



# Preliminary And Quantitative Chemical Profiling Of Pogostemon Benghalensis Leaf Extracts: Phytochemical Screening, Quantification, And Antioxidant Activity

1V. S. Bhavani, 2Dr, Ashutosh Jain

1Ph.D. Scholar, 2Professor

1Malwanchal University,

2Malwanchal University

## ABSTRACT

**Background:** Bioactive components are present in *Pogostemon benghalensis*, which may include flavonoids, tannins, saponins, alkaloids, and terpenoids, and may serve as protective agents against numerous diseases. **Objective:** This study presents both preliminary and quantitative plant chemical profiling of *P. benghalensis* leaf extracts. **Methods:** A soxhlet apparatus extracted dried leaf materials using solvents such as water, methanol, ethyl acetate, chloroform, and petroleum ether. **Results:** Initial phytochemical screening identified steroids, reducing sugars, sugars, alkaloids, phenolic compounds, catechins, flavonoids, saponins, triterpenoids, and tannins. The highest quantitative phenolic content was observed in the methanol extract (15.56 mg GAE/g for 80  $\mu$ L), and the highest flavonoid content was present in the methanol extract (23.00  $\pm$  0.63 mg QE/g for 80  $\mu$ L). In FTIR, different solvents contain more functional groups in *P. benghalensis* leaf, including phenols, alkanes, amines, alkenes, carboxylic acid derivatives, arenes, aldehydes, and ketones. **Conclusion:** The leaf extracts demonstrated promising DPPH scavenging activity. This study will further focus on profiling the plant chemicals from *P. benghalensis*.

**Keywords:** *Pogostemon benghalensis*, Phytochemical analysis, DPPH, antioxidant

Herbal remedies are often regarded as more effective in addressing a wide range of ailments and are perceived to be safer compared to synthetic drugs.<sup>1</sup> Their naturally occurring bioactive components—including flavonoids, tannins, saponins, alkaloids, and terpenoids—serve as protective agents against

numerous diseases. These compounds are recognized for their antioxidant, anti-inflammatory, anti-diarrheal, anti-obesity, and anticancer properties.<sup>2 3</sup>

*Pogostemon benghalensis* (Burm. f.) Kuntze was selected for this study due to its traditional medicinal uses.<sup>4</sup> This aromatic medicinal undershrub, belonging to the family **Lamiaceae** within the order **Lamiales**, thrives in open riverine forests across tropical regions of South Asia, including India, Nepal, China, and Thailand.<sup>5</sup> Historically, its leaves and roots have been used to treat colds, coughs, pneumonia, diarrhea, dysentery, skin disorders, bleeding disorders, respiratory infections, and digestive issues. Additionally, its oil is valued for its styptic and stimulant properties.<sup>6 7</sup>

*P. benghalensis* is a tomentose undershrub characterized by a robust stem and oblong, hairy leaves that possess epidermal hairs and secretory structures arranged in opposite phyllotaxy. The plant bears fragrant bilabiate flowers in a verticillaster inflorescence, typically purple or pinkish-white. The stamens are prominently extended and adorned with violet-purple filament hairs, while the glabrous ovary is paired with a slender style and a bilobed stigma. The reddish-brown, trigonous fruits contain four nutlets.<sup>3</sup>

Within the genus *Pogostemon*, which comprises around 70 species rich in various bioactive compounds, the essential oils and leaf extracts of *P. benghalensis* exhibit a wide spectrum of biological activities, including antioxidant, antibacterial, antifungal, antiviral, larvicidal, and anticancer properties. The oil is widely appreciated for its stimulant and styptic effects, and almost all parts of the plant are utilized in the treatment of various health conditions.<sup>7</sup>

The study employed the conventional and widely adopted **Soxhlet extraction technique**, suitable for bulk extraction with high recovery efficiency. To enhance extraction yields, organic solvents with varying polarity—methanol, ethyl acetate, chloroform, petroleum ether—and distilled water were used.<sup>8</sup>

The therapeutic potential of the plant extract, including its antioxidant, antibacterial, and anti-inflammatory properties, depends on the extraction technique, extraction time, and physico-chemical characteristics that yield secondary metabolites such as alkaloids, phenols, tannins, saponins, carbohydrates, glycosides, flavonoids, and steroids, as determined through qualitative analysis. Phenols and flavonoids are potent antioxidants capable of scavenging free radicals and chelating metal ions. They act as hydrogen-bond donors, forming stable phenoxy radicals while inhibiting the initiation of new chain reactions.<sup>11</sup>

Therefore, this study focused on Soxhlet-assisted extraction of *P. benghalensis* leaves using five solvents. The resulting extracts were then subjected to qualitative and quantitative phytochemical analysis, and FTIR spectroscopy was used to identify functional groups responsible for the plant's biological activities, particularly antioxidant properties.

## MATERIALS AND METHODS

### Plant Material Collection Process

Leaves of *Pogostemon benghalensis* were collected from the Kolli Hills, India. The Botanical Survey of India (BSI), Coimbatore, authenticated the plant as *P. benghalensis* (Burm. f.) Kuntze. A voucher specimen (Code: **BSI/SRC/5/23/2024-25/Tech./603**) has been deposited in the High-Altitude Plant Section, Department of Botany, Bharathidasan University.

### Preparation and Extraction of the Plant Sample

The collected leaves were thoroughly washed under running tap water for 30 minutes and then rinsed with distilled water. The leaves were air-dried for four weeks, coarsely ground using a blender, and stored in a refrigerator for future analysis.

A total of **250 mL** each of petroleum ether, chloroform, ethyl acetate, methanol, and distilled water were used to extract **50 g** of leaf powder over a **24-hour period** in a Soxhlet apparatus, as described by Rapando et al.<sup>10</sup> The extracts were concentrated by allowing the solvents to evaporate at room temperature and were stored at **4°C** until further use (Figure 1).

### Phytochemical Analysis

#### Determination of Extraction Yield

All extracts were stored in a refrigerator until analysis. The organic solvents (petroleum ether, chloroform, ethyl acetate, methanol, and water) were evaporated completely. The extraction yield for each solvent was calculated based on the dry weight of the recovered extract.



Figure 1: Workflow of the current research from the collection of leaves to extractions and their antioxidant activities Preliminary Phytochemical Analysis

The extracts obtained from *Pogostemon benghalensis* leaves were subjected to preliminary qualitative phytochemical screening to determine the presence of major bioactive constituents.<sup>12</sup> This analysis was performed to identify classes of compounds such as alkaloids, phenolics, flavonoids, saponins, tannins, terpenoids, and other secondary metabolites.

## Quantitative Analysis

### *Total Phenolic Content Determination*

Total phenolic content was estimated using the Folin–Ciocalteu reagent with slight modifications based on the method described by Sasadara et al.<sup>13</sup>

A diluted plant extract (40–80 mg/mL) or standard gallic acid solution was mixed with **5 mL of Folin–Ciocalteu reagent** (diluted 1:10 with distilled water) followed by **4 mL of aqueous sodium carbonate**. The reaction mixture was incubated for **15 minutes**, after which absorbance was measured at **765 nm** using a UV–visible spectrophotometer.

The total phenolic content was expressed as **mg of gallic acid equivalent per gram of dry extract (mg GAE/g)**.

### *Total Flavonoid Content Determination*

Total flavonoid content was determined using a colorimetric method involving aluminium chloride, with slight modifications from the standard protocol.<sup>14</sup>

In a 10 mL volumetric flask, the following were added:

- 1 mL of crude extract (1 mg/mL)
- 4 mL of distilled water
- 0.30 mL of 5% sodium nitrite

After **5 minutes**, **0.30 mL of 10% aluminium chloride** was added. After a further **6 minutes**, **2 mL of 1 M sodium hydroxide** was added to the reaction mixture, and the final volume was made up to **10 mL with distilled water**.

Standard quercetin solutions (40–80 µL) were prepared using the same procedure. The absorbance of the test and standard solutions was measured at **510 nm** against a reagent blank.

Fourier-transform infrared spectroscopy (FTIR) analysis was performed using PerkinElmer Spectrum software version 10.03.09 to identify the functional groups present in the leaf extracts of *Pogostemon*



*benghalensis*. Spectral readings were recorded in the range of 400–4000  $\text{cm}^{-1}$ , within which different functional groups exhibit characteristic stretching and bending vibrations depending on their absorption frequencies. These variations produce distinct spectral peaks, enabling structural identification of chemical constituents. Antioxidant activity was assessed using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay following the standard protocol. A 200  $\mu\text{M}$  DPPH solution was prepared in pure methanol, and the extract stock solution was maintained at 1 mg/mL. In a 96-well plate, 200  $\mu\text{L}$  of DPPH was mixed with 100  $\mu\text{L}$  of plant extract at final concentrations of 20, 40, 60, and 80  $\mu\text{L/mL}$ , with methanol serving as both the blank and negative control. All readings were recorded in triplicate, and results were expressed as mean  $\pm$  SD. FTIR analysis revealed the presence of several functional groups across different solvent extracts. In the petroleum ether extract, peaks corresponding to alkanes (2966.18  $\text{cm}^{-1}$ , C–H stretch), primary and secondary amines (3250.14  $\text{cm}^{-1}$ , N–H stretch), and alkyl halides (614.74 and 591.85  $\text{cm}^{-1}$ ) were identified. The chloroform extract showed fifteen functional groups, including carboxylic acids (3219.35  $\text{cm}^{-1}$ ), alkanes (2973.56 and 2880.29  $\text{cm}^{-1}$ ), aromatics (881.22 and 762.15  $\text{cm}^{-1}$ ),  $\alpha,\beta$ -unsaturated esters, and alkynes, as detailed in Table 5 and Figure 3. Ethyl acetate extract exhibited functional groups such as alkanes (3221.30  $\text{cm}^{-1}$ ; 2980.44 and 2883.90  $\text{cm}^{-1}$ ), carboxylic acids (2812.21  $\text{cm}^{-1}$ ), aldehydes (1742.11  $\text{cm}^{-1}$ ), primary amines (1375.23  $\text{cm}^{-1}$ ), aliphatic amines (1243.93 and 1048.16  $\text{cm}^{-1}$ ), and aromatics (771.90  $\text{cm}^{-1}$ ), as shown in Table 7 and Figure 4. Twelve functional groups were identified in the methanol extract, including carboxylic acids (3322.79  $\text{cm}^{-1}$ ), alkanes (2947.50 and 2832.66  $\text{cm}^{-1}$ ), aromatic rings (1408.26  $\text{cm}^{-1}$ ), and alkyl halides (658.85, 596.85, and 505.73  $\text{cm}^{-1}$ ), as noted in Table 7 and Figure 5. The water extract exhibited sixteen functional groups, such as alkynes (3317.11  $\text{cm}^{-1}$ ), aldehydes (2885.55 and 2832.39  $\text{cm}^{-1}$ ),  $\alpha,\beta$ -unsaturated esters, aromatics (1766.13  $\text{cm}^{-1}$ ), aliphatic amines (1246.17 and 1087.31  $\text{cm}^{-1}$ ), and alkyl halides (880.38 and 596.23  $\text{cm}^{-1}$ ), as indicated in Table 8 and Figure 6.

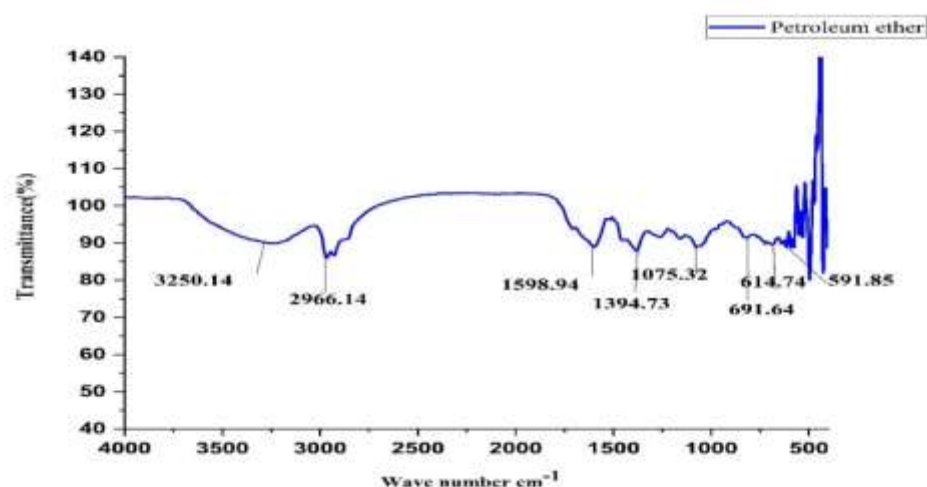
Table 2: Preliminary phytochemical constituents with different solvents from leaf extract of *P. benghalensis*

Experiment	Petroleum ether	Chloroform	Ethyl acetate	Methanol	Water
Steroids	Present	Present	Present	Present	Present
Triterpenoids	Absent	Absent	Absent	Present	Absent
Reducing sugar	Absent	Absent	Present	Present	Present
Sugars	Present	Present	Present	Absent	Absent
Alkaloids	Present	Present	Present	Present	Present
Phenolic	Present	Present	Present	Present	Present
Catechins	Present	Present	Absent	Absent	Absent
Flavonoids	Present	Absent	Absent	Present	Absent
Saponins	Absent	Present	Present	Present	Present
Tannins	Present	Absent	Present	Present	Present
Anthraquinones	Absent	Absent	Present	Absent	Absent
Amino acids	Absent	Absent	Absent	Present	Present

Table 3: Determination of total phenol content in leaf extract of *P. benghalensis* using various solvents

	Total phenol			Total flavonoid		
	40 $\mu$ L	60 $\mu$ L	80 $\mu$ L	40 $\mu$ L	60 $\mu$ L	80 $\mu$ L
Petroleum	15.10 $\pm$	16.20 $\pm$ 0.02	17.20 $\pm$	12.40 $\pm$	13.40 $\pm$ 0.02	15.00 $\pm$
Chloroform	15.60 $\pm$	16.10 $\pm$ 0.13	17.20 $\pm$	13.40 $\pm$	17.20 $\pm$ 0.00	18.70 $\pm$
Ethyl acetate	15.70 $\pm$	16.20 $\pm$ 0.08	17.00 $\pm$	12.50 $\pm$	14.10 $\pm$ 0.06	16.00 $\pm$
Methanol	16.50 $\pm$	17.50 $\pm$ 0.11	18.20 $\pm$	14.70 $\pm$	17.40 $\pm$ 0.10	28.00 $\pm$
Water	15.30 $\pm$	15.70 $\pm$ 0.10	16.30 $\pm$	15.00 $\pm$	15.90 $\pm$ 0.25	23.00 $\pm$

Antioxidant activity of leaf extract of *Pogostemon benghalensis* is of significant interest because evaluating the antioxidant capabilities of natural substances is essential for their potential applications in healthcare, nutrition, and personal care products. Antioxidants play a crucial role in neutralizing free radicals—unstable molecules capable of damaging DNA, proteins, and cellular structures by inducing oxidative stress. Such oxidative damage has been linked to various health issues, including chronic conditions such as cancer, cardiovascular diseases, and neurological disorders. Understanding the antioxidant properties of natural compounds is therefore important, as it can contribute to the development of therapeutic agents aimed at reducing oxidative stress and improving health outcomes.

Figure 2: FTIR spectrum of petroleum ether leaf extract of *P. benghalensis*

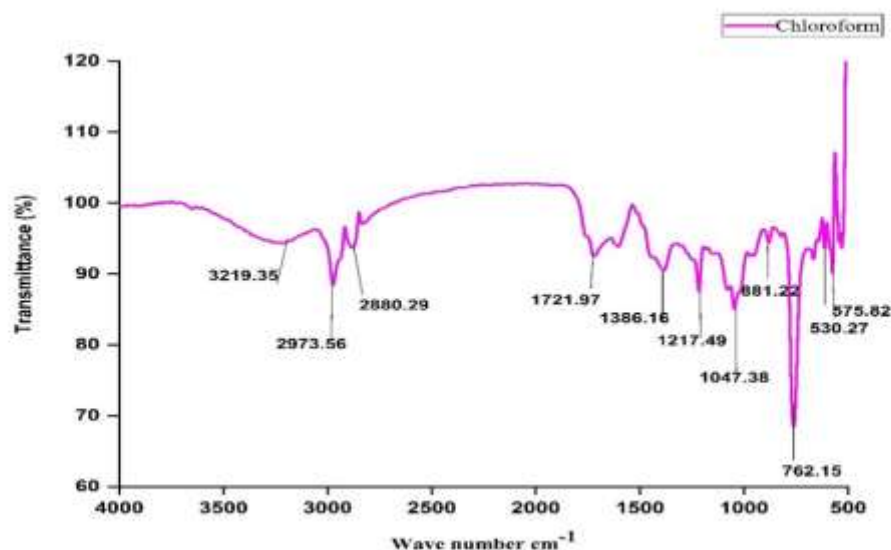


Figure 3: FTIR spectrum of Chloroform leaf extract of *P. benghalensis*

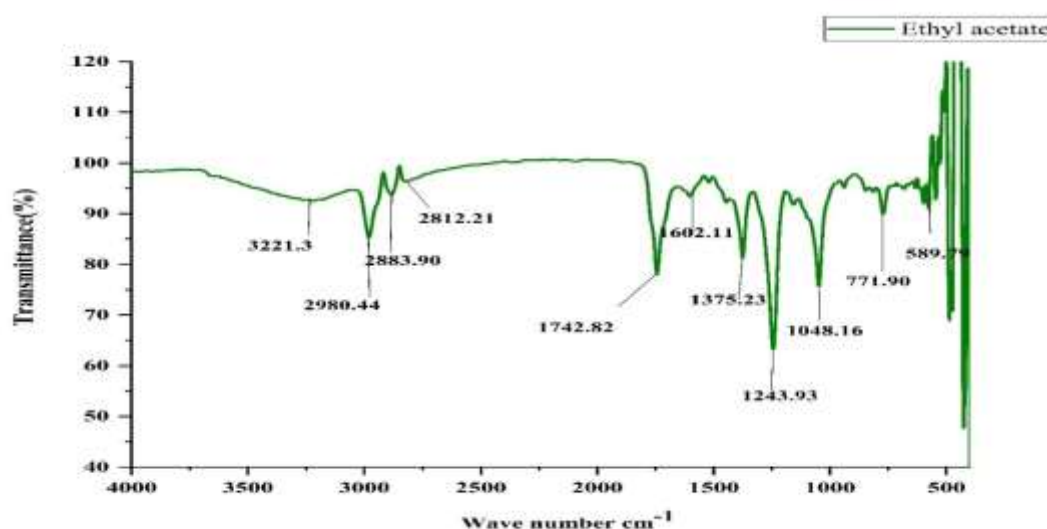


Figure 4: FTIR spectrum of ethyl acetate leaf extract of *P. benghalensis* to the development of new therapeutic agents that help reduce oxidative stress and improve overall health outcomes. Plant-derived compounds such as flavonoids, polyphenols, and vitamins have demonstrated significant antioxidant activity, and extensive research on these natural antioxidants supports their potential use in formulating supplements or medications that strengthen the body's defenses against oxidative damage.<sup>24</sup> Numerous reactive species are generated in living systems as a result of normal metabolic processes as well as exposure to environmental factors. These reactive molecules

Table 4: Functional groups identified in the FTIR spectrum of Petroleum ether leaf extract of *P. benghalensis*

S.No	Wave Number (cm-1)	Molecular Motion	Functional group
1.	3219.35	O–H stretch	carboxylic acids
2.	2973.56	C–H stretch	alkanes
3.	2880.29	C–H stretch	alkanes
4.	1721.97	C=O stretch	$\alpha$ , $\beta$ –unsaturated
5.	1386.16	C–H rock	alkanes
6.	1217.49	C–N stretch	aliphatic amines
7.	1047.38	C–O stretch	alcohols, carboxylic
8.	881.22	C–H “oop”	aromatics
9.	762.15	C–H “oop”	aromatics
10.	614.30	–C≡C–H: C–H bend	alkynes
11.	575.82	C–Cl stretch	alkyl halides

Table 5: Functional groups identified in the FTIR spectrum of Chloroform leaf extract of *P. benghalensis*

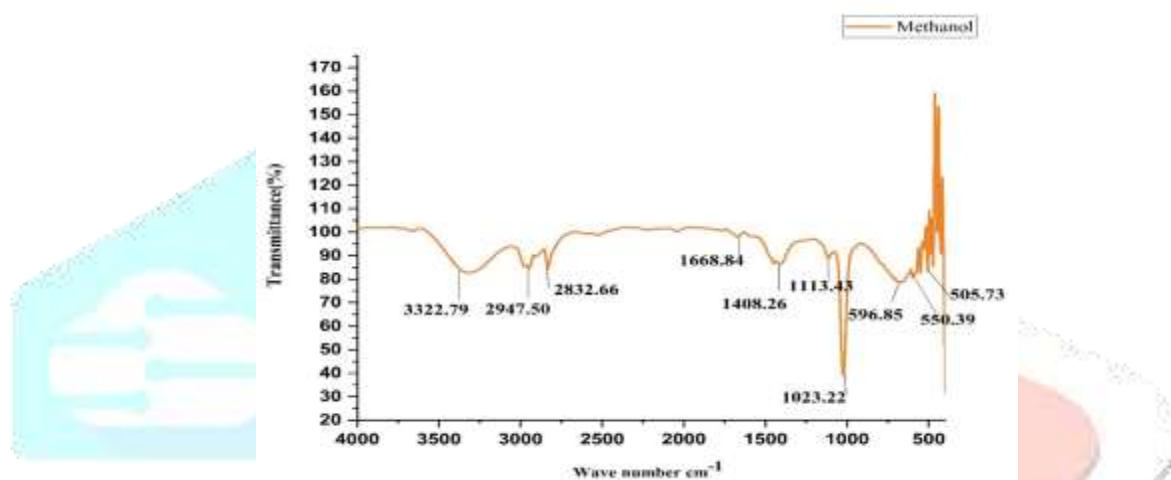
S. No	Wave Number (cm-1)	Molecular Motion	Functional group
1.	3322.79	O–H stretch	carboxylic acids
2.	2947.50	C–H stretch	alkanes
3.	2832.66	C–H stretch	alkanes
4.	1668.84	C=O stretch	$\alpha$ , $\beta$ –unsaturated aldehydes,
5.	1408.26	C–C stretch (in–ring)	aromatics
6.	1113.43	C–N stretch	aliphatic amines
7.	1023.22	C–N stretch	aliphatic amines
8.	658.85	C–Br stretch	alkyl halides
9.	596.85	C–Br stretch	alkyl halides
10.	550.39	C–Br stretch	alkyl halides
11.	505.73	C–Br stretch	alkyl halides

known as reactive oxygen species (ROS), which include various types of free radicals. Elevated levels of ROS can disrupt normal cellular activities, alter biomolecules, and damage structural components, ultimately leading to cellular dysfunction or even cell death. Prolonged increases in ROS contribute to systemic oxidative stress, which has been associated with several health conditions, including cancer, age-related diseases, and cardiovascular disorders.<sup>25</sup> Free radicals are highly reactive species characterized by the presence of unpaired electrons, and they arise as natural byproducts of normal metabolic processes. Oxidative stress develops when the body's antioxidant defense mechanisms are insufficient to neutralize the excessive free radicals generated.<sup>26</sup>



Table 6: Functional groups identified in the FTIR spectrum of ethyl acetate leaf extract of *P. benghalensis*

S. No	Wave Number (cm <sup>-1</sup> )	Molecular Motion	Functional Group
1	3221.3	C–H Stretch	Alkanes
2	2980.44	C–H Stretch	Alkanes
3	2883.90	O–H Stretch	Alcohols ( <i>not alkanes</i> )
4	2812.21	O–H Stretch	Carboxylic acids
5	1742.82	C=O Stretch	Aldehyde (saturated aliphatic)
6	1602.11	N–H Bend	1° Amines
7	1375.23	C–N Stretch	Aromatic amines
8	1243.93	C–N Stretch	Aliphatic amines
9	1048.16	C–N Stretch	Aliphatic amines
10	771.90	C–H “out of plane” (oop)	Aromatics
11	589.79	C–Br Stretch	Alkyl halides

Figure 5: FTIR spectrum of methanol leaf extract of *P. benghalensis*Table 7: Functional groups identified in the FTIR spectrum of methanol leaf extract of *P. benghalensis*Table 8: Functional groups identified in the FTIR spectrum of water leaf extract of *P. benghalensis*

S. No	Wave Number	Molecular Motion	Functional group
1.	3322.79	O–H stretch	carboxylic acids
2.	2947.50	C–H stretch	alkanes
3.	2832.66	C–H stretch	alkanes
4.	1668.84	C=O stretch	$\alpha$ , $\beta$ –unsaturated aldehydes,
5.	1408.26	C–C stretch	aromatics
6.	1113.43	C–N stretch	aliphatic amines
7.	1023.22	C–N stretch	aliphatic amines
8.	658.85	C–Br stretch	alkyl halides
9.	596.85	C–Br stretch	alkyl halides
10.	550.39	C–Br stretch	alkyl halides
11.	505.73	C–Br stretch	alkyl halides

The primary class of free radicals produced within living organisms originates from oxygen and includes species such as superoxide, hydroxyl, peroxy ( $\text{RO}_2\bullet$ ), alkoxy ( $\text{RO}\bullet$ ), and hydroperoxyl ( $\text{HO}_2\bullet$ ) radicals. Collectively, these are known as reactive oxygen species (ROS). In addition, important radicals derived from nitrogen—such as nitric oxide ( $\text{NO}\bullet$ ) and nitrogen dioxide ( $\bullet\text{NO}_2$ )—are classified as reactive nitrogen species (RNS). Both ROS and RNS are natural byproducts of metabolic processes and may exert either beneficial or harmful effects on the organism. At low or regulated concentrations, ROS and RNS help protect the body against infectious agents and play essential roles in cellular signaling pathways. However, excessive production of ROS and RNS can lead to significant damage and impair the normal functioning of cellular lipids, proteins, and DNA, resulting in a condition commonly referred to as oxidative stress or nitrosative stress.<sup>27</sup> The present study evaluates the antioxidant activity of various solvent extracts prepared from the leaves of *Pogostemon benghalensis*. The DPPH assay, a widely used method for assessing radical scavenging activity, employs the stable free radical DPPH to measure the antioxidant potential of compounds, crude drugs, and plant extracts. This test determines the reactivity of the extract toward the DPPH radical based on its ability to donate electrons or hydrogen atoms, indicating its capacity to neutralize free radicals.

Table 9: DPPH radical scavenging activity of different solvents of P. benghalensis leaf extract

**Percentage Inhibition (Mean  $\pm$  SD) at Different Concentrations**

Concentration ( $\mu\text{L}$ )	Petroleum Ether	Chloroform	Ethyl Acetate	Methanol	Water	Standard
20 $\mu\text{L}$	17.20 $\pm$ 0.03	13.10 $\pm$ 0.00	17.90 $\pm$ 0.03	26.80 $\pm$ 0.00	9.10 $\pm$ 0.02	34.20 $\pm$ 0.00
40 $\mu\text{L}$	38.90 $\pm$ 0.00	19.30 $\pm$ 0.00	26.10 $\pm$ 0.01	47.40 $\pm$ 0.04	22.60 $\pm$ 0.05	43.90 $\pm$ 0.02
60 $\mu\text{L}$	51.20 $\pm$ 0.00	45.70 $\pm$ 0.00	42.50 $\pm$ 0.05	56.50 $\pm$ 0.03	38.60 $\pm$ 0.01	68.20 $\pm$ 0.06
80 $\mu\text{L}$	61.80 $\pm$ 0.00	62.30 $\pm$ 0.01	68.50 $\pm$ 0.03	75.20 $\pm$ 0.02	65.00 $\pm$ 0.08	93.60 $\pm$ 0.03

Values represent the mean  $\pm$  SD of three replicates.

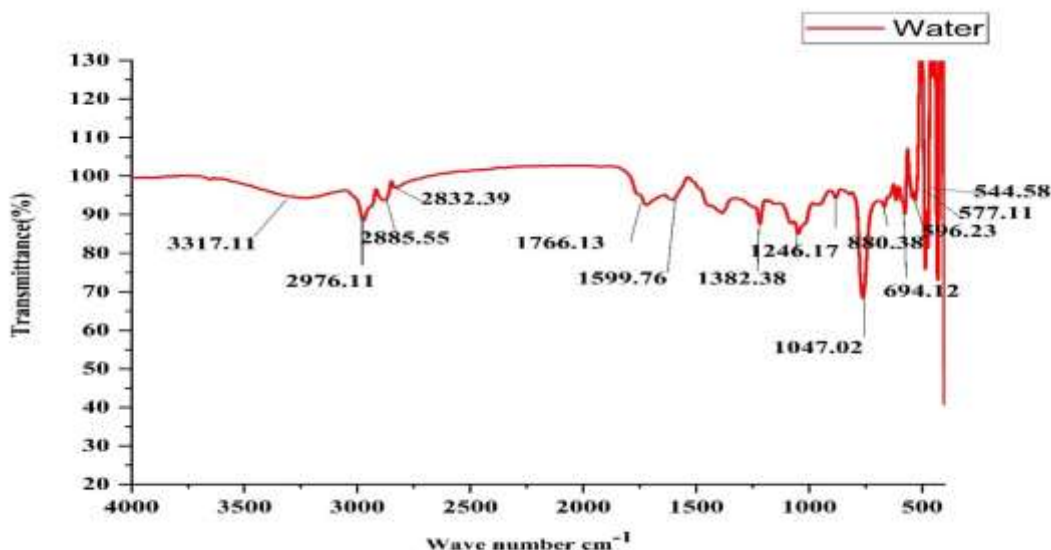


Figure 6: FTIR spectrum of water leaf extract of *P. benghalensis*

The DPPH assay evaluates the reactivity of test substances toward a stable free radical. DPPH, which contains an unpaired electron, exhibits a strong absorption band at 517 nm, producing a deep violet color in visible spectroscopy. When a free radical scavenger is present, electron donation occurs, leading to a reduction in color intensity, which corresponds to stoichiometric decolorization. The scavenging properties of antioxidants are closely associated with their ability to form stable radicals.<sup>28</sup> The DPPH scavenging activities of petroleum ether, chloroform, ethyl acetate, methanol, and water extracts of *Pogostemon benghalensis*, along with ascorbic acid as the standard, are summarized in Table 9. Among the tested solvents, the methanol extract demonstrated the highest antioxidant activity, surpassing even the standard ascorbic acid.

## CONCLUSION

The study demonstrates that methanol extracts of *Pogostemon benghalensis* leaves contain a wide range of secondary metabolites, including steroids, triterpenoids, sugars, alkaloids, phenolic compounds, flavonoids, saponins, tannins, catechins, and amino acids. Methanol showed the highest concentrations of phenols and flavonoids when compared with petroleum ether, ethyl acetate, and water extracts. FTIR analysis further confirmed that petroleum ether, chloroform, ethyl acetate, methanol, and water extracts contained multiple functional groups associated with various bioactive compounds. The DPPH assay revealed that methanol extract exhibited the strongest antioxidant activity among all solvents tested, even higher than ascorbic acid.

Overall, the phytochemical constituents and FTIR characteristics of *P. benghalensis* leaf extracts show promising potential for pharmaceutical and research applications. These findings contribute valuable insights into the chemical composition and therapeutic properties of the plant, supporting its future use in the development of innovative medicinal compounds and formulations.

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