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The estimation of phytochemicals and study of biological activities of *Leucas lavandulifolia* Sm's leaf extract

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Abstract
Medicinal plants have a long-standing history of use as therapeutic agents for the prevention and management of a wide range of diseases. The present study aimed to investigate the phytochemical composition and biological activities of leaf extracts of Leucas lavandulifolia using a series of solvents, including methanol, ethanol, ethyl acetate, dichloromethane (DCM), and hexane. Quantitative analyses were performed to determine the total phenolic content (TPC) and total flavonoid content (TFC) of each extract. Antioxidant activity was assessed via the DPPH radical scavenging assay, while antibacterial efficacy was evaluated against multiple pathogenic strains, along with the determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Cytotoxic potential was examined using the brine shrimp lethality assay (BSLA). Results revealed notable variations in phytochemical composition and bioactivity among the extracts. The methanolic and ethyl acetate extracts exhibited the highest TPC (56.95 ± 0.13 mg GAE/g) and TFC (119.83 ± 0.12 mg QE/g), highlighting a rich presence of polyphenolic compounds. The methanolic extract demonstrated potent antioxidant activity with an IC_{50} value of 221.0 ± 0.15 μg/mL, whereas the ethyl acetate and hexane extracts showed comparatively weak radical scavenging potential. The ethyl acetate extract displayed significant antibacterial activity against Staphylococcus aureus and Shigella sonnei, producing zones of inhibition of 11 mm, compared with those of the positive control neomycin (22 and 23 mm, respectively). Both DCM and ethyl acetate extracts exhibited identical MIC and MBC values against S. sonnei (MIC = 1.56 mg/mL), while the LC_{50} value of the DCM extract was 731.31 μg/mL, indicating moderate cytotoxicity. This comprehensive solvent-dependent comparative study elucidates the influence of extraction media on the phytochemical yield and biological efficacy of L. lavandulifolia, providing a foundational framework for its potential applications in pharmaceutical, nutraceutical, and cosmetic industries.

Keywords
Leucas lavandulifolia, antioxidant, antibacterial, toxicity, phytochemistry.

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1 Introduction

Medicinal plants possess a range of biological characteristics, with their antioxidant properties being particularly noteworthy. This is crucial because the metabolic reactions in our bodies often lead to inconsistency and the generation of free radicals (ROS). These free radicals, which include hydroxyl radicals, peroxide radicals, and superoxide radicals, are implicated in several health issues such as cancer, diabetes, hypertension, DNA damage, and hyperpigmentation. They instigate cellular damage through oxidative chain reactions, resulting in cell shrinkage. The role of antioxidants is paramount in mitigating the detrimental effects of free radicals, thereby maintaining equilibrium between the antioxidant system and ROS. Medicinal plants are esteemed for their robust antioxidant potential, owing to the abundance of phytoconstituents they contain. These natural antioxidants help neutralize ROS and alleviate oxidative stress within the body. Consequently, natural ailments preventing various ailments through their antioxidant attributes [1].

Medicinal plants, known for their significant therapeutic value, have been integral to healthcare since ancient times. Extensive global research has validated their effectiveness, leading, development of plant mediated drugs. The trade for herbal medicines products is substantial, exceeding 100 billion dollars annually worldwide [2].The natural bioactive compounds present in medicinal plants contribute to the creation of various medicines, dietary supplements, and functional foods [3].Medicinal plants are utilized as traditional herbs for treating, preventing, and curing various types of illnesses. Their broad therapeutic applications, accessibility, and relatively safe side effects make natural products highly favored as active ingredients. Many drugs are synthesized from bioactive components found in these plants [4].

Medicinal plants are those that have positive pharmacological effects on either people or animals [5]. With fewer adverse effects, medicinal plants provide accessible and reasonably priced remedies. In many societies, growing and gathering medicinal herbs has great cultural value [6]. Because, phytoconstituents applied in drug development process, plants, also highly important in modern medicine [7]. When screening for possible drug candidates, natural product collections might provide hits due to a variety of structurally defined pharmacophores with extensive stereochemical configuration [8]. Reactive radical species are unpaired electrons that can live independently [9]. When free radicals build up under oxidative stress, they can harm proteins, lipids, and nucleic acids. Numerous illnesses, including diabetes mellitus, cancer, asthma, cataracts, and cardiovascular and neurological disorders, might result from this [10]. Antioxidant compounds stop cellular damage by scavenging free radicals [11]. These antioxidants might be synthetic or natural, enzymatic or non-enzymatic. Fruits, vegetables, and herbs contain huge amount of phytoconstituents [12].

Leucas lavandulifolia Sm., commonly known as "Gumma" or "Thumba," is a perennial herb classified under the Lamiaceae family, naturally found in India terai region of Nepal and widely distributed across the subcontinent, particularly in the Western Ghats and the Eastern Ghats. This plant is valued Ayurvedic and folk medicine. *Leucas lavandulifolia* is characterized by its slender, erect stems that can reach heights of up to 60 cm. The inflorescence of *Leucas lavandulifolia* consists of small, tubular flowers arranged in dense, terminal spikes or clusters. The flowers are typically white to lavender in color, adding to the ornamental appeal, which coincides, the monsoon season in India [13]. Since there hasn't been much information about the phytochemical screening and activity of bark and leaf extracts, toxicity to brine shrimp nauplii and their antioxidant and antibacterial qualities, the suggested study is crucial in bridging the research gap.

2 Materials and methods

2.1 Chemicals

Reagents made by Merck and Sigma are DPPH reagents, gallic acid, quercetin, and tannic acid, have been used in laboratory bioassays. Analytical grade (extra pure) solvents from Scientific Fischer and Merck were obtained, including dichloromethane (DCM), ethanol, methanol, and ethyl acetate. Boric acid, calcium chloride, sodium chloride, potassium iodide, and aluminum chloride anhydrous were obtained from Merck Life Science Limited. EDTA disodium salt dehydrates (RRL), sodium hydrogen carbonate (SQ), potassium chloride purified (CDH), FC reagent (LOBA), mueller hinton broth (HiMedia), nutrient agar (HiMedia), resazurin (LOBA), neomycin (HiMedia), and hexane (LOBA).

2.2 Plant collection and taxonomic identification

Medicinal plant used for this research was identified as *Leucas lavandulifolia* Sm., and it was gathered

from the Madhesh province, Parsa district (Bindabasini Gaupalika wada no. 5). In Nepali, this plant is generally referred to as "Gumma." The identification of this therapeutic plant was assisted by locals and wound healers, who were not aware of its various uses. The date of collection was July 19, 2023. The map of sample collection sites is depicted in Figure 1.

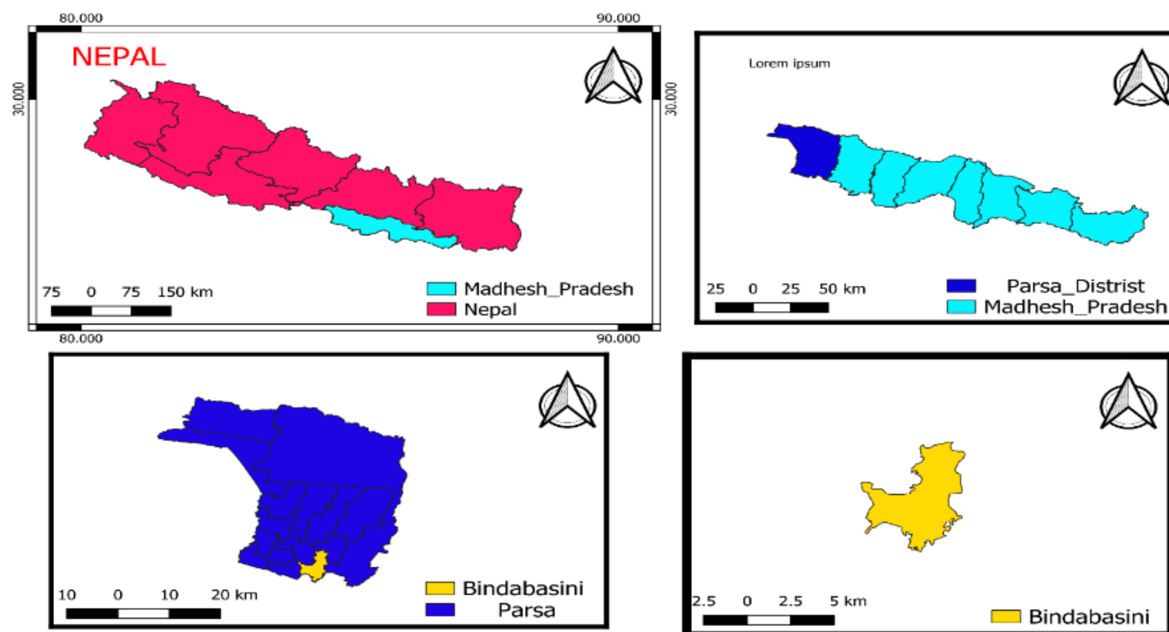


Figure 1: The map of sample collection sites.

2.3 Extract preparation

Fresh leaf sample was gathered, thoroughly cleaned. Following that, the gathered portions were allowed to dry in the shade for around two weeks. After that, they were ground using a rotary mill to create a sample that was finely powdered. Subsequently, the plant material was roughly ground up and kept in a separate water-resistant ziplock bag. Finally, it was labelled for later use, and the sample was preserved. The powder sample that was created was submerged in five distinct solvents, which were arranged in polarity order (MeOH >EtOH>EA>DCM>Hexane) About 60 gm of powdered leaves was filled in 500 ml conical flask and subsequently soaked with 250 ml of solvents as mentioned above. After that, the conical flask's mouth was covered with aluminum foil and shaken once per 24 hours. It was then left for 72 hours. Each soaking sample was filtered using regular filter paper after 72 hours, yielding five filtrates of different solvents. Five different extracts from different solvents were ultimately obtained. Ultimately, stored for later use at 4°C. The yield % was calculated using the following relation

$$\text{Yield\%} = \frac{\text{Crude extract weight}}{\text{Weight of sample}} \times 100 \qquad (1)$$

2.4 Phytochemical screening

The phytochemicals found in the various extracts were qualitatively identified by looking for a change in colour after the extracts were treated with the appropriate reagents. Plant extracts were the subject of phytochemical investigations using several techniques that were based on standard methodology [14–16].

2.5 Estimation of total phenolic content (TPC)

The total phenolic content (TPC) of the plant extracts was quantified using the Folin–Ciocalteu colorimetric assay following the method described by Lu et al. [17]. Briefly, 20 μL of plant extract was dispensed into each well of a 96-well microplate in triplicate. Subsequently, 100 μL of 10% Folin–Ciocalteu reagent (1:10 dilution) and 80 μL of 1 M sodium carbonate (Na_2CO_3) were added to the mixture. The reaction mixture was incubated at room temperature for 30 minutes to allow the formation of a characteristic blue chromophore. The absorbance was then recorded at 765 nm using a spectrophotometer. The total phenolic content was determined using a calibration curve prepared with gallic acid (7.5–100 μg/mL) as the standard and the results were expressed as milligrams of gallic acid

equivalents per gram of dry extract (mg GAE/g).

2.6 Estimation of total flavonoid content (TFC)

The total flavonoid content (TFC) of the plant extracts was quantified using the aluminium chloride colorimetric assay according to the method described by Ahmed et al. [18]. Briefly, 20 μL of plant extract was transferred into each well of a 96-well microplate in triplicate. Subsequently, 100 μL of distilled water and 60 μL of ethanol were added, followed by the addition of 10 μL of 10% aluminium chloride ($AlCl_3$) solution and 10 μL of 1 M potassium acetate (CH_3COOK). The reaction mixture was incubated at room temperature for 30 minutes to allow the formation of a stable flavonoid–aluminium complex. The absorbance was then recorded at 415 nm using a microplate spectrophotometer. The total flavonoid content was calculated from a quercetin standard calibration curve (10–100 μg/mL) and expressed as milligrams of quercetin equivalents per gram of dry extract (mg QE/g).

2.7 Evaluation of antioxidant activity

The antioxidant activity of the crude plant extract was evaluated using a standard protocol [19, 20]. The positive control was serially diluted from 20 μg/mL to 0.625 μg/mL. Each well of a 96-well received 100 μL of either the plant extract or positive control in triplicate. An initial absorbance reading was taken at 517 nm, followed by the addition of 100 μL of DPPH reagent to each well, and the plates were incubated for 30 minutes. The final absorbance was measured at 517 nm. 50% DMSO and methanol served as negative controls. Radical scavenging capacity (RSC) was calculated using the equation given below

$$\mathrm{RSC}(\%) = \frac{\mathrm{Acontrol} - \mathrm{Asample}}{\mathrm{Acontrol}} \times 100 \qquad (2)$$

where Acontrol = absorbance of control and Asample = absorbance of sample.

2.8 Evaluation of antimicrobial activity

The antibacterial activity of the plant extract was evaluated using the agar well diffusion method on Mueller-Hinton Agar (MHA) plates [21, 22]. Test microorganisms, including ATCC 25931 *Shigella sonnei*, ATCC 43300 *Staphylococcus aureus*, ATCC 700603 *Klebsiella pneumoniae*, and ATCC 25312 *Escherichia coli*, were cultured in Mueller-Hinton Broth (MHB) and incubated at 37°C for 24 hours. The turbidity of each culture was adjusted to 0.5 McFarland standard. Wells were created in the agar using a sterile cork borer and filled with 50 μL of the plant extract, 50% DMSO as the negative control, and 50% neomycin as the positive control. After allowing 15 minutes for diffusion, the plates were incubated at 37°C for 18-24 hours. The resulting zones of inhibition were measured to assess antibacterial activity. The zone of clearance was measured and monitored following incubation.

2.9 Determination of minimum inhibitory concentration and minimum bactericidal concentration

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration were evaluated in accordance with the standardized methodology outlined by Sarker et al. [23]. Bacterial inocula were prepared by diluting a 0.5 McFarland turbity standard in Mueller-Hinton broth (MHB) at a 1:100 ratio, yielding a final density of 106 CFU/mL. Subsequently, 5 μL of the bacterial suspension was dispensed into each well of a sterile 96-well microtiter plate. Neomycin served as the positive control, and the plate was sealed and incubated at 37°C for 20-24 hours. Following incubation, 0.003% resazurin solution was added to the wells, and the plate was further incubated at 37°C for 3-4 hours. Wells exhibiting bacterial proliferation transitioned from blue to pink, whereas wells devoid of growth retained the original blue coloration. The MIC was defined as the lowest concentration of the extract that completely inhibited visible bacterial growth. To determine the MBC, aliquots from wells corresponding to and above the MIC were subcultured onto nutrient agar plates and incubated at 37°C for over 18 hours, with the absence of colony formation confirming bactericidal activity.

2.10 Brine Shrimp Lethality Assay (BSLA)

The toxicity of the plant extracts was evaluated following a standardized protocol [24]. The PH of the artificial sea water was maintained between 8-8.5 using 1M NaOH. The plant extracts were prepared in series of graded concentrations, 1000, 800, 500, 250, 125, 100, and 10 μg/mL. Each test tube was then supplemented with 4 mL of artificial seawater, after which 10 nauplii and 500 μL of respective extract were introduced in duplicate. Potassium dichromate served as the positive control, while untreated artificial seawater functioned as the negative control. Following a 24 hour exposure period, the number of deceased nauplii per test tube was recorded, and the percentage mortality was computed using the formula

$$\text{mortality\%} = \frac{\text{Number of dead nauplii}}{\text{Total number of nauplii}} \times 100 \quad (3)$$

The relationship between extract concentration and mortality was further analyzed via probit analysis. The probit values were plotted to derive the linear regression equation $y = mx + c$, where Y represents the probit value at 50% mortality, m is the slope and c is the intercept. The lethal concentration for 50% mortality (LC_{50}) was then extraplotted from this equation with x-corresponding to the LC_{50} value.

2.11 Statistical analysis

Data obtained from the microplate reader were processed and analyzed using Microsoft excel. Antioxidant results are presented as mean ± standard error of the mean (SEM). The inhibitory concentration (IC50) was calculated using Graphpad prism software (Version 8.0.2.263). Comparisons among TPC, TFC, and antioxidants values were performed using a one way ANOVA and differences were considered statistically significant at $p < 0.05$.

3 Results

3.1 Percentage of yield

The extraction yield (%) of the plant extracts is shown in Table 1. The percentage of crude plant extract was found to be higher in ethyl acetate (42.66%), followed by DCM (37.33%), Hexane (33.66%), Ethanol (31.16%) and Methanol (25.33%), Table 3.1.

Table 1: Yield percentages for various solvent extracts of leaves of *Leucas lavandulifolia* Sm.

Source of extraction	**Solvent type**	**% yield**
Leaves	Methanol	25.33
Leaves	Ethanol	31.16
Leaves	Ethyl acetate	42.66
Leaves	Dichloromethane (DCM)	37.33
Leaves	Hexane	33.66

Table 2: Preliminary qualitative phytochemical analysis of *Leucas lavandulifolia* leaf extracts

S.N.	Phytochemicals	Test	Leaf extract
1	Alkaloids	Dragendorff's test	+
2	Carbohydrates	Molish's test	+
3	Reducing Sugars	Fehling's test	+
4	Glycosides	Borntrager's test	+
5	Amino acids	Xanthoproteic test	-
6	Flavonoids	Alkaline reagent test	+
7	Phenols	$FeCl_3$ test	+
8	Tannins	Braymer's test	-
9	Terpenoids	Salkowski's test	+
10	Anthraquinones	Borntrager's test	-
11	Phytosterols	Salkowski's test	-

Table 3: TPC values for various solvent extracts

Extraction source	Solvent type	TPC±SD (mg GAE/g)
Leaves	Methanol	56.95 ± 0.13
	Ethanol	12.29 ± 0.15
	Ethyl acetate	19.83 ± 0.13
	Hexane	7.42 ± 0.15
	DCM	8.87 ± 0.12

3.2 Qualitative phytochemical screening

The results of the preliminary phytochemical screening of *Leucas lavandulifolia* leaf extracts obtained using different solvents are presented in Table 2. In the table, the symbol + indicates the presence of tested phytochemical constituents, whereas − denotes their absence.

3.3 Total phenolic content (TPC)

The total phenolic content (TPC) of the extracts ranged from 7.42± 0.15 to 56.95± 0.13 mg GAE/g, as presented in Table 3. TPC values are significantly different from each other at $p<0.05$. The methanolic extract exhibited the highest TPC value (56.95±0.13 mg GAE/g), whereas the hexane extract showed the lowest TPC (7.42±0.15 mg GAE/g). The gallic acid calibration curve used for the quantification of phenolic content is illustrated

in Figure 2.

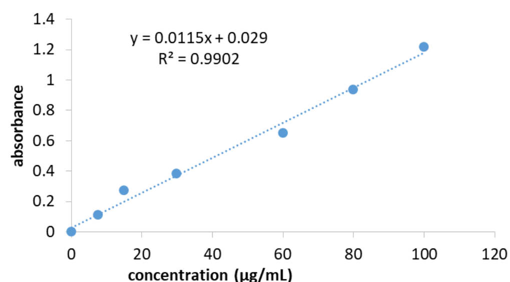


Figure 2: A standard calibration curve of quercetin.

3.4 Total flavonoid content (TFC)

The total flavonoid content (TFC) of the leaf extracts varied between 18.16±0.13 and 119.83±0.12 mg QE/g, as summarized in Table 4. The highest TFC was observed in the ethyl acetate extract (119.83±0.12 mg QE/g), followed by the methanolic extract (102.50±0.16 mg QE/g), hexane extract (46.83±0.13 mg QE/g), dichloromethane extract (30.83±0.14 mg QE/g), and the ethanol extract, which exhibited the lowest TFC (18.16±0.13 mg QE/g). The quercetin standard calibration curve employed for quantification is presented in Figure 3.

Table 4: TFC values of different solvent extracts

Extraction source	Solvent type	TPC±SD (mg GAE/g)
Leaves	Methanol	102.5 ± 0.16
	Ethanol	18.16 ± 0.13
	Ethyl acetate	119.83 ± 0.12
	DCM	30.83 ± 0.14
	Hexane	46.83 ± 0.13

Statistically significant differences were observed in the TFC values across the extracts ($p < 0.05$).

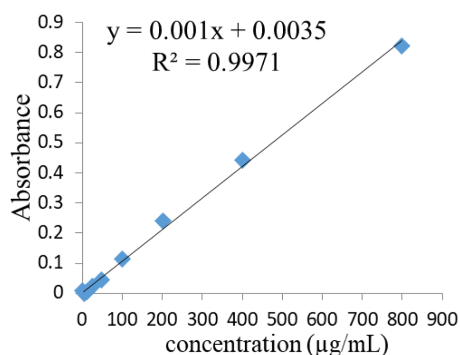


Figure 3: Standard calibration curve of quercetin.

3.5 Antioxidant activity

The methanolic extract had a more potent IC_{50} value, 221.0±0.15 μg/mL than all other plant extracts. The Ethyl acetate and hexane extract had highest IC_{50} value which is greater than 500 μg/mL. The IC_{50} value of different solvent extract of this plant ranges from 221.0±0.15 to 591.3±1.19 μg/mL (Table 5). The percentage inhibition against concentration curve are shown in Figure 4 and IC_{50} versus types of solvent extracts is shown in Figure 5.

Table 5: IC_{50} of different solvent extracts

Part	Solvent extract	IC_{50} (μg/mL)±SEM
Leaves	Methanol	221.0 ± 0.15
	Ethanol	591.3 ± 1.19
	Ethyl acetate	> 500
	DCM	547.2 ± 2.21
	Hexane	> 500
*Quercetin		4.21 ± 1.21

*Quercetin = Positive control

The antioxidant activity values differed significantly among the extracts ($p < 0.05$).

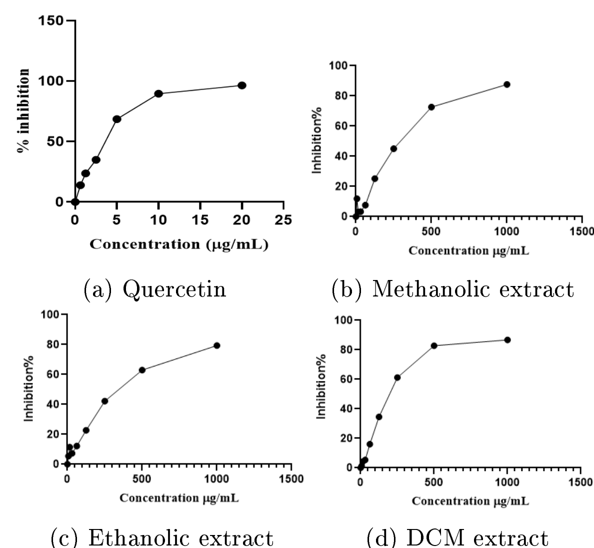


Figure 4: A % inhibition versus concentration plot for (a) standard quercetin (b) methanol (c) ethanol (d) DCM.

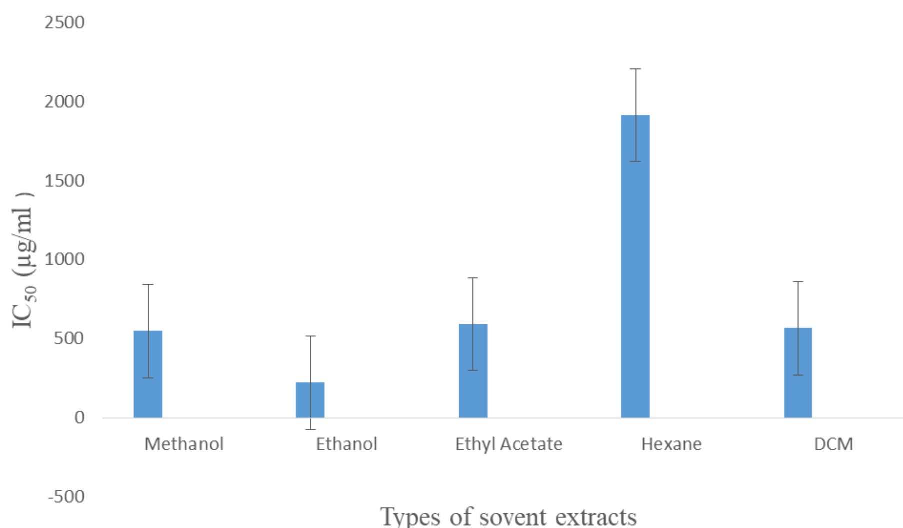
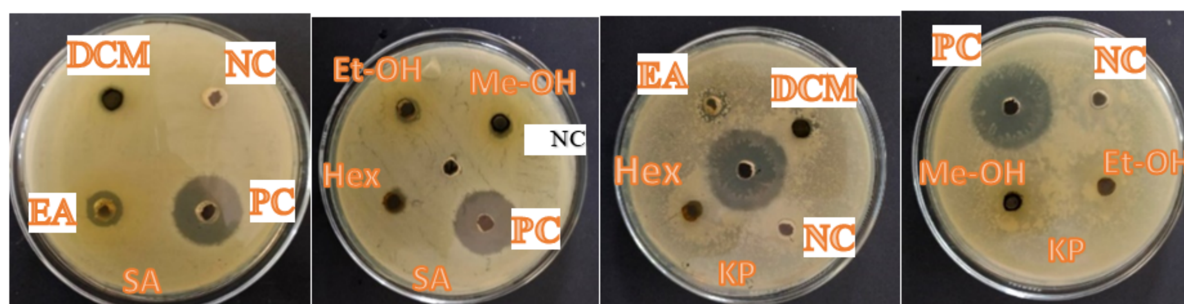
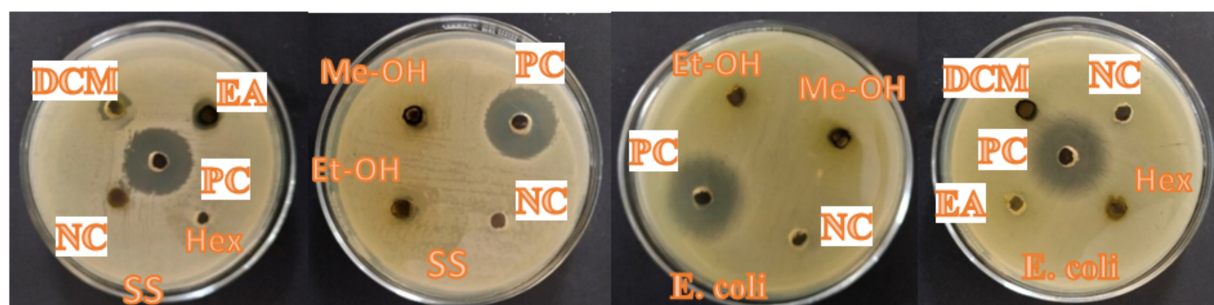


Figure 5: Bar graph displaying the IC_{50} of crude extracts of leaves at different concentrations in various solvents.



(a) Staphylococcus aureus(SA) *(b) Klebsiella pneumoniae (KP)*



(c) Shigella sonnei (SS) *(d) Escherichia coli (E. coli)*

MeOH = Methanol; EtOH = Ethanol; EA = Ethyl acetate; DCM = Dichloromethane; Hex = Hexane

Figure 6: Antimicrobial plate showed zone of inhibition.

3.6 Antibacterial activity

Table 6 displays the antibacterial activity of different solvent extracts of leaf against *E. coli*, *S. aureus*, *K. pneumoniae* and *S. sonnei* in terms of the zone of inhibition. Strong antibacterial activity was shown by the ethyl acetate extract against *Staphylococcus aureus* and *Shigella sonnei* with ZOI values of 11 mm each. This was quite similar to the positive control, which was Neomycin 20 mm for *Staphylococcus aureus* and 23 mm for *Shigella sonnei*. The photos of antimicrobial plates are shown in Figure 6.

Table 6: ZOI shown by crude leaf extracts against *Klebsiella pneumoniae*, *Escherichia coli*, *Shigella sonnei*, and *Staphylococcus aureus*

Part	Solvent extract	Bacteria	ZOI (mm)	ZOI of positive control (mm)
Leaves	Methanol	*Escherichia coli*	6	20
		Staphylococcus aureus	8	22
		Klebsiella pneumoniae	6	27
		Shigella sonnei	7	23
	Ethanol	*Escherichia coli*	6	20
		Staphylococcus aureus	9	22
		Klebsiella pneumoniae	5	27
		Shigella sonnei	8	23
	Ethyl acetate	*Escherichia coli*	6	20
		Staphylococcus aureus	11	22
		Klebsiella pneumoniae	9	27
		Shigella sonnei	11	23
	DCM	*Escherichia coli*	6	23
		Staphylococcus aureus	8	22
		Klebsiella pneumoniae	6	27
		Shigella sonnei	9	23
	Hexane	*Escherichia coli*	7	20
		Staphylococcus aureus	7	22
		Klebsiella pneumoniae	7	27
		Shigella sonnei	6	23

3.7 MIC and MBC

MIC is the lowest concentration of an antimicrobial that inhibits visible bacterial growth, while MBC is the minimum concentration required to prevent regrowth on antibiotic-free media. Using the broth microdilution method with resazurin, the DCM and ethyl acetate extracts were tested against *Klebsiella pneumoniae* (KP) and *Shigella sonnei* (SS). Both extracts showed the lowest MIC against *S. sonnei* (1.56 μg/mL) and an MBC of 3.12 μg/mL. MIC and MBC values against both strains are presented in Table 7.

Table 7: MIC and MBC values of different extracts against selected microorganisms

Plant extract	*K. pneumoniae*		*S. sonnei*	
	MIC (mg/mL)	**MBC** (mg/mL)	**MIC** (mg/mL)	**MBC** (mg/mL)
DCM	6.25	12.5	1.5625	3.125
Ethyl Acetate	6.25	12.5	1.5625	3.125
Positive Control	0.0039	0.0078	0.015	0.031

Table 8: LC_{50} of DCM extract along with percentage mortality for nauplii

S.N.	Concentration (C)	Log(x)	Total number of surviving nauplii after 24 hours				% Mortality	Probit value	LC_{50} (μg/mL)
			T1	T2	T3	Mean			
1	1000	3.0000	4	4	4	4.00	60.0	5.25	731.31
2	800	2.9030	5	3	6	4.67	53.3	5.05	
3	500	2.6989	5	5	3	4.33	46.7	4.92	
4	250	2.3979	7	7	6	6.67	33.33	4.56	
5	100	2.0000	9	9	9	9.00	10.0	3.72	
6	10	1.0000	9	10	10	9.67	3.3	3.12	

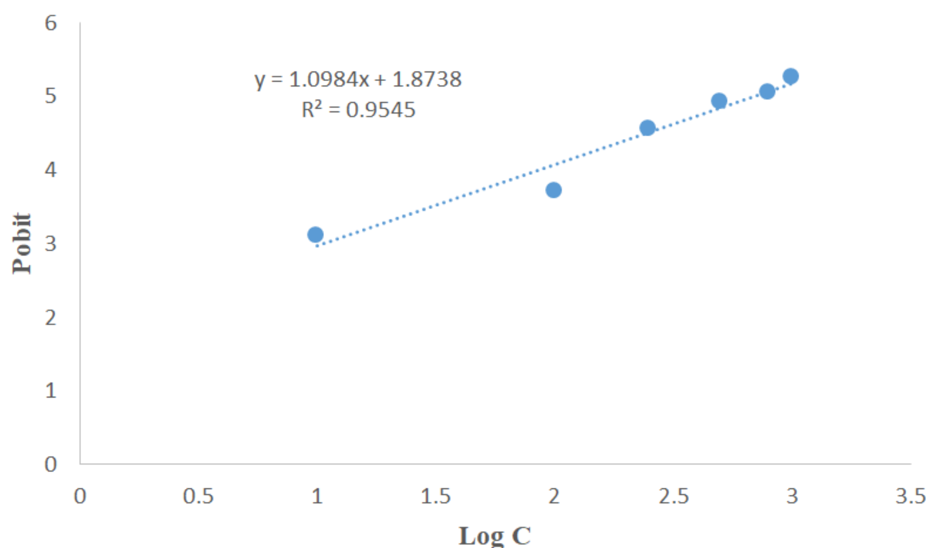


Figure 7: A log concentration (Log C) versus probit plot was constructed to determine the LC_{50} of the DCM crude extract.

3.8 Brine Shrimp Lethality Assay (BSLA)

The DCM extract exhibited the lowest LC_{50} which is 731.31 μg/mL. The negative control was 50% DMSO, potassium dichromate positive control. The positive control (10 nauplii) included all of the dead nauplii, but the 50% DMSO solution contained the same amount of nauplii. The LC_{50} of DCM extract along with % mortality are shown in Table 8. The plot of Log C versus probit value is shown in Figure 7.

4 Discussion

The genus *Leucas* (family Lamiaceae) has been traditionally employed to manage a wide range of ailments, including wounds, snake bites, diabetes, rheumatic pain, and skin disorders. Various species of *Leucas* exhibit diverse pharmacological activities, such as antibacterial, antioxidant, anti-inflammatory, cytotoxic and anticancer, antinociceptive, antidiabetic, antitussive, wound healing, and phytotoxic effects. Phytochemical studies have identified terpenoids, flavonoids, lignans, phenolic glycosides, sterols, and essential oils in different parts of the plants [13].

Previous studies reported the total phenolic content (TPC) of methanolic leaf extract as 3.02 mg GAE/g [25], whereas in the present investigation, the TPC was markedly higher at 56.95±0.13 mg GAE/g. Similarly, the total flavonoid content (TFC) was previously 3.35 mg QE/g, while our study recorded 102.5 ± 0.16 mg QE/g. These discrepancies in phenolic and flavonoid levels may be attributed to factors such as climatic variation, collection period, and geographic origin of the plant material.

Earlier work also evaluated the antioxidant activity of methanolic leaf extract using DPPH assays at concentrations of 25-150 μg/mL, reporting radical scavenging activity ranging from 23.2±1.0% to 87±0.5%, with maximal inhibition observed at 150 μg/mL [25]. In contrast, our results showed a half-maximal inhibitory concentration (IC_{50}) of 221.0±0.15 μg/mL. Variations in antioxidant capacity, TPC, and TFC are likely influenced by environmental and climatic conditions [26]. The distinct functional groups of secondary metabolites in plant extracts further contribute to their potent antioxidant potential [27, 28]. Similar variations in TPC, TFC, and antioxidant activity have been documented in other medicinal plants, such as *Alnus nepalensis* D. Don [29].

In a previous study, the methanolic leaf extract exhibited antibacterial activity against *Klebsiella pneumoniae* and *Staphylococcus aureus*, with zones of inhibition (ZOI) of 17±0.5 mm and 10.3±0.5 mm at 50 µg/mL, respectively [25]. In contrast, the present investigation recorded ZOI values of 6 mm and 8 mm against the same strains. Such differences in antibacterial efficacy may result from variations in phytochemical composition, including vitamins, carotenoids, saponins, enzymes, and minerals, which can also influence phenolic and flavonoid content [30, 31].

Earlier reports indicated MIC values of 65±0.2 μg/mL against *K. pneumoniae* and 58±0.7 μg/mL

against *S. aureus* for the methanolic leaf extract [25]. In this study, the MIC values of the extracts were 1.56 μg/mL against *Shigella sonnei* and 6.25 μg/mL against *K. pneumoniae*. The DCM extract showed a lethal concentration (LC_{50}) of 731.31 μg/mL. Given its diverse biological activities, this plant represents a promising source for the development of novel therapeutic agents and warrants further investigation.

5 Conclusion

Comprehensive analyses of various extracts of *Leucas lavaendulifolia* Sm. have revealed its significant pharmacological potential through evaluations of total phenolic content (TPC), total flavonoid content (TFC), antioxidant activity, antimicrobial efficacy, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and Brine Shrimp Lethality Assay (BSLA). The high levels of TPC and TFC observed in the extracts highlight the plant's promise as a candidate for therapeutic development. The extracts also exhibited strong free radical scavenging activity in the DPPH assay, reflecting substantial antioxidant potential, which is critical for mitigating oxidative stress-related disorders.

The antibacterial potential of *L. lavaendulifolia* is evident against pathogenic strains including *Shigella sonnei*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*, with MIC and MBC values demonstrating particularly strong efficacy against *S. sonnei*. Among the extracts, the methanolic fraction displayed the highest TPC and the most potent antioxidant activity, whereas the ethyl acetate extract showed the greatest TFC and superior antibacterial activity. Both DCM and ethyl acetate extracts exhibited more pronounced MIC and MBC effects against *S. sonnei* compared to *K. pneumoniae*.

Furthermore, the Brine Shrimp Assay's results demonstrating the DCM extract of *Leucas lavaendulifolia*'s cytotoxicity point to potential uses in the treatment or prevention of cancer. All things considered, the thorough investigation carried out for this study indicates that *Leucas lavaendulifolia* Sm. has a promising future of pharmacology. To clarify the mechanisms of action and guarantee *Leucas lavaendulifolia*'s safety for clinical usage, more study is necessary. The bioactivity of *Leucas lavaendulifolia* Sm is clarified by this study, opening the door to possible applications in complementary and alternative medicine, pharmaceuticals, and nutraceuticals.

Abbreviations

- DMSO: Dimethyl sulfoxide
- GAE/g: Gallic acid equivalent per gram
- QE/g: Quercetin equivalent per gram
- TPC: Total phenolic content
- TFC: Total flavonoid content
- IC_{50}: Half-maximal inhibitory concentration
- DPPH: 2,2-diphenyl-1-picrylhydrazyl
- ZOI: Zone of inhibition
- MIC: Minimum inhibitory concentration
- MBC: Minimum bactericidal concentration
- LC_{50}: Lethal concentration for 50% mortality

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Conflicts of interest

The authors declare that there are no conflicts of interest associated with the publication of this research paper.

Authors' contribution

TPC: Performed laboratory work, writing, review, and original draft; **DRJ**: Writing, review, editing, and formal analysis; **SRO**: Writing, review and statistical analysis; **MKT**: Writing, review, and editing; **RCB**: writing, review, editing, supervision, and conceptualisation.

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