RESEARCH ARTICLE

Preliminary and quantitative chemical profiling of *Pogostemon benghalensis* leaf extracts: Phytochemical screening, quantification, and antioxidant activity

Sandhiya Udayakumar, Azhagiyamanavalan Lakshmi Prabha*

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 µL), and the highest flavonoid content was present in the methanol extract (23.00 ± 0.63 mg QE/g for 80 µL). 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

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INTRODUCTION

Herbal remedies are often regarded as more effective for addressing a range of ailments and are perceived to be safer compared to synthetic drugs.[1] 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 benefits.[2, 3]

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

P. benghalensis is a tomentose undershrub characterized by its robust stem and oblong, hairy leaves, which feature epidermal hairs and secretory structures arranged in an opposite phyllotaxy. The plant bears fragrant bilabiate flowers in a verticillaster inflorescence, with hues of purple or pinkish-white. Its stamens are prominently extended and adorned with violet-purple filament hairs, while the glabrous ovary is paired with a slender style and bilobed stigma. The reddish-brown, trigonous fruits contain four nutlets.[3] Within the genus *pogostemon*, which comprises around 70 species rich in 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 plant's oil is widely appreciated for its stimulant and styptic effects, and nearly all parts of the plant are utilized in the treatment of various health conditions.[7]

The study employed the conventional and widely adopted Soxhlet extraction technique, which is suitable for bulk batch extraction with better recovery. To enhance extraction yields, organic solvents of varying polarity were used, specifically methanol, ethyl acetate, chloroform, and petroleum ether.[8] The therapeutic potential, encompassing antioxidant, antibacterial, and anti-inflammatory properties of the plant extract, depends on the extraction technique, 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

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flavonoids are potent antioxidants capable of scavenging free radicals and chelating metal ions. They function as hydrogen bond donors, forming stable phenoxy radical intermediates while inhibiting the initiation of new chain reactions. Therefore, this study focused on Soxhlet-assisted extraction of *P. benghalensis* leaf using five organic solvents.[9] The extracts underwent qualitative and quantitative analysis, and the functional groups conferring the biological activities, particularly the antioxidant property, were determined using FTIR instruments.

MATERIALS AND METHODS

Plant Material Collection Process

The leaf of the plant was collected from the Kolli hills in India. Subsequently, the Botanical Survey of India (BSI) in Coimbatore verified the specimen as *P. benghalensis* (Burm. f.) Kuntze. A voucher specimen, recorded under the code BSI/SRC/5/23/2024-25/Tech./603, has been displayed in the High-Altitude plant section of the Department of Botany at Bharathidasan University.

Preparation and Extraction of the Plant Sample

The gathered leaves were thoroughly washed under running tap water for 30 minutes, followed by a rinse with distilled water. Subsequently, the leaves were air-dried for four weeks, coarsely ground in a blender, and stored in a refrigerator for future use. A total of 250 mL of petroleum ether, chloroform, ethyl acetate, methanol, and water were used to extract 50 g of leaf powder over a 24-hour period using a Soxhlet apparatus, as outlined by Rapando *et al*.[10] The resulting extracts were then allowed to evaporate the solvent at room temperature and were stored in a refrigerator at 4°C until further analysis (Figure 1).

Phytochemical Analysis

Determination of extraction yield

Every extract was stored in a refrigerator. Organic solvents (Petroleum ether, Chloroform, Ethyl acetate, Methanol, Water) were evaporated. The yield of each extraction procedure was expressed as a weight relative to the dried matter of the initial extraction sample, as shown in the table below.[11]

$$The\,extraction\,yield\left(\frac{g}{100g}\,of\,distilled\,water\right)$$

Preliminary phytochemical analysis

This qualitative study established techniques to evaluate the presence of several bioactive components in *P. benghalensis* leaf extracts by preliminary qualitative analysis.[12]

Quantitative Analysis

Total phenolic content determination

Total phenols were assayed using the Folin-Ciocalteu reagent, with minimal modifications by Sasadara *et al*. A diluted extract of each plant (40–80 mg/mL) or gallic acid (a common phenolic component) was mixed with 5 mL of Folin-Ciocalteu reagent (1:10 diluted with distilled water) and 4 mL of aqueous sodium carbonate. After 15 minutes, the total phenols were measured colorimetrically at 765 nm. Total phenol levels are expressed as the amount of gallic acid equivalent (mg/g dry mass).[13]

Total flavonoid content determination

The total flavonoid content was ultimately determined using a colorimetric test with aluminium chloride[14], with some modifications. One mL (1-mg/mL) crude extract, 4 mL distilled water, and 0.30 mL 5% sodium nitrite were added to a 10 mL volumetric flask for the reaction. After 5 minutes, 0.30 mL of 10% aluminium chloride was added. Furthermore, after 6 minutes of incubation, 2 mL of 1M sodium hydroxide was added to the reaction mixture, and the total volume was promptly diluted to 10 mL with distilled water. The same method was used to prepare standard quercetin solutions (40–80 µL). The absorbance of the test and standard solutions was measured at 510 nm using a reagent blank.

Fourier-transform infrared spectroscopy (FTIR) analysis

The spectrum was recorded using PerkinElmer Spectrum version 10.03.09 to analyze and identify the functional groups. The FTIR was measured between 400 and 4000 cm^{-1}. Functional groups of compounds exhibit unique vibrations, bending, and stretching at this wavelength range due to varying absorption frequencies. This is recorded in a spectrum and provides fundamental information about the structure.[15]

Antioxidant activity: 1,1-diphenyl-2-picrylhydrazyl (DPPH)

The DPPH experiment was performed as per the protocol.[16] The DPPH stock solution was 200 µM in pure methanol, and the extract stock solution was 1 mg/mL. In a 96-well plate, 200 µL of DPPH was dispensed, followed by 100 µL of plant leaf samples at final concentrations of 20, 40, 60, and 80 µL/mL. The blank and negative controls were methanol

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

and methanol with DPPH, respectively, while ascorbic acid served as the positive control. The mixtures were incubated at 30°C for 30 minutes in the dark. The absorbance at 517 nm was measured using a Synergy HT Multi-mode Microplate Reader (BioTek Instruments Inc., Winooski, VT, USA).

$$\mathrm{DPPH}(\%) = \frac{\text{Absorbance control OD} - \text{Absorbance Ssample OD}}{\textit{Absorbance control OD} \times 100}$$

Statistical Analysis

Experimental data were presented as mean ± standard deviation (SD) of three replicates. Statistical analysis was conducted using one-way ANOVA and Duncan's Multiple Range Test in SAS 1999 to assess differences among samples. A probability of 0.05 was considered the cutoff value for accepting the significant difference.

RESULTS AND DISCUSSION

Preliminary Phytochemical Analysis

Phytochemistry investigates chemicals derived from plants. These plant-based phytochemicals, which include primary and secondary metabolites, are naturally found in various parts of plants and act as protective agents against numerous diseases.[17] In this study, the methanol solvent yielded the highest amount (10.47 g) from the leaves of *P. benghalensis* compared to other solvents (Table 1). As previously documented, the presence of secondary metabolites in *P. benghalensis* varies depending on the solvents utilized.[18] The initial phytochemical screening of *P. benghalensis* leaf extracts revealed either the presence or the absence of many phytoconstituents in a range of organic solvents, including petroleum ether, chloroform, ethyl acetate, methanol, and water. Secondary metabolites are among these components (Table 2). In the present study, Petroleum ether contained six secondary metabolites: steroids, sugars, alkaloids, phenolic compounds, catechins, and tannins. Similarly, chloroform revealed six secondary metabolites: steroids, sugars, alkaloids, phenolic compounds, catechins, and saponins. Ethyl acetate included eight secondary metabolites. While the methanolic solvent revealed the presence of nine secondary metabolites, notably steroids, triterpenoids, reducing sugar, alkaloids, phenolic compounds, flavonoids, saponins, tannins, and amino acids. Water also demonstrated the presence of eight secondary metabolites. Previous literature reported that *P. benghalensis* leaf extracts from methanol and water solvents contained similar secondary metabolites.[4] Approximately 20% of the secondary metabolites in *Pogostemon* are alkaloids. Alkaloids are renowned for their roles as anesthetics, cardioprotective agents, and anti-inflammatory drugs, with common clinical examples including morphine, quinine, ephedrine, and nicotine.[19] Phenolics are particularly noteworthy due to their significant medicinal benefits.[20] Extensive studies have been conducted on the ethyl acetate leaf extracts to explore their phytoconstituents and bioactive properties.[15,4] Choosing the right extraction solvent is the most crucial step of the solid-liquid extraction process. When selecting a solvent system for phytochemical extraction, it's essential to consider both the solvent and the solubility of the target components.

Table 1: Estimation of percentage yield Extraction

S. No	*Solvent (250ml)*	*Yield of extraction (50g)*
1.	Petroleum ether	3.95g
2.	Chloroform	4.52g
3.	Ethyl acetate	3.79g
4.	Methanol	10.47g
5.	Water	8.2g

Quantitative Analysis

Plants' secondary metabolites, known as phenolic compounds, are generated by the phenylpropanoid pathway or shikimic acid system. These compounds consist of one or more aromatic rings linked to one or more hydroxyl groups. Plant flavonoids principally contribute to the physiological effects related to color, flavor, anti-stress, enzyme protection, and inhibition of fat oxidation.[21] Phenolic compounds are commonly found in plant secondary metabolites and serve as natural antioxidants.[22] In the present study, the phenol content was found to be highest in methanol solvent, with values of 16.58 ± 0.105mg GAE/g for 40 µL, 17.58 ± 0.11 mg GAE/g for 60 µL, and 15.56 mg GAE/g for 80 µL. In contrast, other solvents such as petroleum ether, chloroform, ethyl acetate, and water exhibited lower phenol content (Table 3). The analysis revealed that the highest concentrations of flavonoids were observed in methanol solvent, with values of 14.76 ± 0.08 mg QE/g for 40 µL, 17.41 ± 0.10 mg QE/g for 60 µL, and 28.05 ± 0.15 mg QE/g for 80 µL. In comparison, other solvents such as petroleum ether, chloroform, ethyl acetate, and water exhibited significantly lower flavonoid levels. Specifically, water yielded flavonoid contents of 15.00 ± 0.11 mg QE/g for 40 µL, 15.93 ± 0.25 mg QE/g for 60 µL, and 23.00 ± 0.63 mg QE/g for 80 µL. The flavonoid concentrations were quantified based on quercetin equivalent (mg/g) (Table 4).

Fourier Transform Infrared (FTIR)

The FTIR spectrum, guided by peak values in the IR radiation region, has been utilized to determine the functional groups of the active compounds within the extract.[23] The FTIR analysis was conducted on various solvent extracts, including petroleum ether, chloroform, ethyl acetate, methanol, and water, all rich in phytochemicals. All five solvents contain more functional groups. The FTIR spectra of the leaf extracts revealed highly similar functional groups across the various tested solvents, such as the C–H stretch (alkanes), C–N stretch (aliphatic amines), and O–H stretch (carboxylic acids). In Petroleum ether indicates the presence of eleven functional groups like Alkanes, aromatics, nitro compounds, aliphatic compounds, and alkyl halides (Table 4, Figure 2). The presence

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 compounds	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
Anthraquionones	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 content			*Total flavonoid content*		
	40 µL	*60 µL*	*80 µL*	*40 µL*	*60 µL*	*80 µL*
Petroleum Ether	15.10 ± 0.10	16.20 ± 0.02	17.20 ± 0.02	12.40 ± 0.05	13.40 ± 0.02	15.00 ± 0.07
Chloroform	15.60 ± 0.05	16.10 ± 0.13	17.20 ± 0.07	13.40 ± 0.06	17.20 ± 0.00	18.70 ± 0.09
Ethyl acetate	15.70 ± 0.05	16.20 ± 0.08	17.00 ± 0.02	12.50 ± 0.00	14.10 ± 0.06	16.00 ± 0.10
Methanol	16.50 ± 0.10	17.50 ± 0.11	18.20 ± 0.11	14.70 ± 0.07	17.40 ± 0.10	28.00 ± 0.18
Water	15.30 ± 0.00	15.70 ± 0.10	16.30 ± 0.02	15.00 ± 0.10	15.90 ± 0.25	23.00 ± 0.63

Values represent the mean ± SD of three replicates

of alkanes can be observed at 2966.18 cm^{-1}(C–H stretch). The presence of 1˚ and 2˚ amines can be seen at 3250.14 cm^{-1} (N–H stretch). The peaks at 614.74 and 591.85 cm^{-1} indicate the presence of alkyl halides. In chloroform, fifteen functional groups, such as carboxylic acids, alkanes, α, β-unsaturated esters, aromatics, alkynes, and alkyl halides, are indicated (Table 5, Figure 3). The peaks at 3219.35 cm^{-1}(O–H stretch) indicate the carboxylic acids. The presence of alkanes can be observed at 2973.56 cm^{-1} (C–H stretch) and 2880.29 cm^{-1} (C–H stretch). The peaks at 881.22 cm^{-1} (C–H "oop") and 762.15 cm^{-1} indicate aromatics. The FTIR spectrum of ethyl acetate leaf extract identified functional groups such as alkanes, carboxylic acid, aromatic amines, and aldehyde, saturated aliphatic compounds at specific wavelengths, as illustrated in Table 7 and Figure 4. Alkanes are identified at 3221.3 cm^{-1} through C-H Stretch. Peaks at 2980.44 cm^{-1} (C-H Stretch) and 2883.90 cm^{-1} (O-H Stretch) indicate the presence of alkanes. The carboxylic acid can be seen at 2812.21 cm^{-1} (O-H Stretch). The peak indicates the presence of aldehyde-saturated aliphatic at 1742.11 cm^{-1} (C=O Stretch). 1375.23 cm^{-1} peak indicates the presence of 1o amines, while aliphatic amines can be seen at 1243.93 and 1048.16 cm^{-1} (C-N Stretch). The peak indicates the presence of aromatics at 771.90 cm^{-1}).

Twelve functional groups found in the methanol leaf extract of *P. benghalensis* (Table 7 and Figure 5). The peaks at 3322.79 cm^{-1} indicate the presence of carboxylic acids. Alkanes can be seen at 2947.50, and 2832.66 cm^{-1} (C–H stretch). Aromatics can be seen at 1408.26 cm^{-1} (C–C stretch (in–ring). The peaks at 658.85 cm^{-1} (C–Br stretch), 596.85, 505.73 cm^{-1} presence of indicate the alkyl halides. Sixteen functional groups present in the water extract **(**Table 8 and Figure 6**)** such as alkynes (3317.11 cm^{-1}), aldehydes (2885.55, 2832.39 cm^{-1}), α, β–unsaturated esters, aromatics (1766.13 cm^{-1}), aliphatic amines (1246.17, 1087.31 cm^{-1}), alkyl halides (880.38, 596.23 cm^{-1}).

Antioxidant activity of leaf extract of *P. benghalensis*

Evaluating the antioxidant capabilities of natural substances is essential due to their broad applications in fields such as healthcare, nutrition, and personal care products. Antioxidants are crucial for combating free radicals, which are unstable chemicals that can damage DNA, proteins, and cells by causing oxidative stress. This oxidative damage has been associated with various health concerns, including chronic conditions like cancer, cardiovascular diseases, and disorders affecting the nervous system. In medicine, understanding the antioxidant properties of natural compounds can lead

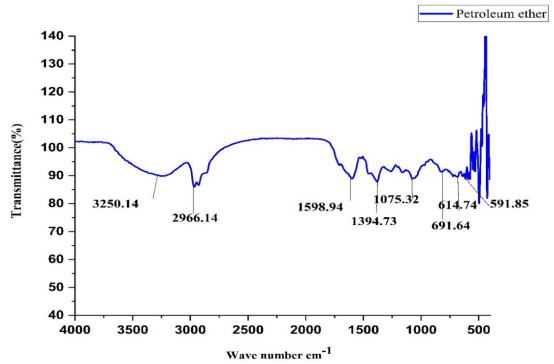

Figure 2: FTIR spectrum of petroleum ether leaf extract of *P.benghalensis*

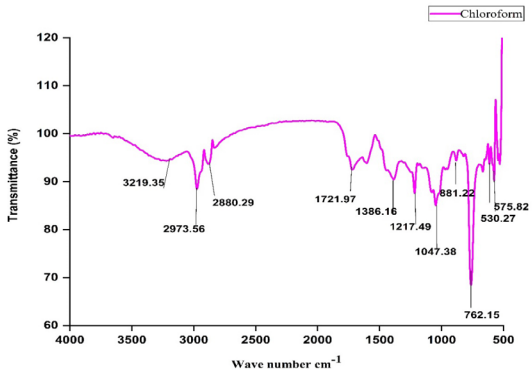

Figure 3: FTIR spectrum of Chloroform leaf extract of *P. benghalensis*

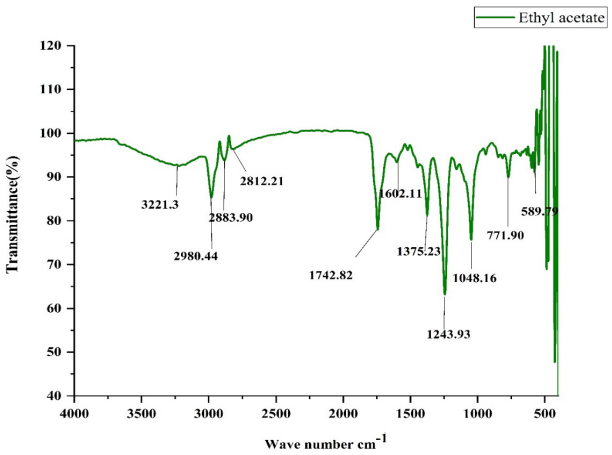

Figure 4: FTIR spectrum of ethyl acetate leaf extract of *P. benghalensis*

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	α, β–unsaturated esters
5.	1386.16	C–H rock	alkanes
6.	1217.49	C–N stretch	aliphatic amines
7.	1047.38	C–O stretch	alcohols, carboxylic acids, esters
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	α, β–unsaturated aldehydes, ketones
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

to the development of new therapeutic agents to help reduce oxidative stress and enhance health outcomes. For example, compounds derived from plants, such as flavonoids, polyphenols, and vitamins, have demonstrated significant antioxidant activity. Research into these natural antioxidants can aid in the formulation of supplements or medications that bolster the body's defenses against oxidative damage.[24] Numerous reactive species arise in living systems due to metabolic processes and environmental factors. These are known as reactive oxygen species (ROS), which include free radicals. Elevated ROS levels can lead to cellular dysfunction and even death by altering the activities of biomolecules and disrupting their structure. Prolonged increases in ROS can result in systemic oxidative stress, presenting as various health problems, including cancer, age-related diseases, and cardiovascular disorders.[25] Free radicals are highly reactive entities with unpaired electrons. These radicals encompass a range of reactive species produced as byproducts of normal cell metabolism. Oxidative stress occurs when the body's antioxidant defenses cannot neutralize the generated free radicals.[26]

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

S. No	Wave Number (cm^{-1})	Molecular Motion	Functional group
1.	3221.3	C-H Stretch	Alkanes
2.	2980.44	C-H Stretch	alkanes
3.	2883.90	O-H Stretch	Alkanes
4.	2812.21	O-H Stretch	Carboxylic acid
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 "oop"	Aromatics
11.	589.79	C-Br Stretch	Alkyl halides

Table 7: Functional groups identified in the FTIR spectrum of methanol 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	α, β–unsaturated aldehydes, ketones
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

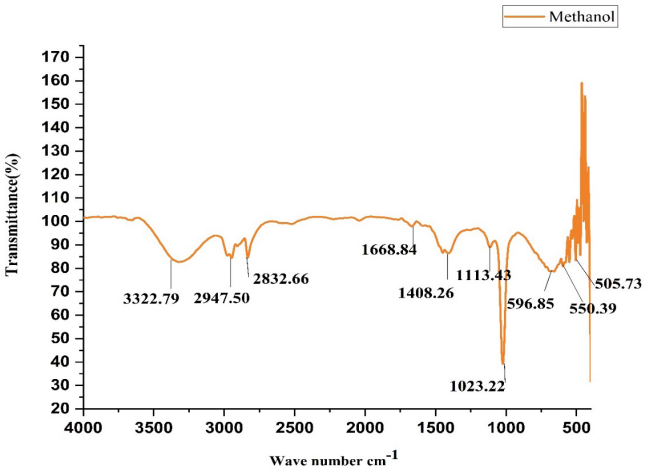

Figure 5: FTIR spectrum of methanol leaf extract of *P. benghalensis*

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

S. No	Wave Number (cm^{-1})	Molecular Motion	Functional group
1.	3317.11	–C≡C–H: C–H stretch	alkynes (terminal)
2.	2976.11	C–H stretch	alkanes
3.	2885.55	H–C=O: C–H stretch	aldehydes
4.	2832.39	H–C=O: C–H stretch	aldehydes
5.	1766.13	C=O stretch	α, β–unsaturated esters
6.	1599.76	C–C stretch (in–ring)	aromatics
7.	1382.38	C–H rock	alkanes
8.	1246.17	C–N stretch	aliphatic amines
9.	1087.31	C–N stretch	aliphatic amines
10.	1047.02	C–O stretch	alcohols, carboxylic acids, esters, ethers
11.	880.38	C–Cl stretch	alkyl halides
12.	694.12	–C≡C–H: C–H bend	alkynes
13.	596.23	C–Cl stretch	alkyl halides
14.	577.11	C–Cl stretch	alkyl halides
15.	544.58	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, peroxyl (RO_2•), alkoxyl (RO•), and hydroperoxyl (HO_2•) radicals. Collectively, these are referred to as reactive oxygen species (ROS). Additionally, significant radicals such as nitric oxide (NO•) and nitrogen dioxide (•NO_2) are derived from nitrogen and are categorized as reactive nitrogen species (RNS). Both ROS and RNS are typical byproducts of metabolic processes and can have either beneficial or harmful effects on the organism. At low concentrations, ROS and RNS serve to protect the body against infectious agents and are involved in various cellular signaling pathways. However, excessive production of ROS and RNS can lead to damage and impair the functionality of cellular lipids, proteins, and DNA, a phenomenon commonly known as oxidative stress or nitrosative stress.[27]

The present study investigates the antioxidant activity of several solvent extracts from the leaves of *P. benghalensis*. DPPH is a free radical commonly used to assess the radical scavenging activity of compounds, drugs, crude drugs, and plant extracts. The DPPH test evaluates the reactivity

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

		Petroleum ether	*Chloroform*	*Ethyl acetate*	*Methanol*	*Water*	*Standard*
Concentration	20 µL	17.20 ± 0.03	13.10 ± 0.0	17.90 ± 0.03	26.80 ± 0.00	9.10 ± 0.02	34.20 ± 0.00
	40 µL	38.90 ± 0.00	19.30 ± 0.0	26.10 ± 0.01	47.40 ± 0.04	22.60 ± 0.05	43.90 ± 0.02
	60 µL	51.20 ± 0.00	45.70 ± 0.0	42.50 ± 0.05	56.50 ± 0.03	38.60 ± 0.01	68.20 ± 0.06
	80 µL	61.80 ± 0.00	62.30 ± 0.01	68.50 ± 0.03	75.20 ± 0.02	65.00 ± 0.08	93.60 ± 0.03

Values represent the mean ± SD of three replicates.

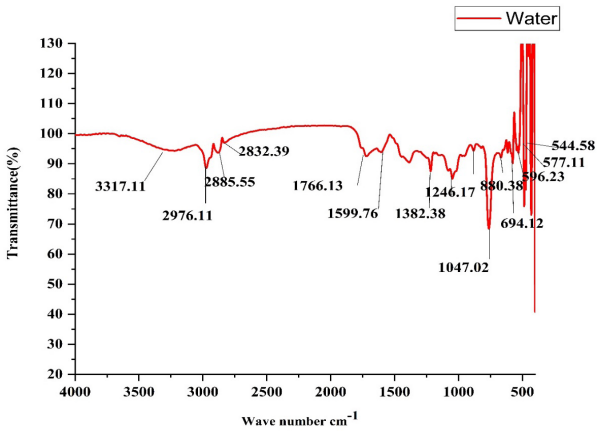

Figure 6: FTIR spectrum of water leaf extract of *P. benghalensis*

of test substances with a stable free radical. DPPH, with its unpaired electron, has a prominent absorption band at 517 nm in visible spectroscopy, resulting in a deep violet color. The presence of a free radical scavenger causes electrons to couple off, leading to stoichiometric decolorization. The scavenging properties of antioxidants are often linked to their ability to produce stable radicals.[28]

The scavenging activities of DPPH induced by the petroleum ether, chloroform, ethyl acetate, and methanol leaf extracts of *P. benghalensis*, along with ascorbic acid, have been summarized in Table 9. Overall, the highest antioxidant presence is in the methanol solvent compared to the standard (ascorbic acid).

CONCLUSION

The study highlights that methanol solvent extracts from *Pogostemon benghalensis* leaf contain numerous secondary metabolites, including steroids, triterpenoids, sugars, alkaloids, phenolic compounds, flavonoids, saponins, tannins, catechins, and amino acids. Methanol exhibited the highest phenol and flavonoid content compared to other solvents such as petroleum ether, ethyl acetate, and water. In FTIR analysis, petroleum ether, chloroform, ethyl acetate, methanol, and water are solvents that contain more functional groups in *P. benghalensis* leaves. The scavenging activities of DPPH induced by petroleum ether, chloroform, ethyl acetate, methanol, and water from leaf extracts of *P. benghalensis*, along with ascorbic acid, have been summarized. Overall, methanol shows the highest presence of antioxidants compared to the standard (ascorbic acid). Therefore, based on the research conducted, the developed phytochemicals and FTIR characteristics hold significant potential for pharmaceutical companies and research institutions in creating innovative medications. These findings contribute to the advancement of drug development by providing valuable insights into the chemical properties of medicinal compounds, enhancing their practical applications in the pharmaceutical field.

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PEER-REVIEWED CERTIFICATION

During the review of this manuscript, a double-blind peer-review policy has been followed. The author(s) of this manuscript received review comments from a minimum of two peer-reviewers. Author(s) submitted revised manuscript as per the comments of the assigned reviewers. On the basis of revision(s) done by the author(s) and compliance to the Reviewers' comments on the manuscript, Editor(s) has approved the revised manuscript for final publication.