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ESSENTIAL OILS EXTRACTION AND IDENTIFICATION OF PHYTOCHEMICAL COMPOUNDS FROM *LAVANDULA ANGUSTIFOLIA* MILL: UNVEILING THEIR POTENTIAL APTITUDE AS ANTIBACTERIAL RESPONSE AGAINST SELECTED STRAINS

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Abstract

Antibiotic resistance is a major global challenge, necessitating the search for new antibacterial agents. This study aims to identify the phytochemical composition and antibacterial properties of essential oils from *Lavandula angustifolia Miller* (lavender). Essential oils from lavender leaves and flowers were extracted using the hydrodistillation method, and Gas Chromatography-Mass Spectrometry (GC-MS) was employed to identify the active compounds. Antibacterial activity was assessed through the disc diffusion assay at different dilutions against *Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. The extracted lavender oils were rich in terpenoids, with monoterpenes being the predominant constituents, accounting for 70.05% in flowers and 61.58% in leaves, while sesquiterpenes constituted 5.75% in flowers and 4.28% in leaves. Lavender essential oils demonstrated significant antibacterial activity, the highest inhibition zone was observed against *Escherichia coli* (28.8 mm ±0.6 for flowers and 25.9 mm ±0.4 for leaves at a 1:1 dilution). These findings suggest that lavender essential oils possess substantial antibacterial potential, warranting further exploration for pharmaceutical applications.

Key words: Essential oils, Hydro-distillation, GC-MS terpenoids, Microbial stains, Antimicrobial assessments, *Lavandula angustifolia*

Introduction

Lavender, an evergreen perennial plant of the family Lamiaceae is widely cultivated around the world. At present, 39 species of the genus *Lavandula* have been identified, but only *Lavandula angustifolia* Mill. (Lavender) is famous for its valuable medicinal and aromatic properties (Yin *et al.*, 2024; Ciocarlan *et al.*, 2021). For decades, lavender has served as an antioxidant, anti-inflammatory, sedative, antidepressant, antifungal, and antibiotic, used to treat muscular dystrophy, body aches, burns, and infections caused by insects and parasites (Perdoi *et al.*, 2018). The main reason for its efficacy is the presence of biologically active compounds in lavender essential oils (Kajjari *et al.*, 2022). However, different phytochemical compositions of essential oils are responsible for targeting different pathogens that affect various body organs (Haban *et al.*, 2023).

The key phytochemical compounds in lavender essential oil include linalool, linalyl acetate, terpin-1-en-4-ol, 1,8-cineole, geranyl acetate, (Z)-ocimene, borneol, (E)-ocimene, camphor, camphene, eucalyptol, caryophyllene oxide, α-terpineol, heptan-2-one, 1,7,7-trimethyl-(IS)-borneol, lavandulyl acetate, octan-3-ol, β-caryophyllene, β-ocimene, terpinen-4-ol, limonene, pinenes, and β-farnesene (Perdoi *et al.*, 2018; Tarek *et al.*, 2014; Dobros *et al.*, 2022; Batiha *et al.*, 2023).

These phytochemicals exhibit potent antibacterial activity, effectively inhibiting pathogen growth (Hameed *et al.*, 2022; Asghar *et al.*, 2022; Saqib *et al.*, 2022; Saqib *et al.*, 2024). However, the phytochemical profile of lavender essential oils from flowers and leaves can vary depending on geographical and environmental factors, as well as the source of the plant seeds. Additionally, essential oil extraction techniques and detection methods strongly influence the phytochemical composition of lavender essential oils (Slimani *et al.*, 2022; Ciocarlan *et al.*, 2021).

Besides its therapeutic applications, lavender essential oil is one of the most suitable essential oils for use in colognes, cosmetics, detergents, and the food industry (Erland *et al.*, 2016; Sharmeen *et al.*, 2021). Furthermore, the ester profile of lavender essential oil, along with other parameters, determines the quality of the extracted oil. High levels of linalool and linalyl acetate, with a minimal amount of camphor, make it particularly suitable for the food industry (Imbrea *et al.*, 2023). Generally, fresh lavender flowers and leaves are used to extract active compounds through conventional methods such as hydro-distillation, solid-liquid extraction, and Soxhlet extraction (Hristova *et al.*, 2022).

At present, the modern medicine industry is grappling with multi-drug resistance as a major complication. This reduction in the effectiveness of antibiotics is directly contributing to high mortality rates (Arshad, 2022; Arshad, 2024). Consequently, healthcare providers are struggling to treat patients with resistant infections. This issue could potentially be addressed through the identification, optimization, and novel combination of known natural biologically active compounds (Arshad, 2024). There is an ongoing need for new, potent therapeutic sources to mitigate pathogen resistance (Ullah *et al.*, 2024; Arshad *et al.*, 2021). The selected bacterial strains in this study are known pathogens that can cause various diseases in humans, including skin, blood, lung, heart, and gastrointestinal

infections, and have been confirmed for multidrug resistance. In this context, natural products or phytochemicals from plant essential oils are often preferred (Amorese *et al.*, 2018), with lavender (Ciocarlan *et al.*, 2021; Todorova *et al.*, 2022) being a notable example. Additionally, blends of lavender essential oil with various antibiotics have been explored (Kwiatkowski *et al.*, 2020; Yap *et al.*, 2013).

This study focuses on identifying and profiling the phytochemical compounds in essential oils extracted from the flowers and leaves of lavender using GC-MS. Additionally, the antibacterial activity and therapeutic effects of lavender essential oils (from both flowers and leaves) are evaluated by applying them to selected pathogenic gram-negative and gram-positive bacterial strains via the disc diffusion method.

Material and Methods

Chemicals: Mueller-Hinton agar, NaCl, and deionized water utilized during the extraction of essential oils and the preparation of aqueous solutions for antibacterial testing were purchased from Merck (Germany). Tween 80, ethanol, and Linalool 97% were purchased from Sigma-Aldrich (Steinheim, Germany). Analytical-grade solvents, chemicals, reagents, and standards were utilized during experimentation. Helium gas was obtained from Merck for analytical work. The same solvent medium was utilized for diluting stock solutions to prepare working standards, which were stored at 4°C.

Sample collection: Lavender plants were acquired from the National Agriculture Research Centre (NARC) Islamabad and cultivated in the natural experimental field of Mohi Uddin Islamic University (MIU), located in a humid subtropical climate in Nerian Sharif, Azad Jammu and Kashmir (33°48′50″N, 73°58′40″E). Fresh leaves and flowers were harvested during the flowering period (June 2020). The flowers and leaves were dried at room temperature (25-32°C) for 4 days, keeping them away from sunlight. Dried flowers and leaves were stored in containers in a cool area for further use. Four bacterial strains, *Escherichia coli* ATCC 33456*, Salmonella typhimurium* ATCC 700720*, Staphylococcus aureus* ATCC 6538*, and Pseudomonas aeruginosa* ACTT 15692*,* were obtained as a generous gift from the microbiology lab of Quaid e-Azam University, Islamabad.

Extraction of essential oil from leaves and flowers: Hydro-distillation, a certified reference method for the quantification of essential oils, was employed for essential oil extraction (Stahl-Biskup & Sáez, 2002). This method is one of the simplest methods for essential oil extraction. Essential oils were distilled from the flowers and leaves using a Clevenger distillation apparatus at NARC, Islamabad. A total of 25 grams of dried flowers and 35 grams of dried leaves, along with 500 mL of deionized water, were added to the distillation apparatus, processed sequentially. After one hour, the essential oils from the flowers and leaves were carefully collected from the apparatus. All details regarding the essential oil yield from lavender flowers and leaves obtained through hydro-distillation are provided in Table 1. The extracted essential oils were stored at 4°C for further experimental use.

Table 1. Essential oil yield from lavender flowers and leaves by hydro-distillation.

Sr. No.	Plant part utilized	Quantity used (g)	Essential oil obtained (m L)
1.	Flower	25	1
2.	Leaf	35	1

Gas chromatography-mass spectrometry (GC-MS) analysis of lavender essential oil: GCMS-QP 2010 Plus, Shimadzu, Japan, coupled with flame ionization detector (FID) 2010 with digital pressure controller (with cylindrical, electrode voltage: ±200V, dynamic range: 10^7 minimum detected quantity: 450°C and nozzle: made of quartz) having split less injector of 1 µL was used for Gas-chromatography and mass-spectroscopy (GC-MS) analysis of lavender essential oil. DB-5 stainless steel capillary column consists of two parts: stationary phase and tubing with MS 30 m × 0.25 mm inner diameter (ID) and 0.25 µm film thickness was used during GC-MS operation. The programming of the column temperature was done in this way; initial temperature 40-90°C that raises up to 90-240°C at the rate of 3°C increases within a minute. When the oven of the column area attained the set temperature and was maintained for 5 minutes. Injector was also held at the same temperature as of column (90-240°C) however, detector temperatures are sustained higher than both column and injector, which was 280°C. Pure (100%) extracted lavender oils F & L were injected without dilution (0.5 µl). Helium gas is flowed at the rate of 1 ml/min as a carrier to perform analysis. Gas chromatography functioned by ionization of electron at 70 eV. While coupled with MS, Compartment was observed from 35-500 AMU to get mass spectra. The lavender essential oils of both flower and leaves were run in triplicate. Linalool 97 % Sigma Aldrich was used as standard curves. GC-MSD Chem Station in build software was used for data analysis of results. Identification of phytochemicals was done by comparing retention time data relative to standard (n-alkanes C8-C20) and mass spectra pre-determined phytochemicals profiles (Umlauf *et al.*, 2004) and with a mass-spectrum library Wiley/NIST database (China Pharmacopoeia Committee, 1999). For quantification of relative amounts of each phytochemical in the flowers and leaves essential oils peak areas, retention time was recorded and presented in percentages.

GC-MS analysis of lavender essential oil was performed using a Shimadzu GCMS-QP 2010 Plus, coupled with a flame ionization detector (FID 2010). The system included a digital pressure controller with a cylindrical electrode (voltage: ±200V), a dynamic range of 10^7, a minimum detected quantity of 450°C, and a quartz nozzle. A splitless injector with a 1 µL capacity was utilized for the analysis.

The analysis employed a DB-5 stainless steel capillary column with a stationary phase and tubing. The column dimensions were 30 m × 0.25 mm, inner diameter (ID) with a film thickness of 0.25 µm. The column temperature was programmed as follows: an initial temperature of 40°C, increasing to 90°C, then ramping up to 240°C at a rate of 3°C per minute. Once the target temperature was reached, it was maintained for 5 minutes. The injector temperature was set to match the column temperature, while the detector temperature was maintained at 280°C.

For the analysis, 100% pure lavender essential oils from flowers and leaves were injected without dilution (0.5 µL). Helium gas was used as the carrier at a flow rate of 1 mL/min. The gas chromatography was performed with electron ionization at 70 eV. Mass spectra were obtained from 35 to 500 atomic mass units (AMU). The essential oils were analyzed in triplicate. A complete schematic representation of the experiment, starting from lavender sample collection, essential oil extraction, and phytochemical compound identification via GC-MS chromatography, is presented in Figure 1.

Linalool (97%, Sigma Aldrich) was used to create standard curves. Data analysis was conducted using the GC-MSD ChemStation software. Phytochemicals were identified by comparing retention times with standard n-alkanes (C8-C20) and by matching mass spectra with the Wiley/NIST database. Relative quantification of each phytochemical component in the essential oils was determined by recording peak areas and retention times, and the results were expressed as percentages.

Antibacterial activity assessment: The disc diffusion test was conducted in accordance with the standardized parameters of the Clinical and Laboratory Standards Institute (Wayne, 2014). Mueller Hinton Agar (MHA) media was prepared and sterilized. Bacterial colonies from four strains *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhimurium*, and *Staphylococcus aureus* were collected and inoculated onto the MHA plates using sterile cotton swabs. Various dilutions of lavender essential oils from leaves and flowers (1:1, 1:2, 1:3, 1:4, and 1:10) were prepared, with 30% ethanol serving as the diluent. Sterile paper discs, 3 mm in diameter, were soaked in 20 microliters of each concentration of the essential oil extracted from flowers and leaves (F & L) and then placed onto the agar plates. The plates were incubated at 37°C for 24 hours, and sterile plastic wrap was used to seal the plates. Each trial was performed in triplicate, and zones of inhibition were measured in millimeters (mm).

Results and Discussion

Essential oil extraction from lavender leaves and flowers: One mL of oil was extracted from 25g of dried flowers and 35 g of dried leaves respectively. Crude oils from lavender flowers and leaves exhibited a very fine aroma. Leaves yielded less oil compared to flowers. The notably lower oil yield from leaves compared to flowers suggests that flower components may be more efficient for essential oil extraction in lavender.

Identification of phytochemical components via GC-MS assay: GC-MS is extensively utilized for the accurate quantification of essential oils. The composition of phytochemical compounds in lavender flower and leaf essential oils was identified using GC-MS. GC-MS analysis of lavender oils revealed thirty-one compounds. The peak number, retention time, and percentage of each identified compound are recorded and presented in Table 2. Thirty-one peaks were generated for the thirty-one compounds. The GC-MS chromatogram of Lavandula essential oils, showing the peaks of the identified phytochemicals, is depicted in Figure 2.

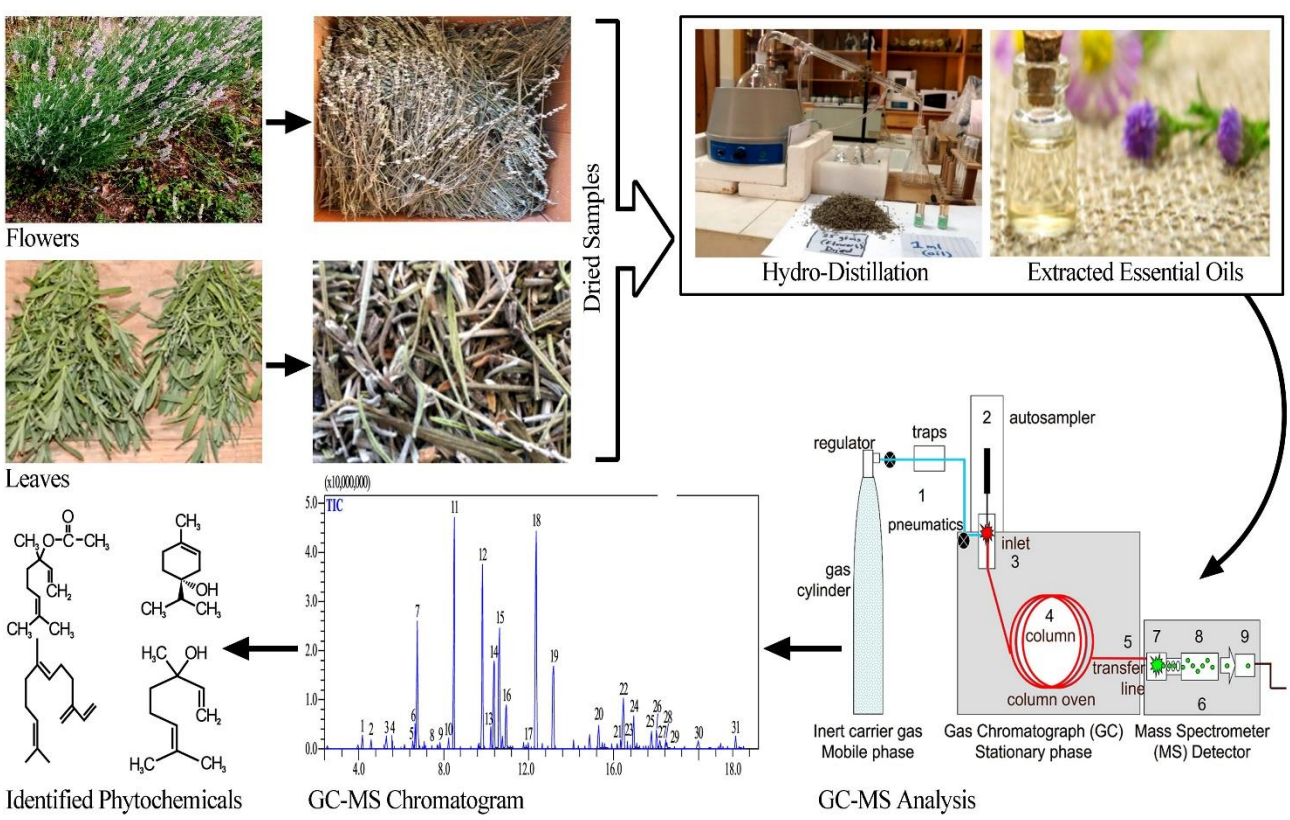

Fig. 1. Schematic representation of lavender sample collection, essential oil extraction and phytochemical compounds identification via GC-MS Chromatography.

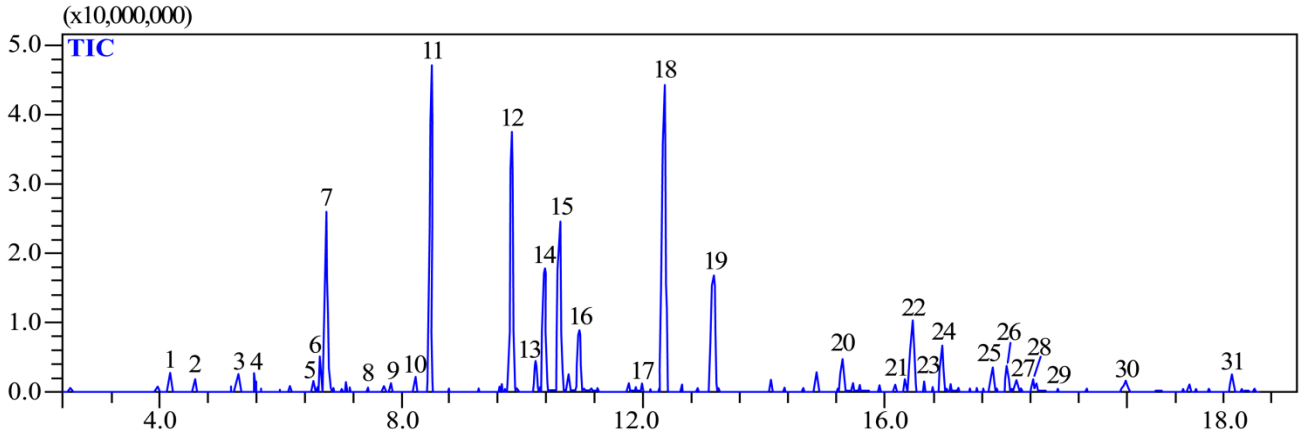

Fig. 2. The GC-MS chromatogram of *Lavandula* essential oils displaying peaks corresponding to the identified phytochemical compounds.

Table 2. Phytochemical compounds of lavender essential oils identified by GC-MS analysis.

Peak No.	Compounds identified from lavender essential oils	Retention time (min)	Content in flower (%)	Content in leaves %
1	1 R-α-Pinene	4.14	0.82	0.42
2	Camphene	4.52	0.76	0.26
3	β-pinene	5.02	0.84	0.54
4	octan -3-1	5.38	1.49	1.19
5	β-Myrcene	5.49	1.06	2.06
6	n-Hexyl acetate	5.92	1.19	0.99
7	p-Cymene	6.27	0.42	0.22
8	Limonene	6.41	0.32	0.12
9	eucalyptol (1,8-cineole)	6.45	3.94	2.99
10	(E)-Ocimene	6.62	4.06	3.52
11	(Z)-Ocimene	6.84	3.14	2.74
12	γ-Terpinene	7.081	0.2	0.11
13	α-Terpinolene	8.14	0.34	0.44
14	1, 6-Octadien-3-ol,3,7-dimethyl-formate (linalool)	8.21	30.89	27.89
15	Oct-1-en-3-yl acetate	8.34	0.34	0.54
16	Camphor	9.308	0.37	0.37
17	Bicyclo [2.2.1] heptan-2-one,1,7,7-trimethyl-, (IS)-Borneol	9.81	2.81	1.91
18	Terpin-1-en-4-ol	10.08	5.37	4.74
19	α-Terpineol	10.48	3.81	2.11
20	Nerol	11.09	0.28	0.18
21	1,6-Octadien-3-ol, 3,7-dimethyl-,2-aminobenzoate - (linalyl ester)	12.32	24.07	21.77
22	Bornyl acetate	13.01	0.12	0.32
23	Lavandulyl acetate	13.18	2.21	1.91
24	Neryl acetate	14.23	0.29	0.19
25	Geranyl acetate	14.85	2.61	2.11
26	β-Caryophyllene	15.84	1.34	1.14
27	α-Bergamotene	16.57	0.21	0.31
28	(E)-β-Farnesene	16.98	4.21	3.14
29	β-Cubebene	17.58	1.07	0
30	Caryophyllene oxide	18.11	0.57	0.49
31	cadinol	18.62	0.17	0.37
	Total %		**99.32%**	**84.39%**

Table 3. Zone of inhibition of selected gram-positive and gram-negative bacterial strains at pre-defined dilutions using the disc diffusion method.

Bacterial strains gram negative (G+) & gram positive (G-)	Zone of inhibition (ZOI) at different Dilution (D)							
	1:1 D (Oil: Ethanol)		1:2 D (Oil: Ethanol)		1:4 D (Oil: Ethanol)		1:10 D (Oil: Ethanol)	
	Flower (mm)	Leaf (mm)	Flower (mm)	Leaf (mm)	Flower (mm)	Leaf (mm)	Flower (mm)	Leaf (mm)
Escherichia coli (G-) ATCC 33456	28.6±0.6	25.9±0.4	25.8±0.5	24.3±0.6	22.1±0.4	20.4±0.2	20.1±0.7	18±0.4
Pseudomonas aeruginosa (G-) ACTT 15692	16.2±0.3	14.6±0.5	13.8±0.7	11.2±0.5	12.5±0.6	10.1±0.3	10.2±0.2	9±0.5
Staphylococcus aureus (G-) ATCC 6538	23.1±0.4	20.2±0.2	20.1±0.5	19±0.3	17.2±0.8	16.7±0.4	15.1±0.7	13.4±0.5
Salmonella typhimurium (G+) ATCC 700720	25.4±0.8	24.7±0.6	23.1±0.4	22.8±0.7	20.6±0.3	19.2±0.6	18.4±0.3	17.6±0.6

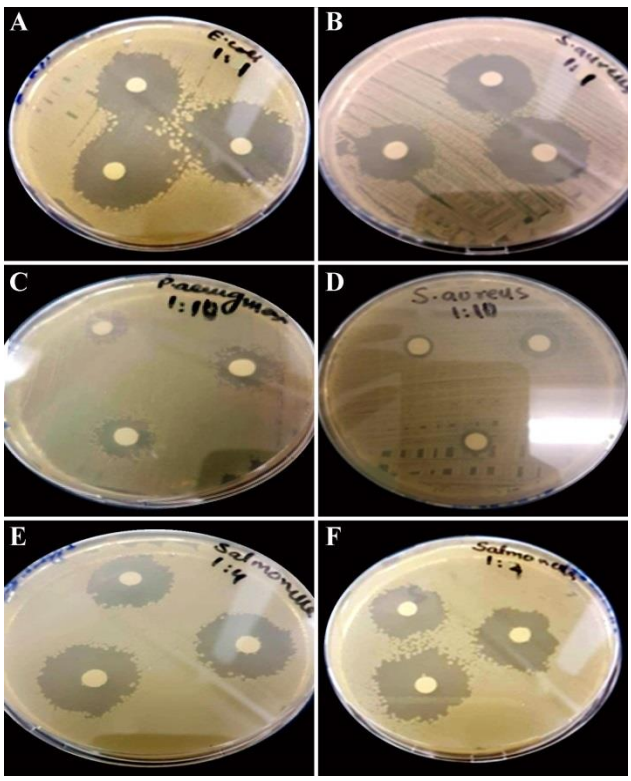

Fig. 3. Zone of inhibition (mm) for lavender essential oils: A, flowers essential oil against *E. coli* at a 1:1 dilution; B, leaves essential oil against *Staphylococcus aureus* at a 1:1 dilution; C, flowers essential oil against *Pseudomonas aeruginosa* at a 1:10 dilution; D, leaves essential oil against *Staphylococcus aureus* at a 1:10 dilution; E, flowers essential oil against *Salmonella typhimurium* at a 1:4 dilution; F**)** Leaves essential oil against *Salmonella typhimurium* at a 1:4 dilution.

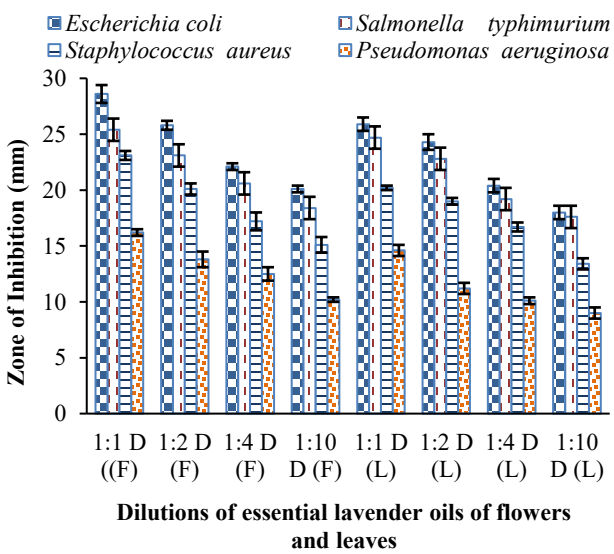

Fig. 4. Graphical representation of the zone of inhibition for bacterial strains at selected dilutions with standard deviation.

The essential oils extracted from *Lavandula* flowers were composed of 1,6-Octadien-3-ol, 3,7-dimethyl- (linalool) (30.89%), linalyl acetate (24.07%), Terpin-1-en-4-ol (5.37%), (E)-β-Farnesene (4.21%), (E)-Ocimene (4.06%), eucalyptol (1,8-cineole) (3.94%), α-Terpineol (3.81%), (Z)-Ocimene (3.14%), Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-(IS)-Borneol (2.81%), Geranyl acetate (2.61%), Lavandulyl acetate (2.21%), octan-3-ol (1.49%), β-Caryophyllene (1.34%), n-Hexyl acetate (1.19%), β-Cubebene (1.07%), β-Myrcene (1.06%), β-Pinene (0.84%), 1,R-α-Pinene (0.82%), Camphene (0.76%), Caryophyllene oxide (0.57%), p-Cymene (0.42%), Camphor (0.37%), Oct-1-en-3-yl acetate (0.34%), α-Terpinolene (0.34%), Limonene (0.32%), Neryl acetate (0.29%), Nerol (0.28%), α-Bergamotene (0.21%), γ-Terpinene (0.20%), cadinol (0.17%), and Boroyl acetate (0.12%). These compounds represent 99.32% of the total phytochemical composition, listed in descending order of abundance.

The essential oils extracted from *Lavandula* leaves contained 1,6-Octadien-3-ol, 3,7-dimethyl- (linalool) (27.89%), linalyl acetate (21.77%), Terpin-1-en-4-ol (4.74%), (E)-Ocimene (3.52%), eucalyptol (1,8-cineole) (2.99%), (Z)-Ocimene (2.74%), α-Terpineol (2.11%), Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-(IS)-Borneol (1.91%), Geranyl acetate (2.11%), β-Myrcene (2.06%), Lavandulyl acetate (1.91%), octan-3-ol (1.19%), β-Caryophyllene (1.14%), n-Hexyl acetate (0.99%), β-Pinene (0.54%), Oct-1-en-3-yl acetate (0.54%), Caryophyllene oxide (0.49%), α-Terpinolene (0.44%), 1,R-α-Pinene (0.42%), Camphor (0.37%), cadinol (0.37%), Boroyl acetate (0.32%), α-Bergamotene (0.31%), Camphene (0.26%), p-Cymene (0.22%), Neryl acetate (0.19%), Nerol (0.18%), Limonene (0.12%), γ-Terpinene (0.11%), and β-Cubebene (0.07%), comprising an overall 84.39% of the phytochemical constituents.

Earlier studies on the identification of phytochemical compounds had shown that *Lavandula* essential oils consisted of triterpenes, hydroxycinnamic acids, coumarins, and trace amounts of flavonoids, depending on the extraction method (Aljaafari *et al.*, 2021). Our analysis of lavender essential oils' phytochemical profile revealed that the flower portion of the plant contained higher quantities of linalool, linalyl ester, and terpenes compared to the leaves. The most abundant terpenic phytochemicals in both the flowers and leaves are monoterpenes and their oxygenated derivatives, accounting for 70.05% and 61.58%, respectively. The phytochemical elements that primarily determine the quality and purity of lavender flower and leaf essential oils, based on the International Standard (ISO 3515, 2002), include linalool (38.0% in flowers and 25.0% in leaves), (E)-ocimene (4.06% in flowers and 3.52% in leaves), camphor (0.37%), linalyl acetate (24.07% in flowers and 21.77% in leaves), 1,8-cineole (eucalyptol) (3.94% in flowers and 2.99% in leaves), (Z)-ocimene (3.14% in flowers and 2.74% in leaves), terpin-1-en-4-ol (5.37% in flowers and 4.74% in leaves), α-terpineol (3.81% in flowers and 2.11% in leaves), and lavandulyl acetate (2.21% in flowers and 1.91% in leaves) (Aljaafari *et al.*, 2021)."

The presence of higher percentage of Linalool and Eucalyptol in lavender oil which has been distilled from locally cultivated lavender plants indicated that lavender essential oils had significant value for the pharmaceutical industry. It is believed that these components have antibacterial anti-inflammatory and antifungal activities (Ciocarlan *et al.*, 2021). All these compounds have antimicrobial properties due to the presence of phenolic

groups. The amount of sesquiterpene hydrocarbons and their oxygenated derivatives is quantified in proper limits within flower 5.75% & 0.57%, and 4.28 & 0.49 leaves respectively. It is also reported that the highly important secondary metabolite (Sesquitepenes) order is: β- caryophyllene > (E) –β- farnesene > caryophyllene oxide in both flowers and leaves (Hellen *et al.*, 2021). Our results in accordance with previously executed research and indicated that linalool and linalyl acetate both were the preeminent constituents of lavender essential oils by Ez zoubi *et al.*, in 2020, Though, chemical transformations in the Lavender F & L essential oil composition are highly associated with geographical area and sample harvesting time of year.

The high percentage of linalool and eucalyptol in lavender oil distilled from locally cultivated lavender plants suggests that these essential oils have considerable potential for the pharmaceutical industry. These components are known for their antibacterial, anti-inflammatory, and antifungal activities (Ciocarlan *et al.*, 2021). The antimicrobial properties of these compounds can be attributed to the presence of phenolic groups. The levels of sesquiterpenes hydrocarbons and their oxygenated derivatives are quantified within acceptable limits: 5.75% & 0.57% in flowers and 4.28% & 0.49% in leaves, respectively. Additionally, the important secondary metabolites (sesquiterpenes) follow the order: β-caryophyllene > (E)-β-farnesene > caryophyllene oxide in both flowers and leaves (Hellen *et al.*, 2021). Our results align with previous research, confirming that linalool and linalyl acetate are the dominant constituents of lavender essential oils, as reported by Ez Zoubi *et al.*, in 2020. The geographical region and the season in which the samples are harvested significantly affect the chemical composition of lavender flower and leaf essential oils.

Antibacterial activity of lavender essential oils against various bacterial strains: Antimicrobial testing of lavender flower and leaf essential oils was performed on selected Gram-negative strains, including *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella typhimurium*, as well as the Gram-positive strain *Staphylococcus aureus*. The preliminary screening results for these bacterial strains at predefined dilutions using the disc diffusion method are summarized in Table 3.

The results indicated a consistent pattern across the tested bacterial strains. Lavender flowers essential oil, diluted with ethanol (1:1), exhibited strong antibacterial activity against all tested bacterial strains. The most sensitive Gram-negative strains were *E. coli* (28.6 mm), *Salmonella typhimurium* (25.4 mm), with the least inhibition observed against *Pseudomonas aeruginosa* (16.2 mm). Similarly, lavender leaves essential oil at a 1:1 dilution demonstrated significant antibacterial activity across all tested strains, with the highest antibacterial response observed against *E. coli* (25.9 mm) and *Salmonella typhimurium* (24.7 mm), and the minimum inhibition recorded against *Pseudomonas aeruginosa* (14.6 mm).

Lavender flowers contain a higher concentration of essential oils compared to the leaves, which may account for the observed differences in antibacterial efficacy. The zones of inhibition (mm) of lavender flowers and leaves essential oils against the bacterial strains are illustrated in Figure 3. The maximum antibacterial activity was observed at the highest concentration (1:1 dilution), while the minimum activity was noted at the lowest concentration (1:10 dilution). The smallest zone of inhibition was recorded for *Pseudomonas aeruginosa*, with 10.2 ± 0.2 mm for lavender flowers essential oil and 9.0 ± 0.5 mm for lavender leaves essential oil.

Our findings indicate that lavender essential oil exhibits a strong bactericidal response against multidrug-resistant clinical strains such as *Escherichia coli* and *Staphylococcus aureus* when diluted 1:1 with methanol. These results are consistent with the findings of Kavanaugh and Ribbeck (2012), Messaoudi Moussii *et al.* (2020), and Diass *et al.* (2023). Representative diagrams illustrating the antibacterial response, as measured by the zone of inhibition (mm), of lavender flower and leaf essential oils against pathogenic Gram-negative and Gram-positive bacteria using the disc diffusion method are presented in Figure 4.

Previous reports have demonstrated that lavender essential oil has significant antimicrobial activity against various bacterial strains, with the most pronounced effects observed against *Escherichia coli* (Saviuc *et al.*, 2016; Predoi *et al.*, 2018), which aligns with our findings. Our results showed that bacterial sensitivity was directly proportional to the concentration of lavender flowers and leaves essential oils, a trend also described by Mardafkan *et al.* (2015) and Bouazama *et al.* (2017).

Our data suggests that the high content of terpenoids, such as linalool, in lavender flowers and leaves contributes to the observed antibacterial potential. Terpenoids exhibit antimicrobial action through several mechanisms. They disrupt bacterial cell membranes, causing structural damage and loss of vital cellular components (Lis-Balchin *et al.*, 1998). Additionally, they inhibit quorum sensing, thereby preventing bacterial communication and resistance formation. Terpenoids also interfere with ATP synthesis, which is essential for energy metabolism, leading to bacterial cell death (Huang *et al.*, 2022). Furthermore, they disrupt protein synthesis pathways, impeding bacterial growth. Terpenoids may also act synergistically with other compounds, enhancing antimicrobial activity by increasing membrane permeability (Di Pasqua *et al.*, 2007; Burt & Reinders, 2003). Together, these combined mechanisms contributed to the broad-spectrum antibacterial effects observed in this study. The zone of inhibition, with standard deviation, for all selected bacterial strains at predefined dilutions using the disc diffusion method is graphically represented in Figure 4.

Conclusion

This study provides comprehensive insights into the extraction, phytochemical profiling, and antibacterial potential of locally cultivated *Lavandula angustifolia* Mill. essential oils from both flowers and leaves against selected bacterial strains. The phytochemical analysis not only supports the traditional uses of *Lavandula*, but also highlights the antibacterial properties of its essential oils. The study suggests that the high content of terpenoids, particularly linalool, plays a key role in the observed antimicrobial effects, including disruption of bacterial cell membranes, inhibition of quorum sensing, and interference

with ATP synthesis. Our findings support the potential use of *Lavandula* essential oils as an alternative to conventional antibiotics, especially against multidrug-resistant strains. Furthermore, this study lays a solid foundation for future research into the therapeutic applications of *Lavandula* essential oils and their role in combating bacterial infections.

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