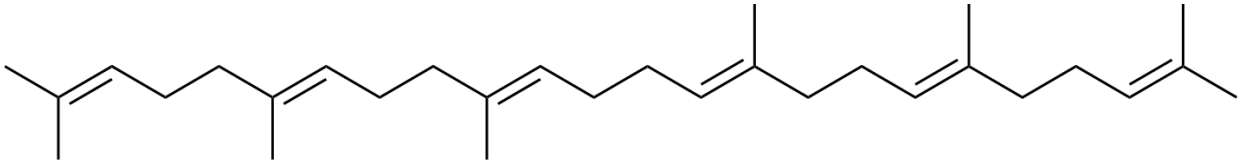


Fig 8: GC-MS chromatogram of the ethanolic fraction of aerial part of *Emilia praetermissa* showing retention times and relative peak areas of volatile and semi-volatile constituents.

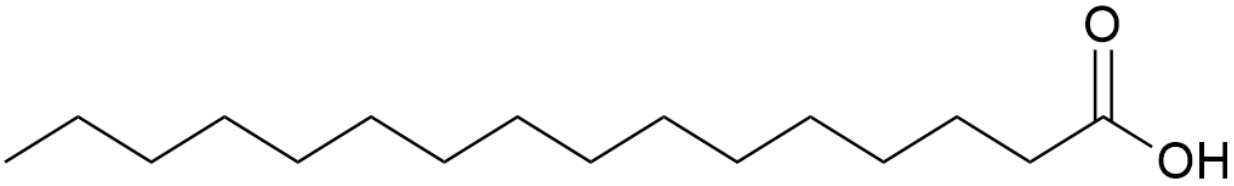


2,4-Di-tert-butylphenol

11-Octadecenoic acid, methyl ester



Squalene



Hexadecanoic acid

Fig 9 Structures of compounds identified through GC-MS analysis

3.3 ANTIBACTERIAL ACTIVITY

Susceptibility assay of *Emilia praetermissa* against selected clinical isolate (measured in mm)

The extract demonstrated moderate inhibitory effects across all tested organisms, with inhibition zones ranging from 15 mm (*Pseudomonas aeruginosa*) to 18 mm (*Enterobacter cloacae* and *Bacillus subtilis*). Tween-80 produced no inhibition, confirming it had no intrinsic antibacterial effect. Ciprofloxacin, as expected, showed markedly higher activity, producing inhibition zones between 28 mm and 40 mm.

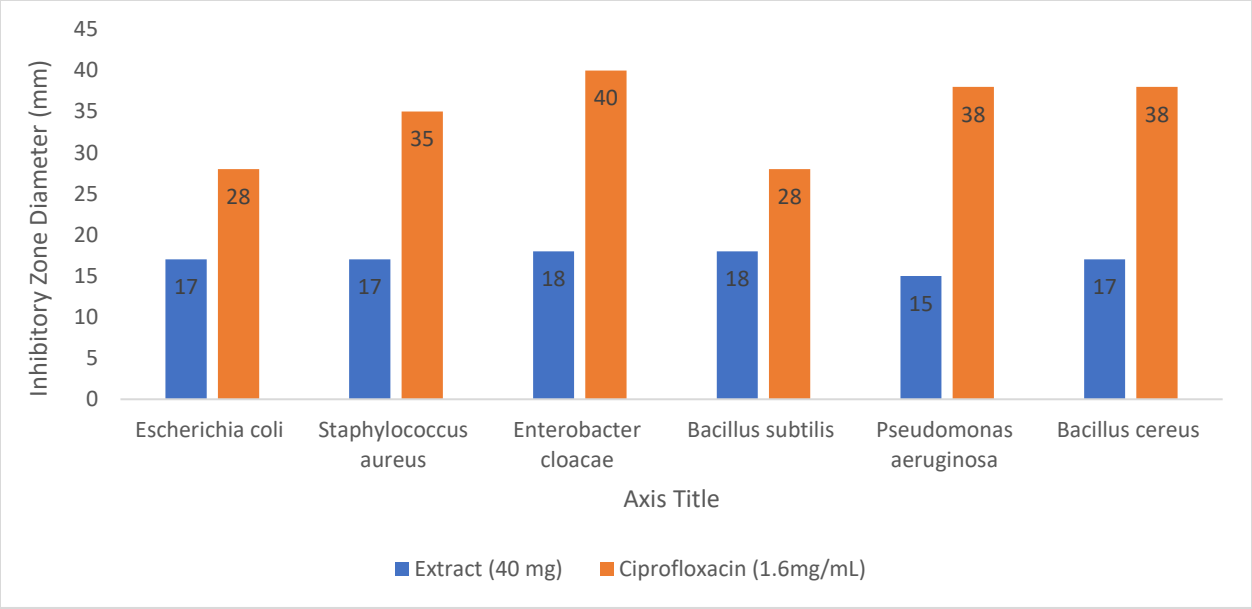


Fig 10: Antibacterial susceptibility of *Emilia praetermissa* ethanolic fraction of *Emilia praetermissa* (inhibition zone diameters in mm).

The antibacterial activity of 40 mg/ml ethanolic fraction of *Emilia praetermissa* was compared to 1.6 mg/ml ciprofloxacin using a paired t-test. The analysis yielded a **p-value of 0.000645**, indicating a statistically significant difference (**$p < 0.001$**) in antibacterial efficacy between the two treatments. This suggests that the ethanolic fraction exhibits potent antibacterial activity, though not necessarily superior to ciprofloxacin.

3.3.1 MINIMUM INHIBITORY CONCENTRATION

The minimum inhibitory concentration (MIC) assay was performed to determine the lowest concentration of *Emilia praetermissa* extract capable of inhibiting visible growth of selected clinical bacterial isolates. Four concentrations (40, 20, 10, and 5 mg/mL) were tested.

At 40 mg/mL, complete growth inhibition was observed for all organisms tested. At 20 mg/mL, inhibition persisted only for *Staphylococcus aureus, Pseudomonas aeruginosa, and Bacillus cereus,* while the remaining isolates exhibited growth. Lower concentrations (10 mg/mL and 5 mg/mL) did not inhibit any of the tested bacteria.

These findings indicate that the extract has broad-spectrum antibacterial activity at higher concentrations, with *S. aureus, P. aeruginosa, and B. cereus* showing greater susceptibility compared to the other isolates.

Table 3: Minimum inhibitory concentration (MIC) results for Ethanolic fraction of *Emilia praetermissa* against clinical bacterial isolates.

Microorganism	Concentrations (mg/mL)			
	40	20	10	5
Escherichia coli	No Growth	Growth	Growth	Growth
Staphylococcus aureus	No Growth	No Growth	Growth	Growth
Enterobacter cloacae	No Growth	Growth	Growth	Growth
Bacillus subtilis	No Growth	Growth	Growth	Growth
Pseudomonas aeruginosa	No Growth	No Growth	Growth	Growth
Bacillus cereus	No Growth	No Growth	Growth	Growth

3.3.2 MINIMUM BACTERICIDAL CONCENTRATION

The minimum bactericidal concentration (MBC) assay was conducted to determine the lowest concentration of the test substance capable of killing the selected bacterial isolates. Two concentrations, 20 mg/mL and 40 mg/mL, were evaluated.

At 20 mg/mL, all tested microorganisms exhibited visible growth, indicating no bactericidal effect at this concentration. At 40 mg/mL, complete bacterial killing was observed for *Staphylococcus aureus, Enterobacter cloacae, Pseudomonas aeruginosa,* and *Bacillus cereus,* while *Escherichia coli and Bacillus subtilis* remained unaffected.

Table 4: Minimum bactericidal concentration (MBC) results for the ethanolic fraction of *Emilia praetermissa.*

Microorganism	Concentration (mg/mL)	
	20	40
Escherichia coli	Growth	Growth
Staphylococcus aureus	Growth	No Growth
Enterobacter cloacae	Growth	No Growth
Bacillus subtilis	Growth	Growth
Pseudomonas aeruginosa	Growth	No Growth
Bacillus cereus	Growth	No Growth

3.3.3 Mode of Antibacterial activity

The following table summarises the minimum inhibitory concentrations (MIC), minimum bactericidal concentrations (MBC), and calculated MBC/MIC ratios for selected clinical isolates treated with the ethanolic aerial fraction of Emilia praetermissa. The MBC/MIC ratio is used to classify the extract's effect as bactericidal (MBC/MIC ≤ 4) or bacteriostatic (MBC/MIC > 4). Values shown reflect the lowest concentrations (mg/mL) that prevented visible growth (MIC) and that produced no growth on subculture (MBC); the ratio and classification indicate whether the extract kills the organism at concentrations close to those that inhibit it, providing a practical measure of likely mode of action in vitro.

Table 5: Mode of antibacterial activity of *Emilia praetermissa*

Organism	MIC	MBC	MBC/MIC	Classification
Staphylococcus aureus	20	40	40/20 = 2	Bactericidal
Enterobacter cloacae	40	40	40/40 = 1	Bactericidal
Pseudomonas aeruginosa	20	40	40/20 = 1	Bactericidal
Bacillus cereus	20	40	40/20 = 2	Bactericidal

3.4 ANTIOXIDANT RESULT

3.4.1 DPPH Antioxidant result

The DPPH assay showed that the hydro-ethanolic aerial fraction of *Emilia praetermissa* has strong radical scavenging activity across the tested concentration range. Percent inhibition increased gradually from 83.09% at 200 µg/mL to 89.36% at 1000 µg/mL, indicating a dose-responsive pool of hydrogen/electron-donating constituents. Ascorbic acid, used as the standard, remained higher and relatively stable ranging between (95.51 to 97.29%) across the same concentrations, confirming the assay's sensitivity and that the extract—while active—does not surpass the reference antioxidant.

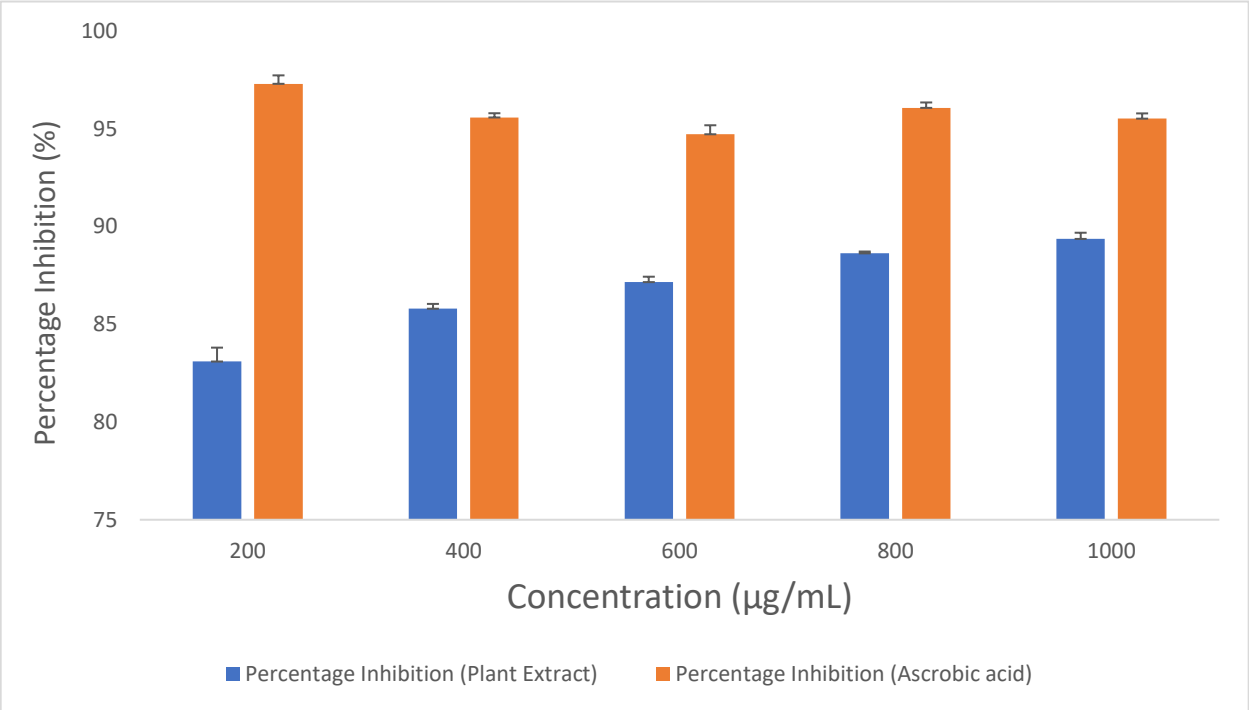


Fig 11. Comparative DPPH % Inhibition at 200–1000 µg/mL of Plant Extract and Ascorbic acid

3.4.2 FRAP Antioxidant Result

The extract exhibits modest ferric-reducing activity in the FRAP assay, with percent values clustered near 39 to 43% and a small rise at higher concentrations (peak of 43.4% at 800 µg/mL). By contrast, ascorbic acid (the standard) displays markedly greater reducing power at all concentrations ranging between 87.91 to 90.07%. These results show the extract has measurable reducing capacity but is substantially weaker than the standard in the FRAP assay.

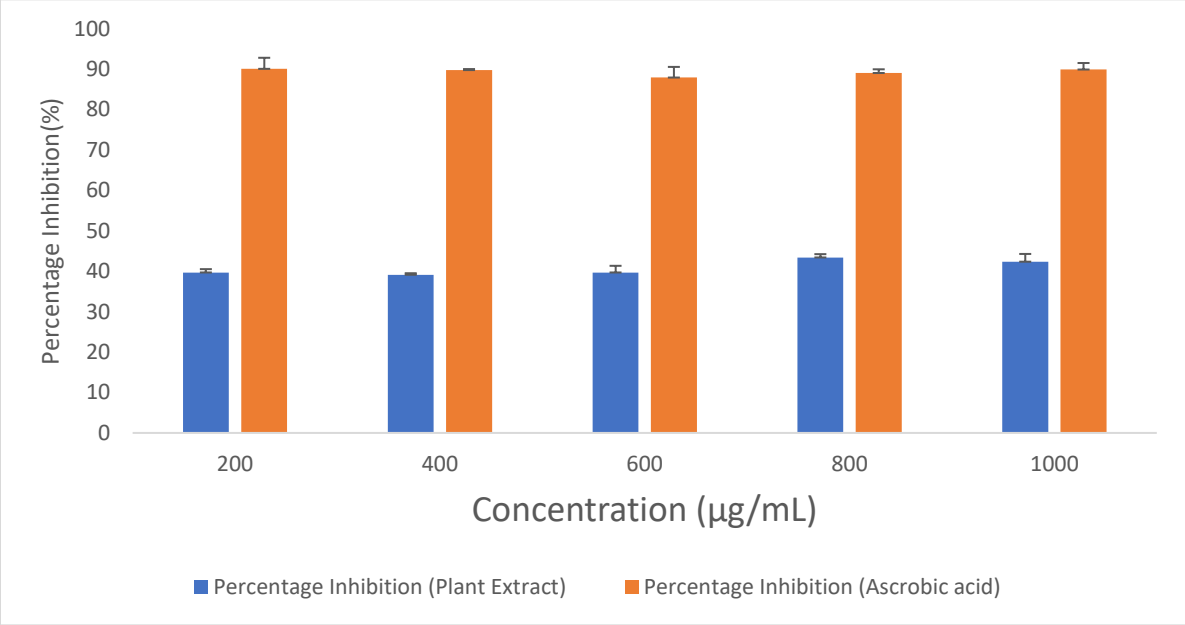


Fig 12. Comparative FRAP % Inhibition at 200–1000 µg/mL of Plant Extract and Ascorbic acid

3.4.3 ABTS Antioxidant result

The ABTS assay produced concentration dependent percent inhibitions for the hydro-ethanolic aerial fraction of *Emilia praetermissa* ranging from 62.78% (200 µg/mL), to 89.67% (1000 µg/mL). Ascorbic acid (standard) maintained consistently high inhibition across the same concentrations (97.13 to 97.85%) outperforming the extract at every point. The extract showed a clear rise in ABTS scavenging from 200 to 800 µg/mL and reached a plateau between 800 and 1000 µg/mL. Overall, the ethanolic fraction demonstrated good ABTS radical-scavenging activity but did not reach the potency of ascorbic acid at the tested concentrations.

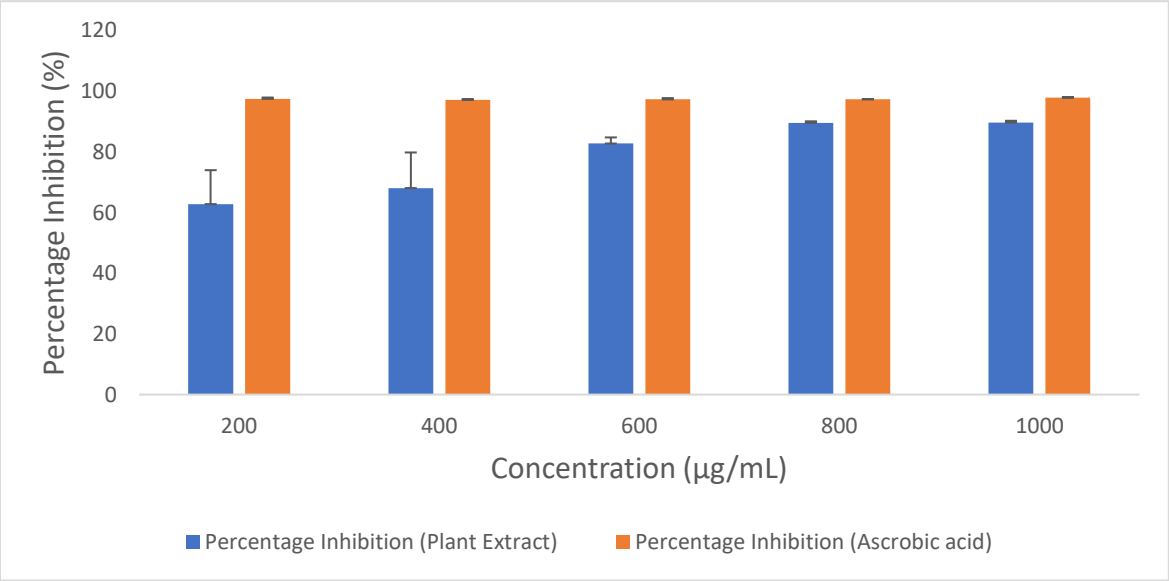


Fig 13. Comparative ABTS % Inhibition at 200–1000 µg/mL of Plant Extract and Ascorbic acid

3.4.4 TAC Antioxidant Result

The extract shows a clear, concentration-dependent increase in total antioxidant capacity, rising from 27.92% at 200 µg/mL to 90.28% at 1000 µg/mL. Ascorbic acid, the standard, maintains very high TAC values between 95.66 to 95.83% across all concentrations and remains superior to the extract. The TAC profile indicates the extract's cumulative antioxidant potential becomes comparable to the standard only at the highest tested doses.

Ascorbic acid was used as the assay standard in all panels and serves as the reference for comparing the extract's antioxidant performance.

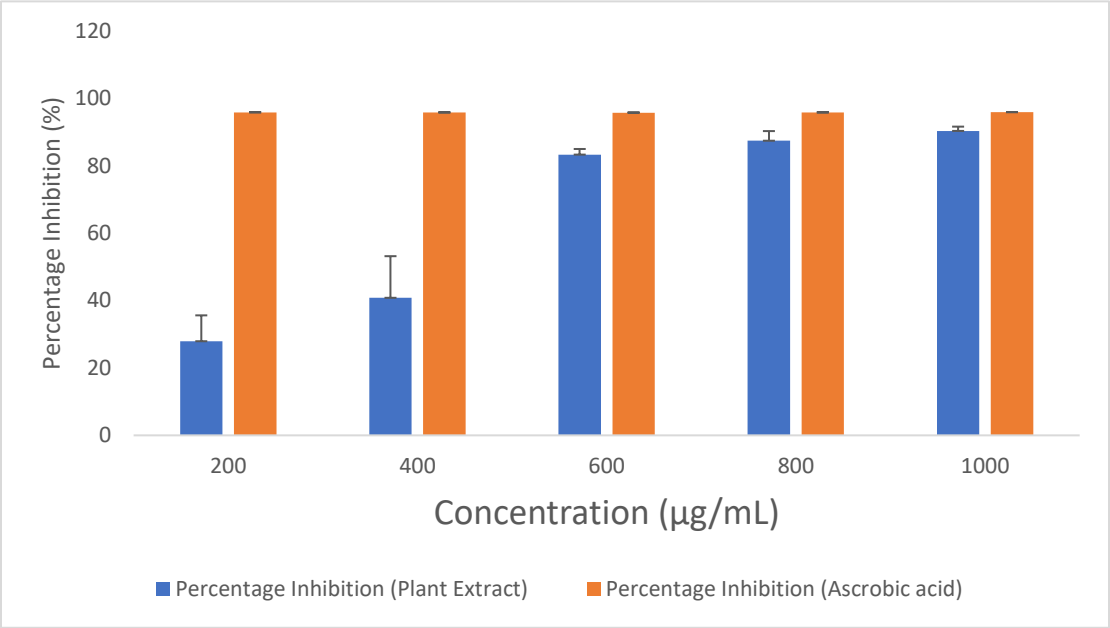


Fig 14. Comparative TAC % Inhibition at 200–1000 µg/mL of Plant Extract and Ascorbic acid

CHAPTER FOUR

4.1 DISCUSSION

The ethanolic extract of the aerial parts of *Emilia praetermissa* yielded a complex phytochemical profile by HPLC with the detection of 20 phytoconstituents across alkaloids, flavonoids, Phytate phenolics, glycosides, steroids and sapogenins with Phytate (19.27 µg/mL), Cyanogenic glycoside (17.16 µg/mL), and Ephedrine (13.57 µg/mL) among the highest concentrations.

High phytate and cyanogenic glycoside content indicates the extract contains polar, phosphorus-containing and defense-related metabolites that may influence bioactivity and toxicity profiles. High phytate could chelate metal ions and modulate redox chemistry and inhibit Fenton reactions (Pires *et al.,* 2023), while cyanogenic glycosides suggest a plant defensive chemistry that may contribute to antibacterial effects but also demands toxicity consideration in therapeutic contexts.

Alkaloids (ephedrine, sparteine) and flavonoids (rutin, resveratrol) detected by HPLC are consistent with reported Emilia alkaloid chemistry and can contribute to antibacterial and anti-inflammatory (Paulo *et al.,* 2010; Tulgar *et al.,* 2018; Hidalgo *et al.,* 2022; Gupta *et al.,* 2024). Ephedrine due to its central nervous effect may partially explain reported antihypertensive/ cardiovascular effects in ethnomedicine through sympathomimetic or adrenergic modulation depending on concentration and matrix.

Flavonoids and phenolics which include; anthocyanin, flavan-3-ol, rutin, resveratrol, phytate, epicatechin, and steroids present in appreciable amounts collectively support antioxidant capacity, free radical scavenging, anti-inflammatory potential, and membrane-targeted antibacterial activity

via disruption of bacterial enzymes and cell walls (Tena *et al.,* 2020; Costa *et al.,* 2022; Pires *et al.,* 2023; Gupta *et al.,* 2024; Sripadung *et al.,* 2025 Taylor *et al.,* 2025).

GC-MS revealed 27 volatile/non-volatile components dominated by fatty acid esters and long-chain lipids, with 11-Octadecenoic acid, methyl ester (27.07%), Octanoic acid, 1-methyltridecyl ester (17.88%), and n-Hexadecanoic acid (8.80%) as the most abundant.

GC-MS profile dominated by fatty acid esters, long-chain hydrocarbons (methyl esters of C16–C22 fatty acids, squalene) and volatile phenolics (2,4-di-tert-butylphenol) suggests the extract contains substantial lipidic constituents and hydrophilic phenols which together create a biphasic chemistry that can act as permeability enhancers, possess mild antibacterial and anti-inflammatory activities, and influence extract solubility and diffusion in agar assays (Zhao *et al.,* 2020; Cheng *et al.,* 2024; Bosson *et al.,* 2025).

The extract produced moderate inhibition zones (15–18 mm) against clinical isolates and showed bacteriostatic/bactericidal effects at higher concentrations. The extract showed complete growth inhibition at 40 mg/mL for all organisms in MIC assays, partial inhibition at 20 mg/mL for some strains, and MBC at 40 mg/mL for S. aureus, E. cloacae, P. aeruginosa, and B. cereus while E. coli and B. subtilis resisted bactericidal killing at that concentration.

The moderate antibacterial zones (15–18 mm) and MIC/MBC pattern are consistent with an extract whose activity arises from a combination of polar phenolics, alkaloids, and amphiphilic saponins rather than a single highly potent antibiotic compound. Flavonoids and tannins likely contribute to bacteriostatic effects which could be due to; enzyme inhibition, metal chelation, and protein precipitation at cell surfaces (Zhuang *et al.,* 2023; Tena *et al.,* 2020). Alkaloids such as Ephedrine

and sparteine compounds are plausible contributors to bactericidal activity against susceptible strains (Tulgar *et al.,* 2018; Hildalgo *et al.,* 2022). Long-chain fatty acid esters may enhance extract penetration into bacterial membranes or exert direct membrane-disturbing effects, explaining activity versus Gram-negative P. aeruginosa at higher concentrations (Cheng *et al.,* 2024)

Traditional uses (antibacterial for otitis/sinusitis, antihypertensive, wound healing, and gastroprotective) align with the detected phytochemicals: antioxidant flavonoids and phenolics support wound healing and mucosal protection; alkaloids and saponins may modulate cardiovascular parameters; and tannins and saponins provide astringent and antibacterial surface activity, supporting topical wound use.

The ethanolic aerial fraction of *Emilia praetermissa* demonstrated concentration-dependent antibacterial activity, inhibiting growth of all six clinical isolates at 40 mg/mL while showing true bactericidal action (no growth on subculture) against *Staphylococcus aureus, Enterobacter cloacae, Pseudomonas aeruginosa and Bacillus cereus* likely reflecting membrane-disruptive and alkaloid-mediated killing at higher concentrations whereas *Escherichia coli and Bacillus subtilis* were only growth-inhibited (bacteriostatic) at that dose, suggesting reduced susceptibility due to intrinsic barrier or efflux mechanisms and indicating the extract's multi-constituent, multi-target mode of action that may require purification or higher doses for consistent bactericidal effects.

Gram-negative organisms (*E. coli, P. aeruginosa, Enterobacter*) and Gram-positive organisms (*S. aureus, Bacillus spp.*) showed differential susceptibility: *P. aeruginosa and S. aureus* were inhibited at 20 mg/mL while *E. coli* resisted bactericidal action at 40 mg/mL. These differences could be due to cell envelope structure and efflux mechanisms. The extract's active constituents appear to overcome outer membrane/efflux barriers in some Gram-negatives but not universally.

The ethanolic aerial fraction of *Emilia praetermissa* shows bactericidal activity against *Staphylococcus aureus, Enterobacter cloacae, Pseudomonas aeruginosa* and *Bacillus cereus*, and only bactericidal activity against *Escherichia coli* and *Bacillus subtilis* under the conditions tested. this pattern is compatible with the presence of constituents that cause rapid or irreversible damage to vital cellular structures

The combination of hydrophilic antioxidants and lipophilic fatty acid esters in the extract may create a biphasic attack: phenolics target intracellular oxidative and enzymatic processes while lipids/saponins disrupt membrane integrity. Such multi-target action reduces the likelihood of single-point resistance but requires higher concentrations to achieve bactericidal effects.

The ethanolic aerial fraction of *Emilia praetermissa* displayed clear antioxidant activity across four in vitro assays (DPPH, FRAP, ABTS, TAC) when tested at 200, 400, 600, 800 and 1000 µg/mL. Ascorbic acid was used as the assay standard in all experiments and served as the reference for comparing extract performance. The pattern of responses shows the extract is rich in radical-scavenging constituents but has variable performance between assays and across concentrations.

The strong DPPH scavenging observed across 200–1000 µg/mL reflects the presence of abundant H-donor antioxidants in the ethanolic fraction most likely flavonoids, proanthocyanidins and related phenolics detected by HPLC which neutralize the DPPH radical via hydrogen-atom or electron transfer Tena *et al.,* 2020; Sripadung *et al.,* 2025). The steady rise in percent inhibition with concentration suggests additive contributions from multiple compounds rather than saturation by a single dominant molecule. That ascorbic acid yields higher and stable values provides a useful potency benchmark and confirms the extract's activity is genuine though weaker than the standard.

FRAP values for the extract were comparatively low (39.69 to 42.36%), showing the extract has measurable single-electron reducing power but is substantially weaker than ascorbic acid (89.9 to 90.07%) in this acidic, ferric-reducing system. A low FRAP but higher DPPH/ABTS suggests the extract contains many effective radical quenchers (multi-step H-donors, flavonoids, glycosides, proanthocyanidins) that perform well in radical scavenging assays but do not transfer a single electron as readily under FRAP's specific redox potential and pH. Therefore, the extract's chemistry is likely skewed toward compounds that neutralise radicals by hydrogen atom donation or complex multi-step reactions rather than by straightforward one-electron reduction of Fe3+ to Fe2+.

The extract neutralized ABTS•+ strongly at increasing concentrations ranging from but declined progressively to 62.78% at 200 µg/mL to 89.67% at 1000 µg/mL. ABTS is more permissive toward both hydrophilic and lipophilic antioxidants and correlates with DPPH assay. This shows the Hydrogen donating antioxidant activity in the ethanolic fraction and it is most likely due to the presence of flavonoids, proanthocyanidins and related phenolics detected by HPLC which neutralize the ABTS•+ radical at 734 nm.

The TAC assay produced a strong, concentration-dependent increase in total antioxidant capacity (27.92% to 90.28% from 200 to 1000 µg/mL). Because TAC measures cumulative reducing equivalents under prolonged, high-temperature acidic conditions, the result shows the extract contains many reductants whose summed capacity becomes substantial at higher doses.

Polyphenolics and flavonoids account for strong radical scavenging (DPPH, ABTS) and rising TAC because they donate H-atoms and can be thermally stable contributors to total reducing equivalents. Saponins and long-chain lipids can alter solubility and form micellar structures or

emulsions at higher extract concentrations, reducing the fraction of free polyphenol available in the aqueous assay medium and producing the non-linear or declining % inhibitions seen in DPPH/ABTS. Phytate and cyanogenic glycosides can chelate metals or alter redox chemistry, potentially modifying FRAP responses.

CHAPTER FIVE

5.1 CONCLUSION

This study successfully demonstrated the phytochemical richness and pharmacological potential of the hydro-ethanolic aerial fraction of *Emilia praetermissa*. Chromatographic profiling via HPLC and GC-MS revealed a diverse array of bioactive compounds, including alkaloids (ephedrine, spartine), flavonoids (flavanone, narigenin), phenolics, tannins, and saponins. These constituents are known for their antibacterial and antioxidant properties.

Antibacterial assays produced moderate broad-spectrum antibacterial activity in vitro that was statistically significant versus ciprofloxacin ($p = 0.0006$), supporting its traditional use for antibacterial and wound-healing purposes, with complete growth inhibition and bactericidal activity observed at 40 mg/mL for several strains. Antioxidant evaluations using DPPH, FRAP, ABTS, and TAC assays further validated the extract's strong radical scavenging capacity.

The findings support the ethnomedicinal use of *Emilia praetermissa* and suggest that its therapeutic effects arise from synergistic interactions among polar and nonpolar phytochemicals.

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APPENDIX A

Table A1: Raw absorbance readings and % inhibition for DPPH assay presented as (mean ± SD, n = 3).

Extract Conc (µg/mL)	Absorbance	DPPH Percentage Inhibition	Ascorbic acid Conc (µg/mL)	Absorbance	DPPH Percentage Inhibition
200	0.275 ± 0.012	83.09	200	0.073 ± 0.004	95.51
400	0.231 ± 0.004	85.79	400	0.064 ± 0.005	96.06
600	0.209 ± 0.005	87.15	600	0.086 ± 0.008	94.71
800	0.185 ± 0.002	88.62	800	0.072 ± 0.004	95.57
1000	0.173 ± 0.005	89.36	1000	0.044 ± 0.007	97.29

Table A2: Raw absorbance readings and % inhibition for FRAP assay presented as (mean ± SD, n = 3).

Extract Conc (µg/mL)	Absorbance	FRAP Percentage Inhibition	Ascorbic acid Conc (µg/mL)	Average absorbance	FRAP Percentage Inhibition
200	0.86 ± 0.012	39.69	200	0.142 ± 0.039	90.07
400	0.868 ± 0.005	39.13	400	0.146 ± 0.004	89.76
600	0.86 ± 0.024	39.69	600	0.172 ± 0.038	87.91
800	0.807 ± 0.012	43.41	800	0.156 ± 0.013	89.04
1000	0.822 ± 0.027	42.36	1000	0.144 ± 0.023	89.9

Table A3: Raw absorbance readings and % inhibition for ABTS assay presented as (mean ± SD, n = 3).

Extract Conc (µg/mL)	Absorbance	ABTS Percentage Inhibition	Ascorbic acid Conc (µg/mL)	Average absorbance	ABTS Percentage Inhibition
200	0.479 ± 0.144	62.78	200	0.028 ± 0.002	97.85
400	0.411 ± 0.151	68.07	400	0.034 ± 0.001	97.33
600	0.222 ± 0,026	82.75	600	0.035 ± 0.006	97.31
800	0.134 ± 0.006	89.59	800	0.037 ± 0.004	97.13
1000	0.133 ± 0.008	89.67	1000	0.032 ± 0.006	97.49

Table A4: Raw absorbance readings and % inhibition for TAC assay presented as (mean ± SD, n = 3).

Extract Conc (µg/mL)	Absorbance	TAC Percentage Inhibition	Ascorbic acid Conc (µg/mL)	Average absorbance	TAC Percentage Inhibition
200	0.146 ± 0.016	27.92	200	2.499 ± 0.017	95.8
400	0.177 ± 0.034	40.79	400	2.476 ± 0.032	95.76
600	0.627 ± 0.062	83.26	600	2.417 ± 0.080	95.66
800	0.832 ± 0.202	87.38	800	2.464 ± 0.057	95.74
1000	1.08 ± 0.140	90.28	1000	2.519 ± 0.037	95.83

APPENDIX B

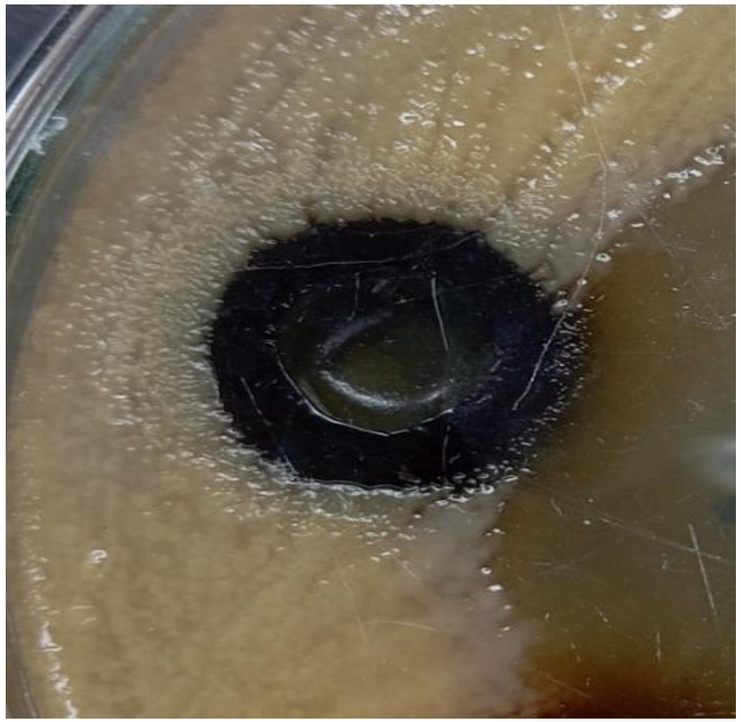

Fig B1 Antibacterial activity of plant extract against *Escherichia coli*

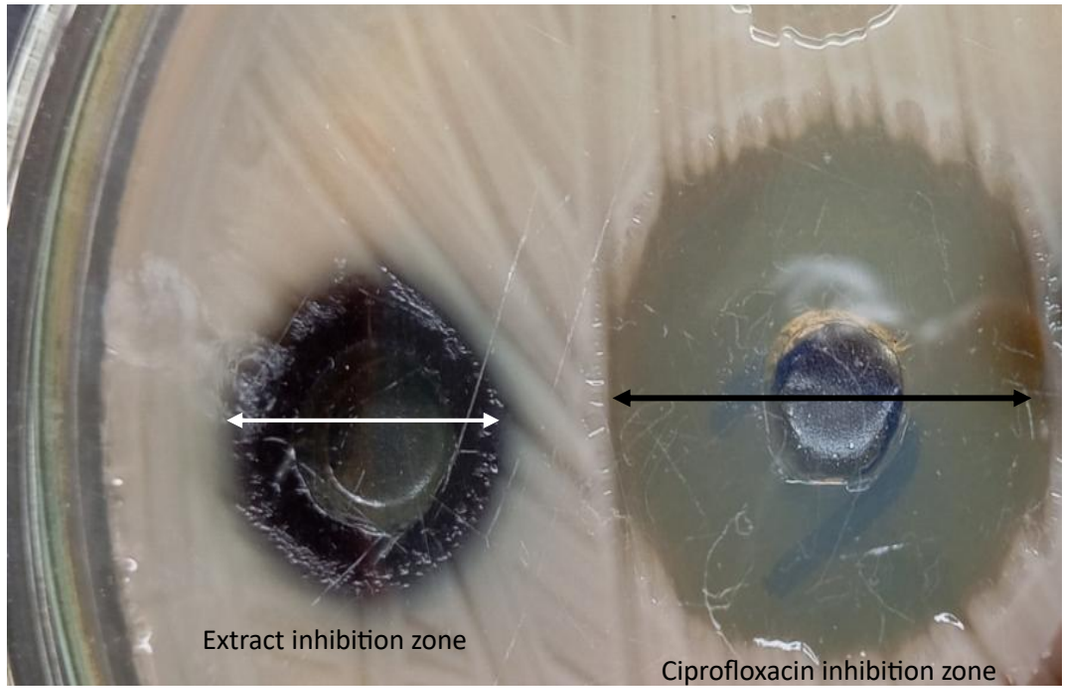

Fig B2 IZD comparison between Ciprofloxacin and extract against *Staphylococcus aureus*