

Full Length Research Paper

Comparative *in-vitro* study on phytochemical profile, antioxidants, and antimicrobial activities of *Pogostemon benghalensis* leaves and stem from two different geographical regions at Lumbini Province of Nepal

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Pogostemon benghalensis stem and leaves have been investigated for their phytochemical constituents, antioxidant and antibacterial activities. For this study, *P. benghalensis* was collected from two different geographical region of Lumbini Province of Nepal. The extraction was carried out by the triple cold maceration process using methanol and ethyl acetate. The quantitative determination of total phenol content (TPC) was accomplished by Folin-Ciocalteu and the highest TPC value was obtained from the leaf Terai of methanolic extract 132.78 ± 1.45 GAE/g and total flavonoid contents (TFC) was accomplished by aluminium chloride colorimetric assay and the highest TFC value was obtained from stem Terai extract 599.75 ± 0.82 mg QE/g. The antioxidant activity of the extract was tested using scavenging activity of 1, 1-diphenyl-2- picrylhydrazyl (DPPH) radical method and the highest antioxidant activity was obtained by stem Terai methanol extract with an IC_{50} value of $7.46 \mu\text{g/mL}$ when compared with antioxidant activity of ascorbic acid ($IC_{50} = 6.72 \mu\text{g/mL}$) as standard. To evaluate their antibacterial properties against the Gram-positive bacteria *Staphylococcus aureus* and Gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*, the disc diffusion method was used and showed that the stem of Terai methanol extract has the highest zone of inhibition (ZOI) (14.15 ± 3.14 mm) against *P. aeruginosa*. This study thus suggests that the leaf and stem of Terai plant species have great pharmacological importance since they have potent biological activities.

Key words: Geographical region, Folin-Ciocalteu, 1, 1-diphenyl-2- picrylhydrazyl (DPPH), colorimetric assay, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, disc diffusion method.

INTRODUCTION

Natural products and secondary metabolites formed by living systems, especially of plant origin, have shown great potential in the treatment of human diseases such

as cancer, coronary diseases, diabetes, and infections (Chew et al., 2011). Medicinal plants have been widely used for their therapeutic value, and many modern drugs

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have been derived from the traditional uses of these plants. This traditional system continues to play a crucial role in healthcare, with about Natural products and secondary metabolites formed by living systems, especially of plant origin, have shown great potential in the treatment of human diseases such as cancer, coronary diseases, diabetes, and infections (Chew et al., 2011). Medicinal plants have been widely used for their therapeutic value, and many modern drugs have been derived from the traditional uses of these plants. This traditional system continues to play a crucial role in healthcare, with about 80% of the world's population primarily relying on traditional medicines for their primary healthcare needs. According to the World Health Organization (WHO), medicinal plants are a valuable source of a variety of medications (Pimpliskar et al., 2021). They represent rich bio-resources for drugs in traditional and modern medicine, including nutraceuticals, dietary supplements, folk medicines, pharmaceutical intermediates, and chemical entities for synthetic drugs. Plants contain various biologically active ingredients; therefore, they are used for the treatment of a large number of infectious diseases. These biologically active ingredients include alkaloids, flavonoids, steroids, glycosides, terpenes, tannins, and phenolic compounds (Lodha and Deshmukh, 2020). In Nepal, over 700 species of plants are used for medicinal purposes in treating different human diseases. However, many of these medicinal plants have not been adequately explored for their therapeutic values and bioactive chemical constituents (Pandey and Sharma, 2022). Phenols and flavonoids, present in fruits, vegetables, medicinal, and aromatic plants, are known for their antioxidant activities. Recently, due to the potential toxicological effects of synthetic antioxidants, natural antioxidants such as phenols and flavonoid compounds from plant origin are gaining popularity (Phuyal et al., 2020).

Pogostemon benghalensis from the Lamiaceae family is a small perennial shrub, 1-2 m tall, commonly known as Rudhilo in Nepal. It grows in open riverine forest areas of tropical climates and is generally found at altitudes between 150 and 1300 m. Native to South Asia, it is widely distributed in India, Nepal, Bangladesh, Myanmar, Sri Lanka, China, Thailand, Vietnam, and Bhutan (Aryal et al., 2019). Traditionally, the leaf extract has been used to alleviate fever, and its paste was given orally for the treatment of body pain. The traditional formulation of leaf juices with Sterculia gum and sesame oil is used to treat piles. Additionally, crushed leaves of *P. benghalensis* were inhaled and placed on the patient's forehead for the treatment of coughs, colds, and headaches, suggesting that the leaves are a highly used part of the plant in folk medicine. Almost all parts of the plant, namely root, leaf, shoot, stem, and bark, are used as medicine, manure for paddy crops, and for other miscellaneous purposes such as scenting woollens, wardrobes, and repelling insects and leeches. The tribals use a poultice of leaves and root

juice as a remedy for snake bites (Lodha and Deshmukh, 2020). This research work was carried out to compare the antibacterial and antioxidant activity of *P. benghalensis* extracts collected from two different geographical regions in Lumbini Province of Nepal: the Hilly and Terai regions, which have significant differences in altitude, atmosphere, and climatic conditions.

MATERIALS AND METHODS

Plant collection and processing

P. benghalensis stem and leaf parts were freshly collected in April-May 2023 from two different geographical regions in Lumbini Province, Butwal-7, Rupandehi (27° 10'N to 27° 45'N latitude and 83° 10'E to 83° 30'E longitude), the Terai Region, and from Wangla-5, Arghakhanchi (27° 45'N to 28° 6'N latitude and 80° 45'E to 83° 23'E longitude), the Hilly Region of Nepal (Rupandehi and Arghakhanchi District, Wikipedia, 2024a,b). The geographical variation between Rupandehi and Arghakhanchi districts in terms of hot and cold climates and weather results in differences in phytochemical constituents. The collected samples were identified and certified taxonomically by the National Herbarium and Plant Laboratories, Godhawari (KATH) Nepal (Letter Number: 080/081/159) (Figure 1). The collected samples of *P. benghalensis* were washed carefully with distilled water and shade-dried for 14 days in the Pharmacognosy Laboratory of Crimson College of Technology at room temperature. The samples were monitored periodically with careful observation. After complete drying, the leaf and stem were coarsely powdered using a conventional method and stored at 4°C for future use. Extraction was performed using the triple cold maceration process. Fifty grams of crude extract from the leaves and stem were individually measured and dissolved in 500 ml of solvent (methanol and ethyl acetate). The process was carried out through triple maceration (1:10 ratio for 72 h with intermittent shaking). A total of eight samples were obtained. After each extraction, the extract was filtered through Whatmann's filter paper. The filtrate obtained from maceration was placed in a rotary evaporator, and the evaporation process was carried out. The ethyl acetate sample was evaporated at 40°C (40 rpm under reduced pressure) and the methanol sample at 50°C (40 rpm under reduced pressure). The concentrated extract was transferred to Petri dishes individually and covered with aluminum foil. The extracts were dried in a desiccator for a few days and refrigerated at 4°C for future use.

Chemicals and standards

The chemicals used were ethanol (Merck, Germany), DPPH (Hi Media Lab Pvt. Ltd.), and ascorbic acid (Thermo Fisher Scientific India Pvt. Ltd.). All other chemicals used were of the highest commercially available grade. For absorption measurement, a double-beam UV spectrophotometer from Shimadzu Company, Japan, was used. For antimicrobial activity, an incubator and autoclave from S.M. Scientific Instruments (P) Ltd., Delhi, and a laminar air flow from Indosati Scientific Lab Equipment were used (Figure 2).

Qualitative phytochemical screening

The preliminary qualitative phytochemical analyses of the methanol and ethyl acetate leaf and stem extracts were carried out using standard procedures to identify the various groups of phytoconstituents (Poudel et al., 2023; Yadav et al., 2017).



A



B

Figure 1. *P. benghalensis* (A: Whole plant; B: Flower with plant).



Figure 2. Shade dried part of *P. benghalensis*.

Determination of total phenolic content

Total phenolic content was determined using the Folin-Ciocalteu method (Phuyal et al., 2020) with minor modifications, using gallic acid as a standard. A standard solution of gallic acid was prepared by dissolving 10 mg in 100 ml of methanol (100 µg/ml). Different concentrations of gallic acid solution in methanol (10, 20, 40, 60, 80, and 100 µg/ml) were prepared from this standard solution. To each concentration, 5 ml of 10% Folin-Ciocalteu reagent and 4 ml of 7% Na₂CO₃ were added to make a final volume of 10 ml. The resulting blue-colored mixture was shaken well and incubated for 30 min. The absorbance was then measured at 760 nm against a blank. All experiments were carried out in triplicates, and the average absorbance values obtained at different concentrations of gallic acid were used to plot the calibration curve. Various concentrations of the plant extracts (10, 20, 40, 60, 80, and 100 µg/ml) were prepared following the same procedure used for the standard gallic acid, and the absorbance for each concentration of the extract was recorded. The total phenolic content of the extracts

was expressed as mg gallic acid equivalents (GAE) per gram of sample in dry weight (mg/g). The total phenol content (TPC) in all the samples was calculated using the formula (Phuyal et al., 2020):

$$C = \frac{cV}{m}$$

where C= total phenolic content mg GAE/g dry extract, c= concentration of gallic acid obtained from calibration curve in mg/ml, V= volume of extract in ml, and m= Mass of extract in gram.

Total flavonoid content

The total flavonoid content was determined using an aluminum chloride colorimetric assay (Phuyal et al., 2020) with slight modifications. A stock solution (1 mg/ml) was prepared by dissolving 100 mg of quercetin in 100 ml of methanol. This standard solution was serially diluted to make various concentrations (100, 200, 400, 600, 800, and 1000 µg/ml). To each test tube containing 4 ml of distilled water, 1 ml of each quercetin concentration was added. Simultaneously, 0.3 ml of 5% NaNO₂ was added to the test tube. After 5 min, 0.3 ml of 10% AlCl₃ was added, and after 6 min, 2 ml of 1 M NaOH was added to the solution. The volume of the mixture was then adjusted to 10 ml by immediately adding 3.4 ml of distilled water. The total flavonoid content was expressed as quercetin equivalents (mg QE/g) using the linear equation based on the calibration curve.

Stock solutions of 1 mg/ml concentration of the extracts in methanol were prepared and serially diluted to make different concentrations (100, 200, 400, 600, 800, and 1000 µg/ml). A blank was prepared without a sample. The same procedure described for quercetin was followed for the extracts, and the absorbance was measured using a spectrophotometer at 415 nm. Readings were taken in triplicate, and the average absorbance value was used to calculate the total flavonoid content.

Antioxidant activity

The antioxidant effect of Leaf and stem of *P. benghalensis* was examined by 2,2'-diphenyl-1-picrylhydrazyl (DPPH) free radical

scavenging activity method (Lamichhane et al., 2023; Sulastrri et al., 2018) with slight modification. In brief, 5 ml of different extract solutions of the sample was mixed with 5 ml of DPPH solution (0.1 mM) and incubated in a dark place. After 30 min, the absorbance of the sample mixture was monitored at 517 nm with the help of a UV spectrophotometer. Methanol and ascorbic acid were chosen as negative and positive controls, respectively. Each test was accomplished in triplicate. The free radical inhibition percentage was determined using the following formula (Phuyal et al., 2020):

$$\text{Percentage radical Scavenged} = \frac{A_0 - A_1}{A_0} \times 100$$

where A_0 was the absorbance of the DPPH Solution and A_1 was the absorbance of the sample. The minimum concentration of sample that can completely scavenge 50% of DPPH free radicals is known as inhibitory concentration (IC_{50}). The trend method in Microsoft Excel was used to determine IC_{50} in all *P. benghalensis* extract samples.

Antibacterial activity

Antibacterial activity was evaluated by measuring bacterial growth inhibition zones using the disc diffusion method (Rahman et al., 2018) with some modifications. For the antimicrobial test, this research used the gram-positive bacterium *Staphylococcus aureus* and the gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*. All strains were obtained from National Path Laboratory, Butwal Pvt. Ltd., Rupandehi, Nepal. Commercially available Gentamycin (for gram-positive bacteria) and Ciprofloxacin (for gram-negative bacteria) were used as bacterial standards.

Preparation of plant extracts samples and sample load

For the preparation of plant extracts from the Hilly and Terai regions, 100 mg of stem and leaf extracts from each sample were dissolved in 100 ml of a prepared solvent (1:1 ratio of DMSO and water). Dried and sterilized filter paper discs (6 mm diameter) were then impregnated with known amounts of the test substances (1 mg/1 ml) using a micropipette. The discs containing the test material were placed on Mueller-Hinton agar (MHA) uniformly seeded with the test microorganisms. Standard antibiotic discs and blank discs (impregnated with DMSO solvents) were used as positive and negative controls, respectively. The plates were incubated at 37°C for 24 h to allow maximum growth of the organisms. The test materials with antibacterial activity inhibited the growth of the microorganisms, resulting in a clear, distinct zone of inhibition surrounding the discs. The antibacterial activity of the test agents was determined by measuring the diameter of the zone of inhibition using a Vernier caliper, expressed in millimeters. All experiments were conducted in triplicates, and the standard deviation was recorded.

Minimum inhibitory concentration (MIC)

According to Maharjan et al. (2019), the MIC value of the crude extract was determined as the lowest concentration that completely inhibited bacterial growth after 48 h of incubation at 37°C. For each extract, eleven small test tubes were sterilized and clearly labeled: nine for the diluted extract solutions, one for the blank control, and one for the negative control. Using a micropipette, 750 μ L of sterile MHA Nutrient Broth medium was transferred to each tube. Nine sterile test tubes were labeled for creating different diluted concentrations of the extract solution. A stock solution was

prepared in the first test tube, consisting of 2 ml of the extract (200 mg extract per ml) mixed with DMSO and water in a 1:1 ratio.

Serial half dilutions were then performed using a diluent composed of an equal mixture of DMSO and water. This process resulted in nine different dilutions, ranging from 200 to 0.78125 mg/ml. Next, 250 μ L of each plant extract solution were meticulously added to the respective broth medium tubes, creating nine distinct extract-broth solutions with concentrations ranging from 50 to 0.1953125 mg/ml (four-fold dilution). The eleventh tube was solely inoculated with the bacterial inoculum and used as a negative control to ensure the suitability of the broth media for bacterial growth. The tenth tube served as the blank control and contained only 1 ml of sterile broth medium. 20 μ L of bacterial suspension (turbidity comparable to the 0.5 McFarland standard) were added to each test tube (except the tenth tube) and properly mixed. All test tubes were incubated at 37°C for 24 h. After this incubation period, the minimum inhibitory concentration (MIC) value of both extracts against the different bacteria was determined.

Minimum bactericidal concentration

According to Maharjan et al. (2019), the minimum bactericidal concentration (MBC) was determined by first preparing MHA Petri dishes without any bacteria, which were activated by incubating them for 45 min at 37°C. The activated Petri dishes were then transferred to a sterile room with a continuous laminar airflow system. Samples from each diluted test tube, prepared similarly to the MIC evaluation, were subcultured onto the activated MHA plates. These subcultured media were then incubated at a constant temperature of 37°C for 24 h. The MBC was determined as the lowest concentration of the plant extract that completely inhibited bacterial growth on the surface of the media.

Statistical analysis

Data were recorded as a mean of three determinations of absorbance for each concentration, from which the linear correlation coefficient (R^2) value was calculated. The regression equation is given as:

$$Y = mx + c$$

where Y is the absorbance of extract, m is the slope from the calibration curve, x is the concentration of extract, and c is the intercept. The regression equation was used to calculate the concentration of extracts. All the experiment was conducted at least 3 times ($n = 3$); the statistical mean was calculated with $\pm$ standard deviation (SD) using Microsoft Excel 2013. Means were compared using chi X^2 -test and values with $p < 0.005$ were considered statistically significant.

RESULTS AND DISCUSSION

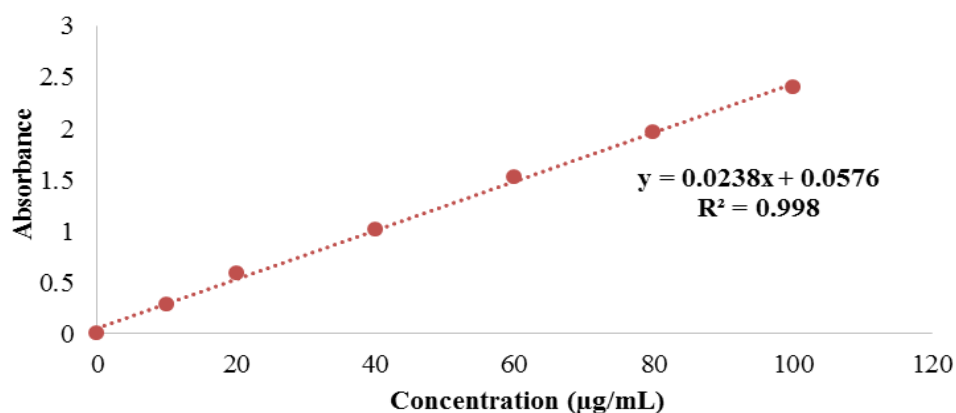
Qualitative phytochemical screening

Phytochemicals play a crucial role in plant defense against predators, microorganisms, and stress, and have been used as drugs for millennia. Therefore, phytochemical screening is an essential initial step in predicting the types of potentially active compounds in plants (Subba et al., 2016). Qualitative phytochemical screening of the extracts of *P. benghalensis* from the Hilly and Terai regions revealed a high presence of various

Table 1. Qualitative phytochemical screening of *P. benghalensis* with different solvent extracts.

Extract	Tests						
	Alkaloid	Saponin	Terpenoid	Flavonoid	Tannin	Glycoside	Resin
PbLTM	+	+	+	+	+	±	+
PbLHM	+	±	+	+	-	±	±
PbSTM	+	±	+	±	+	+	+
PbSHM	±	±	+	±	±	+	+
PbLTEA	±	±	+	+	+	+	+
PbLHEA	±	±	+	+	+	±	+
PbSTEA	±	±	+	±	+	-	+
PbSHEA	±	±	+	±	+	±	+

+ = Significantly present; - = significantly absent; ± = moderately present; PbLTM (LTM)- methanol leaf extract of Tarai; PbLHM (LPM)- methanol leaf extract of hilly region; PbSTM (STM)- methanol stem extract of Tarai; PbSHM (SPM)- methanol stem extract of hilly region; PbLTEA (LTEA)- ethyl acetate leaf extract of Tarai; PbLHEA (LPEA)- ethyl acetate leaf extract of hilly region; PbSTEA (STEa)- ethyl acetate stem extract of Tarai; PbSHEA (SPEA) - ethyl acetate stem extract of *P. benghalensis* of hilly region.

**Figure 3.** Calibration curve of gallic acid for total phenol content determination.

phytochemicals. Tannins were absent in the methanol extract of the stem from the Hilly region, and glycosides were absent in the ethyl acetate extract of the stem from the Terai region. All other extracts showed a high to moderate presence of phytochemicals such as alkaloids, saponins, terpenoids, flavonoids, and resins, as detailed in Table 1. A previous study reported that *P. benghalensis* tested positive for flavonoids, alkaloids, saponins, phenolic compounds, terpenoids, and steroids in methanol extracts (Pandey and Sharma, 2022).

Determination of total phenolic content

Polyphenols are naturally occurring organic compounds composed of multiple phenolic units. Their composition can be significantly affected by internal and external factors such as agro-climatic conditions, harvesting time, cultivation, and origin (Prabhu et al., 2021). The total phenolic content (TPC) of the extracts was calculated

using the regression equation of the calibration curve ($y = 0.0238x + 0.0576$; $R^2 = 0.998$), as shown in Figure 3, and is expressed as mg gallic acid equivalents (GAE) per gram of sample in dry weight (mg/g) in Table 2 and Figure 5.

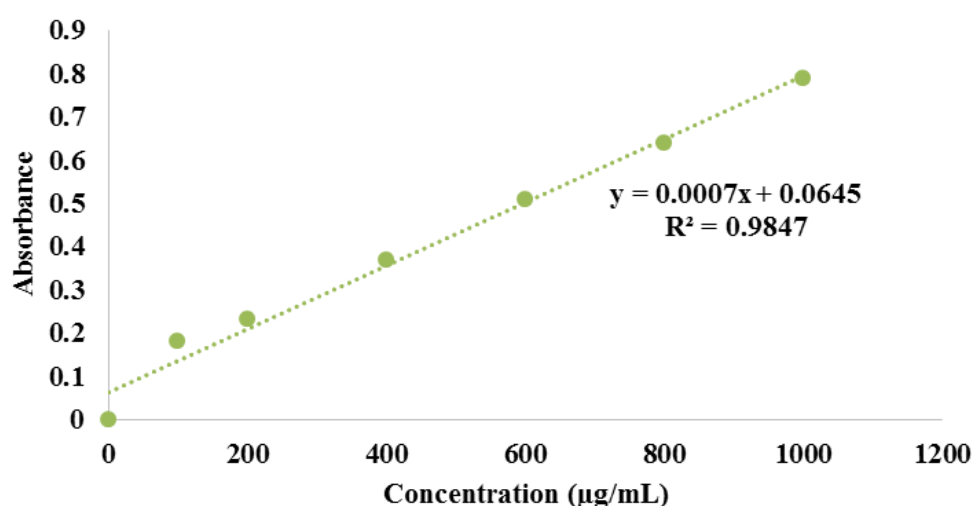
The methanol leaf extract of the Terai species of *P. benghalensis* had the highest TPC at 132.78 ± 1.45 µg GAE/mg, followed by the methanol leaf extract of the Hilly species at 100.16 ± 2.55 µg QE/mg. The lowest TPC values were found in the ethyl acetate extract of the Terai stem at 48.76 ± 1.69 GAE/mg, followed by the ethyl acetate extract of the Hilly stem at 43.86 ± 0.42 µg GAE/mg (Table 2).

Previous studies reported TPC values for methanolic and ethyl acetate leaf extracts of *P. benghalensis* as 159.75 ± 0.08 and 70.08 ± 0.05 mg GAE/g, respectively (Upreti et al., 2023). Pandey and Sharma (2022) found the TPC value of the methanol leaf extract to be 169.43 ± 3.58 mg GAE/g. This study also recorded the highest TPC content in the methanol leaf extract of *P.*

Table 2. Result for the total phenol content and total flavonoid content of leaves and stem of *P. benghalensis* species.

Extract	Total phenol content ($\mu\text{g GAE/mg}$)	Total flavonoid content ($\mu\text{g QE/mg}$)
PbLTM	132.78 ± 1.45	599.75 ± 0.82
PbLPM	100.16 ± 2.55	499.28 ± 1.42
PbLTEA	83.31 ± 2.32	485.0 ± 4.29
PbLHEA	56.12 ± 0.20	476.42 ± 1.42
PbSTM	54.78 ± 0.42	459.76 ± 2.97
PbSHM	52.41 ± 0.65	454.52 ± 4.58
PbSTEa	48.76 ± 1.69	442.61 ± 2.18
PbSHEA	43.86 ± 0.42	361.19 ± 2.97

Data were expressed as mean value $\pm$ standard deviation.

**Figure 4.** Calibration curve of quercetin for total flavonoid content determination.

benghalensis. No significant scientific studies have yet compared the total phenolic content of *P. benghalensis* leaves and stems from the Terai and Hilly regions.

Determination of total flavonoid content

The total flavonoid content of the extracts was calculated from the regression equation of the calibration curve ($Y = 0.0007x + 0.0645$; $R^2 = 0.9847$) and expressed as mg Quercetin equivalents (QE) per gram of sample (mg/g). The antioxidant properties of flavonoid compounds are governed by the hydroxyl positions, which influence their electron or hydrogen donation capacity to free radicals (Pandey and Sharma, 2022). This study conducted a quantitative analysis of eight different extracts of *P. benghalensis* to determine the total flavonoid content. The calibration curve for Quercetin as the standard is shown in Figure 4. Results for the total flavonoid content are detailed in Table 2 and Figure 6.

The highest total flavonoid content was found in the

methanolic extract of Terai stem, with a value of 599.75 ± 0.82 mg QE/g, followed by the methanolic extract of Hilly stem at 499.28 ± 1.42 mg QE/g. The lowest total flavonoid content (TFC) was recorded for the ethyl acetate extract of Hilly leaves, which had a value of 361.19 ± 2.97 mg QE/g (Table 2).

Previous studies reported TFC values for methanolic and ethyl acetate leaf extracts of *P. benghalensis* as 26.41 ± 0.01 and 7.83 ± 0.02 mg QE/g, respectively (Upreti et al., 2023). According to Pandey and Sharma (2022), the TFC value of the methanol leaf extract was 65.2 ± 2.0 mg QE/g. This study also found the highest TFC content in the methanol leaf extract of *P. benghalensis*. No significant scientific studies have yet compared the total flavonoid content of *P. benghalensis* leaves and stems from the Terai and Hilly regions.

Antioxidant activity

The antioxidant activity of the leaves and stem of *P.*

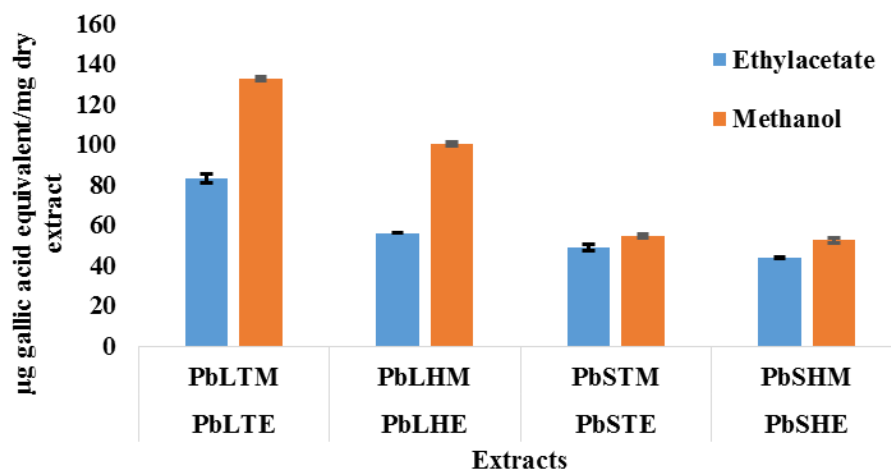


Figure 5. Bar diagram represent the total phenol content of the leaf and stem extracts of *P. benghalensis*

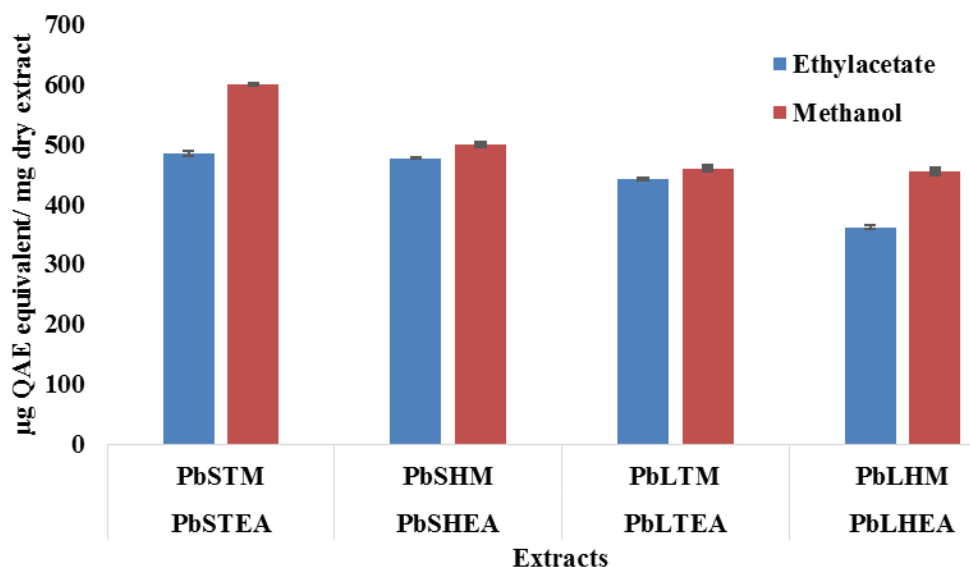


Figure 6. Bar diagram represent the total flavonoid content of the leaf and stem extracts of *P. benghalensis*.

benghalensis was assessed using the DPPH free radical scavenging assay, with results expressed as IC_{50} values, indicating the concentration required to scavenge 50% of DPPH free radicals. The mean percentage of DPPH radical scavenging activity at various extract concentrations is shown in Table 3. The scavenging activity increased with higher concentrations of the extract. Ascorbic acid, used as a standard, had an IC_{50} value of 6.72 $\mu\text{g/ml}$, with a higher IC_{50} value indicating lower radical scavenging activity or antioxidant potential.

Among the solvents tested, the methanolic extract demonstrated the most significant scavenging effect across all concentrations from 10 to 100 $\mu\text{g/ml}$. The

stem extracts had higher antioxidant activity compared to the leaves, with the methanol extracts from both Terai and Hilly regions showing the highest activity, with IC_{50} values of 7.4 and 7.5 $\mu\text{g/ml}$, respectively (Table 4). Conversely, the Hilly leaf ethyl acetate extract exhibited the lowest antioxidant activity, with an IC_{50} value of 87.54 $\mu\text{g/ml}$ (Table 4 and Figure 7).

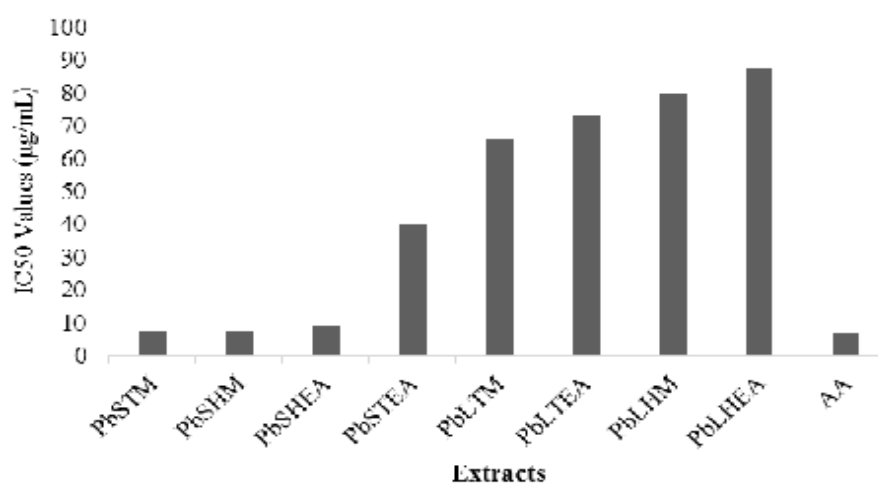
Previous studies have reported varying IC_{50} values for *P. benghalensis*, including 35.92 ± 0.65 $\mu\text{g/ml}$ for methanolic extracts (Pandey and Sharma, 2022), 18 ± 0.90 $\mu\text{g/ml}$ for ethanolic extracts (Pimpliskar et al., 2021), and 150.26 ± 0.02 $\mu\text{g/ml}$ for methanolic extracts (Upreti et al., 2023). This study corroborates that the methanolic

Table 3. Results for the DPPH free radical scavenging effect of *P. benghalensis* leaf and stem.

Sample	% DPPH radical scavenging assay					
	10 (µg/ml)	20 (µg/ml)	40 (µg/ml)	60 (µg/ml)	80 (µg/ml)	100 (µg/ml)
PbSTM	47.84±0.23	57.65±0.24	63.07±0.14	70.97±0.14	79.42±0.24	84.60±0.14
PbSHM	48.33±0.48	56.14±0.49	66.02±0.24	73.20±0.24	81.49±0.28	87.88±0.14
PbSHEA	47.21±0.14	57.09±0.36	65.31±0.24	71.77±0.23	82.06±0.23	89.15±0.14
PbSTEa	29.98±0.96	36.36±0.83	53.19±0.49	62.92±0.24	76.39±0.14	83.97±0.24
PbLTM	27.83±0.27	33.65±0.13	41.15±0.47	46.96±0.27	55.10±0.14	63.16±0.23
PbLHM	3.98±0.14	15.63±0.14	26.39±1.4	39.07±0.14	49.44±0.14	61.48±0.24
PbLTEa	15.95±0.14	20.18±0.36	29.67±0.24	43.06±0.24	54.94±0.14	62.23±0.24
PbLTEa	19.06±1.11	27.19±1.38	36.28±0.14	45.53±0.37	52.47±0.14	66.99±0.14

Table 4. IC₅₀ values of extract of leaf, stem and standard ascorbic acid.

Extract	IC ₅₀ values (µg/ml)
PbSTM	7.46
PbSHM	7.55
PbSHEA	9.30
PbSTEa	40.01
PbLTM	65.82
PbLTEa	73.35
PbLHM	79.94
PbLHEA	87.54
AA	6.72

**Figure 7.** Bar diagram representing IC₅₀ values of leaf, stem extracts, and ascorbic acid.

extract of *P. benghalensis* stems from the Terai and Hilly regions shows higher antioxidant activity across different solvents. A positive correlation between flavonoid content and antioxidant activity was observed, with flavones and flavonoids contributing to antioxidant properties, primarily through their free -OH groups (Pimpliskar et al., 2021). Oil extracted from the leaves of *Pogostemon* possesses

antioxidant activity using the DPPH method (Mittal et al., 2020).

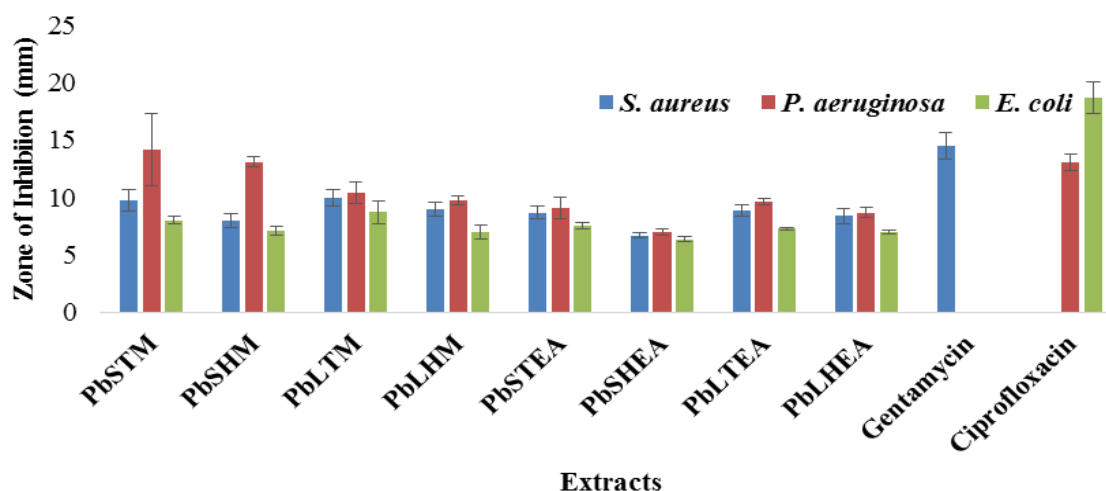
Antimicrobial activity

Eight extracts from the leaves and stems of *P.*

Table 5. Antibacterial activity of *P. benghalensis* against various bacteria in different plant extracts.

Sample	Zone of Inhibition in mm (Mean ± Standard deviation)			p-value (X² test)
	Gram-Positive Bacteria	Gram-Negative Bacteria		
	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>E. coli</i>	
PbSTM	9.73 ± 0.93	14.15 ± 3.14	8.01 ± 0.34	0.006**
PbSHM	7.95 ± 0.59	13.1 ± 0.45	7.08 ± 0.40	
PbLTM	9.95 ± 0.74	10.37 ± 0.93	8.71 ± 1.03	
PbLHM	8.98 ± 0.62	9.73 ± 0.41	7 ± 0.58	
PbSTEa	8.67 ± 0.54	9.08 ± 0.98	7.5 ± 0.29	
PbSHEa	6.70 ± 0.25	7.01 ± 0.27	6.37 ± 0.21	
PbLTEa	8.89 ± 0.48	9.6 ± 0.26	7.27 ± 0.09	
PbLHEa	8.39 ± 0.66	8.67 ± 0.42	7 ± 0.19	
Gentamycin	14.50 ± 1.16	-	-	
Ciprofloxacin	-	13.07 ± 0.74	18.66 ± 1.4	

- Indicate not observable. χ^2 = Chi-square test; P value by the Chi-square test < 0.05, considered to be statically significant.

**Figure 8.** Bar diagram represent the zoi of sensitive extracts of *P. benghalensis* against different bacterial strains.

benghalensis were evaluated for antibacterial activity by measuring the Zone of Inhibition (ZOI) against various bacterial strains. Among the extracts, the methanol extract from the stem of the Terai region exhibited the highest antibacterial activity, showing a ZOI of 14.15 $\pm$ 3.14 mm against *P. aeruginosa*. Conversely, the ethyl acetate extract from the stem of the Hilly region demonstrated the lowest ZOI at 6.37 $\pm$ 0.21 mm. The extracts effectively inhibited both gram-positive and gram-negative bacteria, with the Terai stem methanol extract being the most potent overall. The least inhibition for *S. aureus* was observed in the stem Hilly ethyl acetate extract, with a ZOI of 6.70 $\pm$ 0.25 mm. For *P. aeruginosa*, the lowest ZOI was 7.01 $\pm$ 0.27 mm, and for *E. coli*, it was 7.0 $\pm$ 0.19 mm, both found in the ethyl acetate extract from the Hilly region's leaves. These findings are detailed in Table 5 and Figure 8, with ZOIs measured using a

Vernier caliper, as shown in Figure 9.

Previous studies have highlighted notable antibacterial activities of *P. benghalensis*. Dahiya et al. (2019) reported that essential leaf oil of *P. benghalensis* achieved a ZOI of 39.33 $\pm$ 1.53 mm against *S. aureus*, outperforming the standard drug Gentamicin, which had a ZOI of 35 $\pm$ 1 mm. Similarly, Pimpliskar et al. (2021) observed that the methanolic extract of *P. benghalensis* yielded a ZOI of 15 mm against *S. aureus* and 20 mm against *Escherichia coli*, compared to the standard Streptomycin (10 μ g/disc). In contrast, Rahman et al. (2018) found that *Pogostemon quadrifolius* exhibited a ZOI of 4 $\pm$ 0.81 mm against *P. aeruginosa* with methanolic extract and 1.6 $\pm$ 0.22 mm with ethyl acetate extract, using Chloramphenicol as the standard. Notably, there have been no prior published studies specifically focusing on the antibacterial properties of stem extracts

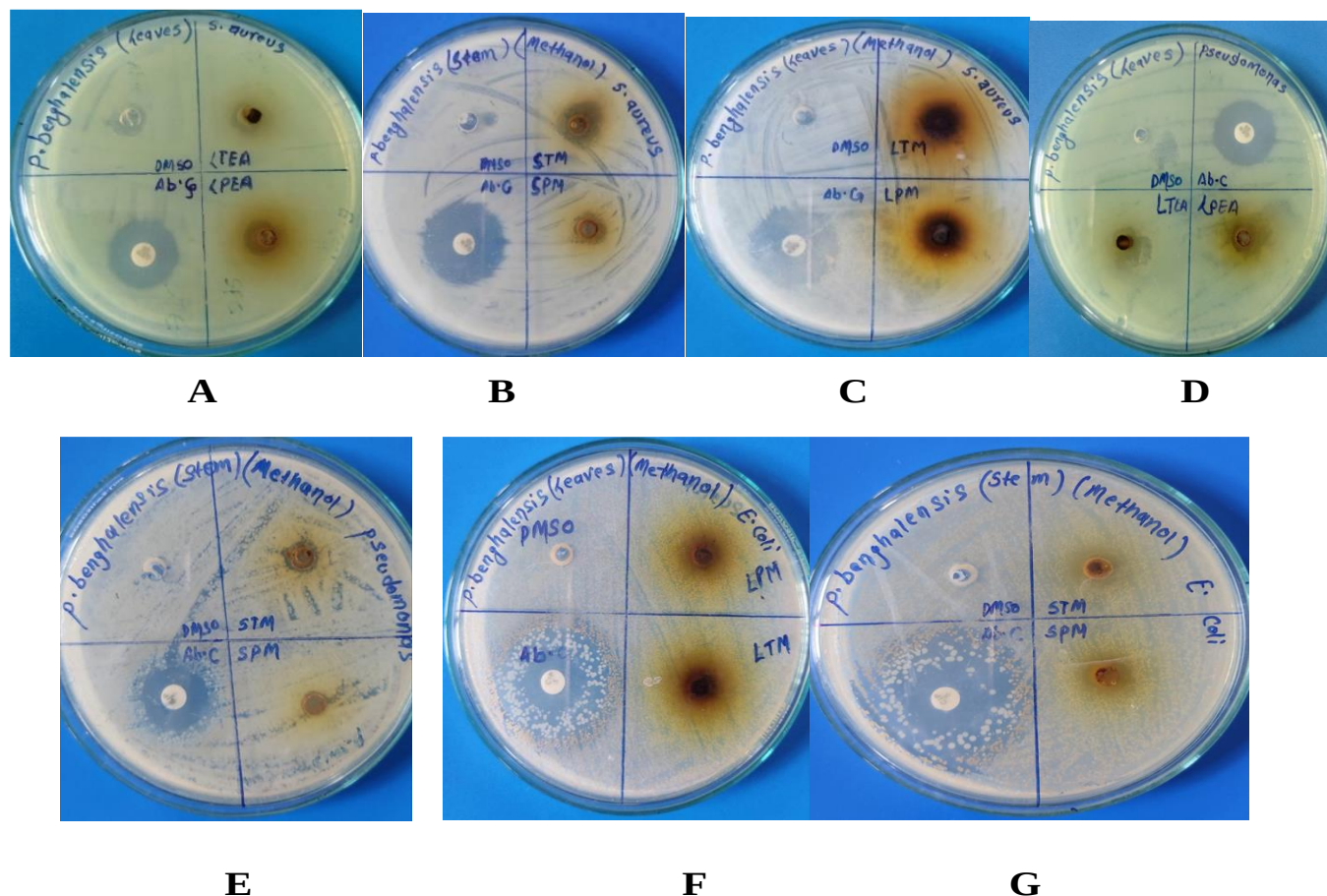


Figure 9. ZOI shown by Tarai and Hilly *P. benghalensis* leaves and stem extract against: *S. aureus*- A: Ethyl acetate extract of leaves; B: Methanolic extract of stem; C: Methanolic leaves extract. *Pseudomonas* - D: Ethyl acetate leaves E: Methanolic extract of stem. *E. coli* - F: Methanolic extract of leaves; G: Methanolic extract of stem.

from *P. benghalensis*.

In this study, all eight extracts with detectable ZOI were further evaluated for their Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). For the first time, MIC values for both leaf and stem extracts of *P. benghalensis* against various bacteria were clearly determined. The MBC values, ranging from 0.39 to 200 mg/ml, are detailed in Table 6, reflecting the efficacy of these extracts in inhibiting bacterial growth.

The study identified the lowest Minimum Bactericidal Concentration (MBC) values for *P. benghalensis* extracts, highlighting notable variations among the extracts. The Hilly leaf methanol extract demonstrated the lowest MBC value of 100 mg/ml against *S. aureus*, while the Tarai leaf methanol extract showed a lower MBC of 50 mg/ml against *E. coli*. Additionally, both Hilly and Tarai leaf ethyl acetate extracts exhibited an MBC of 50 mg/ml against *S. aureus*. The leaf ethyl acetate extract also had an MBC of 50 mg/ml against *P. aeruginosa*, as detailed in Table 6 and illustrated in Figure 10.

Globally, medicinal plants are continually explored for

natural compounds with potential therapeutic applications. This study underscores that the phytochemical profile of *P. benghalensis* varies with its origin and plant parts, suggesting the need for standardized protocols to isolate and purify these compounds. Despite the progress in isolating, purifying, and characterizing various compounds, further research is essential. Future studies should focus on the pharmacokinetics of individual compounds and their mechanisms of action *in vivo*. Additionally, assessing the toxicity and safety of *P. benghalensis* compounds using animal models is recommended (Swamy and Uma, 2015). Climate change also impacts plant life cycles, distribution, and phytochemical composition, affecting medicinal and aromatic plants. Changes in temperature and precipitation due to climate change influence plant growth, flowering, fruiting, and interactions with other species (Kumar et al., 2017).

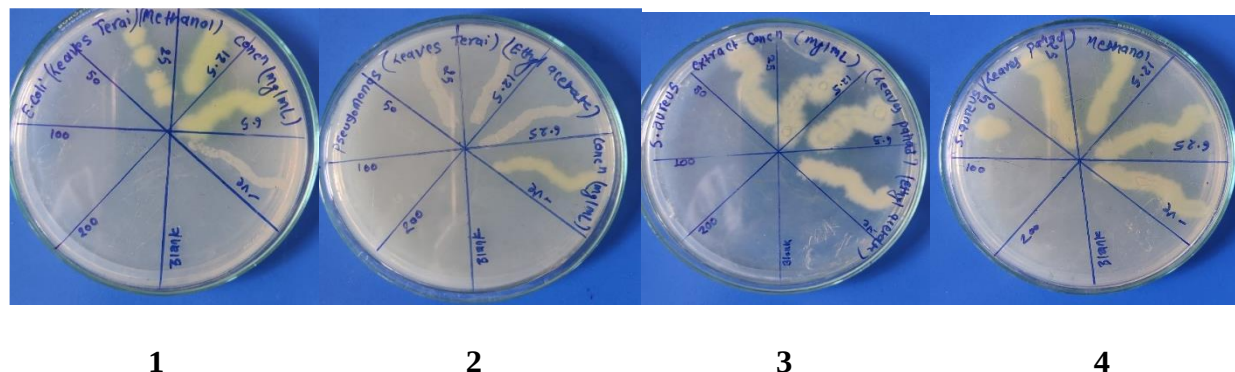
Conclusion

The comparative analysis of leaves and stem extracts of

Table 6. MBC values of leaves extract of *P. benghalensis* against different bacteria.

Bacterial Strain	MBC values of samples (mg/ml)			
	PbLHM	PbLTM	PbLHEA	PbLTEA
<i>Staphylococcus aureus</i>	100	-	50	50
<i>Pseudomonas aeruginosa</i>	-	-	-	50
<i>Escherichia coli</i>	-	50	-	-

- Indicate not observable.

**Figure 10.** MBC of *P. benghalensis* samples. 1: Methanol extract Tarai leaves, *E. coli*; 2: Ethyl acetate extract Tarai leaves, *Pseudomonas aeruginosa*; 3: Ethyl acetate extract Hilly region leaves, *S. aureus*; 4: Methanol extract Hilly region leaves, *S. aureus*).

P. benghalensis from different geographical regions revealed the presence of various phytochemicals across all locations. The study demonstrated significant antibacterial and antioxidant activities in these extracts. Notably, extracts from the Terai region exhibited greater effectiveness against a range of bacteria compared to those from the Hilly region. Among the plant parts, stem extracts consistently showed higher antibacterial activity than leaf extracts. These findings highlight the potential of *P. benghalensis* for further biological research and its application in treating diseases such as urinary tract infections, malaria, pneumonia, and hemorrhage.

RECOMMENDATION

Further investigations into the antiviral, antifungal, and anti-analgesic properties of *P. benghalensis* stem are needed, as current research is limited. Conducting *in-vivo* clinical trials is crucial to verify the safety and efficacy of this plant. Additionally, exploring other plant parts, such as roots and flowers, could provide more comprehensive insights. Expanding antioxidant activity assessments using methods like TRAP, ORAC, and TOSC would offer a broader understanding of the plant's potential.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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