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Research Article

Estimation of Secondary Metabolites and Antibacterial Potential of *Perilla frutescens* (L.) Britton Leaf Extract against Human Pathogens

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ABSTRACT

The present investigation was undertaken to explore the potential of Bhangjeera [*Perilla frutescens* (L.) Britton], family Lamiaceae as a source of antibacterial agents against human pathogenic bacteria, along with qualitative and quantitative estimation of its phytochemical constituents. The experiment was carried out on six germplasm (PF1 to PF6) in Botany laboratory of Shri Guru Ram Rai University, Uttarakhand, India using ethanol as a solvent. The yield of crude ethanolic extract varied from 2.4% to 3.04%, with the highest extract yield observed in germplasm PF3 (3.04%) and the lowest in PF1 (2.4%). Qualitative phytochemical screening revealed the presence of carbohydrate, protein, amino acids, glycoside, phenolic compounds, flavonoids, while saponins were absent. The total phenolic content ranged from 99±0.80 mg GAE/g to 225.06±0.80 mg GAE/g, and the total flavonoid content ranged from 83.25±0.80 mg GAE/g to 115.29±0.48 mg GAE/g. Antibacterial activity was estimated against *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Staphylococcus aureus* and *Bacillus pumilis* known to cause human diseases. Antibacterial assays indicated that most of the studied germplasm exhibited the highest zone of inhibition against *Pseudomonas aeruginosa*, followed by *Klebsiella pneumoniae*. Minimum Inhibitory Concentration (MIC) was determined against *Pseudomonas*, showing a zone of inhibition till a concentration of 31.25 µg/mL. Among Gram-positive bacteria, PF3 demonstrated the maximum zone of inhibition, while PF4 showed the highest activity against Bacillus pumilis. The findings of this study highlight the potential of *Perilla frutescens* as a natural source of antibacterial agents. Its bioactive compounds can be further developed for commercial applications in pharmaceuticals for the treatment and prevention of bacterial infections in humans.

Keywords: Flavonoids, Phenol, Antioxidative activity, Phytoconstituents, Antimicrobial activity, Minimum Inhibitory Concentration.

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INTRODUCTION

For thousands of years, plants have served as vital sources of nutrition and healthcare products. They are diverse reservoirs rich in multifunctional bioactive constituents and have been traditionally used either in crude preparations or as purified active constituents. Utilizing locally available plant resources whether domesticated or wild offers a sustainable alternative to synthetic formulations.[1] Approximately 90% of medicinal plants used in industry are harvested from the wild, with over 70% of these involving destructive practices such as the extraction of roots, stems, bark, or wood. This possesses a significant threat to both the genetic diversity and conservation of medicinal plant species. In response, recent years have seen increased interest in replacing synthetic drugs, which may cause adverse effects, with plant-derived natural compounds. Plants are known to produce a variety of phytochemical constituents such as phenols, flavonoids, saponins, and tannins often associated with essential oils that show wide range of therapeutic potential including antioxidant, antimicrobial, and anti-inflammatory effects. The resurgence of interest in plant-based compounds as alternatives to antibiotics is particularly promising. Owing to their evolutionary development of chemical defenses against pathogens, many plant-derived compounds demonstrate unique antibacterial mechanisms distinct from those of conventional antibiotics.[2,3] Emergence of antimicrobial resistance as a consequence of the overuse of antibiotics has spurred renewed scientific interest in herbal therapeutics.[1,4] Due to growing reliance on herbal products necessitates careful evaluation of their safety, efficacy, and potential for drug resistance.

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Bangjeera [*Perilla frutescens* (L.) Britton], family Lamiaceae is native to Asian countries, where *Perilla* plant is widely used in cooking and culture healing traditions.[5,6] Outside of Asia, *Perilla* is less recognized and not cultivated on a significant scale. In places like the United States of America and Ukraine, it has naturalized as an annual weed commonly found in waste areas, pastures, and along roadsides. In India, particularly in the hilly states ranging from Kashmir to Bhutan at elevations of 457-2590 m *Perilla* is commonly grown in wild.[5] *Perilla* seeds are notable for their high oil content, comprising approximately 35-45%, and are an excellent source of polyunsaturated fatty acids, especially linolenic acid (54-64%).[6] *Perilla* oil is significantly valued for its high omega-3 fatty acid content, with α-linolenic acid constituting over 60% of the total fatty acids in its triacylglycerols.[7,8] *Perilla* has been reported to possess various pharmacological properties including antibacterial, and anticarcinogenic activity.[9]

Based on reviewed literature survey, the present investigation endeavor to explore the potential of *Perilla* plant to possess as a natural anti-bacterial activity for treating infections caused by various pathogenic bacteria along with qualitative and quantitative estimation of phyto-constituents present in germplasm collected from wild in Uttarakhand state in India.

MATERIALS AND METHODS

Experimental Material

The diverse germplasm of Perilla collected from Garhwal and Kumaun regions of Uttarakhand, India was used for the experimental work (Table 1).[10]

Methodology

Experimental site and collection of fresh plant material

The experiment trial was conducted in farm of the School of Agricultural Sciences, Shri Guru Ram Rai University, Dehradun, Uttarakhand, India. The crop growth period spanned eight months, from March to October during the years 2021 to 2024. Seeds were sown in March at a depth of 1.0-1.5 cm at a line sowing. All standard agronomic practices were followed to ensure healthy crop development.

Fresh, disease-free plants of *Perilla frutescens* were collected from the agricultural field. The samples were cleaned, and the leaves were dried in the shade at 25°C to 30°C for approximately 10-12 days in the Botany laboratory. The dried leaves were then crushed into a coarse powder using a grinder. The powdered leaf material was stored in paper bags for subsequent preliminary phytochemical and antibacterial analyses.

Phytochemical investigation of crude plant extract in *P. frutescens*

The air-dried leaf powder (50 grams) was macerated in 100 mL of ethanol, covered with aluminium foil, and left undisturbed for 48 hrs. After the initial extraction, the sample was removed, dried in shade. The extracts were then concentrated on a water bath to dryness, stored in containers at low temperature until for determination of primary and secondary metabolites-carbohydrate, protein, amino acid, glycoside, phenol, flavonoid and saponin and screening for antibacterial activity (Table 2).[11]

Quantitative tests for phyto-constituents of plant samples

Quantification of Total Phenolic Content (TPC)

Folin-Ciocalteu assay was utilized for the determination of TPC with slight changes.[17] Briefly, 0.1 mL extract of the sample was mixed with 50 µL of 2 N Folin-Ciocalteu reagent in a 5 mL volumetric flask. After incubating for 3-5 min at room temperature, 0.3 mL of 20% (w/v) solution of sodium carbonate was added. Following vortex mixing, the reaction mixture was left to incubate for 15 min before it was made up to a final volume of 5 mL with distilled water. The formed blue color was read at 725 nm in comparison to a reagent blank by a UV-visible spectrophotometer. Quantification was carried out against a gallic acid standard curve, and results were given as grams of Gallic Acid Equivalents (GAE) per 100 g extract. All analyses were carried out in triplicate.

Quantification of Total Flavonoid Content (TFC)

The flavonoid content of the *Perilla* sample extracts was quantified through the Aluminium Chloride ($AlCl_3$) colorimetric method with quercetin as the reference compound.[15] A 0.25 mL aliquot of the extract was combined with 1.25 mL of double distilled water. To this combination, 75 µL of 5% Sodium Nitrite ($NaNO_2$) solution was added and the reaction mixture was left to incubate at room temperature for 5 min. Then 0.15 mL of 10% Aluminium Chloride ($AlCl_3$) solution was added, followed by addition of 0.5 mL of 1 mM Sodium Hydroxide (NaOH). After an additional incubation of 6 min at room temperature, the reaction mixture was diluted with 5 mL of double distilled water. The resulting mixture was incubated for 20 min at room temperature and was determined at 510 nm with a UV-visible spectrophotometer. The flavonoid content was calculated from a quercetin calibration curve, and was expressed in terms of milligrams of Quercetin Equivalents (mg QE) per gram sample.

Antibacterial Activity

Antibacterial activity of *Perilla frutescens* extract was determined with a modified disc diffusion test following Singh and Bist (2017).[18] The research utilized six bacterial strains, four Gram-negative (*Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Salmonella typhi*) and two Gram-positive (*Staphylococcus aureus, Bacillus pumilis*) species (Table 3). Nutrient agar medium was set up by dissolving NaCl (5 g), beef extract (3 g), peptone (5 g), and agar (15 g) in 1000 mL of distilled water, and then sterilization at 121°C for 15 min. The medium was

inoculated on sterile Petri dishes (3-4 mm depth) under laminar airflow and left to solidify. Bacterial suspensions (10^6 CFU/mL, 100 µL) were plated onto the agar surface and left to absorb for 15 min. Sterile paper discs (6 mm) saturated with *P. frutescens* extract (dissolved in DMSO) were put on the inoculated agar, and streptomycin (30 mg/disc) and DMSO were used as positive and negative controls, respectively. The plates were incubated at 37°C for 24 hr, and the inhibition zones were read in millimeters after incubation. The experiment was carried out in triplicate with three separate replicates for reproducibility. Active compounds were initially identified through preliminary screening, followed by concentration-dependent testing and Minimum Inhibitory Concentration (MIC) determination. Glassware was autoclaved (121°C for 15 min) before use to sterilize them. DMSO was established to be non-toxic to test microorganisms at the concentrations used.

Minimum Inhibitory Concentration (MIC) analysis

In this study, MIC values were determined only for those bacterial strains that exhibited high sensitivity during preliminary antibacterial screening. The analysis was conducted using the serial dilution method of the active extract. The extract was first Dissolved in Dimethyl Sulfoxide (DMSO) to obtain a range of concentrations from 1000 mg/mL down to 15.62 mg/mL. For disc diffusion assays, extract solutions of different concentrations were placed on filter discs and applied to agar plates inoculated with bacterial cultures. Following incubation, the one of inhibition (the clear area around the disc where bacterial growth is suppressed) were measured. The MIC was identified as the lowest concentration of the extract that produced a significant zone of inhibition, indicating effective antimicrobial activity against the largest number of bacterial strains, in comparison to a standard antimicrobial drug.

RESULTS AND DISCUSSION

In recent years, *Perilla* plant has attracted increasing attention owing to its medicinal value and bio-active substances. Analysis of *Perilla* seeds has showed numerous biological activities- anticancer, anti-diabetic, anti-asthma, antimicrobial, anti-inflammatory, antioxidant, and cardio-protective effect etc.[19] Owing to its high nutrient content and bioactive substances, *P. frutescens* L. has been extensively studied in medicine, food, healthcare industries, and chemical industries with strong potential for future utilization.[10] The present study comprehensively investigates the phytochemical composition of the ethanolic extract of *Perilla frutscens* L. along with its antibacterial activity potential presented under the following headings:

Extraction yield and physical properties of the crude extract

In present study, 50 g of powder from each sample of *P. frutescens* was processed with ethanol-based extraction to yield the crude extract. The extracts from the plant samples harvested from different places in Uttarakhand, India, differed in color from green to dark green. The overall yield of crude extract ranged from 2.4% to 3.04%. The highest crude extract yield was in PF 3 accession (3.04 %). Whereas the lowest extract yield was in PF 1 (2.4 %) (Table 4).

Qualitative phytochemical screening

Phytochemicals are plant-based chemical compounds that occur naturally. They are not considered essential nutrients, but they have great impact on human health. Alkaloids, terpenoids, tannins, saponins, flavonoids, and glycosides are the six primary classes of phytochemicals.[20-22] Phytochemicals have several

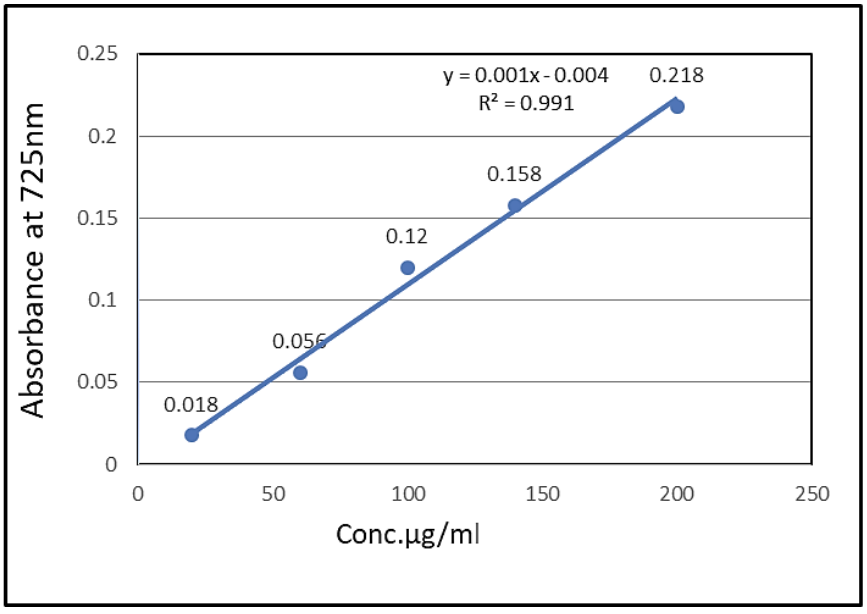

Figure 1: Standard curve represent concentration of gallic acid (µg/mL) against absorbance.

basic functions in plant life and mechanisms of defence as they protect plants against pathogens and herbivores. In *Perilla* plants, the major phytochemicals consist of phenolic compounds, flavonoids, phytosterols, tocopherols, and fatty acids. Perilla seed oil is especially rich in essential fatty acids, especially α-linolenic acid (54-64%) and linoleic acid (14%). Historically, this oil has been employed for food and medicinal purposes in different cultures.

Phyto chemical composition of *Perilla* has been linked to various biological activities, including anticancer, antioxidant, and anti-inflammatory activities. *Perilla* constituents are hence seen as useful in the prevention and management of diseases like cardiovascular diseases, cancer, inflammatory diseases, and rheumatoid arthritis, and their contributions are immense to human health and well-being.[23]

The extract was subjected to qualitative and quantitative estimation. On the qualitative estimation, phenolic compounds, flavonoids, carbohydrate, protein, glycoside, amino acid were present whereas, saponin was absent in all studied *Perilla* germplasm. Polyphenols consist of structurally diverse natural compounds with widespread distribution among plants and foods. Flavonoids, the most notable subclass, have been well studied. Numerous plant polyphenols are currently sold as dietary supplements or herbal remedies, a testament to their increasing therapeutic and commercial relevance.[24] The results of preliminary qualitative phytochemical screening of extract of *Perilla frutescens* L. confirms the presence of carbohydrate, protein, amino acid, glycoside, phenol, and flavonoids. Saponin was absent in all the samples (Table 5).

Total phenolic and flavonoid concentration

According to the previous report, *P. frutescens* leaves were found to be rich in phenolic and flavonoid components which were the important secondary metabolites of many plants and exhibited a variety of biochemical and biological activities.[25]

Phenolics are one major class of phytonutrients that have been widely studied, thus they are well known antioxidants compounds that work in multiple ways to prevent diseases.[26] In the present study, the phenolic content was evaluated from the regression equation of the calibration curve (y=0.001x-0.004, R2=0.991), expressed in GAE as milligrams per gram of extract (mg GAE/g extract). The total phenolic content of the six-germplasm showed large variations. It ranged from 99±0.80mg GAE/g to 225.06±0.80 mg GAE/g (Table 6 and Figure 1). The highest amount of phenol was found in the PF3 (225±0.80 mg GAE/g extract) and lowest amount of phenol was found in PF2 (99.60±0.80) mg GAE/g).

Total flavonoid content was evaluated from the regression equation of the calibration curve (Y=0.001x-0.032, R2=0.991), expressed in GAE as milligrams per gram of extract (mg GAE/g extract). The total flavonoid content of the six germplasm showed large variations ranged from 83.25±0.80 GAE/g to 115.29±0.48mg GAE/g (Table 6 and Figure 2). The highest amount of flavonoid was found in the PF1 leaf extract of *Perilla frutescens* (115±0.48 mg GAE/g extract) and lowest amount of flavonoid was found in PF2 leaf extract of *Perilla frutescens* (83.25±0.80 mg GAE/g). The studies conducted by Tantipai Boonwong *et al.*, in 2023 on extraction, yield, physical appearance, and phytochemical content of the Thai *Perilla* Leaf Extracts (PLEs) revealed that dry plant

Table 1: Collected germplasm from the different places of Uttarakhand (Garhwal and Kumaun region), India.

Sl. No.	Accession	Location	Altitude
1	PF1	Champawat	1615 m
2	PF2	Pithoragarh	1627 m
3	PF3	Almora	1642 m
4	PF4	Rudraprayag	895 m
5	PF5	Chamoli	694 m
6	PF6	Uttarkashi	1158 m

Table 2: Qualitative tests for phytochemical investigation of plant samples collected from different locations of Uttarakhand, India.

Phyto-constituents	Test	Observations (Indicating Positive Test)	References
Protein	Biuret test	A pink colour solution (In ethanolic layer)	[12,13]
Carbohydrates	Molish test	A violet ring	[13,14]
Flavonoid	Lead acetate test	A yellow precipitate	[12,14,15]
Glycoside	Bromine water test	A yellow precipitate	[16]
Amino acid	Ninhydrin test	A purple colure solution	[15,12]
Phenol	Ferric chloride test	Dark/green bluish black solution	[13,15]
Saponins	Foam test	Formation of 2 cm thick layer of foam	[13]

Table 3: Detail characteristics of bacterial strains used for the present studies.

Sl. No.	Bacterial Strain	Family	Characteristics	Disease caused
1.	*Pseudomonas aeroginosa* ATCC 424	Pseudomonadaceae	Aerobic, saprophyte, Gram negative rod, motile nonsporing.	Wound, burn and eye infection, UTI, infertile diarrhoea.
2.	*Klebsiella pneumoniae* ATCC 109	Enterobacteriaceae	Aerobic, saprophytic, Gram-negative, non-motile, non-sporing	Pneumonia, UTI, and other pyogenic infection such as abscess, Septicemia
3.	*Escherichia coli* ATCC433	Enterobacteriaceae	Gram-negative, facultative anaerobic and non-sporulation	Gastroenteritis, Urinary Tract Infection, and neonatal meningitis
4.	*Salmonella typhi* MTCC1255	Enterobacteriaceae	Aerobic, facultative anaerobic, motile, parasite, Gram-negative rod.	Enteric fever such as typhoid and paratyphoid, gastro-intestinal and septicimia
5.	*Staphylococcus aureus* MTCC 737	Staphylococcaceae	Non-motile Gram-positive cocci, arranged in grape-like clusters	Catheters
6.	*Bacillus pumilis* MTCC 1607	Bacillaceae	Gram-positive, aerobic, motile	Food poisoning

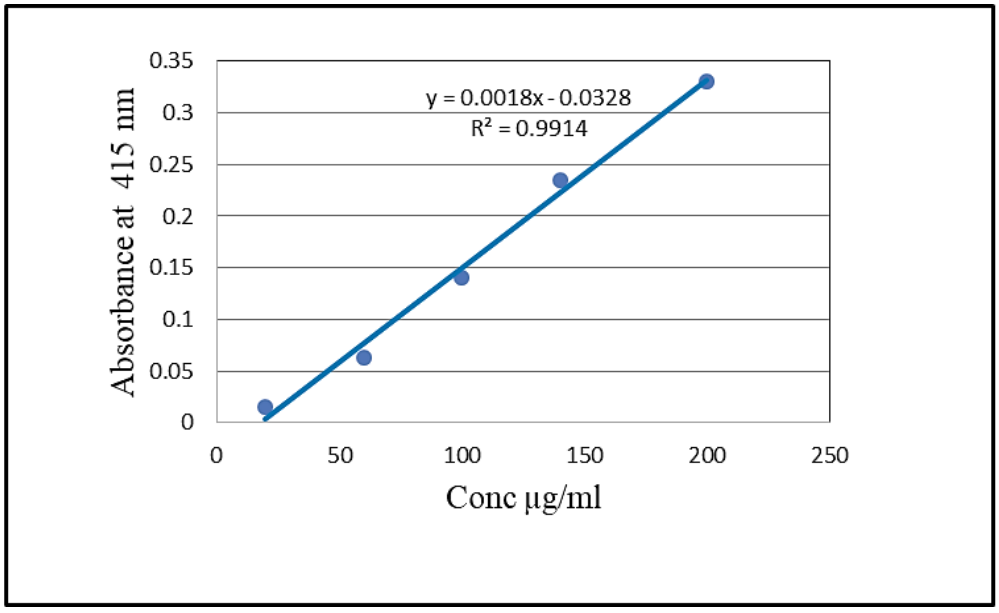

Figure 2: Standard curve represent concentration of quercetin (µg/mL) against absorbance.

extract contain more phenolic and flavanoid content as compare to fresh leaf extract.[27]

Antibacterial Activity

All ethanolic *Perilla* extracts were tested for initial antibacterial activity at 1000 µg/mL against six bacteria, including two Gram-positive and four Gram-negative species. The extracts showed different levels of antibacterial activities against all the tested bacteria (Table 7).

Germplasm PF3 has exhibited maximum zone of inhibition and PF4 showed minimum zone of inhibition against *Pseudomonas aeruginosa*. PF1 illustrated maximum zone of inhibition and PF 4 and PF5 show minimum zone of inhibition against gram negative bacteria *Klebsiella pneumoniae*. Germplasm PF3, PF4 and PF6 has showed maximum zone of inhibition and germplasm PF2show minimum zone of inhibition in Gram negative bacteria *Escherichia coli*.PF4 germplasm maximum zone of inhibition against gram negative bacteria *Salmonella typhi* whereas germplasm PF5, PF6 showing minimum zone of inhibition (Figure 3). This finding implies that most of studies germplasm showed maximum inhibitory activities against *Pseudomonas aeruginosa* followed by *Klebsiella pneumoniae* against 4 gram negative bacterial strains.

Table 4: Yield and Appearance of crude extract in Ethanol solvent.

Accessions	Appearance of Extract	Quality of Plant material(g)	Weight of extract (g)	Percentage yield
PF1(Champawat)	Green	50 g	1.20 g	2.4
PF2(Pithoragarh)	Dark green	50 g	1.44 g	2.88
PF3(Almora)	Dark green	50 g	1.52 g	3.04
PF4(Rudraprayag)	Green	50 g	1.40 g	2.8
PF5(Chamoli)	Green	50 g	1.26 g	2.52
PF6(Uttarkashi)	Dark green	50 g	1.32 g	2.64

Table 5: Preliminary Qualitative phytochemical screening of *Perilla frutescens*.

Germplasm	Test						
	Protein	Carbohydrate	Flavonoid	Glycoside	Amino acid	Phenol	Saponin
PF1	+	+	+	+	+	+	-
PF2	+	+	+	+	+	+	-
PF3	+	+	+	+	+	+	-
PF4	+	+	+	+	+	+	-
PF5	+	+	+	+	+	+	-
PF6	+	+	+	+	+	+	-

*(+) and (-) signs indicate presence or absence of the compound respectively.

In case of Gram-positive bacteria, germplasm PF3 showed maximum zone of inhibition and PF5, PF6 showed minimum zone of inhibition against *Staphylococcus aureus.* PF4 germplasm showed the maximum zone of inhibition and PF5, PF6 showing minimum zone of inhibition against *Bacillus pumilis.* These findings align with the studies conducted by He *et al.*, 2020 on *Perilla* against *Staphylococcus aureus* which is an important cause of food-borne illness in humans and animals.[28] The inhibitory effect on *S. aureus* through cell membrane permeabilization which was associated with generalized membrane-disrupting effects.

Ready to use antibiotics impregnated disc i.e. Streptomycin (15 mcg) were used as a positive control in order to check the sensitivity of the bacterial cultures. All cultures tested had visible zones of inhibition, showing high sensitivity to the antibiotic. DMSO (99% purity) served as the negative control. Results obtained that were showing sensitivity of the bacteria against positive and negative control were presented in Table 7.

Results of Minimum Inhibitory Concentration (MIC) analysis

The Minimum Inhibitory Concentration (MIC) is the lowest antimicrobial agent concentration able to inhibit all visible microbial growth after incubation overnight. It is a fundamental measurement used in microbiological and pharmaceutical studies to assess the efficacy of antimicrobial compounds. MIC values are crucial in both clinical diagnostics to confirm microbial resistance and in research for monitoring the effectiveness of new antimicrobial agents.[11,18,24]

Table 6: Total phenol and flavonoid content in Perilla germplasm.

Plant Germplasm	Total phenols (mg GAE/g)	Total flavonoids (mg QE/g)
PF1	107.18	115.29
PF2	99.60	83.25
PF3	225.06	95.11
PF4	108.39	85.66
PF5	213.24	104.55
PF6	159.60	90.29

The Minimum Inhibitory Concentration (MIC) is a crucial diagnostic tool in microbiology, used to determine microbial resistance to antimicrobial agents and to assess the potency of novel compounds.[24] In this study, the MIC values were determined using a two-fold serial dilution method, with the concentrated aqueous extract prepared in pure DMSO to achieve final concentrations ranging from 1000 mg/mL to 15.62 mg/mL.

Among the six plant extracts tested for antibacterial activity, the largest inhibition zone towards Gram-negative bacteria was seen for *Pseudomonas aeruginosa*, and in Gram-positive bacteria, the greatest zone of inhibition was seen for *Staphylococcus aureus*. The extracts were screened against two Gram-positive (*Staphylococcus*

Table 7: **The zone of inhibition against human pathogenic bacterial strains in *Perilla frutescens* L. with Streptomycin (positive control) and DMSO (negative control)**

Plant samples	Zone of inhibition in mm					
	Pseudomonas aeruginosa ATCC424 (Gram-ve)	*Klebsiella pneumoniae* ATCC109 (Gram-ve)	*Escherichia coli* ATCC433 (Gram-ve)	*Salmonella typhi* MTCC1255 (Gram-ve)	*Staphylococcus aureus* MTCC737 (Gram+ve)	*Bacillus pumilis* MTCC1607 (Gram+ve)
PF1	13	15	01	9	14	10
PF2	17	12	13	11	16	10
PF3	18	14	14	12	18	10
PF4	16	13	14	14	14	11
PF5	10	13	01	12	7	7
PF6	15	12	14	12	7	7
Streptomycin (+control)	20	14	18	12	20	16
DMSO (-ve Control)	0	0	0	0	0	0

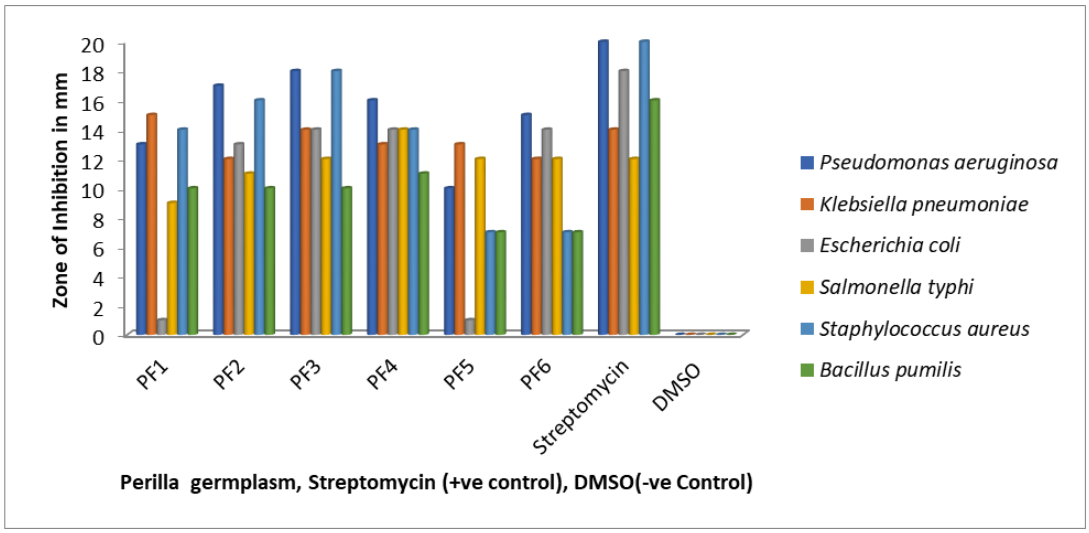

Figure 3: Zone of inhibition (mm) of Perilla germplasm, Streptomycin (+ve control) and DMSO (-ve Control).

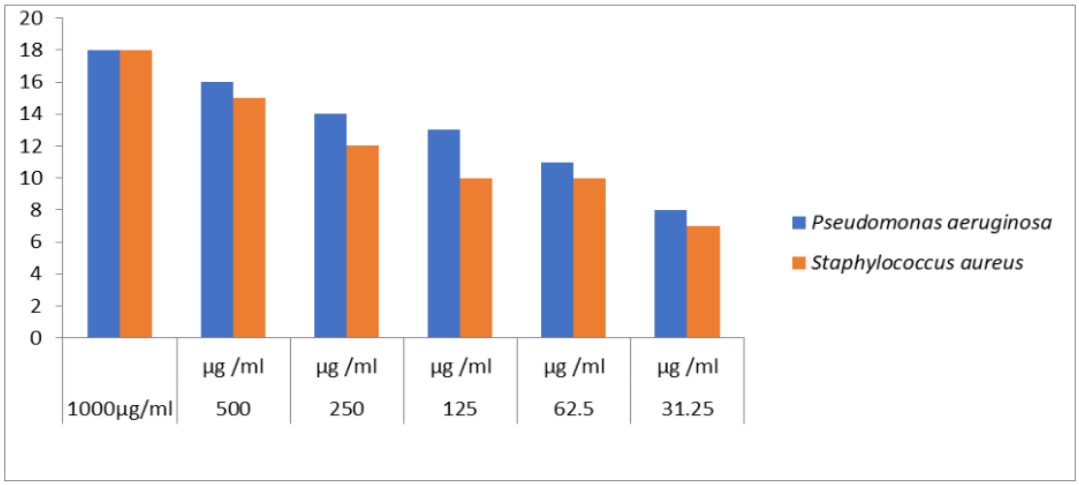

Figure 4: Minimum Inhibitory Concentration (MIC) shown by PF3 sample of *Perilla frutescence* L.

Table 8: Minimum inhibitory concentration (MIC) of *Perilla frutescence* L. against *Pseudomonas aeruginosa* and *Staphylococcus aureus*

Plant Used	1000 µg/mL	500 µg /mL		250 µg /mL	125 µg /mL	62.5 µg /mL	31.25 µg /mL	15.625
Pseudomonas aeruginosa								
PF3	18	16		14	13	11	8	-
Staphylococcus aureus								
PF3	18	15		12	10	10	7	-

aureus and *Bacillus pumilis*) and four Gram-negative (*Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae*, and *Salmonella typhi*) strains. Consequently, MIC determination was performed specifically on the plant extract that showed significant inhibition of *P. aeruginosa*, with activity observed down to a concentration of 31.25 µg/mL (Figure 4). Similarly, MIC analysis for *S. aureus* was carried out based on the data presented in Tables 7, 8 and Figure 4. The studies conducted by Kang *et al.*, (1992),[29] highlights the antimicrobial efficacy of phytochemicals found in the *Perilla* plant, particularly perillaldehyde, limonene, β-caryophyllene, α-bergamotene, and linalool. Among these, perillaldehyde is the most abundant and demonstrates moderate inhibitory effects against a broad spectrum of bacteria and fungi. Additionally, perillaldehyde exhibits synergistic antimicrobial effects with polygodial, enhancing activity against both Gram-positive and Gram-negative bacteria as well as fungi. These synergistic interactions are typically assessed using the Fractional Inhibitory Concentration (FIC) method.

CONCLUSION

The current research demonstrates the promising potential of *Perilla frutescens* as a natural antibacterial agent in response to the rising challenge of bacterial resistance to synthetic antibiotics. The investigation explored the antibacterial properties of the plant's crude ethanolic extract and the phyto-constituents present in it. Major phytochemical sach as phenolic compound. The phytochemical investigation revealed that the total yield of the crude ethanolic extract varied between 2.4% and 3.04%. The extract contained important phytochemicals such as phenolic compounds, flavonoids, carbohydrates, proteins, glycosides, and amino acids, although saponins were absent in all germplasms studied. The antibacterial effect was tested on both gram-negative and gram-positive bacterial strains, including *Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Staphylococcus aureus*, and *Bacillus pumilis*. The most significant antibacterial activity was observed against *Pseudomonas aeruginosa* and *Staphylococcus aureus*, which are known to cause severe human infections. The findings suggest that *Perilla frutescens* could be an effective natural alternative for combating bacterial infections, particularly in the face of rising antibiotic resistance. In future, phyto-constituents can be isolated and purified for determination of its biological activities for human welfare.

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ABBREVIATIONS

DMSO: Dimethylsuphoxide; **MIC:** Minimum Inhibitory Concentration.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Maneesha Singh, Suman Khanduri, Chandni Pundir, Seema Gupta and Neha Kumari. The first draft of the manuscript was written by Suman Khanduri and Maneesha Singh commented on previous versions of the manuscript. All authors read and approved the final manuscript.

SUMMARY

This investigation seeks to determine the antibacterial activity and phytochemical content of *Perilla frutescens* L. (Bhangjeera), which is an underexploited Asian medicinal herb. The experiment was carried out on six germplasms (PF1-PF6) grown in a Latin square system using ethanolic extracts. The crude extract content varied from 2.4% (PF1) to 3.04% (PF3), reflecting differences in concentration of bioactive compounds. Phytochemical screening was consistent with the presence of phenolic, flavonoid, carbohydrates, proteins, glycosides, and amino acids, but no saponins were detected in all the germplasm tested. Spectrophotometric analysis showed high phenolic (99-225.06 mg GAE/g) and flavonoid (83.25-115.29 mg GAE/g) content, indicating potential antioxidant and antimicrobial activity. Antibacterial tests against disease-causing strains showed substantial inhibition, notably against Gram-negative *Pseudomonas aeruginosa* (MIC: 31.25 µg/mL) and *Klebsiella pneumoniae*, and Gram-positive *Staphylococcus aureus* and *Bacillus pumilis*. The PF3 germplasm showed the highest antibacterial potential, giving further boost to its use as a natural

substitute for synthetic antibiotics. Considering the increasing problem of antibiotic resistance, this paper brings *Perilla frutescens* into limelight as a new source of potential antimicrobial compounds. Isolation and purification of active phytoconstituents like phenolics and flavonoids should be addressed in future research to derive pharmacological therapeutics from plants for disease control in human beings. The results emphasize the necessity of conducting more in vivo and clinical experiments in order to prove its efficacy and safety for therapeutic purposes.

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