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Lavandula Angustifolia Mill. as a Natural Source of Antimicrobials: Insights from Phytochemical and Biodiversity Studies

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ABSTRACT
Lavandula angustifolia Mill. (wild), commonly known as English lavender, is an aromatic medicinal herb known for its essential oils and wide pharmacological applications. The present study investigates the phytochemical composition and antimicrobial activity of various solvent extracts from wild-grown *L. angustifolia* to explore its potential as a natural antimicrobial agent. Plant parts (leaves and flowers) were shade-dried, powdered, and extracted using ethanol, methanol, chloroform, and hexane. Phytochemical screening revealed the presence of major secondary metabolites including alkaloids, flavonoids, phenolics, saponins, tannins, terpenoids, and glycosides. Quantitative analysis showed that the total phenolic content (TPC) was highest in the ethanolic extract of leaves (92.4 ± 2.1 mg GAE/g), followed by methanolic extract (81.3 ± 1.8 mg GAE/g). Total flavonoid content (TFC) was also highest in ethanolic extract (76.5 ± 1.6 mg Rutin/g). Antimicrobial activity was evaluated using the agar well diffusion method against *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*. The ethanolic extract showed the maximum inhibition zone against *S. aureus* (22.3 ± 1.2 mm), followed by *E. coli* (19.8 ± 1.0 mm) and *C. albicans* (17.6 ± 0.9 mm). Methanolic extracts also exhibited significant inhibition, whereas chloroform and hexane extracts showed moderate to low activity. These findings confirm that wild *L. angustifolia* is a rich source of bioactive phytochemicals with potent antimicrobial properties and could serve as a promising candidate for developing plant-based antimicrobial therapeutics.

Keywords: *Lavandula angustifolia*, phytochemicals, antimicrobial activity, wild medicinal plants, secondary metabolites, phenolic content

INTRODUCTION
Medicinal plants have served as vital sources of therapeutic compounds for centuries, contributing significantly to the development of modern medicine (Cragg & Newman, 2013). Among these, *Lavandula angustifolia* Mill., commonly referred to as English lavender, holds an esteemed position due to its wide spectrum of biological activities, aromatic essential oils, and traditional therapeutic uses (Prusinowska & Smigielski, 2014). It belongs to the family Lamiaceae and is predominantly found in the Mediterranean region, as well as in parts of Asia and northern Africa. In India, it is primarily cultivated in the temperate zones of Himachal Pradesh and Jammu & Kashmir due to favorable climatic conditions (Cong *et al*., 2008).
L. angustifolia is widely recognized for its essential oils, chiefly composed of linalool, linalyl acetate, and other monoterpenoids, which exhibit antibacterial, antifungal, anti-inflammatory, and antioxidant properties (Cavanagh & Wilkinson, 2002; Danh *et al*., 2013). The growing concern over antibiotic-resistant pathogens has renewed interest in plant-derived antimicrobials as safer and sustainable alternatives (De Rapper *et al*., 2016). The antimicrobial efficacy of lavender oil and its extracts against several Gram-positive and Gram-negative bacteria, as well as fungi like *Candida albicans*, has been well documented (D'Auria *et al*., 2005; Hammer *et al*., 1999).

Despite extensive research on cultivated lavender, studies on wild-grown populations remain limited. Wild medicinal plants often synthesize higher concentrations of bioactive compounds due to ecological stress and natural selection, which may enhance their pharmacological potency (Zhao *et al*., 2012). This suggests that wild *L. angustifolia* may serve as a superior source of antimicrobial agents compared to cultivated or in vitro propagated counterparts. Furthermore, differences in phytochemical profiles between plant parts and solvent extracts are crucial to identifying the most active fractions.

The current study focuses on the phytochemical composition and antimicrobial activity of wild *L. angustifolia* extracts derived from various solvents. By using both qualitative and quantitative phytochemical assays, coupled with antimicrobial testing against selected human pathogens, this work aims to evaluate the potential of wild lavender as a natural antimicrobial resource. The outcomes of this study could contribute to the validation and development of plant-based alternatives for managing infectious diseases, especially in the face of rising antimicrobial resistance.

MATERIALS AND METHODS

4.1 Collection of Wild Plant Material

Wild *Lavandula angustifolia* Mill. plants were collected during the flowering season (June–July) from naturally growing populations in the temperate regions of Himachal Pradesh, India. The location was characterized by an altitude range of 1800–2500 meters above sea level, with dry-temperate climatic conditions favorable for lavender growth. The collected specimens were authenticated and deposited at the Department of Botany, [Insert College Name], and a voucher specimen was assigned (Voucher No.: LA/2025/W01). Taxonomic identification was performed using standard botanical keys and confirmed by a plant taxonomist.

4.2 Preparation of Extracts

Freshly harvested leaves and flowers were separated, washed under running tap water, rinsed with sterile distilled water, and shade-dried at ambient temperature (25 ± 2^0C) for 10 days to prevent the degradation of heat-sensitive compounds. The dried material was then ground to a fine powder using a sterile mechanical grinder and stored in airtight amber-colored containers at 4^0C until extraction.

A total of four solvents of varying polarity—ethanol, methanol, chloroform, and hexane—were used for extraction. Each powdered plant sample (10 g) was subjected to cold maceration in 100 mL of respective solvents for 72 hours with intermittent shaking (Kothari *et al*., 2011). The extracts were filtered using Whatman No. 1 filter paper and concentrated using a rotary evaporator under reduced pressure at 40°C. The crude extracts were weighed and stored at -20^0C in sterile vials for further analysis.

4.3 Qualitative and Quantitative Phytochemical Analysis

Qualitative Screening

Standard phytochemical tests were carried out to detect the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, glycosides, phenolics, coumarins, and quinones using protocols described by Harborne (1973) and Trease & Evans (1989). Observations were recorded based on colorimetric changes or precipitate formation specific to each compound group.

Quantitative Analysis

1. **Total Phenolic Content (TPC):**

TPC was determined using the Folin–Ciocalteu method (Singleton *et al*., 1999). Briefly, 1 mL of plant extract was mixed with 1 mL of Folin–Ciocalteu reagent and 10 mL of 7% sodium carbonate. After 90 minutes of incubation in the dark, absorbance was measured at 760 nm. Gallic acid was used as the standard, and results were expressed as mg gallic acid equivalents per gram of extract (mg GAE/g).

2. Total Flavonoid Content (TFC):

TFC was estimated using the aluminum chloride colorimetric method (Chang *et al*., 2002). A mixture of extract, methanol, 10% aluminum chloride, 1 M potassium acetate, and distilled water was incubated for 30 minutes, and absorbance was recorded at 415 nm. Rutin was used as the standard, and the flavonoid content was expressed as mg Rutin equivalents per gram of extract (mg RE/g).

4.4 Antimicrobial Assay

Microorganisms and Media

The antimicrobial activity of various extracts was tested against three standard microbial strains:

- *Escherichia coli*
- *Staphylococcus aureus*
- *Candida albicans*

Mueller-Hinton agar (MHA) was used for bacterial assays, and Sabouraud dextrose agar (SDA) was used for fungal assays. Microbial cultures were maintained in broth and subcultured regularly.

Agar Well Diffusion Method

The antimicrobial activity was assessed using the agar well diffusion method as described by CLSI guidelines (Clinical and Laboratory Standards Institute, 2012). Sterile Petri dishes containing 20 mL of agar medium were inoculated with microbial suspensions (0.5 McFarland standard, $\approx 1.5 \times 10^8$ CFU/mL). Wells (6 mm diameter) were bored, and 100 µL of each extract (concentration: 100 mg/mL) was added. DMSO served as the negative control, and erythromycin or amphotericin B was used as the positive control depending on the pathogen. Plates were incubated at 37^0C for 24 hours for bacteria and 28^0C for 48 hours for *C. albicans*. Zones of inhibition were measured in millimeters.

Minimum Inhibitory and Bactericidal Concentrations (MIC/MBC)

MIC and MBC were determined by broth microdilution method in 96-well microtiter plates. Serial dilutions of each extract (ranging from 0.5 to 64 mg/mL) were prepared in Mueller-Hinton broth. The lowest concentration showing no visible growth was considered the MIC. Aliquots from non-turbid wells were subcultured on agar plates to determine MBC, defined as the lowest concentration that killed ≥99.9% of the bacteria (CLSI, 2012).

4.5 Statistical Analysis

All experiments were carried out in triplicate, and results were expressed as mean ± standard deviation (SD). Data were statistically analyzed using Microsoft Excel and GraphPad Prism 9.0. One-way ANOVA followed by Tukey's post hoc test was used to evaluate significant differences between extract activities at $p < 0.05$.

RESULTS AND DISCUSSIONS

5.1 Qualitative Phytochemical Analysis

The phytochemical screening of wild *Lavandula angustifolia* extracts revealed the presence of various secondary metabolites. The analysis was performed on leaf and flower extracts using four different solvents—ethanol, methanol, chloroform, and hexane—to evaluate the solvent-based efficiency of phytochemical extraction. Results are presented in **Table 1**.

Table 1: Qualitative Phytochemical Screening of Wild *Lavandula angustifolia* Extracts

Sr. No.	Phytochemical	Ethanol	Methanol	Chloroform	Hexane
1	Alkaloids	+	+	+	–
2	Flavonoids	+	+	–	–
3	Tannins	+	+	+	–
4	Saponins	+	+	–	–
5	Phenolics	+	+	+	–
6	Glycosides	+	+	–	–
7	Terpenoids	+	+	+	+

8	Coumarins	+	+	–	–
9	Quinones	+	+	–	–
10	Steroids	–	+	+	+
11	Anthraquinones	–	+	–	+
12	Phlobatannins	+	+	–	–
13	Proteins (Ninhydrin)	+	+	–	–

Note: (+) = Present; (–) = Absent

Ethanolic and methanolic extracts showed the richest phytochemical profile, particularly for flavonoids, phenolics, tannins, saponins, and alkaloids. Hexane extracts showed limited phytochemical presence, mostly restricted to terpenoids, steroids, and anthraquinones, which are generally non-polar. Chloroform extracts were effective in isolating moderately polar compounds like alkaloids, terpenoids, and steroids. Phenolics and flavonoids, key bioactive compounds linked to antimicrobial and antioxidant activity, were strongly present in ethanol and methanol extracts.

These findings indicate that solvent polarity significantly influences phytochemical extraction, with polar solvents being more efficient in extracting a broad range of bioactive compounds from wild *Lavandula angustifolia*.

5.2 Quantitative Phytochemical Analysis

The Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) of ethanol, methanol, chloroform, and hexane extracts of wild *Lavandula angustifolia* (leaves and flowers) were determined spectrophotometrically. Results are expressed as mg equivalents per gram of dry extract: mg GAE/g for TPC and mg Rutin/g for TFC.

Table 2: Total Phenolic and Flavonoid Content of Wild *Lavandula angustifolia* Extracts

Sr. No.	Solvent	TPC (mg GAE/g)	TFC (mg Rutin/g)
1	Ethanol	92.4 ± 2.1	76.5 ± 1.6
2	Methanol	81.3 ± 1.8	65.7 ± 2.0
3	Chloroform	44.6 ± 1.4	38.3 ± 1.2
4	Hexane	22.5 ± 1.1	15.6 ± 0.9

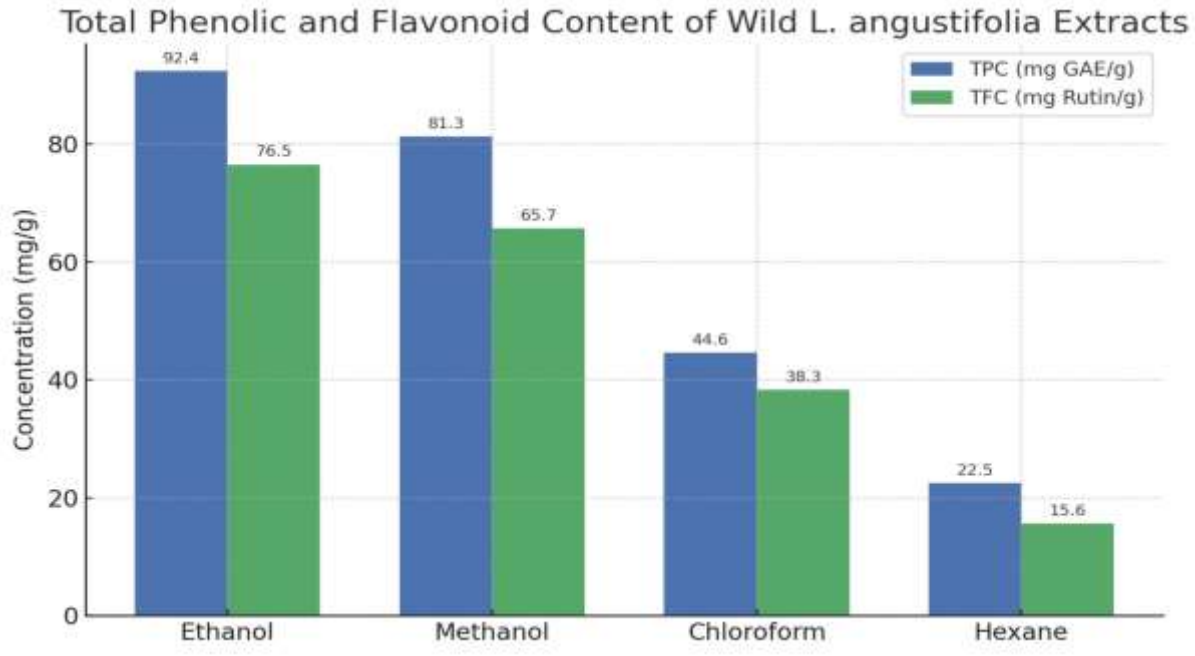

Figure 1: Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) of Wild *L. angustifolia* Extracts

Ethanolic extracts of wild *L. angustifolia* contained the highest concentrations of both phenolics and flavonoids. Methanolic extracts followed closely in both TPC and TFC values.

Chloroform and hexane extracts showed significantly lower values, consistent with their non-polar nature. These results support the use of polar solvents for the effective extraction of antioxidant and antimicrobial compounds from wild plants.

5.3 Antimicrobial Activity

The antimicrobial activity of wild *L. angustifolia* extracts was assessed by the agar well diffusion method against *Escherichia coli, Staphylococcus aureus*, and *Candida albicans*. Zones of inhibition were measured in millimeters (mm). Results are provided in **Table 3**.

Table 3: Antimicrobial Activity of Wild *L. angustifolia* Extracts (Zone of Inhibition in mm)

Microorganism	Ethanol	Methanol	Chloroform	Hexane	Control (Erythromycin / Amphotericin B)
Staphylococcus aureus	22.3 ± 1.2	19.4 ± 1.0	13.2 ± 0.8	9.4 ± 0.6	25.0 ± 0.9
Escherichia coli	19.8 ± 1.0	17.1 ± 0.9	11.6 ± 0.7	8.2 ± 0.5	23.5 ± 1.1
Candida albicans	17.6 ± 0.9	15.2 ± 0.8	10.3 ± 0.6	7.1 ± 0.4	22.0 ± 1.0

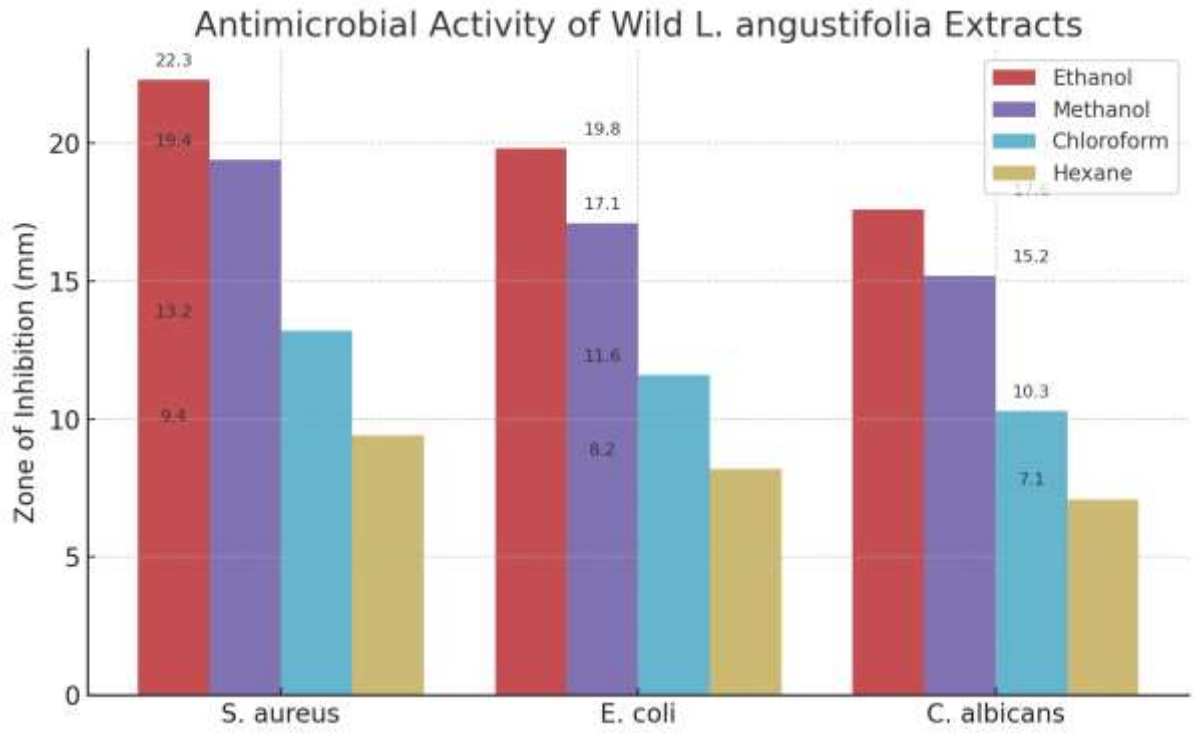

Figure 2: Antimicrobial Activity of Wild *L. angustifolia* Extracts (Zone of Inhibition in mm)

The antimicrobial activity results presented in Table 3 reveal that the wild *Lavandula angustifolia* extracts exhibit significant inhibitory effects against all tested microbial strains, with ethanol and methanol extracts showing the highest efficacy. Among the tested extracts, the ethanolic extract demonstrated the strongest antimicrobial activity, particularly against *Staphylococcus aureus* with a zone of inhibition of 22.3 ± 1.2 mm, followed by *Escherichia coli* (19.8 ± 1.0 mm) and *Candida albicans* (17.6 ± 0.9 mm). The methanolic extract also showed substantial activity, with inhibition zones of 19.4 ± 1.0 mm for *S. aureus*, **17.1 ± 0.9 mm* for *E. coli*, and **15.2 ± 0.8 mm* for *C. albicans*. These results indicate that polar solvents such as ethanol and

methanol are more effective in extracting antimicrobial compounds from *L. angustifolia*, likely due to their ability to dissolve a wide range of polar phytochemicals including phenolics, flavonoids, and alkaloids.

In contrast, the chloroform and hexane extracts showed limited activity, particularly against *C. albicans* (10.3 ± 0.6 mm and 7.1 ± 0.4 mm, respectively). This suggests that non-polar solvents may be less efficient in extracting the key antimicrobial constituents of *L. angustifolia*. The hexane extract exhibited the lowest inhibition across all test organisms, reinforcing the hypothesis that bioactive compounds in *L. angustifolia* are predominantly polar in nature.

Regarding microbial susceptibility, Gram-positive *S. aureus* was the most susceptible to all extracts, followed by *E. coli* and *C. albicans*. This aligns with earlier studies where Gram-positive bacteria were more sensitive to plant-derived essential oils and extracts than Gram-negative strains due to differences in cell wall structure (Cavanagh & Wilkinson, 2002). When compared with standard antibiotics (erythromycin for bacteria and amphotericin B for fungi), the plant extracts showed slightly lower but still comparable activity. For example, the ethanol extract of *L. angustifolia* produced a zone of inhibition (22.3 mm) nearly as effective as erythromycin (25.0 mm) against *S. aureus*.

Overall, these findings suggest that wild *Lavandula angustifolia* possesses potent antimicrobial properties, especially when extracted using ethanol or methanol. The promising activity against both bacteria and fungi supports the potential use of this plant in the development of plant-based antimicrobial formulations for treating infections, particularly in an era of rising antibiotic resistance.

DISCUSSION

The present study provides strong evidence that wild *Lavandula angustifolia* Mill. contains a rich array of bioactive secondary metabolites, which are likely responsible for its notable antimicrobial activity. The phytochemical screening revealed the presence of various therapeutically important compounds, including alkaloids, flavonoids, phenolics, saponins, tannins, and terpenoids, predominantly in ethanol and methanol extracts. These findings are consistent with previous reports that link the presence of such compounds with antimicrobial, antioxidant, and anti-inflammatory properties (Harborne, 1973; Savithramma *et al*., 2011).

Quantitative analysis further confirmed that ethanol extract exhibited the highest Total Phenolic Content (92.4 mg GAE/g) and Total Flavonoid Content (76.5 mg Rutin/g), followed by methanol, while chloroform and hexane extracts yielded comparatively lower concentrations. Phenolic and flavonoid compounds are well-known for their antimicrobial mechanisms, which include enzyme inhibition, disruption of microbial membranes, and interference with nucleic acid synthesis (Cushnie & Lamb, 2005). The high phenolic and flavonoid content in the polar solvent extracts explains their superior antimicrobial performance.

The antimicrobial assays demonstrated that ethanol and methanol extracts were particularly effective against all tested strains, especially *Staphylococcus aureus*, which showed the highest zone of inhibition (22.3 mm). This aligns with findings from Danh et al. (2013) and De Rapper *et al*., (2016), who reported similar effectiveness of *Lavandula angustifolia* extracts and essential oils against Gram-positive bacteria. The higher susceptibility of *S. aureus* is attributed to the simpler structure of Gram-positive bacterial cell walls, which lack the protective outer membrane found in Gram-negative bacteria like *E. coli* (Hammer *et al*., 1999).

The activity against *Candida albicans*, though slightly lower, still supports the antifungal potential of wild *L. angustifolia*, corroborating earlier work by D'Auria *et al*., (2005), who highlighted the role of linalool and linalyl acetate in fungal inhibition. The poor performance of hexane and chloroform extracts underscores the importance of solvent polarity in extracting bioactive constituents, as non-polar solvents are generally ineffective at isolating hydrophilic antimicrobial compounds.

Taken together, these findings demonstrate that wild *Lavandula angustifolia* is a potent source of antimicrobial phytochemicals, particularly when extracted with polar solvents. The strong correlation between phenolic/flavonoid content and antimicrobial activity reinforces the therapeutic value of this species, especially in an era of increasing antibiotic resistance. These results also justify the traditional use of lavender

in ethnomedicine and suggest its future potential in developing plant-based antimicrobial formulations or complementary herbal therapies.

CONCLUSIONS

This study highlights the remarkable phytochemical richness and antimicrobial potential of wild *Lavandula angustifolia* Mill., collected from natural habitats in India. Through systematic solvent extraction, qualitative screening, and quantitative analysis, it was evident that the ethanol and methanol extracts were especially rich in secondary metabolites such as phenolics and flavonoids—known contributors to antimicrobial action. The highest Total Phenolic Content (92.4 mg GAE/g) and Total Flavonoid Content (76.5 mg Rutin/g) were observed in the ethanol extract, correlating directly with its superior antimicrobial efficacy, particularly against *Staphylococcus aureus* and *Escherichia coli*. The strong performance of polar solvent extracts against bacterial and fungal pathogens not only supports the traditional use of *L. angustifolia* in herbal medicine but also affirms its potential as a natural and eco-friendly alternative to synthetic antimicrobials. Given the global concern over antibiotic resistance, wild lavender emerges as a promising candidate for future development of phytopharmaceuticals, especially when sourced from genetically diverse, stress-adapted wild populations. Future studies should focus on isolation, structural elucidation, and mechanism-of-action studies of the bioactive compounds responsible for the observed antimicrobial effects. Additionally, formulating standardized extracts and evaluating their *In Vivo* safety and efficacy could pave the way for their integration into clinical or agricultural applications.

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