Phytochemical analysis anti-microbial and anti-oxidant activity evaluation of hobet leaf (*persicaria senegalensis*) extracts

Riyad Aman Shifa

A Thesis Submitted to

The Department of Applied Biology

College of Applied Natural Science

Presented in Partial Fulfillment of the Requirement for the Degree of Master's in Applied Biology (Applied Microbiology)

School of Post Graduate Studies

Adama Science and Technology University

April, 2025

Adama, Ethiopia

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Advisor: Seid Mohammed (PhD, Associate professor)

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DECLARATION

I hereby declare that this Master Thesis entitled **"Phytochemical analysis anti-microbial and anti-oxidant Activity *evaluation* of *P. senegalensis* leaf extract"** is my original work. That is, it has not been submitted for the award of any academic degree, diploma, or certificate in any other university. All sources of materials that are used for this thesis have been duly acknowledged through citation.

RIYAD AMAN SHIFA		**06/04/2025**
Name of the student	**Signature**	**Date**

RECOMMENDATION OF SUPERVISOR

I, the major advisor/supervisor of this Thesis, hereby certify that I have closely read the revised version of the thesis entitled "**Phytochemical analysis anti-microbial and anti-oxidant Activity evaluation of Hobet Leaf (*P. senegalensis*) extracts** "prepared under my guidance by **Riyad Aman Shifa** submitted in partial fulfillment of the requirements for the degree of Master of Science in Applied Microbiology. Therefore, I recommend the submission of the revised version of the thesis to the department following the applicable procedures.

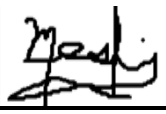
Seid Mohammed (PhD)		07-02-2025
Major- Supervisor	**Signature**	**Date**

APPROVAL PAGE

I, the advisor of the thesis entitled **"Phytochemical analysis anti-microbial and anti-oxidant Activity evaluation of Hobet Leaf (*P.senegalensis*) extract"** and developed by **Riyad Aman Shifa**, hereby certify that the recommendation and suggestions made by the board of examiners are appropriately incorporated into the final version of the thesis.

Dr. Seid Mohammed (Assoc. Prof)		
Major Advisor	Signature	Date

We, the undersigned, members of the Board of Examiners of the thesis by **Riyad Aman Shifa,** have read and evaluated the thesis entitled **"Phytochemical analysis anti-microbial and anti-oxidant Activity evaluation of Hobet Leaf (*P.senegalensis*) extract"** and examined the candidate during open defense. This is, therefore, to certify that the thesis is accepted for partial fulfillment of the requirement of the degree of Master of Science in Applied Microbiology.

Dr.Ketema Tafess (Assoc. Prof)		
Chairperson	Signature	Date
Aman Dekebo (PHD, Prof)		
Internal Examiner	Signature	Date
Dr. Mesfin Tafesse (Assoc. Prof)		**06/04/2025**
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College Dean	Signature	Date
Graduate School Dean	Signature	Date

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Table of Contents

LIST OF TABLES

LIST OF FIGURES

LIST OF ACRONYMS

CC	Column Chromatography
DPPH	2,2-diphenyl-1-picrylhydrazyl
EO	Essential oil
FTIR	Fourier-transform Infrared Spectroscopy
GC-MS	Gas Chromatography and Mass Spectrometry
HPLC	High Performance Liquid Chromatography
MeOH	Methanol
MBC	Minimum Bactericidal Concentration
MHA	Muller Hinton Agar
MIC	Minimum Inhibitory Concentration
NMR	Nuclear Magnetic Resonance
SNNPR	South Nations, Nationalities, and Peoples Region
TLC	Thin Layer Chromatography
TMP	Traditional medicinal plant
UV	Ultra Violet

ABSTRACT

Hobet leaf (P. senegalensis) is a perennial herbal species in the Polygonaceae family that has a growth habit that supports it. In this study, the main aim was to investigate the chemical components of P. senegalensis leaf and evaluate their antimicrobial activities against bacterial and fungal strains.The solid-liquid extraction technique was utilized as a general method for extraction. Phytochemical screening analysis was conducted following standard procedures.EOs was extracted from the plant by hydro-distillation and analysed by GC-MS. Antimicrobial activities was condacted using paper disc-diffusion assay. The antioxidant activities was evaluated using (DPPH) radical scavenging assay in vitro. The results indicated the presence of alkaloids, flavonoids, Saponins, tannins, steroids, phenols, and terpenoids in the extracts but alkaloids, tannins, and phenols were absent in the n-hexane extract. Citral (44.9%), hexacosane (9.96%), stearyl alcohol (9.23%), diisobutyl phthalate (8.12%), and β-Caryophyllene (2.6%) were major constituents of the EOs. Among the extracts the MeOH: Chloroform(1:1) extract showed maximum zone (17.3 ±0.25 mm) of inhibition against E.coli ATTC 25922^T at a concentration of 200 mg/ml and The minimum zone of inhibition (8.4 ± 0.38 mm) was recorded for test Hexane extract against E.coli ATTC 25922^T at a concentration of 25 mg/ml. The essential oil showed a maximum inhibiting effect (19.8±0.29 mm) against S. pyogenes ATCC 12204^T at a concentration of 50 mg/ml while the minimum zone of inhibition (11.8± 0.25 mm) was recorded against E. coli ATTC 25922^T at a concentration of 6.25 mg/ml. also showed a maximum inhibiting effect (19.8±0.25mm) and a minimum of (13.3±0.25mm) against C. albicans ATCC 10231^T.The extracts and essential oil exhibited more effective antibacterial activities against these selected bacterial strains and the zone of inhibition ranged from (8.4 to 19.8±0.29) mm. The essential oil exhibits the highest antioxidant activity, with %RSA values reaching 97.77% at 1mg/mL, followed by the methanol extract with %RSA values reaching 90.55%, while the chloroform and hexane extracts show lower activities with %RSA values of 74.65% and 79.07%, respectively. The MeOH: Chloroform extract also demonstrates significant antioxidant potential, with %RSA values peaking at 94.44%. Thus, the findings of this study show that leave of P.senegaensis posses medicinale properties. Additional research efforts are also recommended to be conducted on other parts of the plant such as on the root, seed, and stem.

Key words: Antimicrobial, Antioxidant, Hobet, Phytochemical, *Persicaria*, *Polygonacea*, Crude extract.

1. INTRODUCTION

1.1. Background of the study

Natural remedies have been the cornerstone of disease therapy, including plants and the substances obtained from them. People have become familiar with plants over the millennia and have utilized them in several ways. Modern therapeutic medications are developed using the conventional knowledge of the medical system that has existed since the dawn of humanity. About half of today's medications come either directly or indirectly from plant sources (Fabricant & Farnsworth, 2001).

A recent analysis found that, from the 1940s to 2020, 64.9% of authorized anticancer medications were either derived from natural products or natural product mimics (Newman & Cragg, 2020). Research on natural products has benefited from contributions from a variety of ethnic groups; these interactions have increased our understanding of the intimate connection between a compound's chemical structure and its biological effects. (Swargiary et al., 2021)These factors make medicinal plants crucial for the research of their traditional usage through the confirmation of pharmacological effects, and they can operate as new anti-infectious agents through natural composite sources. (Batool et al., 2021).

Indigenous plants remain a vital resource in Africa, serving as the main source of medicine, craft materials, and tools a tradition that stretches back centuries. In Ethiopia, a country with diverse landscapes ranging from tropical to temperate zones, the introduction of imported plants has enriched its already varied flora. The climate is shifting back toward subtropical conditions, supporting a wide range of plant life. Ethiopians have long been interested in harnessing these natural resources, not only for practical uses but also to influence local ecosystems, including the development of new animal species. This rich environmental diversity highlights the use of plant-based substances to combat widespread diseases, reflecting the deep connection between the region’s abundant plant and animal life and its people.(Lakew, 2015).

There has been an increase in interest in creating new plant-based medications during the past few decades. In many regions of the world, several studies have been conducted to examine the pharmacological qualities of traditional medicines (TM) and medicinal plants. Findings from studies on the antibacterial activities of numerous native plants are helpful. Then, these plants began to produce substances with possible medicinal use against human

pathogens like bacteria, fungi, or viruses. Various phytochemicals found in plants can be used to cure diseases such as Malaria, cancer, diabetes, and helminthiasis are just a few of the illnesses that the phytochemicals and secondary metabolites can treat *P. senegalensis* have been used for the treatment of ringworm in traditional practice in the West Shewa zone, Ethiopia (Swargiary et al., 2021).

P. senegalensis (Hobet leaf) is a perennial herbal species in the Polygonaceae family that grows along the banks of lakes, drains, and canals, and they have a growth type that can support themselves. The Polygonaceae family has long been used historically to treat a variety of illnesses. *P. senegalensis* is one of the species of the genus *Persicaria*. The species *P. senegalensis*, also known as *Polygonum senegalense Meissner*. It is indigenous to southern and tropical regions of the world. Locals in tropical African countries combine the plant's leaves with other plants to treat syphilis and anemic individuals (Mathole & King, 2023).

*P. senegalens*is remarkably rich in chemicals derived from surface exudates. Chalcones are one prominent family of chemical components, their heterocyclic compounds, pyrazolines, their derivatives, phenolics, and flavonoids present in *P. senegalensis*. It is also well known to possess a range of biological properties, including antimicrobial, antibacterial, antitumor, and anti-inflammatory properties. Since they have long been used to treat a variety of inflammatory illnesses, including arthritis, amenorrhea, gout, knee pain, menstrual discomfort, and rheumatoid *Persicaria* species play a key role in complementary medicine. These species contain biochemically active components that have tyrosinase inhibition, antioxidant, analgesic, antileukemic, antibacterial, and anti-tumor activities. They are also regarded as conventional medicines for the treatment of conditions like dyspepsia, hemorrhoids, diarrhea, and itchy skin. These species have been demonstrated to have potential medicinal properties and include phenolic acids and flavonoid chemicals (Kenand & Omosa, 2017).

1.2. Statement of the problem

Traditional medicine remains a widely accepted healthcare alternative, but the rising prevalence of emerging and re-emerging infectious diseases, coupled with growing microbial resistance to conventional antibiotics. despite the development of new antibiotics by pharmaceutical companies over the past three decades (Batool et al., 2021). In southern Ethiopia, *P. senegalensis* is commonly used as a TMP; however, there is little scientific data

on its phytochemistry and biological properties, with no studies in Ethiopia, to our knowledge, examining the chemical composition of *P. senegalensis* extract or its antimicrobial, antioxidant, or other properties. This study aims to bridge this knowledge gap through a comprehensive phytochemical analysis and evaluation of the antimicrobial and antioxidant properties of *P. senegalensis*.

1.3. Objective of the study

1.3.1. General objective

- To evaluate the antimicrobial and antioxidant activities of *P. senegalensis* (Hobet leaf*)* extract against selected human pathogenic microorganisms and to analyse its chemical composition.

1.3.2. Specific objectives

- To perform phytochemical screening test on the extract of *P. senegalensis* to identify and measure the bioactive chemicals present.
- To Examine the antibacterial activities of *P. Senegalensis* against bacteria and fungus pathogens.
- To assess the radical scavaging activities of *P.senegalensis* leaves extracte and EOs
- To extract essential oil of the plant by hydro-distillation and analyse it by GCMS.
- To characterize isolated compound of *P. senegalensis* using various analytical techniques (e.g., NMR, GC-MS and FTIR).

1.4. Scope of the study

The sampling area for this study was the southern part of Ethiopia around Werabe town. The study was conducted at Adama Science and Technology University. This research was mainly focused on the phytochemical investigation and evaluation of the antibacterial and antioxidant activity of Hobet leaf on diseases caused by bacterial and fungi species that may cause severe illness to human beings.

1.5. Significance of the study

The significance of this study is to give information on the phytochemical composition antioxidant and antibacterial properties of *P. senegalensis,* which is found in Ethiopia. The study can detect and quantify the bioactive chemicals contained in these plants by

performing a phytochemical analysis. This knowledge can shed light on their therapeutic potential and help with the creation of pharmaceutical or natural treatments made from *P. senegalensis*.

2. LITERATURE REVIEW

2.1. Medicinal plants

Plants traditionally utilized for therapeutic purposes are referred to as medicinal plants sometimes known as medical herbs. These plants include a variety of bioactive substances with therapeutic characteristics including alkaloids, terpenoids, and phenolic compounds. Many conventional medical systems, including Ayurveda, Traditional Chinese Medicine, and Indigenous Medicine, have utilized medicinal plants for healing and treating illnesses for thousands of years(Agidew, 2022). Only a few of the chemical elements present in medicinal plants that have clear physiological effects on people are alkaloids, tannins, sugars, terpenoids, and flavonoids. These chemicals are produced by living things primarily or more precisely by secondary metabolism. They are widely used in human therapy as well as veterinary, agricultural, and academic research. Several phytochemicals belonging to a variety of chemical classes have in vitro inhibitory effects on all microorganism kinds (Yadav & Agarwala, 2011). Medicinal herbs hold significant importance for Ethiopians and residents of other developing countries in Asia and Africa. These individuals highly value the use of medicinal plants, which serve as alternative or complementary remedies to compensate for the often limited access to modern medical treatments. Moreover, the utilization of medicinal plants contributes to the enhancement of overall health and safety within the local communities. These plants play a crucial role in the daily lives of people and establish profound connections with diverse populations(Agidew, 2022).

The presence of phytochemical components such as tannins, phenolic compounds, flavonoids, caffeoylquinic acid, and terpenoids is closely associated with the antibacterial properties of plant extracts. Several plant extracts have been scientifically confirmed to possess antimicrobial characteristics and serve as antioxidants and preservatives. These antimicrobial agents have played significant roles in the domains of food and health products, primarily due to their non-toxic nature and high safety profiles (Myint et al., 2022).

2.2. Polygonaceae family

2.2.1. Distribution and traditional uses of the Polygonaceae family

The Polygonaceae family, commonly referred to as the knotweed or smartweed family, encompasses over 1200 species of flowering plants. Notable genera within this family include *Eriogonum* (with 2410 species), *Rumex* (200 species), *Cocoloba* (120 species), and

Persicaria (100 species). Throughout history, this plant family has been utilized for its medicinal properties in treating various ailments. These include but are not limited to asthma, bronchitis, cough, diarrhea, dysentery, eczema, earaches, inflammation, leprosy, paralysis, toothaches, ulcerative colitis, intestinal parasites, and other health conditions (Vasas et al., 2015) While Polygonaceae family is found across various regions globally, its distribution is particularly prominent in the North Temperate Zone. Some genera within the family exhibit a tropical or subtropical habitat, but the majority are restricted to Northern temperate climates. This indicates that while the family is widely distributed, its primary concentration is within the Northern temperate regions of the world. (Ayodele & Olowokudejo, 2006)

2.2.2. Genus *persicaria*

The genus *Persicaria* was initially described by Miller in 1754; however, no typification was established at that time. Subsequently, Britton and Brown (1913) lectotypified the genus based on *P. persicaria* L. Currently, there are 150 recognized species of *P. persicaria L.* worldwide. This genus exhibits a wide distribution, with a predominant presence in temperate zones. Additionally, certain species can be found in tropical and subtropical regions, ranging from sea level to high altitudes. The majority of taxa within the genus, including the extensively cultivated and highly regarded *P. odorata* (Vietnamese coriander), are perennial plants (Kantachot et al., 2010).

2.2.3. *Persicaria* Species

Persicaria species within the Polygonaceae family have significant importance as complementary medicines, owing to their long-standing utilization in the treatment of various conditions. These species have long been used to treat inflammatory conditions like colic, rheumatic pain, knee pain, gout, menstrual pain, and amenorrhea. Moreover, they have been used to treat skin diseases such as ringworms, boils, abscesses, and scabies. Additionally, *Persicaria* species are frequently employed in the treatment of diseases like hemorrhoids, dyspepsia, itchy skin, and diarrhea. *Persicaria* species have been found to contain phytochemicals such as terpenoids, anthraquinones, flavonoids, and apianen lactones. The therapeutic properties of the species, such as its anticancer, antioxidant, analgesic, antileukemic, antibacterial, and tyrosinase-inhibiting effects, are attributed to these active biochemical components. The presence of these phytochemicals enhances the medicinal value of *Persicaria* species, making them valuable resources in the field of natural medicine.(Vasas et al., 2015).

2.3. *P. senegalensis*

P. senegalensis is a plant species classified under the genus *Persicaria* within the family Polygonaceae. It naturally occurs in tropical and subtropical regions of Africa and Asia (Figure 1). This plant species has a history of being utilized for diverse medicinal applications. It has been traditionally employed in the treatment of wounds, inflammation, pain management, and even malaria. *P. senegalensis* holds significance as a medicinal plant due to its therapeutic properties and has been valued for its potential health benefits in various traditional healing practices. (Hussein et al., 2017).The genus *Persicaria* is well-known for its extensive array of therapeutic properties, which are attributed to its capacity to synthesize a diverse range of secondary metabolites. These metabolites include phenylpropanoids, phenolic compounds containing tannins, coumarins, anthraquinones, flavonoids, neoflavonoids, triterpenes, lignans, and various other phenolic compounds that do not fall under the subclass of flavonoids. The presence of these bioactive compounds contributes to the medicinal potential of *Persicaria* genera, allowing for a wide range of therapeutic applications in traditional and modern medicine (Seimandi et al., 2021).

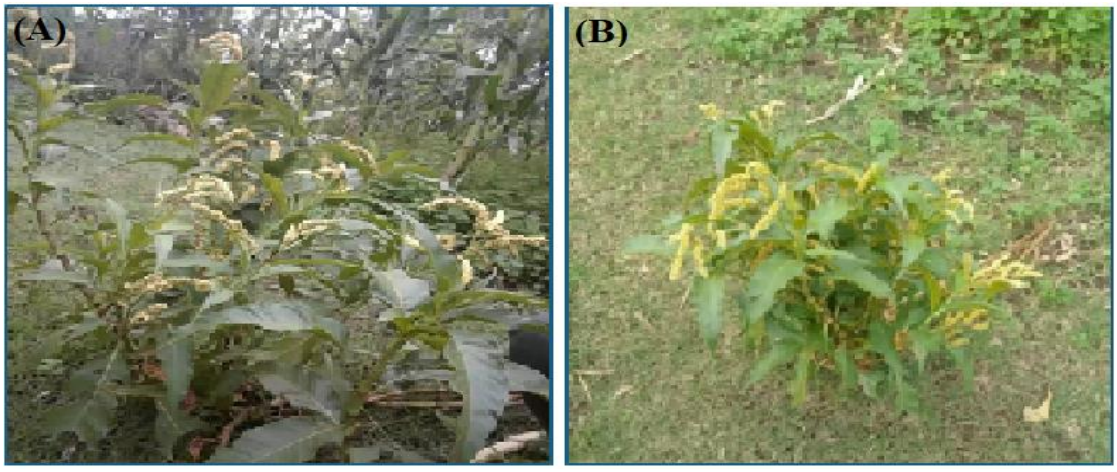

Figure 1: *P. senegalensis* (Hobet) (A)Matured form of leave and (B) young form of leave Photo taken by Riyad Aman on September 12, 2023, from Werabe, southern Ethiopia.

2.4. Pharmacological active compounds and their biological activity

The pharmacological properties exhibited by numerous plant species can be attributed to their phytoconstituents, which encompass a wide range of compounds such as saponins, glycosides, alkaloids, tannins, terpenoids (including monoterpenes, diterpenes, and sesquiterpenes), steroids, and flavonoids. A significant proportion, approximately 80%, of the global population relies on traditional medicines as a primary source of healthcare.

Medicinal plant extracts contain various components that possess diverse biological activities, including virucidal, bactericidal, fungicidal, anti-inflammatory, analgesic, sedative, spasmolytic, and local anesthetic effects, among others. These biological activities contribute to the therapeutic potential of medicinal plants and support their utilization in traditional and complementary medicine practices worldwide. (Batiha et al., 2020).

2.4.1. Techniques of isolation and purification of bioactive compound

Modern methods of isolating, separating, and purifying substances are precise thanks to sophisticated techniques. These methods also aid in the creation and accessibility of a wide range of complex bioassays. Finding appropriate techniques to quickly, easily, and accurately screen source materials for bioactivities, like cytotoxicity, antioxidant capacity, or antibacterial qualities, is the goal in the hunt for bioactive compounds. By using these techniques, scientists can find valuable compounds with desired biological properties by efficiently assessing the potential bioactivity of various substances.(Altemimi et al., 2017).

Medicinal plants are obtained through harvesting and subsequently prepared for experimentation or direct consumption as herbal or traditional medicine. The preparation of medicinal plants for experimentation involves several steps, including timely and accurate collection of plant material, expert authentication, proper drying, and grinding. Following these steps, the bioactive compounds are extracted, fractionated, and isolated, if applicable. This process also involves determining the quantity and quality of the bioactive compounds present. The extraction of medicinal plants entails selecting an appropriate solvent and following standardized extraction procedures to separate the active plant material, such as alkaloids, flavonoids, terpenes, saponins, steroids, and glycosides, from (Abubakar & Haque, 2020a) the inert or inactive components. These extraction methods are essential for obtaining the desired bioactive compounds for further analysis and potential application in medicine. Plant extracts have been analyzed using a variety of methods, such as high-performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS), and TLC. Although TLC-densitometry has two advantages over HPLC, which is still widely used, they have greater sensitivity and can process a large number of samples quickly (Altemimi et al., 2015).

2.4.2. Chemical composition of *P. senegalensis*

P. senegalense exhibits a remarkable abundance of chemicals derived from surface exudates. Among the notable chemical components phenolics, and flavonoids. These compounds are known to possess various biological properties, including antimicrobial, antibacterial, antitumor, and anti-inflammatory activities. *P. senegalense* has a longstanding history of traditional use in the treatment of diverse inflammatory conditions, such as arthritis, amenorrhea, gout, knee pain, menstrual discomfort, and rheumatoid ailments. The presence of these bioactive compounds contributes to the therapeutic potential of *P. senegalense* in managing inflammatory illnesses (Kenand & Omosa, 2017). *P.senegalensis* extract of leaves, flowers, and stems had high concentrations of tannins, phenols, and saponins, while the roots had only trace amounts of these compounds. Glycosides were found to be absent from the roots but to be present in moderate concentrations everywhere other than the leaves. There were no terpenoids detected in any of the plant sections (Mathole & King, 2023). Phenolic acids and flavonoid compounds are also present and have shown potential therapeutic effects. Phenolics and flavonoids demonstrate pharmacological features in the form of antifungal, antibacterial, anti-inflammatory, and anti-tumor properties (Youssef & El-Swaify, 2018b).

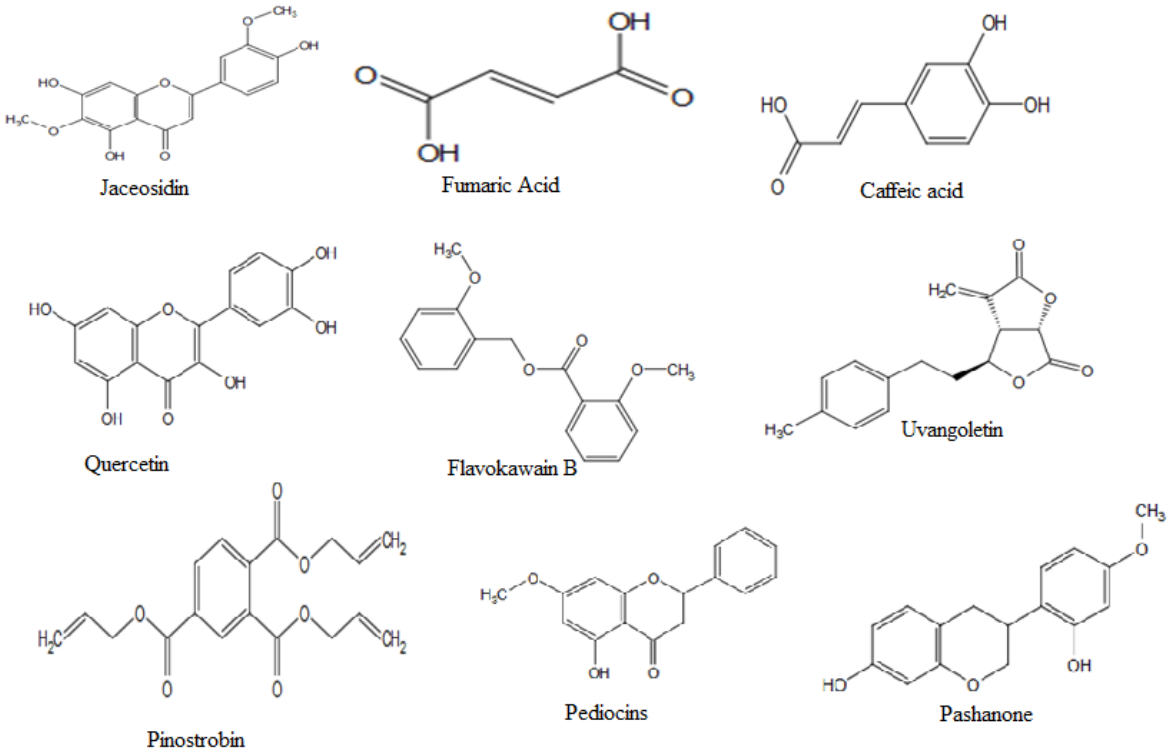

Figure 2: Phytochemical compounds isolated from *P. senegalensis* (Meisn.) Sojak (Ayman et al., 2024)

2.5. Biological Activities of *P. senegalensis*

2.5.1. Antimicrobial Activity of *P. senegalensis*

Plants have developed defense systems in the form of secondary metabolites to effectively protect against infections in humans. These defense mechanisms are a result of the plants' evolutionary adaptations. It is estimated that approximately 14-28% of higher plant species are used for medicinal purposes. The utilization of plant-based antimicrobials offers various advantages, including cost-effectiveness, improved patient tolerance, absence of adverse effects, enhanced bioavailability of active agents, and effective therapeutic action at lower doses. The antimicrobial properties of *Persicaria* species have been demonstrated against a diverse range of bacterial and fungal strains, making them a potential treatment option for bacterial and fungal infections in humans. *P. senegalensis* has shown multiple biological activities, including cytotoxic, immunosuppressive, insecticidal, amoebicidal, antiviral, antibacterial, and anticancer effects (Seimandi et al., 2021).

In a previous study, three primary bioassays were employed to detect antimicrobial activities in various extracts of *Polygonum* or *Persicaria* species, along with their bioactive compounds. These bioassays included measuring the percentage of inhibition of microorganism growth and determining the Minimum Inhibitory Concentration (MIC) for

each tested sample, whether it was an extract or an isolated compound. The MIC determination is a recognized standard method for assessing antimicrobial susceptibility, thus enhancing the credibility and reliability of the obtained results from conducting these bioassays(Derita et al., 2013). Promising activities were observed in the extracts of *P. acuminata* which contained the sesquiterpene polygonal, as well as in the extracts of *P. ferruginea*, which contained the chalcones cardamonin and pashanone. Additionally, *P.senegalensis* exhibited both antifungal and antibacterial properties. Notably, the isolated compounds confertifolin and drimenol from *P. hydropiper* essential oil, as well as polygodial from its chloroformic extract, displayed significant MIC values ranging from 0.39 to 125 µg/mL, which were comparable to those of standard drugs (Duraipandiyan et al., 2010).

2.5.2. Antioxidant activity of *P. senegalensis*

The presence of free radicals, including reactive oxygen species (ROS) and reactive nitrogen species (RNS), results in a highly reactive condition that can cause damage to numerous biomolecules. This damage is associated with the development of various chronic diseases, including diabetes mellitus, atherosclerosis, inflammation, and cancer. However, in response to this, antioxidants play a crucial role in counteracting these harmful effects and preventing the progression of such pathologies. It is noteworthy that natural antioxidant extracts derived from plants contain a diverse array of potent bioactive compounds, such as phenolic acids, flavonoids, and tannins, which are known for their antioxidant activity (Chansiw et al., 2019).

The effects of antioxidants can be attributed to various functions. These functions include the suppression of active species formation by reducing hydroperoxides and hydrogen peroxide, as well as the sequestering of metal ions. Additionally, antioxidants scavenge active free radicals and aid in the repair and/or clearance of damage (Noguchi & Niki, 2000). Phenolics have been found to have beneficial effects on color, taste, scent, and flavor in addition to their positive health effects; these aspects all contribute to the nutritional value and quality of food. Several studies demonstrate the vital role that phenolic compounds play in scavenging free radicals. Flavonoids also have a major effect on human nutrition and health and show antioxidant activity. They function by either chelating or scavenging substances(Liu, 2003).

The assessment of antioxidant activity can be conducted using various methods, both *in vitro* and *in vivo.* One particularly popular *in vitro* assay is the DPPH-based method, which is

widely employed due to its simplicity, rapidity, and cost-effectiveness (Alam et al., 2013) The *Persicaria* and *Polygonum* genera encompass species that exhibit notable antioxidant activity. Certain compounds found in these genera possess the ability to eliminate excessive free radicals within the body, thereby sustaining normal metabolic processes. Furthermore, it has been observed that the antioxidant activity of these compounds is dependent on the dosage. Additionally, phenolic compounds are widely distributed throughout the plant kingdom and are renowned for their role as antioxidants, providing protective benefits to plants (Seimandi et al., 2021c).

3. MATERIALS AND METHODS

3.1. Chemicals and instruments

The research work employed the following chemicals and reagents. N-hexane (99.9%, Pentokey Organy, India), ethyl acetate (99.9%, Sisco Research Laboratories, India), chloroform, methanol, and DMSO (99.8%, Loba Chemie, India), ciprofloxacin (Wellona Pharma, India), anhydrous Na_2SO_4, ascorbic acid, Mueller Hinton Agar (Micro express, India), DPPH (98.5%, China), distilled water, and silica gel for column chromatography (60-200 mesh, Merck, India). All the solvents and reagents used were in analytical grade.

The apparatus and instruments which have been utilized in the study were: mortar and pestle, electric blender (Shanghai Jingke, JK-HSG-100A, China), polyethylene bag, electronic balance, beakers (different size), shaker (Gemmy Industries, VRN-200, Taiwan), filter paper (Whatman No.1 filter paper, 125 mm diameter, India)), column chromatography set up, separatory funnel, vials, oven, stove, heating mantle, TLC plate (thickness; 0.25 mm, size; 20 x 20 cm Aluminum coated with high-grade silica gel, 230–400 mesh, pore size 60 Å, Merck Grade 64271, Darmstadt, Germany), capillary tube, water bath, UV lamp (UV4AC6/2, CBIO Bioscience and Technologies, China), Clevenger's apparatus (Aarson Scientific Works, India), flasks (different size), measuring cylinder, rotary evaporator (DW-RE-3000, China), aluminum foil, glass rod, chromatographic chamber, ruler, pencil, spatula, distillation flask, petri dish, refrigerator, holder, incubator, digital melting point (Japson-type apparatus, JA90161, India) and UV-VIS spectrophotometer (Cecil CE4001, UK) and GC-MS (Santa Clara, CA, USA).

3.2. Description of the study area

The plant sample used for this study was collected from Werabe, which is 172 km away from Addis Ababa. This town is in the (SNNPR). Werabe town is geographically located in south-central Ethiopia. The City has a latitude and longitude of 8°1′N 38°20′E and an elevation of 2,113 m(6,932 ft) above sea level. Siltie is one of the central zones of the region, with large plains, mountainous areas, and plateaus. The climate of the zone has three different agro-climatic conditions: high and mid-land, consisting of 37 and 63% respectively. The average temperature ranges from 12 to 26 °C, and the average annual rainfall ranges from 780 to 1818 mm. 95.5% of the population is engaged in agriculture (Bekele et al., 2021) (Figure 3).

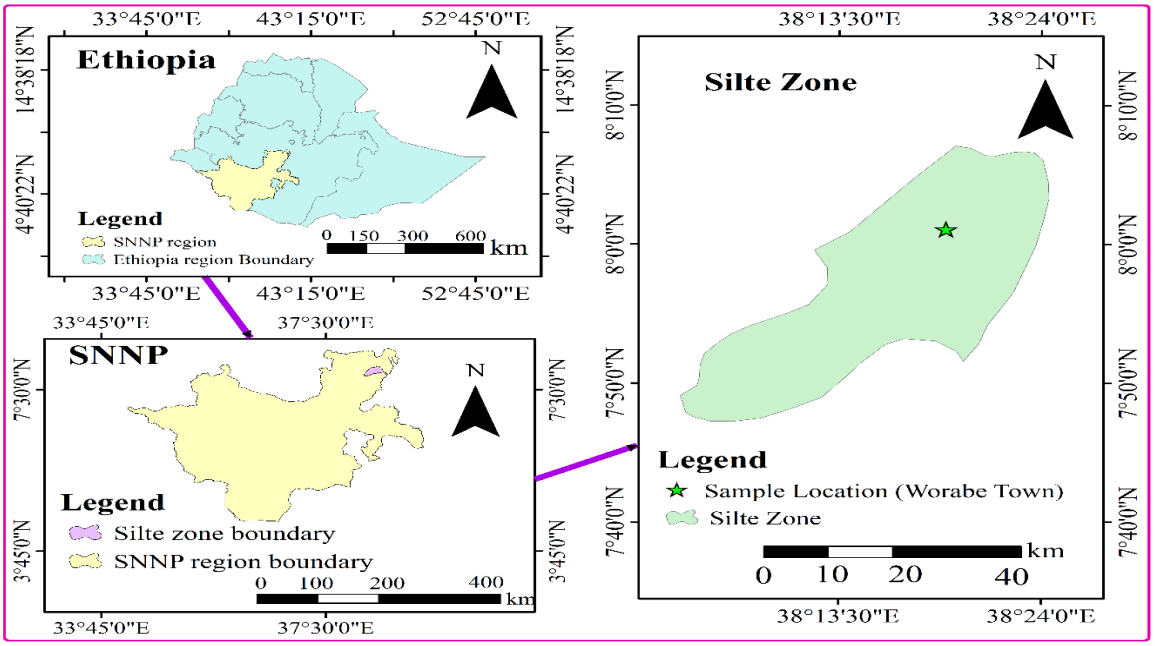

Figure 3: Global information system (GIS) for sample collection area of *P. senegalensis*

3.3. Study samples and sampling techniques

3.3.1. Collection of Experimental Plant and Identification

A 10 kg leaves of *P. senegalensis (Hobet)* were collected from the town of Werabe in the Silte Zone of the South Nations, Nationalities, and Peoples Region (SNNPR) in Ethiopia. The fresh samples were packed in plastic bags and transported to Adama Science and Technology University. To facilitate taxonomic identification, a voucher specimen was taken and pressed on a newspaper. The taxonomic identification was conducted by Mr. *Melaku Wendafrash* (chief botanist). A voucher number of the specimen was designated by an accession number (RA001) and deposited at the National Herbarium of the Biology Department, Addis Ababa University, and a voucher specimen was deposited there.

3.3.2. Preparation of experimental plant for extraction

The solid-liquid extraction technique was utilized as a general method for extraction. Plant samples were washed thoroughly with distilled water to remove dirt, dust, and insects, and dried at room temperature in a shaded area, without being exposed to direct sunlight, to reduce the high moisture content for approximately three weeks. Following the drying period, the dried plant materials were ground using an electronic grinder to obtain a fine powder of the plant. To achieve complete extraction of the plant material, a mixture of organic solvents and plant material was prepared in a 1:5 ratio. Specifically, 0.4 kg of dried

and ground plant leaves material was extracted with 2 L of n-hexane. The mixture was macerated at room temperature on a shaker for 72 hours and then filtered using Whatman No. 1 filter paper. The solvents were freed using a rotary evaporator maintaining the temperature at 40 °C. The residual plant material left after n-hexane extraction was undergoing further extraction using chloroform, methanol/chloroform (1:1), and methanol following the same procedure, and the filtrates were concentrated in vacuo on a rotary evaporator at 40 °C to generate 4.5g, 7.69g, 11.25g and 20.5 g of n-hexane, $CHCl_3$, CH_3OH: $CHCl_3$ (1:1) and methanol extracts, respectively. The yield of the crude extract was calculated (Abubakar & Haque, 2020b).

$$\text{Yield } \% = \frac{\text{Weight of extract obtained}}{\text{Weight of plant sample}} \times 100 \quad (1)$$

3.3.3. Phytochemical screening

Phytochemical examinations were conducted on *P. senegalensis* extracts according to standard procedures.

Test for alkaloids: To test for the presence of alkaloids involve the addition of 1 ml of Hydrochloric acid to 2 mg of the methanolic extract, in addition to a few drops of Wagner's reagent. The detection of a yellow or brown precipitate served as an indication of the presence of alkaloids (Joshi et al., 2013).

Test for flavonoids: To test for the presence of flavonoids, 2 mg of the extract was treated with 2 ml of 0.1 N sodium hydroxide solutions and 0.1 N hydrochloric acid. Following this procedure, the presence of flavonoids was indicated by the color change from yellow to colorless in the solutions (Edema & Alaga, 2012).

Test for phenols: To detect the presence of phenols, 1 mg of extract was combined with 2 ml of distilled water, followed by the addition of five drops of 10% ferric chloride. The presence of phenols is indicated by the formation of blue or green color. (Sha'a et al., 2019).

Test for Saponins: To determine the presence of saponins, a 1 mg extract was vigorously mixed with a few drops of distilled water. The presence of saponins was indicated by the formation of persistent foam (Joshi et al., 2013).

Test for steroids: To determine the presence of steroids, 2ml of acetic anhydride was added to the 2mg extracts, which have been dissolved in an appropriate solvent. Alongside this, 2 ml of concentrated sulfuric acid was added. The test tube was then carefully inverted to form

a distinct layer underneath. The test tube was observed for the appearance of a green coloration, which indicates the presence of steroids (Auwal et al., 2014).

Test for Tannins: To determine the presence of tannins, a few drops of a 10% Ferric chloride solution (light yellow) were added to 2 ml of an aqueous solution (W/V) of the extract. The appearance of a blackish-blue color served as an indication of the presence of tannins (Auwal et al., 2014).

Test for terpenoids: To test for the presence of terpenoids, 5 mg of the extract was combined with 2 ml of chloroform. Following that, 3 ml of sulfuric acid was added cautiously to create a distinct layer. The occurrence of a reddish-brown coloration at the interface served as an indication of the presence of terpenoids (Takaidza et al., 2018).

3.3.4. Characterization of Bioactive compounds

3.3.4.1. TLC analysis

TLC analysis was used to facilitate the identification of compounds present in the four crude extracts. This was done by putting a spot of each sample on a thin-layer chromatography (TLC) plate and allowing the solvent to migrate up the plate. The distance covered by the solvent front and the distances traveled by individual spots were recorded. Once the solvent front approaches the top of the plate, the plate is removed from the beaker to prevent further solvent migration. The positions of the spots were marked by drawing a line with a pencil to ensure accurate identification and documentation.

3.3.4.2. Liquid-liquid partitioning

The methanol and MeOH:Cloroform(1:1) extracts exhibited similar TLC profiles in different solvent systems (10-80% EtOAc in n-hexane) and were mixed to give 30g of MeOH and Met: Chlo (1:1) extracts were dissolved in 200 ml of distilled water and 200 ml MeOH consecutively partitioned with equal volumes of n-hexane, chloroform, and ethyl acetate (EtOAc). Each solvent fraction was filtered and concentrated with a rotary evaporator at 35 °C. All crude extracts were stored in the refrigerator at 4 °C until required for further analysis. The extracts were analyzed with TLC for further fractionation and the ethyl acetate extract was subjected to silica gel column chromatography for further isolation of compounds (Sasidharan et al., 2011a).

3.3.4.3. Column Chromatography Technique

The leaves of *P. senegalensis* were extracted on maceration with n-hexane, Chloroform, Methanol/chloroform 1:1, and MeOH. A (5 g) of the ethyl acetate fraction was adsorbed on an equal amount of silica gel (mesh size 60-120) and subjected to silica gel column chromatography (165 g of silica gel) using n-hexane for initial packing. Elution was carried out with an increasing gradient of ethyl acetate in n-hexane followed by an increasing gradient of methanol in chloroform. Silica gel served as the stationary phase, and various solvent combinations were used as the mobile phase. Each fraction obtained was collected, labeled, and subjected to TLC analysis to assess its purity. For further purification of the isolated compounds, repeated column chromatography, was employed. The purified compounds were then submitted for FTIR analysis to determine their structures, and biological activity tests to evaluate their respective biological activities (Mawire et al., 2021).

3.3.5. Extraction of the volatile oils

Essential oils (EOs) were extracted from the leaves of *P. senegalensis* (Hobet leaf) via the hydro distillation method. About 100 g of the dried powder of *P. senegalensis* was placed in the round bottom flask (1000 mL). Then, 400 mL of distilled water was added and mixed thoroughly with the powdered sample. The flasks were fitted with a Clevenger-type apparatus, a glass condenser, and heated using a heating mantle. Subsequently, the mixture was hydro-distilled at atmospheric pressure for 3 h. The oil part of the extract was separated from the aqueous layer by adding 100 mL of chloroform in a separatory funnel. A small amount of aqueous left with the organic layer was dried by adding 5 g of anhydrous Na_2SO_4 and filtered using Whatman No 1. Finally, the extract was concentrated *in vacuo* using a rotary evaporator and the essential oils were kept in a refrigerator at 4 °C until further analysis (Elyemni et al., 2019).

3.3.6. GC-MS analysis

GC-MS analysis of plant essential oils was performed by a GC (7890B, Agilent Technologies, USA) coupled with an MS (5977A Network, Agilent Technologies). The GC had an HP-5MS column (30mm × 250 μm internal diameter (i.d.) and 0.25 μm). Helium was used as a carrier gas with (1ml/min flow rate). The oven temperature was set to 100 °C for 2 min before being raised to 280 °C at a rate of 10 ºC/min (inlet 250° C; detector 280 °C; split less injection/purge time 1.0min), solvent delay 4.00 min Mass spectra were recorded in an electron-impact mode, with ionization energy of mode at 70 eV, scanning the 33-550

m/z range (Gülçin et al., 2004). The volatile compounds in the oil were identified by comparing the mass spectra of the compounds in the oils with those in the database of NIST11 GC-MS libraries (Hanuš et al., 2008).

3.3.7. Biological Activity

3.3.7.1. Bacterial strains

The bacterial strains used in this study were American-type culture collections (ATCCs). Four bacterial strains including two Gram-negative (*E. coli* (ATCC 25922^{T}) and *P. aeruginosa* (ATCC 27853^{T})) and two Gram-positive (*S. aureus* ATCC 25923^{T}) and *S. pyogene* (ATCC 19615^{T}) and fungi strain *C. albicans ATCC 10231^{T}*. The strains were obtained from laboratory stock of Adama Regional Laboratory, Adama, and cultured in Mueller Hinton broth (MHB) overnight at 37°C. The microorganisms were handled and transported aseptically. The strains were grown and preserved in test tubes containing nutrient broths at 4 °C in a refrigerator until required for the bioassay.

3.3.7.2. Preparation of inoculum, culture media, and plates

The Mueller Hinton broth (MHB), Mueller Hinton Agar (for bacteria), and Sabouraud dextrose broth (for fungi) were organized as per the directions of the manufacturers. The media were sterilized at 121 °C and 20 mL of it was poured into sterilized Petri dishes to form the Mueller Hinton media with a uniform thickness of about 3 mm. The MHA plates were equal-segmented to confirm that the disks were not very close to 24 mm on the MHA. Each bacterial strain was cultured overnight at 37 °C in Petri dishes containing the MHA. The bacterial inoculums were prepared by the direct colony suspension method (Sengera et al., 2023).

3.3.7.3. Antimicrobial activity

The extracts against the strains were evaluated by paper disc-diffusion assay as per the standard protocols of the Clinical and Laboratory Standards Institute (CLSI) (Balouiri et al., 2016). Bacterial strain colonies were transferred into a saline solution using an incubating loop and grown in Mueller-Hinton Broth (MHB). The turbidity of the bacterial strains was attuned to about 0.5 McFarland standard solution (1.5 x 10^{8} CFU/mL) and fungal cultures grown in Sabouraud Dextrose Broth (SDB) at 30°C were adjusted at 2 ml with a concentration of 10^{6} spores/ml (Andrews, 2001). The bacterial and fungal suspensions were transferred by swapping with a sterilized cotton swap onto the Petri dishes containing the

media (MHA) (Micro express, India) (g/L) (Beef extract, 2.0 g/L, Acid Hydrolysate of casein, 17.5; Starch, 1.5; and Agar: 17.0) plates. Medium for bacteria (solidified 20-25 mL) and Sabouraund Dextrose agar (SDA) (g/mL) (peptones: 10; Dextrose: 40 and Agar: 15) for fungi. Whatman No.1 filter paper discs (6 mm in diameter) were then prepared using a puncher for the impregnation of the test samples and sterilized at 121 °C for 15 min. Stock solutions of the crude extracts (50 mg/mL) and EOs (10 mg/mL), were prepared in 4% DMSO (v/v) followed by the preparation of different concentrations by serial dilution. Accordingly, four different concentrations of the crude extracts (were prepared in four sets of dilutions: 200, 100, 50, and 25 µg/ml, and four different concentrations of the EOs (50, 25, 12.5, and 6.25 µl/ml) were prepared from their respective stock solutions.

A standard antibiotic (ciprofloxacin, 0.25 mg/mL for bacteria and ketoconazole, 100 µg/mL for fungi) and DMSO were used as positive and negative controls, respectively. Part of each solution (100 μL) was impregnated onto the sterilized paper discs of about 6 mm in diameter which were placed over the petri dishes containing the bacterial culture inoculated MHA. The Petri dishes were left for 30 min for diffusion and incubated at 37 °C for 24 h. The antibacterial activities were evaluated by measuring the zone of inhibition (mm) of the extracts using the digital zone of inhibition measuring caliper against the test organism. The entire tests were performed in triplicate and the results were recorded as mean ± standard error of the mean (SEM) using statistical analysis software (Balouiri et al., 2016). After the completion of the assay, the used bacterial inoculums, petri dishes, swabs, and other disposable materials were autoclaved and discarded into trash.

3.3.7.4. Antioxidant activity

The crude extracts and EOs of *P. senegalensis* were screened for their antioxidant efficacy using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay *in vitro*. The free radical trapping potential of the extracts along with a reference drug (ascorbic acid) was tested against DPPH radicals using the protocol described by (Khorasani Esmaeili et al., 2015) Five different concentrations (1000, 500, 250, 125, and 62.5 μg/mL) of each extract, EOs, and ascorbic acid (AA) were prepared from the corresponding stock solutions (1 mg/mL in methanol). To each prepared concentration, freshly prepared DPPH solution (1 mL, 0.04% w/v in methanol) was added followed by place it in dark area for 30 min at room temperature. After incubation, the absorbance of each solution was measured at 517 nm using the UV-Vis spectrophotometer in triplicate. Sample-free DPPH solution in methanol

was used as a negative control. The DPPH radical scavenging potential of each sample was expressed in terms of the percent of scavenging activity (Khorasani Esmaeili et al., 2015).

$$\% \text{ DPPH scavenging} = \frac{\text{Control absorbance - extract absorbance}}{\text{Control absorbance}} \times 100 \quad (2)$$

Where, A and A_0 signify the absorbance of the containing sample in methanol, and DPPH radical in methanol, respectively.

Finally, the DPPH free radical scavenging power of the extracts was compared to AA, and the IC_{50} (the concentration needed to scavenge the total DPPH radicals by 50%) values were calculated from the relationship curves of the plots of concentration versus the mean percentages of the antioxidant activity.

3.4. Determination of Minimum Inhibitory Concentration (MIC)

3.4.1. MIC determination of crude extracts and Essential oil

The broth dilution method was used to determine the minimum inhibitory concentration (MIC). First adjusted microbial suspension was diluted to concentrations of 1.5×10^8 CFU/ml of bacteria. Prepared stock solutions of *P.senegalensis* leaf extracts at a concentration of 400 mg/ml. Then two-fold serial dilutions in 25 ml beakers, creating solutions with 200, 100, 50, 25, and 12.5 mg/ml concentrations. Similarly, a 100 mg/ml solution of essential oil was prepared. Two-fold serial dilutions to obtain solutions at lower concentrations (50, 25, 12.5, 6.25, and 3.125 mg/ml). Using a micropipette, 100 Mg of each crude extract and essential oil were transferred into test tubes containing 5ml of Mueller Hinton Broth (MHB) each marked with their respective concentrations. Subsequently, 10 ml of inoculum adjusted to 0.5 McFarland of selected pathogenic microbes (i.e. *S. aureus* ATCC 25923^T, *S. pyogene* ATCC 12204^T, *P. aeruginosa* ATCC 27853^T, and *E. coli* ATTC 25922^T) were added to each marked test tube, following a slightly modified version of the previously adopted method. The test tubes were incubated at 37 ºC for 24 h (Koeth et al., 2015). After incubation, the absorbance of the culture was measured at 517 nm. The minimum inhibitory concentration (MIC) was determined as the lowest concentration of the extract that prevents visible growth of the organism (Trentin et al., 2013).

3.4.2. Determination of MBC

After MIC values were determined, the minimum bactericidal concentrations were evaluated. Aliquots from test tubes were subcultured on the surfaces of Muller Hinton Agar (MHA) plates in the absence of visible bacterial growth, as identified by the MIC evaluation.

Every subculture was incubated for 24 h at 37°C to promote bacterial growth. Following the incubation time, the Minimum Bactericidal Concentration (MBC) values were determined to be the lowest concentrations of each plant extract and essential oil that inhibited the growth of bacterial cells, (Akinduti et al., 2019).

3.5. Data analysis

The experimental results were expressed as mean ± standard deviation (SD) of three replicates. Where applicable, the data was subjected to SPSS 16 one-way analysis of variance (ANOVA), and the results were presented using tables and graphs. P values less than 0.05 were considered statistically significant.

4. RESULTS AND DISCUSSIONS

4.1. Extraction yields

Extraction yield refers to the percentage of desired compounds successfully extracted from a given plant material. It's a critical metric that reflects the efficiency of the extraction process and can provide insights into the potential bioactivity of the extracts. The plant materials (leaves) of *P. senengalensis* were successively extracted and, yielded 4.5g greenish n-hexane extract, 7.69 g blackish chloroform extract, 11.25 g dark blackish, CH_3OH: $CHCl_3$ (1:1) extract, and 20.5 g brownish methanol extracts. (Figure.4) The maximum (5.125%) and minimum (1.125%) contents of extractable matter were obtained from the methanol extract of the leaves of *P. senegalensis* and n-hexane extract respectively. This suggests that the plant leaves of *P.senegalensis* contain more polar secondary metabolites that are better extracted using polar solvents like methanol, compared to less polar compounds extracted by non-polar solvents like n-hexane.

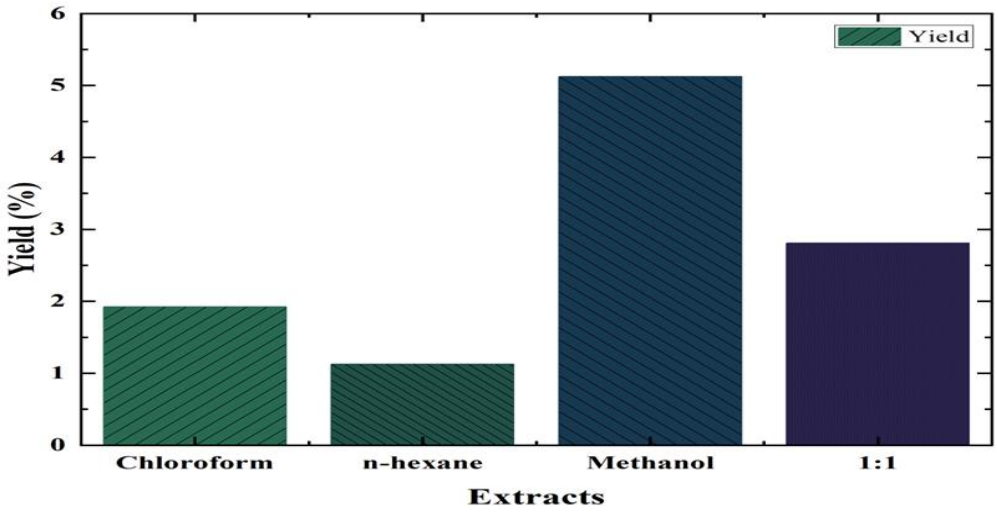

Figure 4: Percentage yield of *P. senegalensis* from leave extracts

4.2. Phytochemical screening

Phytochemical screening analysis was conducted to identify the secondary metabolites in the crude extracts following standard experimental procedures. These tests revealed the presence of alkaloids, flavonoids, Saponins, tannins, steroids, phenols, and terpenoids in chloroform, Methanol: Chloroform (1:1), and methanol extracts. Phytochemical screening of the extracts was positive almost for all the phytochemical tests analyzed within the crude extracts except for n-hexane extract alkaloids, tannins, and phenols, which were absent as

shown (Table 1, Figure 5). Qualitative phytochemical tests showed a significant indication of the presence of major secondary metabolites in *P. senegalensis*.

The study undertaken by Payne & Lall, (2024) confirmed the presence of alkaloids, flavonoids, saponins, tannins, steroids, phenols, and terpenoids in this plant these bioactive compounds, indicating their potential medical value, including biological activities, such as antioxidant, antimicrobial, and antidiabetic properties. In line with our findings, Markos et al., (2023)s analysis of *P. senegalensis* methanol extract also identified active components, with a similar profile except for the absence of steroids. The composition of a plant's secondary metabolites isn't fixed but rather can vary due to several factors. These include a plant's genetic makeup, as different varieties within a species may have inherent differences in their metabolite profiles. Additionally, the climate where the plants are grown, including temperature, sunlight, and water availability, can significantly impact the types and quantities of secondary metabolites produced. Finally, even the way plant parts are collected, dried, and extracted can influence the final composition of the extracted compounds (Dewijanti et al., 2019; Onuminya et al., 2017).

This research suggests *P. senegalensis* leaves contain bioactive compounds with potential medicinal applications. The identified phytochemicals are likely responsible for the plant's biological activities. Plants possess antimicrobial properties due to a variety of phytochemicals, including flavonoids, phenolic saponins, tannins, compounds, and essential oils (Rahman et al., 2011). Flavonoids are well known for their diverse biological activities, including antibacterial, antioxidant, and anti-inflammatory properties. These properties are believed to contribute to a plant's defense mechanisms against disease (Dhiman et al., 2012; Hafidh et al., 2011). Thus, the presence of these classes of compounds in this plant confirms its potential to treat various diseases such as microbial infections, a traditional medicinal plant.

Table 1: Phytochemical constituents of the leaf extracts from *P. senegalensis*

Phytochemical Screening	Observation	Extracts			
		MeOH	Chloroform	MeOH:Chloroform(1:1)	Hexane
Alkaloids	Green coloration	+	+	+	-
Flavonoids	reddish-brown	+	+	+	+
Saponins	persistent foam	+	+	+	+
Phenol	Blue colors	+	+	+	-
Tannin	Blackish - blue color	+	+	+	-
Terpenoids	Reddish-brown	+	+	+	+
Steroid	green coloration	+	+	+	+

Key: (+) presences (-) absences

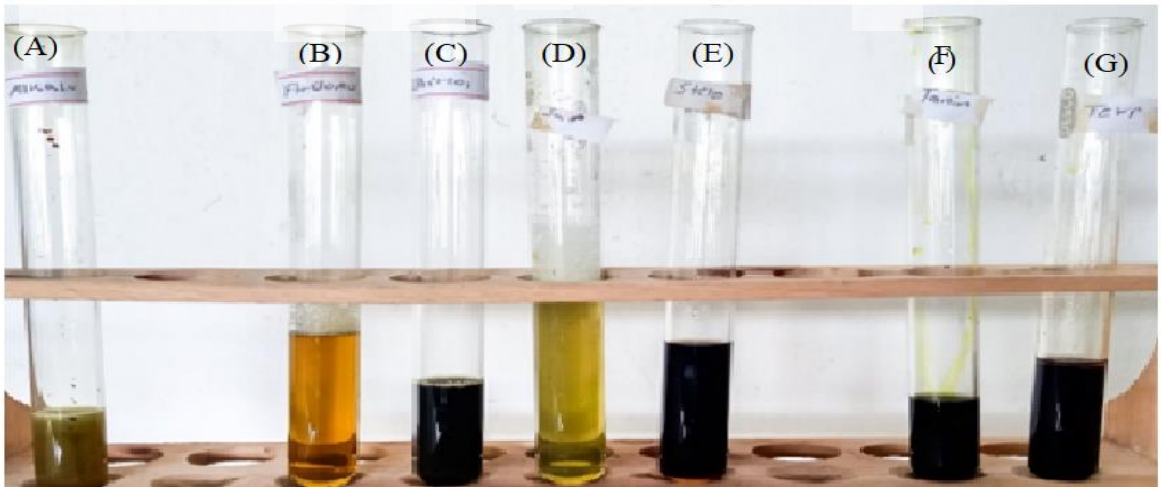

Figure 5: Phytochemical components, which predicted against the crude extract of *P. senegalensis* (A) Indicated as alkaloids, (B) predicted to be flavonoids, (C) suggested to be phenol, (D) Indicated saponins, (E) Indicated to be steroid, (F) characterized as tannin, and (G)Predicted to be terpenoids

4.3. Characterization of compound1

The elucidation of the compound's structure was achieved through the combined analysis of ^{1}H NMR and FTIR spectroscopic data. The ^{1}H NMR spectrum, recorded at 600 MHz in $CDCl_3$, revealed a series of chemical shift values that provided insight into the molecular structure. Signals were observed at 7.59, 7.50, 7.49, 7.38, 7.37, 7.07, 7.06, 7.05, 7.02, 6.84, 6.83, 6.81, 6.61, and 6.41 ppm, which were characteristic of aromatic protons, suggesting the presence of one or more benzene rings. Additional signals appeared at 4.41, 4.30, 3.90, 3.89, 3.88, 3.87, 3.83, 3.80, 3.79, 3.78, 3.77, 3.75, 3.74, 3.43, and 3.41 ppm, indicative of protons adjacent to oxygen atoms, potentially from ether, alcohol, or ester functionalities. Further signals at 3.32, 2.62, 2.66, 2.54, 2.02, 1.29, and 1.22 ppm pointed to aliphatic protons, likely from methyl, methylene, or methine groups.

Additionally, in the NMR data, the FTIR spectrum exhibited absorption bands at 3315 cm^{-1}, 2942 cm^{-1}, 2830 cm^{-1}, 1450 cm^{-1}, 1021 cm^{-1}, 754 cm^{-1}, and 662 cm^{-1}. These bands were assigned to O-H stretching, asymmetric and symmetric C-H stretching in alkanes, C-H bending, C-O stretching in alcohols, -CH_2 rocking, and C-O-H bending, respectively. The combined spectroscopic data suggested that the compound contained aromatic rings, aliphatic chains, and alcohol functional groups. Based on this information, a possible structure was proposed as a benzene ring substituted with an alcohol group, such as a benzyl alcohol derivative, and an aliphatic chain.

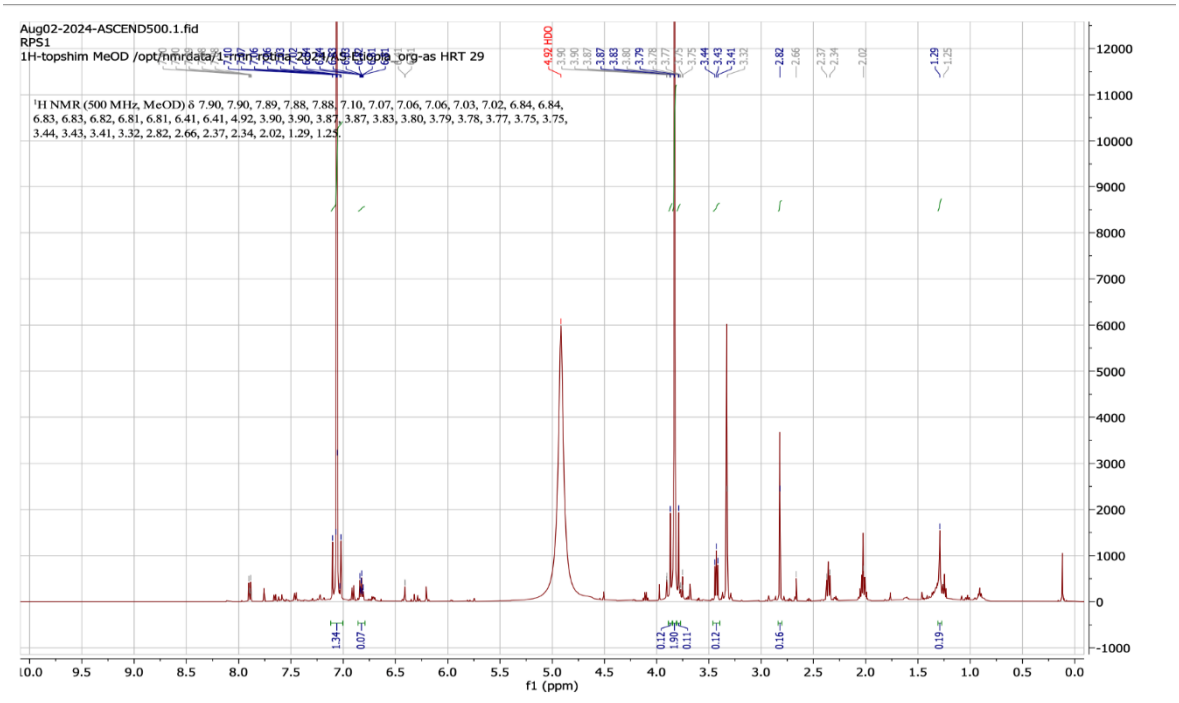

Figure 6: ^{1}H NMR Spectra of Compound 1

Table 2: ^{1}H NMR data of Compound 1 Compared with literature report

ppm	Compound 1	**Propafenone** (Holzer, 2001)
7.5–8.0	7.89, 7.88, 7.87	7.6–7.9
7.0–7.5	7.07, 7.06, 7.05, 7.03, 7.02	7.0–7.4
6.5–7.0	6.84, 6.83, 6.81, 6.41	6.7–6.9
3.5–4.5	4.41, 3.90, 3.80, 3.78, 3.75, 3.43, 3.32	3.8–4.2
2.0–3.0	2.62, 2.51, 2.02	2.2–2.8
1.0–2.0	1.29, 1.23	1.1–1.5

4.3.1.TLC Analysis of the Extracts of the Leaves of *P. senegalensis*

After the Hexane, Chloroform, Methanol: Chloroform (1:1), and Methanol extractions had been analyzed for their TLC profile using different solvents as a mobile phase, the results of the TLC profile of the crude extract demonstrated the presence of different spots, confirming the existence of different compounds in the crude extract.

4.3.2. Liquid-liquid partitioning

Liquid-liquid partitioning methods are essential for separating biomolecules based on their solubility in two immiscible liquids. The liquid-liquid partitioning approach is typical for extracting chlorophyll and other polar and medium-polar secondary metabolites. This partitioning method is effective in isolating molecules based on their polarity. The n-hexane fraction contains non-polar chemicals and has a greenish color due to the presence of non-polar pigments. The chloroform fractions contain rarely polar compounds and have a brownish color due to the presence of other medium-polar pigments. And the ethyl acetate fraction contains polar compounds. And have a brownish color due to the presence of polar compounds as shown in (Figure 7). This technique is in line with the study done by Sasidharan et al., (2011a), which employed to extract pigments such as chlorophyll before plant extracts are put via column chromatography. The chlorophyll-free "degreed" extract in the lower phase is used for further fractionation and isolation of target compounds.

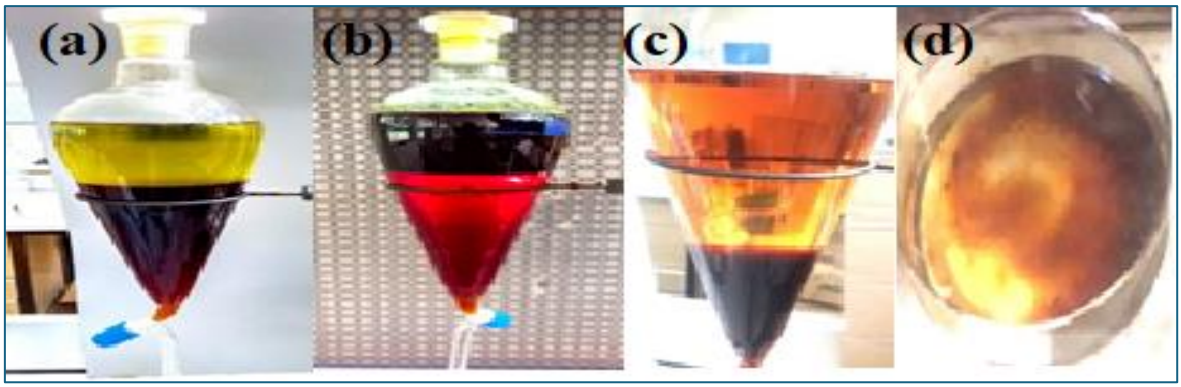

Figure 7: (a) n-hexane, (b) chloroform, and (c)ethyl acetate (EtOAc partitions from liquid-liquid extraction (d) final extract for isolation of a compound

4.3.3. Column Chromatography Technique

Subsequent column chromatography, employing an increasing gradient of ethyl acetate in n-hexane followed by methanol in chloroform, resulted in the collection of 288 fractions, which were analyzed for purity using Thin Layer Chromatography (TLC) as indicated in Additional File; Figure A_2. TLC results indicated that fractions 71-79 (eluted with a 4:6 ratio of Petroleum/ EtOAc) contained pure compound.

4.3.4. Fourier-transform infrared spectroscopy (FTIR)

FTIR analysis revealed significant absorption peaks corresponding to functional groups such as hydroxyl (O-H), carbonyl (C=O), and alkenes (C=C) (Figure 8). Fractions 71-79 (eluted with a 4:6 ratio of Petroleum/ EtOAc) afforded compound 1 (37 mg) and had a yellowish color. The unique FTIR spectra generated for pure compounds serve as distinct molecular "fingerprints." By comparing the spectrum of an unknown substance with a database of known compounds, one can often identify its composition (Sasidharan et al., 2011b).

FTIR analysis of Fractions 71-79 (eluted with a 4:6 ratio of Petroleum/ EtOAc) revealed Broad Peak around 3315 cm^{-1}: This indicates the presence of O-H stretching, likely from alcohols or carboxylic acids. Peaks around 2942, 2830 cm^{-1}: These suggest the presence of C-H stretching, characteristic of aliphatic hydrocarbons.Peaks around 1450cm^{-1}: These are associated with C-H bending vibrations, further supporting the presence of aliphatic groups. Peaks around 1114, 1021, and 754 cm^{-1}: various functional groups, including C-O stretching (as in ethers or esters) or C-N stretching (as in amines or amides).

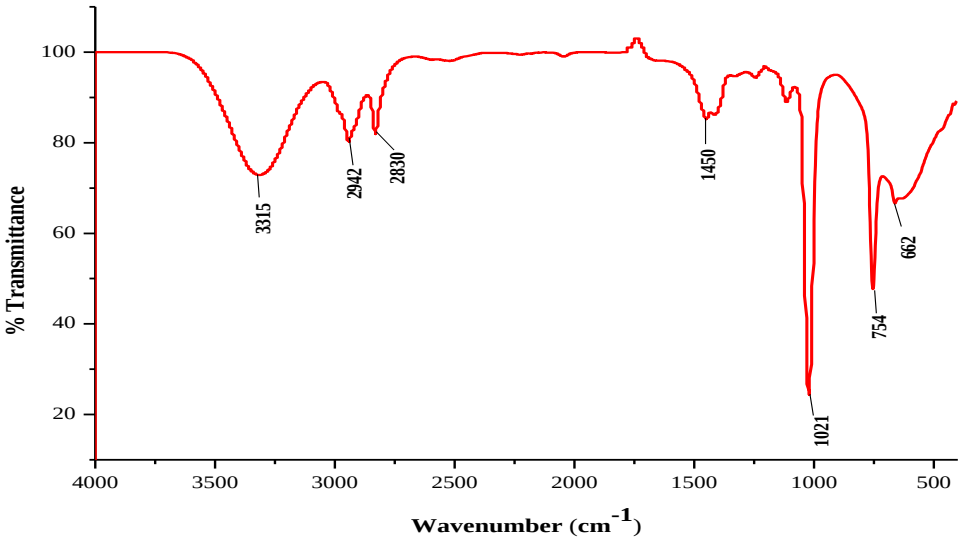

Figure 8: FTIR analysis and corresponding results. Chemical bonding, covalent stability analysis by FTIR spectroscopic technique

Table 3: Typical FTIR absorption frequencies of compound1

Observed frequency (cm^{-1})	Possible Frequency range (cm^{-1})	Assignments
3315	3600-3200	O-H stretching in alcohol
2942	3000-2850	Asymmetric -C-H stretching of alkanes
2830	2950-2800	Symmetrical -C-H stretching of alkanes
1450	1470-1350	-C-H bending for alkanes
1021	1300-1000	-C-O stretching of alcohols
754	760-720	$-CH_2$ rocking in methylene chains
662	680-620	-C-O-H bending in alcohols

4.4. GC-MS analysis of *P.senegalensis* leaves

GC-MS is a powerful tool that is increasingly used to study medicinal plants. This method combines the capabilities of gas chromatography, which separates volatile compounds, and mass spectrometry, which provides detailed information about the molecular structure of these compounds. This technique is especially good at finding certain chemicals, including those in essential oils, fats, and alkaloids. GC-MS analysis can be applied in various fields,

including pharmaceuticals, cosmetics, and food industries (Ramya, 2022). The leaves of *P. senegalensis* were hydrodistilled, yielding 0.3% (v/w) of light yellowish essential oils with a specific odor. The chemical composition of the essential oils from *P. senegalensis* leaves, including their retention time (RT), molecular formula, and percentage area, is presented in Table 4. GC-MS analysis revealed a total of 29 chemicals and the major components, accounting for 75% of the total composition. Constituents were as follows: 1.Citral- 44.9%, 2. Hexacosane - 9.96%, 3. Stearyl alcohol - 9.23%, 4. diisobutyl- 8.12%, and 5. β-Caryophyllene - 2.631%, sequentially (Figure 8). The remaining constituents ranged from 0.6 to 1.645% (Additional File; Figure A_5).

The findings of the present work on the chemical composition of essential oils from *P. senegalensis* leaves are partially in agreement with the GC-MS analysis of *P. cognatum* conducted by Demirpolat (2022). Both studies identified compounds such as (E)-β-Myrcene, dodecanal, β-caryophyllene, geranyl acetate, and undecanal. However, the percentage composition of these compounds differs between the two studies. For instance, (E)-β-Myrcene was reported at 9.49% in *P. cognatum*, while it is not listed among the major constituents of *P. senegalensis*. Similarly, dodecanal was found at 14.01%, β-caryophyllene at 11.92%, geranyl acetate at 9.49%, and undecanal at 7.35% in *P. cognatum*, but their percentages are lower in the present study.

The comparison with most literature regarding the chemical composition of the essential oil of *P. senegalensis* from other areas showed substantial differences. For instance, a study conducted by Youssef and El-Swaify (2018) identified 29 compounds in *P. senegalensis* that were not found in this study. Specifically, the study reported the presence of hexadecenoic acid (33.3%), oleic acid (16.3%), 1-[(2-aminoethoxy) hydroxyphosphinyl] oxy] methyl]-1, 2-ethanediol ester (16.5%), octadecanoic acid, 2,3-hydroxypropyl ester (6.25%), and lucenin (3.6%) in the essential oil of *P. senegalensis*. These differences highlight the variability in essential oil composition that can occur due to factors such as geographical location, environmental conditions, and extraction methods. The identification of specific compounds can support the traditional uses of these plants in treating various ailments and can lead to the development of new therapeutic agents.

Table 4: GC-MS analysis of essential oils of *P. senegalensis*

No	Compound Name	RT	Molecular formula	Composition (%)
1	β-Myrcene	6.2	$C_{10}H_{16}$	0.696
2	α-Copaene	7.23	$C_{10}H_{16}$	0.607
3	linalool	8.231	$C_{10}H_{18}O$	1.344
4	2-phenyl ethanol	8.532	$C_8H_{10}O$	0.759
5	Geraniol	10.835	$C_{10}H_{18}O$	1.645
6	Citral	11.716	$C_{10}H_{16}O$	44.879
7	Thymol	12.059	$C_{10}H_{14}O$	0.696
8	Benzenol	11.533	$C_{10}H_{14}O$	0.696
9	Dodecanol	13.89	$C_{12}H_{26}O$	0.696
10	Carvacrol	16.574	$C_{11}H_{16}O_2$	0.696
11	diethyl phthalate	17.524	$C_{12}H_{14}O_4$	0.696
12	β-Caryophyllene	19.063	$C_{15}H_{26}O$	2.631
13	di isobutyl phthalate	21.804	$C_{16}H_{22}O_4$	8.12
14	Stearyl alcohol	31.68	$C_{18}H_{38}O$	9.243
15	Hexacosane	34.73	$C_{26}H_{54}$	9.963

The essential oil of *P. senegalensis* has not been extensively studied in isolation, but related species within the *Polygonum* genus have shown promising chemical profiles. For instance, essential oils from other Polygonum species have been characterized by compounds such as (E)-β-farnesene, dodecanal, β-caryophyllene, and caryophyllene oxide, contributing to their biological activities. While specific data on the essential oil composition of *P. senegalensis* is limited, it is likely to share similar properties due to its taxonomic relationship.

4.5. Biological activity of crude extracts and essential oil from *P. senegalensis*

4.5.1. Antibacterial Activity of Crude Extracts and Essential Oil from *P. senegalensis*

The antibacterial potential of *P. senegalensis* leaves extracts Hexane, chloroform, MeOH: Chl(1:1), methanol, and essential oils were examined using the disk diffusion assay for their antibacterial activity in different concentrations against four pathogenic bacterial strains, (i.e. *E. coli* ATTC 25922^T*, S. aureus* ATCC 25923^T*, P. aeruginosa* ATCC 27853^T*, and S. pyogenes*) compared to ciprofloxacin as positive control and DMSO as negative control. The mean zone of inhibition values indicated that all the plant extracts and essential oil exhibited antibacterial activities ranging from 8.4 mm to 19.8±0.29 mm (Table 5e, Figure 9). In general, the inhibitory activity of the extracts was found to be dose-dependent, where

the zone of inhibition increased with the increasing concentrations. This trend was observed across different extracts and bacterial strains. The results were quantified by measurements of the zone of inhibitions in mm and presented in Table 5_{a-e} and Figure 9.

Among the extracts the Methanol: Chloroform (1:1) extract showed maximum zone (17.3 ±0.25 mm) of inhibition against *E.coli* ATTC 25922^{T}, the chloroform extract showed maximum inhibition (16.5±0.5 mm) against *P. aeruginosa* ATCC 27853^{T} ATCC 27853^{T}, While Hexane extract showed maximum inhibition (15.5±0.5 mm) against *S. pyogenes* ATCC 12204^{T} and methanol extracts showed good activity (16.5±0.5 mm) against *P. aeruginosa* ATCC 27853^{T} at concentration of 200 mg/mL compared to ciprofloxacin with 21.2±0.76, 18.5±0.5, 24±1 and 20.3±0.58 mm, respectively at a concentration of 10 µg/ml (Table 5_{a-e}).

Table 5a: Antibacterial activity (zone of inhibition) of Hexane, Chloroform, MeOH: Chloroform (1:1), MeOH Extracts, and Essential Oil of *P. senegalensis* determined by disc diffusion assay.

S.N.	Measurable zone of inhibition of hexane extract (mm)								
	Concentration (mg/mL)	***E. coli* ATTC 25922^T**	**%RI**	***S. aureus* ATCC 25923^T**	**%RI**	***P. aeruginosa* ATCC 27853^T**	**%RI**	***S. pyogene* ATCC 12204^T**	**%RI**
1.	200	13.2± 0.76	66.6	14.3±0.38	69.7	15.1±0.14	71.9	15.5±0.5	64.6
2.	100	11 ± 0.25	55.5	13±0.25	63.4	13.5±0.5	64.3	13.5±0.5	56.3
3.	50	9.3 ± 0.25	46.9	11.3±0.25	55.1	12.3±0.25	58.6	11.8±0.25	49.2
4.	25	8.4 ± 0.38	42.4	9.8±0.25	47.8	10.4±0.38	49.5	10.3±0.25	42.9
5.	PC	19.8 ±0.25	100	20.5±0.5	100	21± 1	100	24±1	100

Table 5b: Antibacterial activity (zone of inhibition) of Hexane, Chloroform, MeOH: Chlololoform (1:1), MeOH Extracts, and Essential Oil of *P. senegalensis* determined by disc diffusion assay.

S.N.	Measurable zone of inhibition of chloroform extract (mm)								
	Concentration (mg/mL)	***E. coli* ATTC 25922^T**	**%RI**	***S. aureus* ATCC 25923^T**	**%RI**	***P.aeruginosa* ATCC 27853^T**	**%RI**	***S.pyogene* ATCC 12204^T**	**%RI**
6.	200	15.3± 0.25	74.6	15.4±0.38	73.3	16.5±0.5	89.2	16.5±0.5	70.8
7.	100	14.1 ±0.14	68.8	14.4±0.38	68.6	14.4±0.38	77.8	12.1±038	51.9
8.	50	13 ±0.25	63.4	12.6±0.52	60	12.4±0.38	67	11.3± 0.25	48.5
9.	25	11.3 ±0.25	55.1	10.4±0.38	49.5	10.3±0.29	55.7	10.3±0.25	44.2
10.	PC	20.5 ± 0.5	100	21±1	100	18.5±0.5	100	23.3±0.76	100

Table 5c: Antibacterial activity (zone of inhibition) of Hexane, Chloroform, MeOH: Chloroform (1:1), MeOH Extracts, and Essential Oil of *P. senegalensis* determined by disc diffusion assay.

S.N.	Measurable zone of inhibition of Methanol: Chlolroform (1:1) extract (mm)								
	Concentration (mg/mL)	***E. coli* ATTC 25922^T**	**%RI**	***S. aureus* ATCC 25923^T**	**%RI**	***P.aeruginosa* ATCC**	**%RI**	***S.pyogene* ATCC 12204^T**	**%RI**
11.	200	**17.3 ±0.25**	**81.6**	**14.8±0.25**	**72.2**	**16.8±0.25**	**63.4**	**15±1**	**62.5**
12.	100	15.8 ±0.25	74.5	12.3±0.25	60	14±0	52.8	13.3±0.25	55.4
13.	50	12.8 ±0.25	60.4	11.5±0.5	56.1	13.2±0.14	49.8	12.4±0.38	51.6
14.	25	10.8 ±0.25	50.9	10±0.25	48.8	12.4±0.38	46.8	10.4±0.38	43.3
15.	PC	21.2±0.76	100	20.5±0.5	100	26.5±0.5	100	24±1	100

%RI: Percent of relative inhibition, PC: Positive control, Zone of inhibition was expressed in Mean± Standard deviation.

Table 5d: Antibacterial activity (zone of inhibition) of Hexane, Chloroform, MeOH: Chloroform (1:1), MeOH Extracts, and Essential Oil of *P. senegalensis* determined by disc diffusion assay.

S.N.	Measurable zone of inhibition of MeOH Extracts (mm)								
	Concentration (mg/mL)	***E. coli***	**%RI**	***S. aureus***	**%RI**	***P. aeruginosa***	**%RI**	***S. pyogene***	**%RI**
16.	200	14.3 ±0.25	66.8	15.5±0.25	76	16.5±0.5	81.3	14.9±0.38	67.7
17.	100	13.1 ±0.38	61.2	14.3±0.25	70.1	13.3±0.25	65.5	13.4±0.38	60.9
18.	50	11.3 ±0.25	52.8	12.3±0.38	60.3	12.3±0.25	60.6	10.8±0.66	49.1
19.	25	9.5 ± 0.25	44.4	10.3±0.25	50.5	10.3±0.25	50.7	10.5±0.25	47.7
20.	PC	21.4±0.38	100	20.4±0.38	100	20.3±0.58	100	22±0.5	100

%RI: Percept of relative inhibition, PC: Positive control, Zone of inhibition was expressed in Mean± Standard deviation.

Table 5e: Antibacterial activity (zone of inhibition) of Hexane, Chloroform, MeOH: Chloroform (1:1), MeOH Extracts, and Essential Oil of *P. senegalensis* determined by disc diffusion assay.

S.N.	Measurable zone of inhibition of Essential Oil extract (mm)								
	Concentration (mg/mL)	***E. coli* ATTC 25922^T**	**%RI**	***S. aureus* ATCC 25923^T**	**%RI**	***P. aeruginosa* ATCC 27853^T**	**%RI**	***S. pyogene* ATCC 12204^T**	**%RI**
21.	50	18 ±0.5	70.6	17.3±0.25	69.5	19.8±0.29	84.6	15.3±0.25	62.9
22.	25	14.6±0.52	57.3	15.3±0.25	61.4	18.4±0.38	78.6	14.4±0.38	59.3
23.	12.5	13.3±0.25	52.2	14.1±0.38	56.6	17.3±0.25	73.9	13.5±0.25	55.5
24.	6.25	11.8± 0.25	46.3	12.3±0.25	49.4	15.3±0.25	65.4	12.5±0.5	51.4
25.	PC	25.5 ±0.5	100	24.9±0.38	100	23.4±0.25	100	24.3±0.38	100

%RI: Percent of relative inhibition, PC: Positive control, Zone of inhibition was expressed in Mean± Standard deviation

The essential oil showed maximum inhibition (19.8±0.29 mm) against *S. pyogenes* ATCC 12204^T at a concentration of 50 mg/ml (Figure 9). In this study, essential oil showed high antimicrobial activity at lower concentrations than crude extract. The EO of *P.senegalensis* showed moderate to high inhibitory activity against the tested bacterial strains notably, it displayed stronger activity against the gram-negative bacteria, compared to the gram-positive bacteria, *P. aeruginosa* ATCC 27853^T (Pa) (84.6%) and *E. coli* ATTC 25922^T (Ec) (%70.6), *S. aureus* ATCC 25923^T (Sa) (69.5%) and *S. pyogene* ATCC 12204^T (Sp) (62.9%) respectively (Figure 9, Table 5_{a-e}).

In another study, the extracts of *P. senegalensis* were found to be prominently active against the tested microorganisms (*S. aureus* ATCC 25923^T, *E. coli* ATTC 25922^T, *S. pyogene* ATCC 12204^T, and *P. aeruginosa* ATCC 27853^T). Research indicates that different extraction methods influence the antibacterial efficacy of *P. senegalensis*. For instance, according to the study by Seimandi et al., (2021c) methanol and methylene chloride extracts have shown notable antibacterial activity against both Gram-positive and Gram-negative bacteria. The methanol extract typically exhibits the highest inhibition zones, suggesting a strong antibacterial effect due to its rich content of bioactive compounds, including phenolics and flavonoids that agree with the present study.

The Extracts and EOs of *P. senegalensis* showed significantly larger inhibition zones against the evaluated bacterial strains at 50 mg/ml and 200mg/ml for EOs and crude extract respectively. The p-values for all comparisons involving the essential oil are ($P<0.05$). This indicates that the essential oil consistently produced significantly larger inhibition zones compared to all the plant extracts tested. The p-values for comparisons between the different plant extracts vary. Some comparisons show significant differences, while others do not. For example, Chloroform extract against S. pyogenes has a significant difference(P-value<0.05) compared to Hexan extract and Methanol: chloroform (1:1) extract, but not compared to MeOH extract (Additional File; Table A_1-A_4).

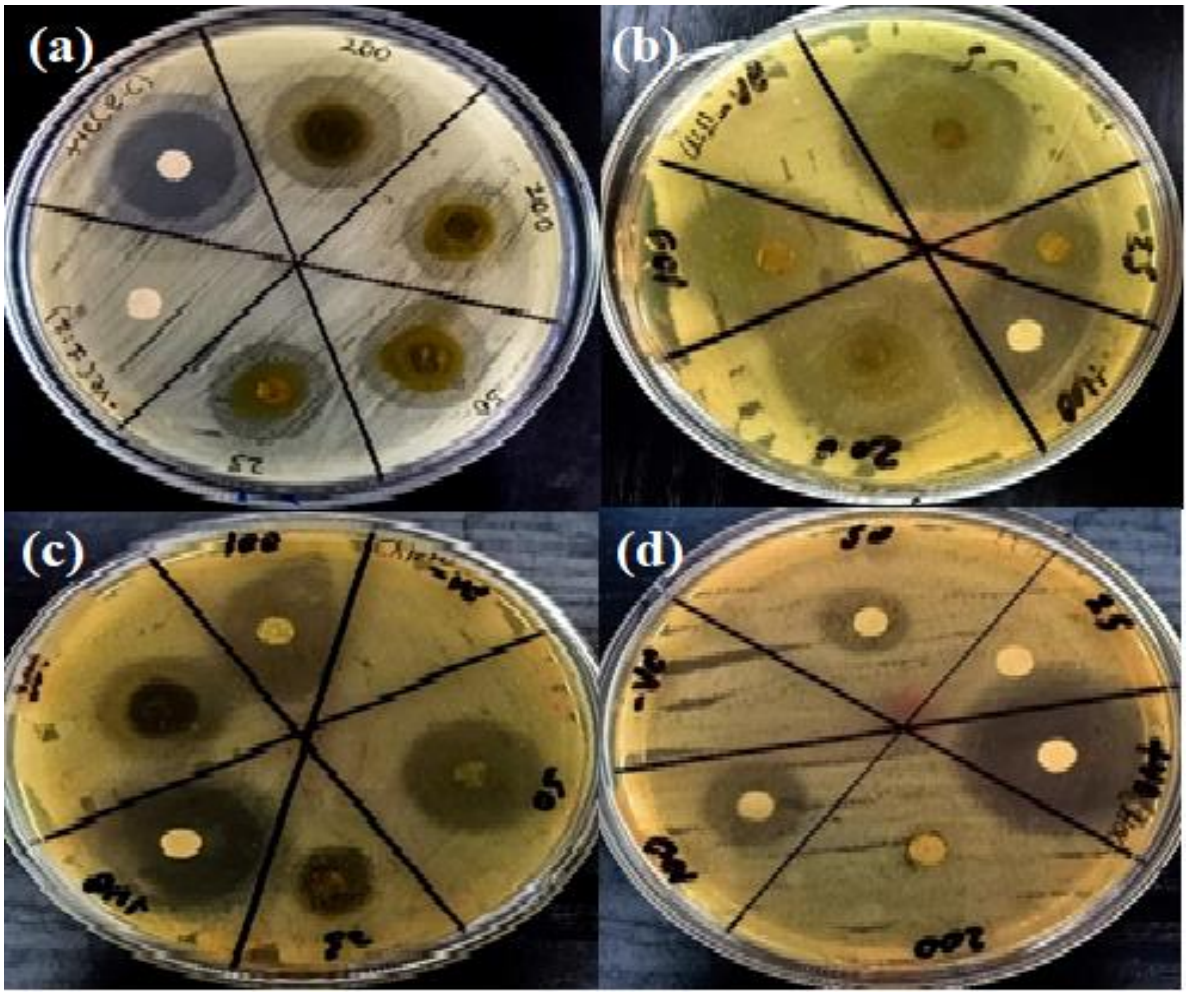

Figure 9: Zone of inhibition of Crude extracts against two gram-negative (a) *E. coli* ATTC 25922^{T}, (b) *P. aeruginosa* ATCC 27853^{T}, and two gram-positive (c) *S. aureus* ATCC 25923^{T}, (d) *S. pyogene* ATCC 12204^{T}.

4.5.2. Antifungal Activity of Crude Extracts and Essential Oil from *P. senegalensis*

The extracts (hexane, chloroform, MeOH: Chloroform (1:1), methanol, and essential oil) were tested for their antifungal activity at different concentrations against *C.albicans* and compared to ketoconazole as the positive control, while DMSO was used as a negative control. The mean zone of inhibition values indicated that all the plant extracts and essential oils exhibited antifungal activities ranging from 12.5 to 23.5 mm (Table $6_{a\text{-}b}$, Figure 10). Activity increased in a dose-dependent manner. Among the extracts, the chloroform extract showed the maximum zone of inhibition of 23.5 ± 0.5 mm, and the hexane extract showed 22.8 ± 0.75 mm at a 200 mg/ml concentration, respectively (Table 6_{a}, Figure 10).

The MeOH: Chloroform (1:1) extract showed an 18.5 ± 0.5 mm inhibition on *C.albicans* at 200 mg/ml concentration. The methanol extract showed a 19.6 ± 0.38 mm inhibition on

C.albicans at 200 mg/ml. The essential oil showed a 19.8 ± 0.25 mm inhibition on *C.albicans* at a concentration of 50 mg/ml, compared to ketoconazole at 25.4 ± 0.38 mm. The hexane extract exhibited significant antifungal activity against *C. albicans* ATCC 10231^T, with a maximum zone of inhibition of 26.5 mm at 100 mg/mL, which decreased to 15.7 mm (59.2% relative inhibition) at 25 mg/mL; the chloroform extract showed a similar trend, with a peak inhibition of 25.2 mm at 100 mg/mL, dropping to 15 mm (59.5% RI) at 25 mg/mL; the mixed methanol-chloroform extract exhibited a maximum inhibition of 25.1 mm at 100 mg/mL, which diminished to 12.5 mm (49.8% RI) at 25 mg/mL; the methanol extract had the lowest peak inhibition among the extracts at 24.6 mm (100 mg/mL), with a minimum of 13.8 mm (56.1% RI) at 25 mg/mL; in contrast, the essential oil maintained relatively high inhibition across concentrations, with a maximum of 25.4 mm at 100 mg/mL and 15.5 mm even at the lowest tested concentration of 12.5 mg/mL (Table 6, Figure 10).

This result reveals that the essential oil showed good antifungal activity at a lower concentration than the crude extracts. The active constituents in *P. senegalensis* include phenolic compounds and flavonoids, which are known for their antifungal, antibacterial, and anti-inflammatory properties. These compounds are often responsible for the observed biological activities in various medicinal plants, including the antifungal effect(Youssef & El-Swaify, 2018c)

The findings align with the Hussain et al., (2010) statement that methanol and essential oil extracts from *Persicaria* species often exhibit significant antifungal activity against Candida species. And provides additional evidence supporting the antifungal properties of *Persicaria* species, specifically *P. maculosa*, against *C. albicans* ATCC 10231^T. This study is in line with Wong et al., (2014) that suggest zone of inhibition decrease with lower concentrations aligns with established patterns in phytochemical studies, where higher concentrations of plant extracts typically yield greater antimicrobial effects. The P-values in the table provide insights into the statistical significance of the differences in inhibition zones between various extracts and essential oils against *C. albicans* ATCC 10231^T (Additional File; Table A_5).

Except for MeOH extract the three extracts and EOs have significant differences against *E. coli* ATTC 25922^T and *C. albicans* ATCC 10231^T. (P-value < 0.05). EOs have significant differences with all extracts against *S. pyogene* ATCC 12204^T (P-Value <0.05). Other extracts have similar activities. All extracts and EOs have similar activity against *P. aeruginosa* ATCC 27853^T (P-value >0.05) Hexan, Chloroform: Methanol (1:1) extracts and

EOs have significant differences against *S. aureus* ATCC 25923^{T}(P-value<0.05) (Additional File; Table A_{1-5}).

Table 6a: Antifungal activity (zone of inhibition) of Hexane, Chloroform, MeOH: Chloroform (1:1), MeOH Extracts, and Essential Oil of *P. senegalensis* determined by disc diffusion assay (Cont…)

S.N.	Measurable zone of inhibition of Hexane extract (mm)			Measurable zone of inhibition of Chloroform extract (mm)			Measurable zone of inhibition of MeOH: Chloroform (1:1)extract (mm)		
	Concentration (mg/mL)	*C. albicans* ATCC 10231^T	% RI	Concentration (mg/mL)	*C. albicans* ATCC 10231^T	% RI	Concentration (mg/mL)	*C. albicans* ATCC 10231^T	% RI
26.	200	23.5±0.5	88.7	200	22.8±0.76	90.5	200	18.5±0.5	**73.7**
27.	100	20.4±0.38	77	100	19.5±0.5	77.4	100	15.4±0.38	61.4
28.	50	17.4±0.52	65.7	50	17.5±0.5	69.4	50	13.8±0.25	54.9
29.	25	15.7±0.25	59.2	25	15±0.25	59.5	25	12.5±0.5	49.8
30.	PC	26.5±0.5	100	PC	25.2±0.29	100	PC	25.1±0.38	100

Table 6b: Antifungal activity (zone of inhibition) of Hexane, Chloroform, MeOH: Chloroform (1:1), MeOH Extracts, and Essential Oil of *P. senegalensis* determined by disc diffusion assay (Cont…)

S.N.	Measurable zone of inhibition of MeOH extract (mm)			Measurable zone of inhibition of Essential Oil (mm)		
	Concentration (mg/mL)	*C. albicans* ATCC 10231^T	% RI	Concentration (mg/mL)	*C. albicans* ATCC 10231^T	% RI
31.	200	19.6±0.38	79.7	50	19.8±0.25	77.9
32.	100	18.3±0.25	74.4	25	17.3±0.25	68.1
33.	50	15.6±0.38	63.4	12.5	15.5±0.5	61
34.	25	13.8±0.25	56.1	6.25	13.3±0.25	52.4
35.	PC	24.6±0.38	100	PC	25.4±0.38	100

%RI: Percept of relative inhibition, PC: Positive control, Zone of inhibition was expressed in Mean± Standard deviation.

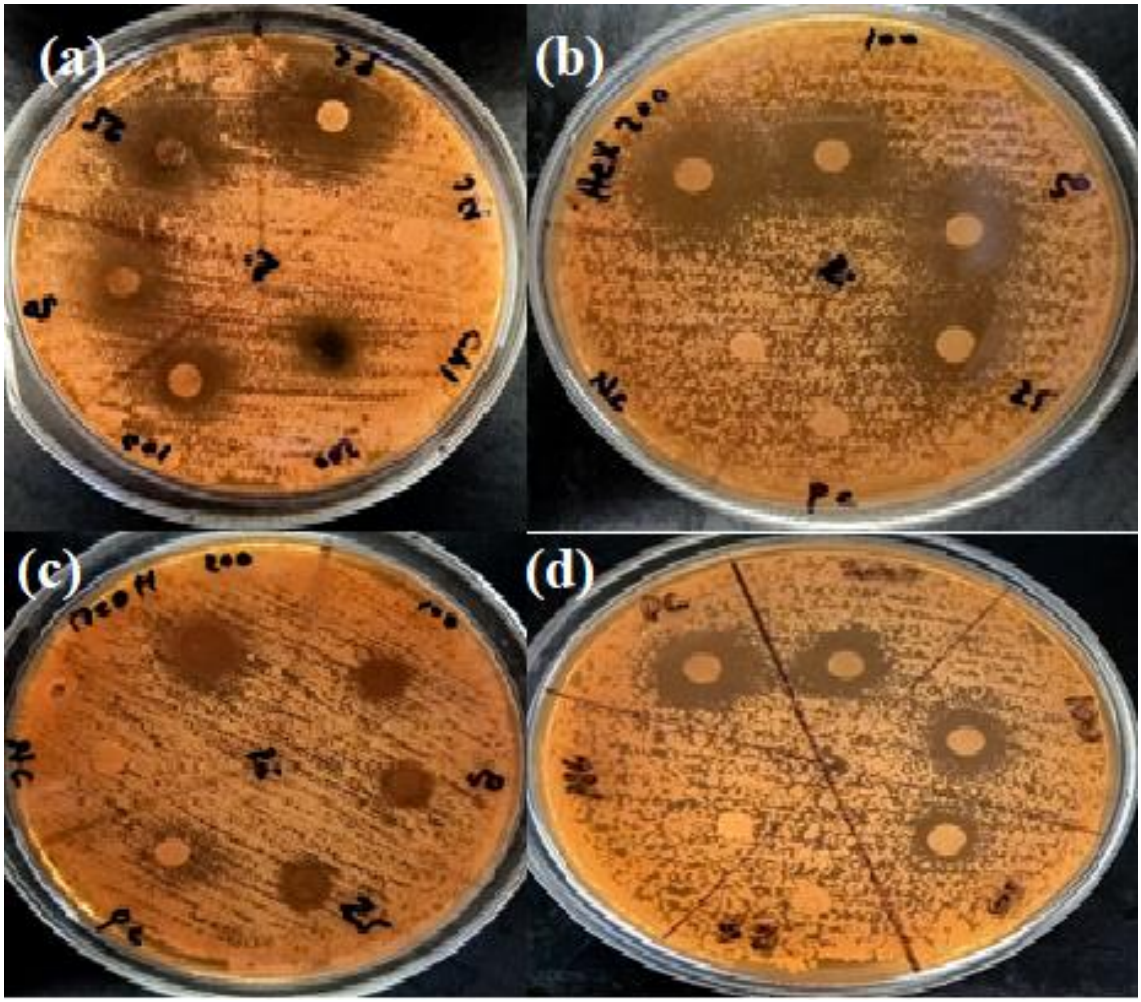

Figure 10: Zone of inhibition of (a) Chloroform, (b) Hexane, (c) MeOH, and (d) MeOH: Chloroform extracts against fungal species *C. albicans* ATCC 10231^{T}

4.5.3. Minimum Inhibition Concentration (MIC) and Minimum Bactericidal Concentration (MBC) analysis

The MIC is the lowest concentration of a substance needed to prevent the visible growth of a bacterium, while the MBC is the lowest concentration required to kill the bacterium. Thus, the smallest concentration value indicates the material's effectiveness at low concentrations. A tolerance test provides information about the potency of the test materials; higher values indicate bacteriostatic action, while lower values indicate bactericidal action (Applerot et al., 2012). In the present study, Essential oil showed a difference in the inhibitory concentrations against the test bacteria. *E.coli, P. aeruginosa* ATCC 27853^{T}*, S. aureus* ATCC 25923^{T}*, and S. pyogene* ATCC 12204^{T}, were measured by minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and tolerance values (TV).

In the present study, the MIC value obtained from methanol extract also showed notable efficacy, with MIC values of 12.5 mg/mL for *E. coli* A TTC 25922^T and *S. pyogene* ATCC 12204^T, with MBC values of 25 mg/mL; for *P. aeruginosa* ATCC 27853^T, the MIC was 12.5 mg/mL, but the MBC was significantly higher at 100 mg/mL, indicating a reduced bactericidal effect, and the TV varied, being 2 for *E. coli* ATTC 25922^T and *S. pyogene* ATCC 12204^T, and 4 for *P. aeruginosa* ATCC 27853^T. In contrast, the hexane extract had higher MICs and MBCs, ranging from 25 to 50 mg/mL, indicating weaker antibacterial effects. Exhibited MIC values ranging from 25 mg/mL for *E. coli* ATTC 25922^T and *P. aeruginosa* ATCC 27853^T to 50 mg/mL for both *S. aureus* ATCC 25923^T and *S. pyogene* ATCC 12204^T, with MBC values mirroring the MICs, indicating that higher concentrations were required to achieve bactericidal effects; the tolerance value (TV) was consistent at 2 for all strains.

The chloroform extract showed lower MICs, with values of 12.5 mg/mL against *E. coli* ATTC 25922^T and *S. pyogene* ATCC 12204^T, and 50 mg/mL for *P. aeruginosa* ATCC 27853^T, and 25mg/ml for S.aureus while MBC values were higher, particularly at 100 mg/mL for *P. aeruginosa* ATCC 27853^T, with a TV remaining at 2 for all strains, suggesting similar resistance levels. The Methanol: Chloroform extract had MIC values ranging from 25 mg/mL for *E. coli* ATTC 25922^T to 50 mg/mL for *S. aureus* ATCC 25923^T and *S. pyogene* ATCC 12204^T, with MBC values consistently at 50 mg/mL for *E. coli* ATTC 25922^T and *S. pyogene* ATCC 12204^T, indicating a strong bactericidal effect at higher concentrations; the TV was stable at 2 across all strains. The essential oil extract exhibited the strongest antibacterial activity, with MIC values as low as 6.25 mg/mL against *S. aureus* ATCC 25923^T and *S. pyogene* ATCC 12204^T, and MBC values ranging from 12.5 mg/mL to 25 mg/mL; the TV was consistent at 2 for *E. coli* ATTC 25922^T and *S. pyogene* ATCC 12204^T but increased to 4 for *P. aeruginosa* ATCC 27853^T (Table 7).

Table 7: The MIC, MBC, and TV values of the crude extracts and EO

MIC and MBC (mg/ml) with TV of crude extracts

Bacterial strains tested	*E. coli* ATTC 25922^T			*P. aeruginosa* ATCC 27853^T			*S. aureus* ATCC 25923^T			*S. pyogene* ATCC 12204^T		
	MIC	MBC	TV	MIC	MBC	TV	MIC	MBC	TV	MIC	MBC	TV
Hexane	25	50	2	25	50	2	50	100	2	50	100	2
Chl	12.5	50	4	50	100	2	25	50	2	12.5	25	2
MeOH: Chl	25	50	2	50	100	2	50	100	2	25	50	2
MeOH	12.5	25	2	12.5	50	4	12.5	25	2	25	100	4
Ess. oil	12.5	25	4	12.5	25	2	6.25	25	4	6.25	12.5	2

Key note: TV: tolerance values of the bacteria, MIC: minimum inhibition concentration (mg/mL), MBC: minimum bactericidal concentration (mg/mL).

4.6. Antioxidant Activity of Crude Extracts and Essential Oil from *P. senegalensis leaves*

After the antioxidation potential of the Hexane, Chloroform, Methanol: Chloroform(1:1), MeOH, and Oil extracts of *P.senegalensis* were assayed and evaluated using different concentrations. The results were expressed as %RSA compared to ascorbic acid as indicated in Figure 11.

The DPPH radical scavenging activities (%) of crude extracts and essential oil were 79.07, 74.65, 89.57, 90.55, and 94.44 % for the Hexane, chloroform, Methanol: Chloroform (1:1), Methanol and oil extracts (1000 µg/ml) respectively (Table 8, Figure 11). It was observed that the DPPH scavenging activity increased with increasing concentration of the samples in the assay (Figure 11). For the various concentrations, oil extracts exhibited the highest percent inhibition of the DPPH as compared to the other extracts. Ascorbic acid, a positive control, showed a percent scavenging of 97.77, 95.9, 94.67, 93.44, and 92.85% at concentrations of 1000, 500, 250, 125, 65.5 µg/ml respectively (Table 8, Figure 11).

The data reveals the antioxidant activity of hexane, chloroform, methanol, a mixed extract of methanol and chloroform (MeOH: Chlo), and essential oil, extracts measured by their % RSA (Percentage of Radical Scavenging Activity) at different concentrations. The essential oil exhibits the highest antioxidant activity, with %RSA values reaching 97.77% at 1000 µg/mL, followed by the methanol extract at 90.55%, while the chloroform and hexane

extracts show lower activities at 74.65 and 79.07%, respectively. The MeOH: Chlo extract also demonstrates significant antioxidant potential, with %RSA values peaking at 94.44%.

The IC_{50} is a common measure of how well a substance can act as an antioxidant. It shows the amount of the substance required to reduce a specific amount of free radicals by half. Lower IC_{50} values indicate that the substance is a more potent antioxidant (Rivero-Cruz et al., 2020). In terms of IC_{50} values, which indicate the concentration needed to inhibit 50% of free radicals, the essential oil has the lowest IC_{50} at 2.46 µg/mL, indicating superior efficacy, followed by the methanol extract at 4.69 µg/mL, the MeOH: Chloroform extract at 8.35 µg/mL, the chloroform extract at 20.75 µg/mL, and finally, the hexane extract at 30.73 µg/mL. This data underscores the essential oil's remarkable antioxidant capabilities, suggesting its potential applications in food preservation and pharmaceuticals.

This study shows a higher antioxidant capacity, compared to previous research studies done on the antioxidant activity of Hexane extracts. According to Telrandhe et al., (2022) hexane extract displayed inhibition of DPPH radical scavenging activity in the range of 22.27, 36.97, 37.30, 52.37, and 56.64% with concentrations of 0.1, 0.3, 0.5, 0.7, and 1 µg/ml respectively. In this study, the essential oil of *P.senegalensis* exhibits a greater scavenging effect. This finding does not go along with the previous report of Al-Reza et al., (2016) The scavenging effect of the essential oil and organic extracts of methanol, ethyl acetate, and chloroform on DPPH radicals increased with increasing concentration and was 75.78, 65.51, 67.24, and 52.48% at a concentration of 100 µg/ml, respectively (Figure 11), while ascorbic acid was the highest DPPH radical scavenging activity (83.23%).

According to Al-Reza et al., (2016), the essential oil had the strongest free radical-scavenging activity with an IC_{50} value of 24.19 µg/ml. The methanol and ethyl acetate extract showed moderate activity with IC_{50} values of 33.64 and 49.38 µg/ml, respectively, these results agree with this study indicating that both essential oil and extracts have a noticeable effect on scavenging free radicals. According to the study undertaken by Quesada-Romero et al., (2020), The antioxidant activity of various extracts from *P. maculosa* was assessed through their IC_{50} values, which indicate the concentration needed to inhibit 50% of free radicals. The methanol extract exhibited the strongest antioxidant activity, with an IC_{50} value of 2.46 µg/mL, demonstrating a high level of efficacy in scavenging free radicals. Following closely, the dichloromethane extract showed moderate antioxidant activity with an IC_{50} of 4.69 µg/mL. In contrast, the methanol extract had a

significantly higher IC_{50} value of 20.75 µg/mL, suggesting lower antioxidant efficacy compared to both the methanol and chloroform extracts. Lastly, the hexane extract demonstrated the least antioxidant activity, with an IC_{50} of 30.73 µg/mL.

It has been reported that the scavenging effect of the extract antioxidants causes a color change from purple to yellow in all tested samples except for the Hexane samples in the high concentration. This color change causes a proportional decrease in absorbance as the radical is scavenged by antioxidants through a donation of hydrogen to form the stable DPPH-H molecule (Dhifi et al., 2018). Based on the data obtained from this study, all crude extracts, and the essential oil are free radical inhibitors or scavengers, which may limit free radical damage occurring in the human body.

Table 8: Antioxidant activities of crude extracts and essential Oil from *P. senegalensis*

Conμg/mL)	Control	Crude Extracts										Positive control	
		Hexane		Chloroform		Methanol		MeOH: Chlo		Essential oil		Ascorbic	
		A	%RSA	A	%RSA	A	%RSA	A	%RSA	A	%RSA	A	%RSA
1000	1.002	0.212	79.07	0.256	74.65	0.096	90.55	0.111	89.57	0.077	94.44	0.024	97.77
500	1.002	0.243	75.89	0.296	70.59	0.139	86.26	0.122	88.51	0.101	92.05	0.043	95.90
250	1.002	0.291	71.19	0.323	67.96	0.196	80.64	0.127	87.94	0.115	90.69	0.055	94.67
125	1.002	0.334	66.86	0.355	64.73	0.241	76.11	0.143	86.39	0.137	88.52	0.067	93.44
62.5	1.002	0.381	62.14	0.489	51.36	0.312	69.02	0.154	85.29	0.211	81.14	0.074	92.85
IC50		30.73		20.75		4.69		8.35		2.46		0.05	

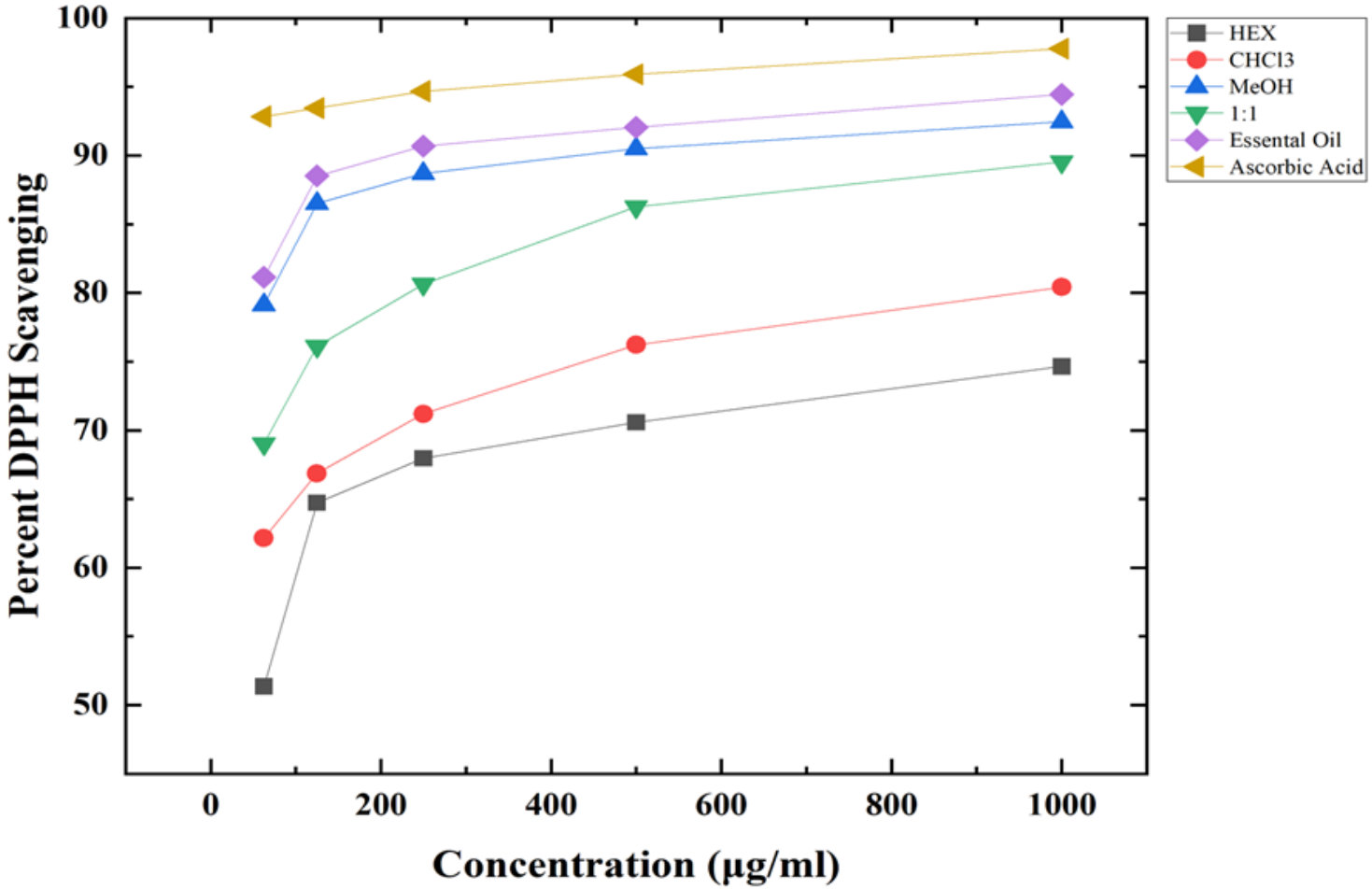

Figure 11: Antioxidant activity of *P. senegalensis* Crude extracts, Essential oil, ascorbic acid, and standard control determined by the DPPH free radical scavenging assay.

5. CONCLUSIONS AND RECOMMENDATIONS

5.1. Concussions

In the present study, the following points were concluded. Extraction of leaves *P. senegalensis* were carried out, 4.5g greenish n-hexane extract,7.69 g blackish chloroform extract, 11.25 g dark blackish, CH_3OH: $CHCl_3$ (1:1) extract, 20.5 g brownish methanol yields were obtained. The highest yield of crude extract was obtained with methanol. indicating the presence of secondary metabolites in the plant, such as alkaloids, Flavonoids, Saponins, Phenol, Tannin, Terpenoids, and steroids, were detected from *P. senegalensis leaves* using various solvents. *Based on* FTIR analysis, hydroxyl (O-H), carbonyl (C=O), and alkenes (C=C) were predicted with various absorption peaks from the crude extracts of *P. senegalensis* leaf extract. This study, essential oils (EOs) of the plant was analysed by GC-MS and showed presence of major compounds such as β-myrcene, thymol, carvacrol, hexacosane, benzenol, β-caryophyllene, di isobutyl phthalate, 2-phenyl ethanol, linalool, geraniol, citral, thymol, diethyl phthalate, and carvacrol. These compounds inhibit human pathogens such as *E. coli* ATTC 25922^T, *P. aeruginosa* ATCC 27853^T, *S. aureus* ATCC 25923^T, and *S. pyogene* ATCC 12204^T. The crude extracts of various solvents at different concentrations were found to inhibit the growth of these pathogens. In this study, 200 mg/ml concentrations of crude extracts of *P. senegalensis* appeared to be effective against these human pathogens with a considerable amount of zone of inhibitions. The same crude extract of *P. senegalensis* exhibited minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) against these human pathogens. This study's lowest MIC was at 6.25 mg/ml crude extracts of *P. senegalensis* against *S. pyogene* ATCC 12204^T and *S.aureus* ATCC 25923^T. However, the highest MIC was recorded at 50 mg/ml crude extracts of *P. senegalensis* against human pathogens. The highest and lowest MBCs were also obtained for the crude extracts of *P. senegalensis* against these human pathogens. The crude extracts and EOs of *P. senegalensis* leaves have demonstrated antioxidant activity in the presence of various solvents, including hexane, chloroform, methanol: chloroform mixture (1:1), and methanol, at concentrations ranging from 62.5 to 1mg/mL. In conclusion, the leaves crude extracts of *P. senegalensis* exhibited antibacterial, antifungal, and antioxidant activities.

5.2. Recommendations

Based on the findings of the research the following recommendations are made:

- More research is needed on the plant to fully understand its chemical composition, and biological activities, such as (anticancer, and cytotoxicity) on crude extracts, isolated compounds, and essential oil is needed in the future.
- A detailed study on 2D, NMR, and MS is also recommended as essential to characterize the isolated compounds' structures fully.
- Additional research efforts are also recommended to be conducted on other parts of the plant such as on the root, seed, and stem.

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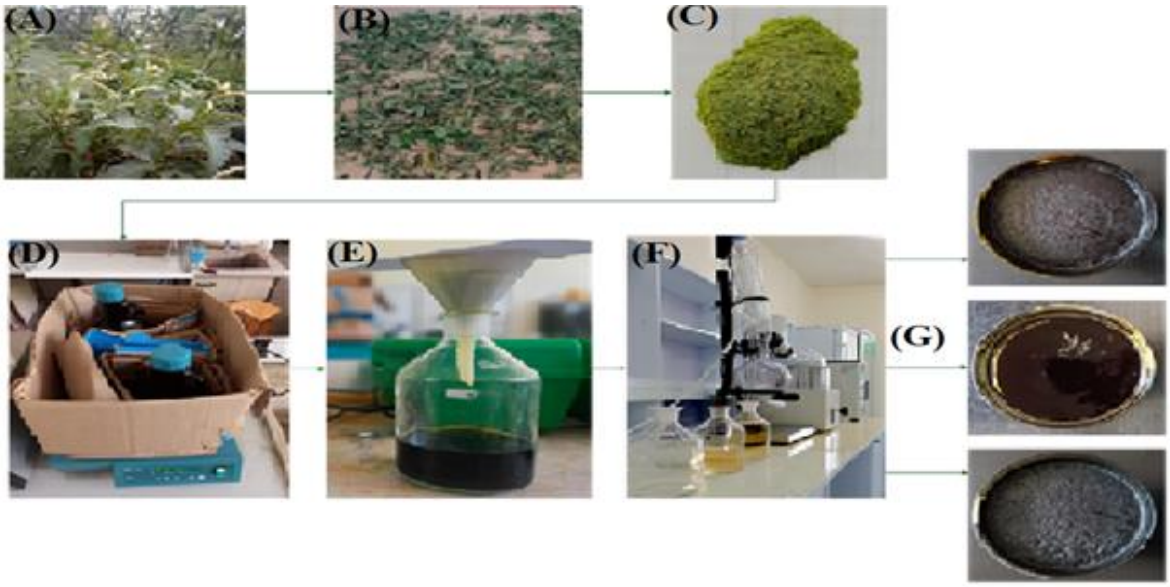
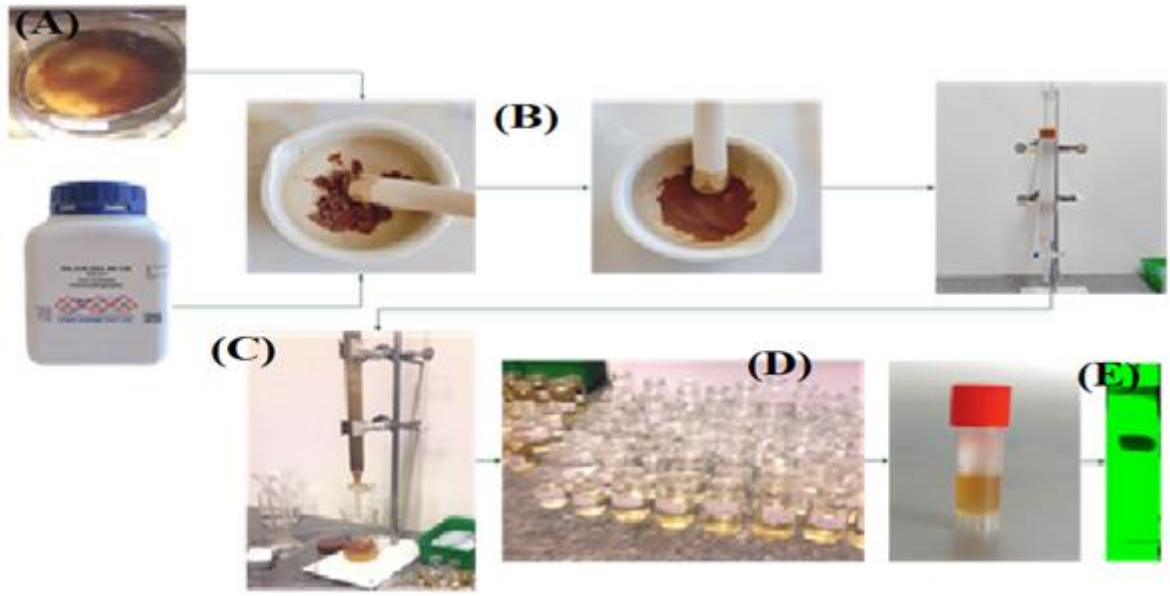
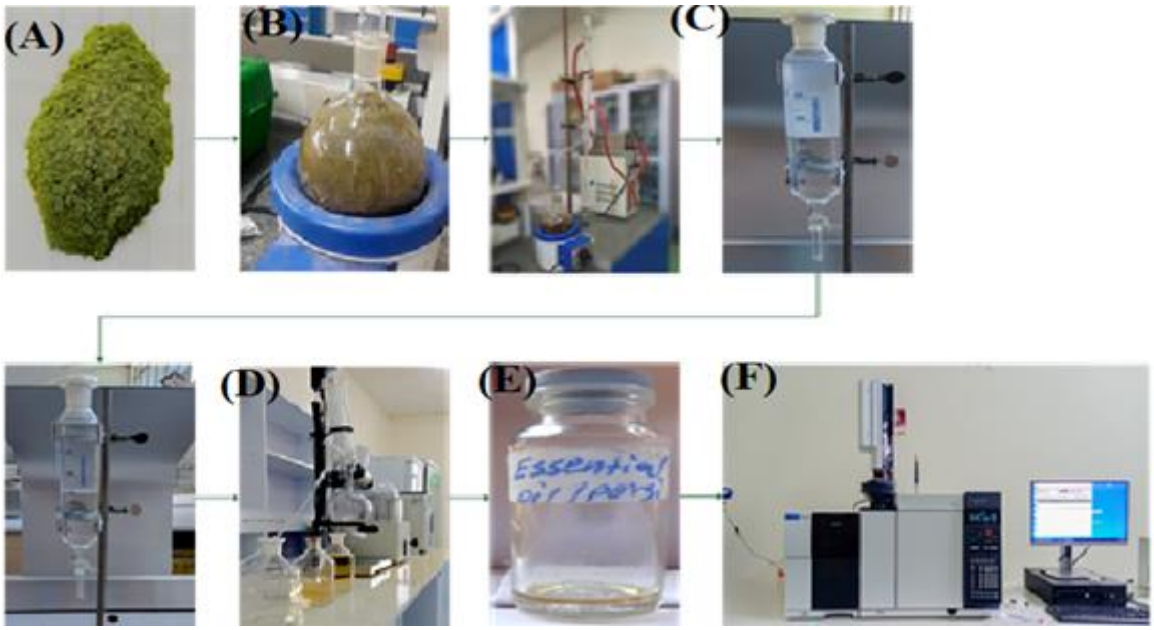
8. APPENDIXES



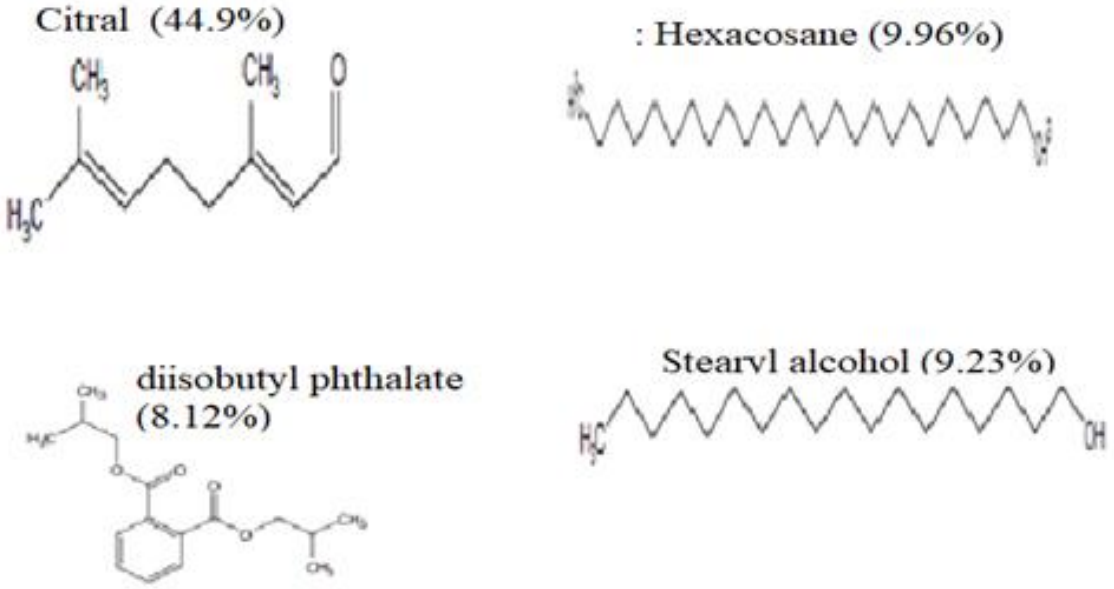
FigureA$_1$: Flowchart of Extraction Process (A) *P. sengalensis* harb, (B) Sample while drying,(C) Powder of sample,(D) sample with solvent mixing by shaker, (F) Extracts were concentrated *in vacuo* using a rotary evaporator,(G) Final extracts



FigureA$_2$: Flowchart of Column Chromatography Technique Process in Applied Biology Laboratory. (A) Ethyl extract, (B) Ethyl extract mixed with silica gel (c) column chromatography set up, (D) Collected Fractions, (E) TLC plate result inside UV lamp.



FigureA$_3$: Flowchart of essential oil extraction (A) Powered of *P. senegalensis*,(B) Clevenger's apparatus With sample,(C) (EOs) with Chloroform,(D) EOs were concentrated *in vitro* using a rotary evaporator, (E) Extracted Eos, (F) GC-MS analysis



FigureA$_4$: GC of Essential oil of *P. senegalensis* 1: Citral (44.9%); 2: Hexacosane (9.96%);3: Stearyl alcohol (9.23%);4: diisobutyl phthalate (8.12%).

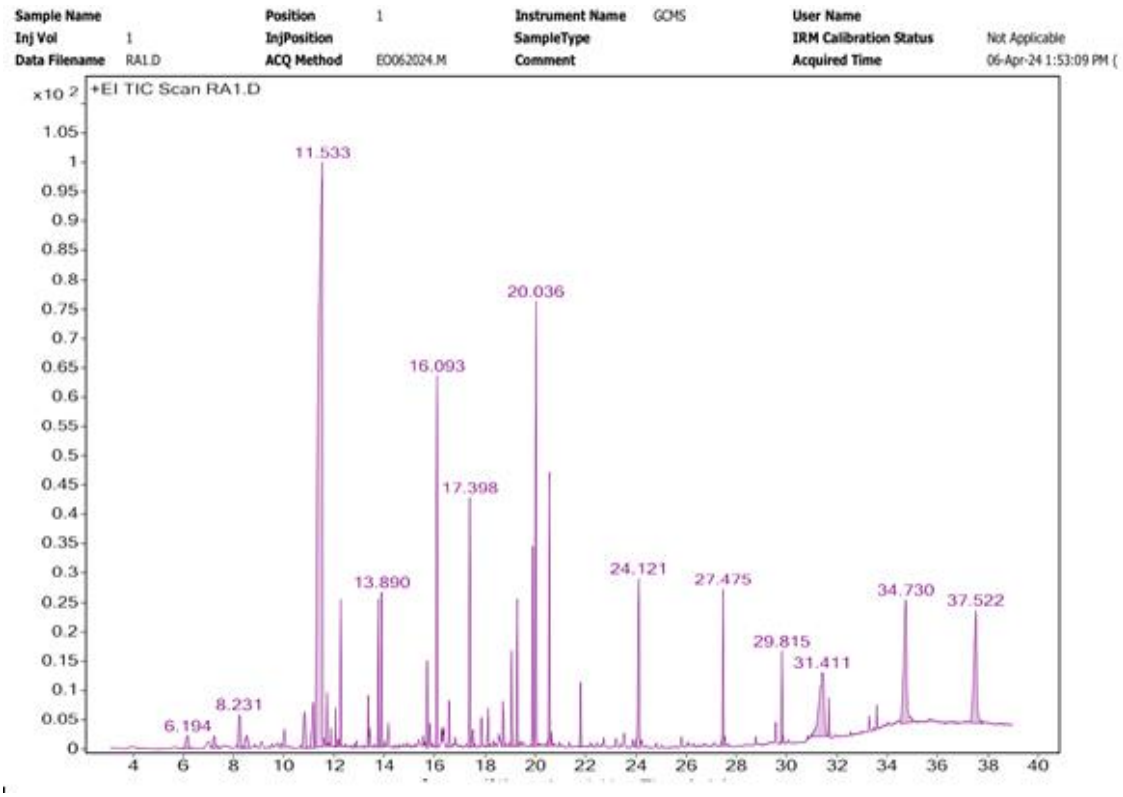
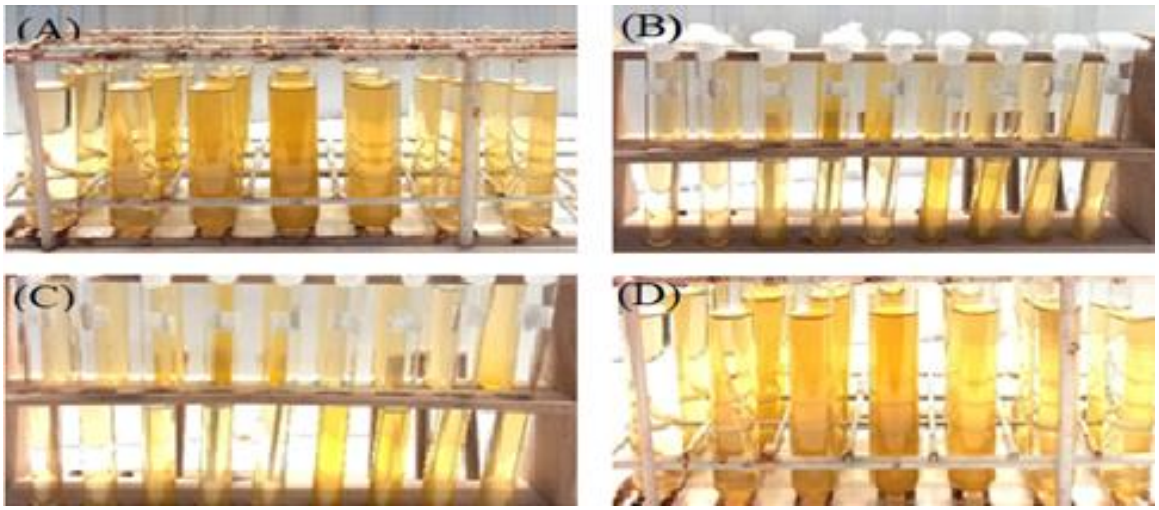
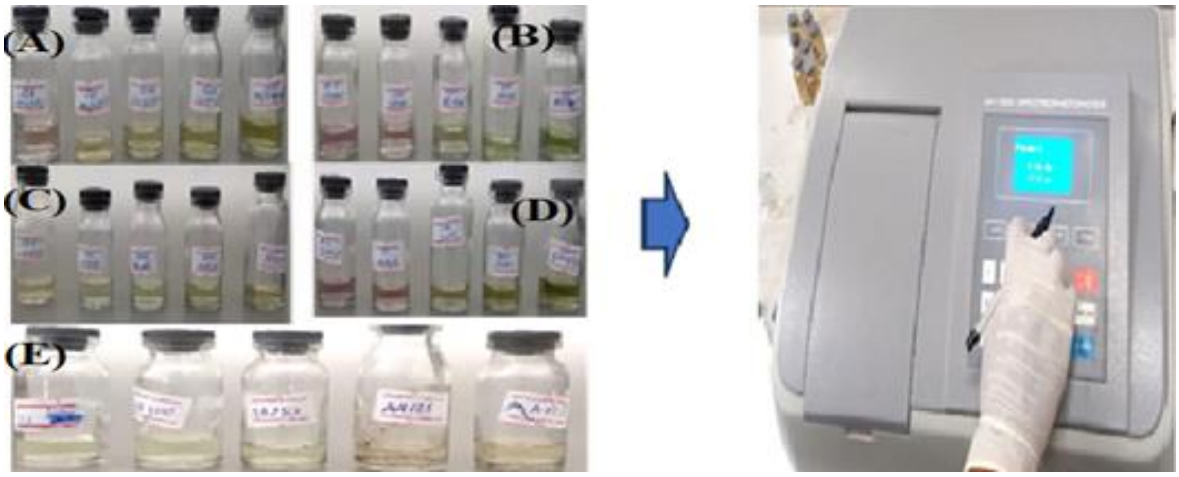


Figure A5: Structures of the Major compounds of essential oil from *P. senegalensis* leaf

FigureA6: Antimicrobial activity process in applied biology laboratory



FigureA$_7$: Visible growth inhibition of the (A) Hexane, (B) Chloroform, (C) MeOH: Chloroform, and (D) MeOH extracts on the Bacterial strains tested.



FigureA$_8$: Antioxidant process of (A) Methanol extract, (B) Chloroform extract, (C) (MeOH: Chloroform) extract, (D) Hexane extract, and (E) Ascorbic acid

Table 9A$_1$: Multiple comparisons for Hobet leaf (*P. senegalensis*) extracts against *E. coli* ATCC 25922^T using One-Way-ANOVA at LSD test in terms of Inhibition zone (mm).

						95% Confidence Interval	
	Plant extracts against. *E. coli* ATCC 25922^T	Plant extracts against *E. coli* ATCC 25922^T	Mean Difference	Std. Error	Sig.	Lower Bound	Upper Bound
LSD	Hexane against *E. coli* ATCC 25922^T	Chl against *E. coli* ATCC *25922^T*	-2.95833*	.86367	.001	-4.6813	-1.2354
		Chl: MeOH(1:1)against *E. coli* ATCC 25922^T	-3.85417*	.86367	.000	-5.5771	-2.1312
		MeOH against *E. coli* ATCC *25922^T*	-1.52083	.86367	.083	-3.2438	.2021
		EOs against *E. coli* ATCC 25922^T	-4.00000*	.86367	.000	-5.7230	-2.2770
		Ciprofloxacin against *E. coli* ATCC *25922^T*	-11.07917*	.81935	.000	-12.7137	-9.4446
	Chl against *E. coli* ATCC 25922^T	Hexane against *E. coli* ATCC 25922^T	2.95833*	.86367	.001	1.2354	4.6813
		Chl: MeOH(1:1)against *E. coli* ATCC 25922^T	-.89583	.86367	.303	-2.6188	.8271
		MeOH against *E. coli* ATCC 25922^T	1.43750	.86367	.101	-.2855	3.1605
		EOs against *E. coli* ATCC 25922^T	-1.04167	.86367	.232	-2.7646	.6813
		Ciprofloxacin against *E. coli* ATCC 25922^T	-8.12083*	.81935	.000	-9.7554	-6.4863
	Chl: MeOH(1:1)against *E. coli* ATCC 25922^T	Hexane against *E. coli* ATCC *25922^T*	3.85417*	.86367	.000	2.1312	5.5771
		Chl against *E. coli* ATCC 25922^T	.89583	.86367	.303	-.8271	2.6188
		MeOH against *E. coli* ATCC 25922^T	2.33333*	.86367	.009	.6104	4.0563
		EOs against *E. coli* ATCC 25922^T	-.14583	.86367	.866	-1.8688	1.5771
		Ciprofloxacin against *E. coli* ATCC 25922^T	-7.22500*	.81935	.000	-8.8596	-5.5904
	MeOH against *E. coli* ATCC 25922^T	Hexane against *E. coli* ATCC *25922^T*	1.52083	.86367	.083	-.2021	3.2438
		Chl against *E. coli* ATCC 25922^T	-1.43750	.86367	.101	-3.1605	.2855
		Chl: MeOH(1:1)against *E. coli* ATCC 25922^T	-2.33333*	.86367	.009	-4.0563	-.6104
		EOs against *E. coli* ATCC 25922^T	-2.47917*	.86367	.005	-4.2021	-.7562
		Ciprofloxacin against *E. coli* ATCC 25922^T	-9.55833*	.81935	.000	-11.1929	-7.9238
	EOs against *E. coli* ATCC 25922^T	Hexane against *E. coli* ATCC 25922^T	4.00000*	.86367	.000	2.2770	5.7230
		Chl against *E. coli* ATCC 25922^T	1.04167	.86367	.232	-.6813	2.7646
		Chl: MeOH(1:1)against *E. coli* ATCC 25922^T	.14583	.86367	.866	-1.5771	1.8688

		MeOH against *E. coli* ATCC 25922^{T}	2.47917*	.86367	.005	.7562	4.2021
		Ciprofloxacin against *E. coli* ATCC 25922^{T}	-7.07917*	.81935	.000	-8.7137	-5.4446
	Ciprofloxacin against *E. coli* ATCC 25922^{T}	Hexane against *E. coli* ATCC 25922^{T}	11.07917*	.81935	.000	9.4446	12.7137
		Chl against *E. coli* ATCC *25922^{T}*	8.12083*	.81935	.000	6.4863	9.7554
		Chl: MeOH(1:1)against *E. coli* ATTC *25922^{T}*	7.22500*	.81935	.000	5.5904	8.8596
		MeOH against *E. coli* ATCC *25922^{T}*	9.55833*	.81935	.000	7.9238	11.1929
		EOs against *E. coli* ATCC *25922^{T}*	7.07917*	.81935	.000	5.4446	8.7137
*. The mean difference is significant at the 0.05 level.							

Table 10A2: Multiple comparisons for Hobet leaf (*P. senegalensis*) extracts against *S. pyogene* ATCC 12204^T using One-Way-ANOVA at LSD test in terms of Inhibition zone (mm)

			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
	Plant extract against *S. pyogene* ATCC 12204^T	Plant extract against *S. pyogene* ATCC 12204^T				Lower Bound	Upper Bound
LSD	Hexane against *S. pyogene* ATCC 12204^T	Chl against *S. pyogene* ATCC 12204^T	.35417	.76603	.645	-1.1740	1.8824
		Chl: MeOH(1:1)against *S. pyogene* ATCC 12204^T	.10417	.76603	.892	-1.4240	1.6324
		MeOH against *S. pyogene* ATCC 12204^T	.41667	.76603	.588	-1.1115	1.9449
		EOs against *S. pyogene* ATCC 12204^T	-4.64583*	.76603	.000	-6.1740	-3.1176
		Ciprofloxacin against *S. pyogene* ATCC 12204^T	-10.47500*	.72672	.000	-11.9248	-9.0252
	Chl against *S. pyogene* ATCC 12204^T	Hexane against *S. pyogene* ATCC 12204^T	-.35417	.76603	.645	-1.8824	1.1740
		Chl: MeOH(1:1)against *S. pyogene* ATCC 12204^T	-.25000	.76603	.745	-1.7782	1.2782
		MeOH against *S. pyogene* ATCC 12204^T	.06250	.76603	.935	-1.4657	1.5907
		EOs against *S. pyogene* ATCC 12204^T	-5.00000*	.76603	.000	-6.5282	-3.4718
		Ciprofloxacin against *S. pyogene* ATCC 12204^T	-10.82917*	.72672	.000	-12.2789	-9.3794
	Chl: MeOH(1:1)against *S. pyogene* ATCC 12204^T	Hexane against *S. pyogene* ATCC 12204^T	-.10417	.76603	.892	-1.6324	1.4240
		Chl against *S. pyogene* ATCC 12204^T	.25000	.76603	.745	-1.2782	1.7782
		MeOH against *S. pyogene* ATCC 12204^T	.31250	.76603	.685	-1.2157	1.8407
		EOs against *S. pyogene* ATCC 12204^T	-4.75000*	.76603	.000	-6.2782	-3.2218
		Ciprofloxacin against *S. pyogene* ATCC 12204^T	-10.57917*	.72672	.000	-12.0289	-9.1294
	MeOH against *S. pyogene* ATCC 12204^T	Hexane against *S. pyogene* ATCC 12204^T	-.41667	.76603	.588	-1.9449	1.1115
		Chl against *S. pyogene* ATCC 12204^T	-.06250	.76603	.935	-1.5907	1.4657
		Chl: MeOH(1:1) against *S. pyogene* ATCC 12204^T	-.31250	.76603	.685	-1.8407	1.2157
		EOs against *S. pyogene* ATCC 12204^T	-5.06250*	.76603	.000	-6.5907	-3.5343
		Ciprofloxacin against *S. pyogene* ATCC 12204^T	-10.89167*	.72672	.000	-12.3414	-9.4419
	EOs against *S. pyogene* ATCC 12204^T	Hexane against *S. pyogene* ATCC 12204^T	4.64583*	.76603	.000	3.1176	6.1740
		Chloro against *S. pyogene* ATCC 12204^T	5.00000*	.76603	.000	3.4718	6.5282
		Chl: MeOH (1:1) against *S. pyogene* ATCC 12204^T	4.75000*	.76603	.000	3.2218	6.2782

		MeOH against *S. pyogene* ATCC 12204^{T}	5.06250*	.76603	.000	3.5343	6.5907
		Ciprofloxacin against *S. pyogene* ATCC 12204^{T}	-5.82917*	.72672	.000	-7.2789	-4.3794
	Ciprofloxacin against *S. pyogene* ATCC 12204^{T}	Hexane against *S. pyogene* ATCC 12204^{T}	10.47500*	.72672	.000	9.0252	11.9248
		Chl against *S. pyogene* ATCC 12204^{T}	10.82917*	.72672	.000	9.3794	12.2789
		Chl: MeOH(1:1) against *S. pyogene* ATCC 12204^{T}	10.57917*	.72672	.000	9.1294	12.0289
		MeOH against *S. pyogene* ATCC 12204^{T}	10.89167*	.72672	.000	9.4419	12.3414
		EOs against *S. pyogene* ATCC 12204^{T}	5.82917*	.72672	.000	4.3794	7.2789
*. The mean difference is significant at the 0.05 level.							

Table 11A$_3$: Multiple comparisons for Hobet leaf (*P. senegalensis*) extracts against *P. aeruginosa* ATCC 27853^T using One-Way-ANOVA at LSD test in terms of Inhibition zone (mm)

Dependent Variable:							
	(I) Plant_extracts_against_ *P. aeruginosa* ATCC 27853^T	(J) Plant extracts against *P. aeruginosa* ATCC 27853^T	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	Hexane against *P. aeruginosa* ATCC 27853^T	Chl against *P. aeruginosa* ATCC 27853^T	-.60417	.90141	.505	-2.4024	1.1941
		Chl:MeOH(1:1)against *P. aeruginosa* ATCC 27853^T	-1.27083	.90141	.163	-3.0691	.5274
		MeOH against *P. aeruginosa* ATCC 27853^T	-.25000	.90141	.782	-2.0483	1.5483
		EOs against *P. aeruginosa* ATCC 27853^T	-1.10417	.90141	.225	-2.9024	.6941
		Ciprofloxacin against *P. aeruginosa* ATCC 27853^T	-9.30417*	.85515	.000	-11.0101	-7.5982
	Chl against *P. aeruginosa* ATCC 27853^T	Hexane against *P. aeruginosa* ATCC 27853^T	.60417	.90141	.505	-1.1941	2.4024
		Chl:MeOH(1:1)against *P. aeruginosa* ATCC 27853^T	-.66667	.90141	.462	-2.4649	1.1316
		MeOH against *P. aeruginosa* ATCC 27853^T	.35417	.90141	.696	-1.4441	2.1524
		EOs against *P. aeruginosa* ATCC 27853^T	-.50000	.90141	.581	-2.2983	1.2983
		Ciprofloxacin against *P. aeruginosa* ATCC 27853^T	-8.70000*	.85515	.000	-10.4060	-6.9940
	Chl:MeOH(1:1)against *P. aeruginosa* ATCC 27853^T	Hexane against *P. aeruginosa* ATCC 27853^T	1.27083	.90141	.163	-.5274	3.0691
		Chl against *P. aeruginosa* ATCC 27853^T	.66667	.90141	.462	-1.1316	2.4649
		MeOH against *P. aeruginosa* ATCC 27853^T	1.02083	.90141	.261	-.7774	2.8191
		EOs against *P. aeruginosa* ATCC 27853^T	.16667	.90141	.854	-1.6316	1.9649
		Ciprofloxacin against *P. aeruginosa* ATCC 27853^T	-8.03333*	.85515	.000	-9.7393	-6.3274

	MeOH against *P. aeruginosa* ATCC 27853^{T}	Hexane against *P. aeruginosa* ATCC 27853^{T}	.25000	.90141	.782	-1.5483	2.0483
		Chl against *P. aeruginosa* ATCC 27853^{T}	-.35417	.90141	.696	-2.1524	1.4441
		Chl:MeOH(1:1)against *P. aeruginosa* ATCC 27853^{T}	-1.02083	.90141	.261	-2.8191	.7774
		EOs against *P. aeruginosa* ATCC 27853^{T}	-.85417	.90141	.347	-2.6524	.9441
		Ciprofloxacin against *P. aeruginosa* ATCC 27853^{T}	-9.05417*	.85515	.000	-10.7601	-7.3482
	EOs against *P. aeruginosa* ATCC 27853^{T}	Hexan against *P. aeruginosa* ATCC 27853^{T}	1.10417	.90141	.225	-.6941	2.9024
		Chl against *P. aeruginosa* ATCC 27853^{T}	.50000	.90141	.581	-1.2983	2.2983
		Chl:MeOH(1:1)against *P. aeruginosa* ATCC 27853^{T}	-.16667	.90141	.854	-1.9649	1.6316
		MeOH against *P. aeruginosa* ATCC 27853^{T}	.85417	.90141	.347	-.9441	2.6524
		Ciprofloxacin against *P. aeruginosa* ATCC 27853^{T}	-8.20000*	.85515	.000	-9.9060	-6.4940
	Ciprofloxacin against *P. aeruginosa* ATCC 27853^{T}	Hexane against *P. aeruginosa* ATCC 27853^{T}	9.30417*	.85515	.000	7.5982	11.0101
		Chl against *P. aeruginosa* ATCC 27853^{T}	8.70000*	.85515	.000	6.9940	10.4060
		Chl:MeOH(1:1)against *P. aeruginosa* ATCC 27853^{T}	8.03333*	.85515	.000	6.3274	9.7393
		MeOH against *P. aeruginosa* ATCC 27853^{T}	9.05417*	.85515	.000	7.3482	10.7601
		EOs against *P. aeruginosa* ATCC 27853^{T}	8.20000*	.85515	.000	6.4940	9.9060
*. The mean difference is significant at the 0.05 level.							

Table 12A4: Multiple comparisons for Hobet leaf (*P. senegalensis*) extracts against *S. aureus* ATCC 25923^T using One-Way-ANOVA at LSD test

	Plant extracts against *S. aureus* ATCC 25923^T	Plant extracts against *S. aureus* ATCC 25923^T	Mean Difference	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	Hexane against *S. aureus* ATCC 25923^T	Chl against *S. aureus* ATCC 25923^T	-1.12500	.78515	.156	-2.6913	.4413
		Chl: MeOH(1:1)against *S. aureus* ATCC 25923^T	-.04167	.78515	.958	-1.6080	1.5247
		MeOH against *S. aureus* ATCC 25923^T	-1.00000	.78515	.207	-2.5663	.5663
		EOs against *S. aureus* ATCC 25923^T	-2.62500*	.78515	.001	-4.1913	-1.0587
		Ciprofloxacin against *S. aureus* ATCC 25923^T	-9.38333*	.74486	.000	-10.8693	-7.8974
	Chl against *S. aureus* ATCC 25923^T	Hexane against *S. aureus* ATCC 25923^T	1.12500	.78515	.156	-.4413	2.6913
		Chl: MeOH(1:1)against *S. aureus* ATCC 25923^T	1.08333	.78515	.172	-.4830	2.6497
		MeOH against *S. aureus* ATCC 25923^T	.12500	.78515	.874	-1.4413	1.6913
		EOs against *S. aureus* ATCC 25923^T	-1.50000	.78515	.060	-3.0663	.0663
		Ciprofloxacin against *S. aureus* ATCC 25923^T	-8.25833*	.74486	.000	-9.7443	-6.7724
	Chl:MeOH(1:1)against *S. aureus* ATCC 25923^T	Hexane against *S. aureus* ATCC 25923^T	.04167	.78515	.958	-1.5247	1.6080
		Chl against *S. aureus* ATCC 25923^T	-1.08333	.78515	.172	-2.6497	.4830
		MeOH against *S. aureus* ATCC 25923^T	-.95833	.78515	.226	-2.5247	.6080
		EOs against *S. aureus* ATCC 25923^T	-2.58333*	.78515	.002	-4.1497	-1.0170
		Ciprofloxacin against *S. aureus* ATCC 25923^T	-9.34167*	.74486	.000	-10.8276	-7.8557
	MeOH against *S. aureus* ATCC 25923^T	Hexane against *S. aureus* ATCC 25923^T	1.00000	.78515	.207	-.5663	2.5663
		Chl against *S. aureus* ATCC 25923^T	-.12500	.78515	.874	-1.6913	1.4413
		Chl: MeOH(1:1)against *S. aureus* ATCC 25923^T	.95833	.78515	.226	-.6080	2.5247
		EOs against *S. aureus* ATCC 25923^T	-1.62500*	.78515	.042	-3.1913	-.0587
		Ciprofloxacin against *S. aureus* ATCC 25923^T	-8.38333*	.74486	.000	-9.8693	-6.8974
	EOs against *S. aureus* ATCC 25923^T	Hexane against *S. aureus* ATCC 25923^T	2.62500*	.78515	.001	1.0587	4.1913
		Chl against *S. aureus* ATCC 25923^T	1.50000	.78515	.060	-.0663	3.0663
		Chl: MeOH(1:1)against *S. aureus* ATCC 25923^T	2.58333*	.78515	.002	1.0170	4.1497
		MeOH against *S. aureus* ATCC 25923^T	1.62500*	.78515	.042	.0587	3.1913
		Ciprofloxacin against *S. aureus* ATCC 25923^T	-6.75833*	.74486	.000	-8.2443	-5.2724

	Ciprofloxacin against *S. aureus* ATCC 25923^{T}	Hexane against *S. aureus* ATCC 25923^{T}	9.38333*	.74486	.000	7.8974	10.8693
		Chl against *S. aureus* ATCC 25923^{T}	8.25833*	.74486	.000	6.7724	9.7443
		Chl: MeOH(1:1)against *S. aureus* ATCC 25923^{T}	9.34167*	.74486	.000	7.8557	10.8276
		MeOH against *S. aureus* ATCC 25923^{T}	8.38333*	.74486	.000	6.8974	9.8693
		EOs against *S. aureus* ATCC 25923^{T}	6.75833*	.74486	.000	5.2724	8.2443
*. The mean difference is significant at the 0.05 level.							

Table 13A5: Significance of Extracts and Essential Oil against *C. albicans ATCC 10231^T*.

Dependent Variable: Inhibition zone (mm)							
						95% Confidence Interval	
	(I) Plant extracts against *C. albicans ATCC 10231^T*	(J) Plant extracts against_ *C. albicans ATCC 10231^T*	Mean Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
LSD	Hexane against *C. albicans ATCC 10231^T*	Chl against *C. albicans* ATCC *10231^T*	-2.87500*	.86550	.001	-4.6016	-1.1484
		Chl: MeOH(1:1) against *C. albicans* ATCC *10231^T*	-3.60417*	.86550	.000	-5.3308	-1.8775
		MeOH against *C. albicans* ATCC *10231^T*	-1.50000	.86550	.088	-3.2266	.2266
		EOs against *C. albicans* ATCC *10231^T*	-3.87500*	.86550	.000	-5.6016	-2.1484
		Ciprooxacin against *C. albicans* ATCC *10231^T*	-11.14583*	.82109	.000	-12.7839	-9.5078
	Chloro against *C. albicans ATCC 10231^T*	Hexane against *C. albicans ATCC 10231^T*	2.87500*	.86550	.001	1.1484	4.6016
		Chl: MeOH(1:1) against *C. albicans ATCC 10231^T*	-.72917	.86550	.402	-2.4558	.9975
		MeOH against *C. albicans ATCC 10231^T*	1.37500	.86550	.117	-.3516	3.1016
		EOs against *C. albicans ATCC 10231^T*	-1.00000	.86550	.252	-2.7266	.7266
		Ciprofloxacin against *C. albicans ATCC 10231^T*	-8.27083*	.82109	.000	-9.9089	-6.6328
	Chloro: MeOH(1:1) against *C. albicans ATCC 10231^T*	Hexane against *C. albicans ATCC 10231^T*	3.60417*	.86550	.000	1.8775	5.3308
		Chl against *C. albicans ATCC 10231^T*	.72917	.86550	.402	-.9975	2.4558
		MeOH against *C. albicans ATCC 10231^T*	2.10417*	.86550	.018	.3775	3.8308
		EOs against *C. albicans ATCC 10231^T*	-.27083	.86550	.755	-1.9975	1.4558
		Ciprofloxacin against *C. albicans ATCC 10231^T*	-7.54167*	.82109	.000	-9.1797	-5.9036
	MeOH against *C. albicans ATCC 10231^T*	Hexane against *C. albicans ATCC 10231^T*	1.50000	.86550	.088	-.2266	3.2266
		Chloro against *C. albicans ATCC 10231^T*	-1.37500	.86550	.117	-3.1016	.3516
		Chloro: MeOH(1:1) against *C. albicans ATCC 10231^T*	-2.10417*	.86550	.018	-3.8308	-.3775
		EOs against *C. albicans ATCC 10231^T*	-2.37500*	.86550	.008	-4.1016	-.6484
		Ciprofloxacin against *C. albicans ATCC 10231^T*	-9.64583*	.82109	.000	-11.2839	-8.0078
	EOs against *C. albicans ATCC 10231^T*	Hexane against *C. albicans ATCC 10231^T*	3.87500*	.86550	.000	2.1484	5.6016
		Chloro against *C. albicans ATCC 10231^T*	1.00000	.86550	.252	-.7266	2.7266
		Chloro: MeOH(1:1) against *C. albicans ATCC 10231^T*	.27083	.86550	.755	-1.4558	1.9975
		MeOH against *C. albicans ATCC 10231^T*	2.37500*	.86550	.008	.6484	4.1016

		Ciprofloxacin against *C. albicans ATCC 10231^T*	-7.27083*	.82109	.000	-8.9089	-5.6328
	Ciprofloxacin against *C. albicans ATCC 10231^T*	Hexane against *C. albicans ATCC 10231^T*	11.14583*	.82109	.000	9.5078	12.7839
		Chloro against *C. albicans ATCC 10231^T*	8.27083*	.82109	.000	6.6328	9.9089
		Chloro: MeOH(1:1) against *C. albicans ATCC 10231^T*	7.54167*	.82109	.000	5.9036	9.1797
		MeOH against *C. albicans ATCC 10231^T*	9.64583*	.82109	.000	8.0078	11.2839
		EOs against *C. albicans ATCC 10231^T*	7.27083*	.82109	.000	5.6328	8.9089
*. The mean difference is significant at the 0.05 level.							

Table 14A$_6$: Average absorbance values and percentage growth (%G) of bacterial culture treated with different concentrations of crude extract and EO.

Extract	concentration(mg/ml)	Average Absorbance values ± SD			
		E. coli ATTC 25922^T	*S. aureus* ATCC 25923^T	*P. aeruginosa* ATCC 27853^T	*S. pyogene* ATCC 12204^T
Hexane	**200**	0.118±0.02	0.103±0.01	0.398±0.01	0.103±0.02
	100	0.287±0.01	0.176±0.01	0.442±0.02	0.176±0.02
	50	0.415±0.01	0.215±0.02	0.465±0.02	0.247±0.03
	25	0.531±0.04	0.410±0.01	0.629±0.03	0.372±0.01
	12.5	0.612±0.01	0.507±0.03	0.723±0.02	0.483±0.01
	Control	0.65±0.03	0.623±0.01	0.823±0.02	0.582±0.01
Chloroform	**200**	0.545±0.02	0.121±0.02	0.143±0.01	0.165±0.02
	100	0.204±0.03	0.188±0.04	0.227±0.01	0.232±0.01
	50	0.313±0.01	0.234±0.02	0.335±0.02	0.301±0.01
	25	0.422±0.02	0.356±0.03	0.524±0.02	0.368±0.02
	12.5	0.537±0.04	0.414±0.06	0.656±0.03	0.471±0.02
	Control	0.679±0.03	0.623±0.01	0.823±0.02	0.582±0.01
MeOH: ChL (1:1)	**200**	0.121±0.02	0.129±0.01	0.102±0.01	0.158±0.02
	100	0.232±0.02	0.204±0.02	0.158±0.02	0.211±0.06
	50	0.318±0.01	0.256±0.02	0.369±0.02	0.303±0.01
	25	0.464±0.01	0.342±0.02	0.451±0.03	0.365±0.01
	12.5	0.557±0.03	0.445±0.01	0.534±0.01	0.464±0.01
	Control	0.679±0.03	0.623±0.01	0.823±0.02	0.582±0.01
MeOH	**200**	0.163±0.02	0.192±0.06	0.248±0.01	0.146±0.09
	100	0.235±0.01	0.231±0.01	0.333±0.02	0.259±0.02
	50	0.357±0.01	0.352±0.02	0.412±0.01	0.346±0.02
	25	0.462±0.03	0.405±0.01	0.436±0.02	0.413±0.01
	12.5	0.567±0.01	0.437±0.09	0.525±0.01	0.453±0.01
	Control	0.679±0.03	0.623±0.01	0.823±0.01	0.582±0.01
Essential oil	**50**	0.246±0.01	0.175±0.01	0.356±0.01	0.168±0.06
	25	0.356±0.02	0.298±0.02	0.476±0.02	0.245±0.02
	12.5	0.456±0.03	0.378±0.01	0.565±0.01	0.334±0.01
	6.25	0.678±0.01	0.546±0.02	0.679±0.01	0.574±0.02
	3.125	0.789±0.02	0.659±0.01	0.879±0.03	0.653±0.02
	Control	0.977±0.01	0.897±0.03	1.023±0.02	0.769±0.01

%G, percent growth relative to control which was taken as 100%. Concentrations corresponding to the highlighted absorbance values are taken as MIC.