

Weed to wonder: antioxidant and antibacterial investigation of *Parthenium hysterophorus* L.

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The overuse of modern synthetic pesticides for controlling insect-pest and plant diseases has led to environmental pollution, health risks, and the development of resistant bacterial strains, creating an urgent need for sustainable and eco-friendly alternatives. *Parthenium hysterophorus*, a widely distributed invasive weed, is known for its harmful effects but also contains bioactive compounds such as phenolics, flavonoids, tannins, and alkaloids. This study explores its potential as a natural source of antioxidant and antibacterial agents to address crop protection challenges. Aqueous leaf and flower extracts were analyzed for phytochemicals and bioactivity. Qualitative and quantitative profiling confirmed a high level of phenolics (13.5±9.49 mg GAE/g), flavonoids (22.60±0.52 mg QE/g), tannins (11.26±1.70 mg TAE/g), and alkaloids (717.27±211mg/g) in leaf extracts. Antioxidant evaluation revealed strong radical scavenging activity, with leaf extracts exhibiting lower IC₅₀ values in DPPH (604.88 µg/mL) and ABTS (644.9 µg/mL) assays compared to flower (1,028.74 µg/mL and 900 µg/mL, respectively). Reducing power was higher in leaves (256.92 mg AAE/g) than flowers (210.30 mg AAE/g). Fourier transform infrared spectroscopy (FTIR) revealed a strong broad peak at 4000–400 cm⁻¹, corresponding to O–H stretching, which is characteristic of phenolic and alcoholic compounds, and Gas chromatography mass spectroscopy (GC-MS) exhibited the Dimethyl sulfone as a major bioactive compound in both leaf (24.31%) and flower (32.83%) extracts. Antibacterial activity assessed by agar well diffusion demonstrated significant inhibition against *Pectobacterium carotovorum* (18 mm at 60 mg/mL) and *Ralstonia solanacearum* (11mm at 60 mg/mL), outperforming flower extracts. These findings highlight *P. hysterophorus* as a rich source of bioactive compounds with potent antioxidant and antibacterial properties, supporting its transformation from an invasive weed into a sustainable biocontrol agent for eco-friendly crop protection.

Keywords: crop protection, free radical scavenging, Gram-negative pathogens, *Parthenium hysterophorus*, phytochemicals, plant-derived metabolites

Introduction

Global agriculture is seriously threatened by the rising prevalence of plant illnesses brought on by phytopathogenic bacteria, which results in large output losses and decreased crop quality¹. Plant pathogens, *Ralstonia solanacearum* and *Pectobacterium carotovorum* are two Gram-negative bacteria. *R. solanacearum* yield losses are strongly influenced by the host, cultivar, climate, soil type, cropping pattern, and pathogen strains and reported yield losses ranging from 0–91% in tomatoes, 33–90% in potatoes, 10–30% in tobacco, 80–100% in bananas, and up to 20% in groundnuts, according to a

global analysis collated from numerous locations, including Asia and Africa². *P. carotovorum* caused bacterial soft rot has emerged as a significant worldwide problem affecting a range of vegetable crops and ornamental plant species, including tomato, onion, garlic, cucumber, potato, carrot, and cabbage³. This pathogen has resulted in notable production losses; estimates from 20% to 30% for cucumbers in China to over 40% for potato plants in Serbia. Another issue is storage losses; according to one study, they account for about 7.5% of potato production in the USA⁴. Chemical bactericides and antibiotics are frequently used extensively in traditional management tactics, which not only endanger the environment and non-target organisms but also fuel the development of resistant bacterial

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strains. As a result, the demand for environmentally friendly, sustainable substitutes for efficient plant disease management is rising⁵. Plant-based bioactive chemicals present promising substitutes in this regard. *P. hysterophorus*, a rapidly spreading invasive weed, is one such contender as it has a rich phytochemical profile that includes flavonoids, phenolics, and alkaloids, while being well-known for its detrimental impacts on agriculture, biodiversity, and human health, despite its invasive character, it has a great antioxidant potential owing to its high concentration of polyphenolic chemicals that scavenge free radicals⁶. Although *P. hysterophorus* is known to be a toxic and invasive plant that can cause respiratory and dermatitis in people, these risks are reduced by its control by utilizing its bioactive chemicals in the form of aqueous extract. Since the extracts are produced through water-based maceration and lyophilization, no hazardous solvent residues remain. Prior researches have shown that extract of *P. hysterophorus* can be used to protect plants when applied in controlled doses besides no negative impact non-target organisms⁵. The goal of the current study is to identify major functional groups and compounds in different parts of *P. hysterophorus* and to assess *P. hysterophorus* aqueous (water-based) extract's antibacterial activity against *R. solanacearum* and *P. caratovorum*.

Materials and methods

All chemicals used in this study, including sodium carbonate, ammonium molybdate, sulfuric acid, gallic acid, sodium phosphate, ascorbic acid, potassium persulfate, ferric chloride, potassium acetate, ammonium hydroxide, potassium dichromate, potassium ferricyanide, trichloroacetic acid (TCA), quercetin, DPPH, ABTS, and methanol, were procured from Hi-Media, India. Folin-Ciocalteu reagent and aluminum chloride were obtained from Central Drug House (P) Ltd., India. All chemicals and solvents were of analytical grade. A stock solution (1mg/mL) was prepared in methanol and double-distilled water and subsequently diluted to obtain the desired concentrations for working standards and sample analysis.

Identification and plant collection

Fresh leaves and flowers of *P. hysterophorus* were collected from the campus fields of the Indira Gandhi National Tribal University (IGNTU), Anuppur, Madhya Pradesh, India. A taxonomic expert, Dr. Arti

Garg, of the Botanical Survey of India, Allahabad, identified the plant specimen (BSI/RGC/GOI/2022-23/283). The leaves and flowers of *P. hysterophorus* were carefully rinsed to remove impurities, then shade-dried, finely pulverized into a powder, and stored at 4°C for future use.

Bacterial stains

The phytopathogenic bacterial strains were obtained from the Indian Type Culture Collection (ITCC), Division of Plant Pathology, ICAR-Agricultural Research Institute (IARI), New Delhi, India. *P. carotovorum* (ITCC No. BL0010) and *R. solanacearum* (ITCC No. BI 0004).

Plant extract preparation

A ten percent plant extract (leaf and flower) was prepared using distilled sterile water through the maceration process, further mixture was passed through Whatman No.1 filter paper, and the resulting aqueous extracts were concentrated through lyophilization (Labconco, USA), which involved freeze-drying to preserve the bioactive compounds present in the extract, and the extraction yield was calculated which was 14.2% for leaf extract and 11.6% for flower extract. The dried samples were stored and labeled as LE (leaf extract) and FE (flower extract).

Phytochemical analysis

Different methods have been employed for phytochemical analysis of leaf and flower extracts to determine the presence of phenol, flavonoid, tannin, glycosides, terpenoids, saponins, and quinone⁷.

Phenolic content

The phenolic content was assessed with a spectrophotometric method⁸. An aliquot of 500 µL, from each extract was combined with Folin-Ciocalteu reagent (10%) and Na₂CO₃ (7.5%), ensuring thorough mixing. A blank sample was also prepared as control. After 45 min. standing in the dark, a spectrophotometer measured absorbance at a wavelength of 730 nm. Each measurement was conducted thrice and expressed as GAE mg/g dry weight.

Flavonoid content

The total flavonoid content was estimated using the AlCl₃ colorimetric method⁹. Extracts were diluted to 1 mg/mL, and a calibration curve with quercetin concentrations of 20 to 100 µg/mL was established. The mixture of 2 mL diluted extracts, quercetin, 10% AlCl₃, and 0.1 mM CH₃COOK was incubated at 30°C

for 40 min. Optical density was measured at $\lambda_{\text{max}} = 415\text{nm}$. QE mg/g dw(dry weight) of extract was utilized to determine the overall flavonoid concentration.

Total tannin content

The total tannin content (TTC) was determined using the Folin–Ciocalteu method¹⁰. A 100 μL aliquot of the sample extract was mixed with 7.5 mL of distilled water and 0.5 mL of Folin–Ciocalteu reagent. Subsequently, 1 mL of 35% sodium carbonate (Na_2CO_3) solution was added, and the final volume was made up to 10 mL with distilled water. The mixture was thoroughly shaken and incubated at 37 °C for 45 minutes. Tannic acid standard solutions (20–100 $\mu\text{g}/\text{mL}$) were prepared following the same procedure. The absorbance was measured at $\lambda_{\text{max}} = 700\text{ nm}$, and the total tannin content was expressed as mg tannic acid equivalent (TAE) per gram of dw.

Total alkaloid content

Preparation of Reagents

To prepare a standard solution, 0.1mg/mL of atropine was prepared using D/W. A bromocresol green (69.8) solution was prepared with 3 mL of 2N sodium hydroxide and 5 mL of D/W, followed by dilution to a final volume of 1 liter with D/W further, a Phosphate buffer of pH 4.7 was prepared¹¹.

Alkaloid separation

One milliliter of the filtrate was placed in a separatory funnel and rinsed three times with 10 mL of chloroform. Afterward, 5 mL each of bromocresol green and phosphate buffer was added. The mixture was shaken thoroughly to form a complex, which was then extracted with 1-4mL of chloroform. The chloroform phase was collected, and its absorbance was recorded at 470nm using a UV spectrophotometer¹¹.

Antioxidant assay

DPPH assay

A DPPH assay was used to assess free radical scavenging activity. A 0.004% DPPH solution in methanol (0.98 ± 0.02 at 515 nm) was mixed with 0.2 mL of the sample or standard (20–260 $\mu\text{g}/\text{mL}$) and incubated in the dark at room temperature for 30 min. A control used methanol instead of the sample, and ascorbic acid was used as the positive control¹². The scavenging activity was calculated as:

$$\frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

ABTS assay

The ABTS assay was carried out with slight modifications. A 7 mM ABTS solution was mixed with 2.45 mM $\text{K}_2\text{S}_2\text{O}_8$ and kept in the dark for 16–18 hours to generate the ABTS^+ radical. Afterward, 3mL of ABTS^+ solution was added to 300 μL of the leaf and flower extracts, and the OD was measured at 734 nm. Ascorbic acid was used as the positive control¹³. Scavenging% was calculated as

$$\frac{\text{Absorbance of control} - \text{absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Reducing power assay

Reducing power was assessed by mixing 1mL of extract with phosphate buffer and potassium ferricyanide, then incubating at 50°C for 20 min. After adding trichloroacetic acid, the mixture was centrifuged, and the supernatant was treated with FeCl_3 . Absorbance was measured to evaluate reducing power, a calibration curve was prepared using ascorbic acid standard (20-100 $\mu\text{g}/\text{mL}$), and the absorbance of the sample was plotted against this curve. The reducing power of extracts was expressed as mg AAE/g¹⁴

Fourier transform infrared spectroscopy

Fourier transform infrared (FTIR) spectroscopy was employed to identify the functional group present in the leaf and flower extracts of *P. hysterothorus*. The analysis was performed using a Bruker FTIR spectrophotometer equipped with an attenuated total reflectance (ATR) accessory. A small amount of the dried sample was placed directly to the ATR crystal without any further preparation. The spectra were recorded in the mid-infrared region, ranging from 4000 to 400 cm^{-1} , with a resolution of 4 cm^{-1} . Each spectrum was obtained by averaging 32 scans to improve the signal-to-noise ratio. Background correction was performed prior to sample analysis to eliminate atmospheric interference. The characteristic absorption peaks were analyzed to identify various functional groups, including hydroxyl (O-H), carbonyl (C=O), alkyl (C-H), and aromatic groups, present in the extract¹⁵.

Gas chromatography-mass spectroscopy (GC-MS)

Gas chromatography-mass spectrometry analysis was carried out using a Shimadzu GC-MS-QP2020 NX system to characterize the chemical constituents of the samples. The prepared extracts were filtered through a 0.22 μm syringe filter and transferred into autosampler vials for analysis. One microliter (1 μL)

of the sample was injected in splitless mode, with the injector temperature set to 250°C. The analysis was performed using a capillary column (RTX-5MS, 30 m × 0.25 mm internal diameter, 0.25 µm film thickness). Helium was employed as the carrier gas at a constant flow rate of 1.5mL/min, with a column pressure of 88kPa and a total flow of 19.5mL/min. The purge flow was maintained at 3 mL/min with a split ratio of 10:1. The oven temperature program was initiated at 50°C with a 2 min hold, increased to 200°C at a ramp rate of 20°C/min and hold for 2 min, and finally raised to 280°C at the same ramp with a final hold time of 5min. The interface temperature was maintained at 280°C, and the ion source temperature was set at 230°C. The mass spectrometer operated in electron ionization mode, scanning in the m/z range of 35-500 with a scan speed of 1666 amu/sec. The solvent cut time was set to 3 min. to eliminate the solvent interface. The compound identification was carried out by comparing the acquired mass spectra with reference data from the NIST 20 mass spectral library.

Antibacterial activity

Antibacterial activity was evaluated using the agar well diffusion method¹⁶ against *P. carotovorum* and *R. solanacearum*. Luria-Bertani agar (LBA) medium was poured into a sterile Petri plate, and bacterial cultures incubated for 24 hours were utilized to inoculate. Fifty microliters of the bacterial suspension were disseminated onto each LBA plate. A sterile cork borer of 5 mm diameter was used to create wells once the agar plate had solidified. Furthermore, 90 µL of the plant extract at varying dosages (30 and 60mg/mL) was added to the corresponding wells. The plates were subsequently kept at 37°C for 12-24 hours. The antibacterial effectiveness was assessed by measuring the diameter of the inhibition zone around the wells.

Statistical analysis

Results were presented as mean ± SD (Standard deviation) from triplicate experiments using GraphPad Prism software (8.0.1 version).

Results

Assessment of phytochemicals qualitatively

Medicinal plants have attracted considerable attention from the global medical sectors due to their enhanced biological activities and fewer side effects. The cosmetic and pharmaceutical industries harness bioactive compounds such as vitamins, dyes, antioxidants, and oils, along with various micro and macro-nutrients extracted from different parts of these plants. In this study, the qualitative phytochemical analysis of aqueous leaf and flower extracts of *P. hysterophorus* revealed the presence of various phytoconstituents, including alkaloids, tannins, phenols, glycosides, terpenoids, quinones, flavonoids, and saponins. The results revealed a diverse phytochemical composition, with phenols, tannins, glycosides, and alkaloids notably more abundant in leaf than in flower extracts (Table 1).

Assessment of phytochemicals quantitatively

The total alkaloid content in the leaf extract was significantly higher at 717.27±21 mg/g dw compared to the flower extract (598.15±45 mg/g dw). The leaf extract also contained more phenolics, 13.5±9.49 mg/g dw, and flavonoids, 22.60±0.52 mg/g dw, than the flower extract with 13.26±1.00 mg/g dw and 18.50±0.06, respectively. Additionally, tannin content was more significant in the leaf (11.26±1.70 mg/g dw) as compared to flower extract (8.65±0.40 mg/g dw) (Fig. 1 & Table 2).

Antioxidant assay (DPPH, ABTS, and FRAP)

Our study demonstrated considerable antioxidant activity in the leaf and flower extracts of

Table 1 — A qualitative study of the compounds identified in *P. hysterophorus*.

Phytochemicals	Leaf	Flower	Test/Method
Alkaloids	+	-	Mayers test
Tannins	++	+	Ferric chloride
Phenol	++	++	Ferric chloride
Glycosides	++	+	Bontrager's test
Terpenoids	-	+	Salkowski test
Quinone	-	-	Sulphuric acid test
Flavonoids	++	+	Alkaline reagent test
Saponin	++	+	Foam test

++ represents the highest amount of phytochemicals, + represents the moderate amount of phytochemicals, - represents the absence of phytochemicals

P. hysterophorus, as measured using the DPPH, ABTS, and ferric-reducing power assays. In the DPPH experiment, the leaf extract displayed a greater inhibition percentage (22.81%) as compared to the flower extract (13.13%) at the highest dosage of 260 µg/mL. The IC₅₀ values further corroborated this trend, with the leaf extract showing a lower IC₅₀ (604.88 µg/mL) than the flower extract (1028.74 µg/mL), indicating a stronger free radical scavenging capacity. Similarly, in the ABTS results, both extracts demonstrated equal inhibition rates, with the leaf extract showing 29.80% and the floral extract 29.50% inhibition at 260.00 µg/mL (Fig. 2). The IC₅₀ values were 644.9 µg/mL (leaf extract) and 900.09 µg/mL

(flower extract), indicating the stronger antioxidant potential of the leaf extract. Furthermore, the ferric reducing power assay demonstrated that the reducing capacity of the leaf extract (256.92 AAE/g) was greater than flower extract (210.30 AAE/g), as assessed in ascorbic acid equivalents per gram of extract. These data collectively indicated that *P. hysterophorus* leaf extract had greater antioxidant capacity than its flower extract, underscoring its usefulness as a potent regulator of free radicals.

Fourier transform infrared spectroscopy

Fourier Transform Infrared (FTIR) spectroscopy is a widely used analytical technique for identifying pharmacologically active molecules in natural products by analyzing the peak values in the FTIR spectrum. This technique provides a unique chemical fingerprint of bioactive compounds, as no two molecules exhibit identical FTIR spectra. In the present study, FTIR analysis was conducted on *P. hysterophorus* both leaf and flower parts to elucidate the functional groups present in these extracts. Both spectra displayed multiple absorption peaks corresponding to various functional groups (Table 3) (Fig. 3 & 4). The similarity in the IR profile of leaf and flower extracts suggested the presence of common polar metabolites in both extracts. The FTIR analysis of *P. hysterophorus* flower and leaf extracts revealed a range of functional groups indicative of diverse bioactive metabolites.

Gas chromatography mass spectrometry

The GC-MS analysis of the leaves and flowers of *P. hysterophorus* revealed a diverse array of

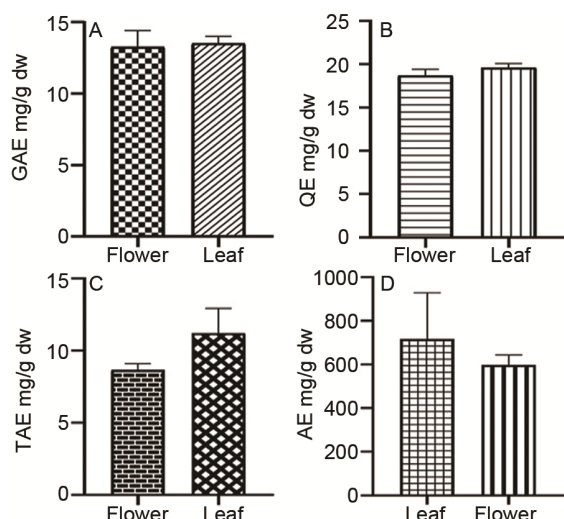


Fig. 1 — Quantitative assay of *P. hysterophorus* Leaf and flower extract (A) Total phenolic content (B) Total flavonoid content (C) Total tannin content (D) Total alkaloid content.

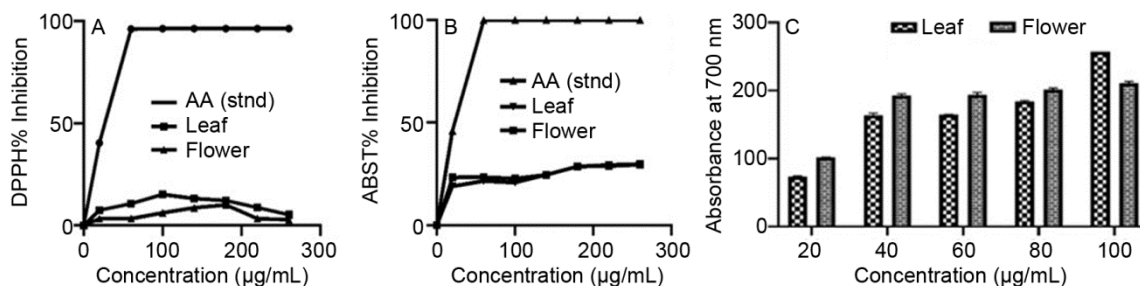


Fig. 2 — The radical scavenging activity of *P. hysterophorus* was assessed, (A). DPPH assay (B). ABTS assay, and (C). Reducing power assay expressed as absorbance at 700nm, values were also converted to mg AAE/g using an ascorbic acid calibration curve.

Table 2 — Antibacterial activity of *P. hysterophorus* (leaf and flower extract)

Sample name	Concentrations	<i>P. carotovorum</i>	<i>R. solanacearum</i>
Leaf extract	30mg/ml	13±0	6±1
	60mg/ml	18±1.4	11±1.4
Flower extract	30mg/ml	13±0.5	0
	60mg/ml	16±2.8	6±2.0

Table 3 — FTIR absorption bands of *P. hysterophorus* leaf and flower extracts with corresponding functional group and literature references. The wavenumber values represent the characteristic vibrational frequencies associated with specific chemical bonds indicative of phenolics, alcohols, esters, alkanes, aldehydes, and amines present in the extract

Leaf extract Wavelength (cm ⁻¹)	Flower extract Wavelength (cm ⁻¹)	Functional group	References
3399.74	3401.51	O-H stretching (phenol, alcohol)	18
2999-2927	2992-2921,2822	C-H stretching (alkanes, aldehydes)	19
1653	1677	C=O stretching (carbonyl compounds, ketones, esters)	20
1453-1244	1453-1240	C-H bending, C-O stretching (esters, phenolics)	18
1015-531	1019-532	C-N stretching (amines, aromatics)	21

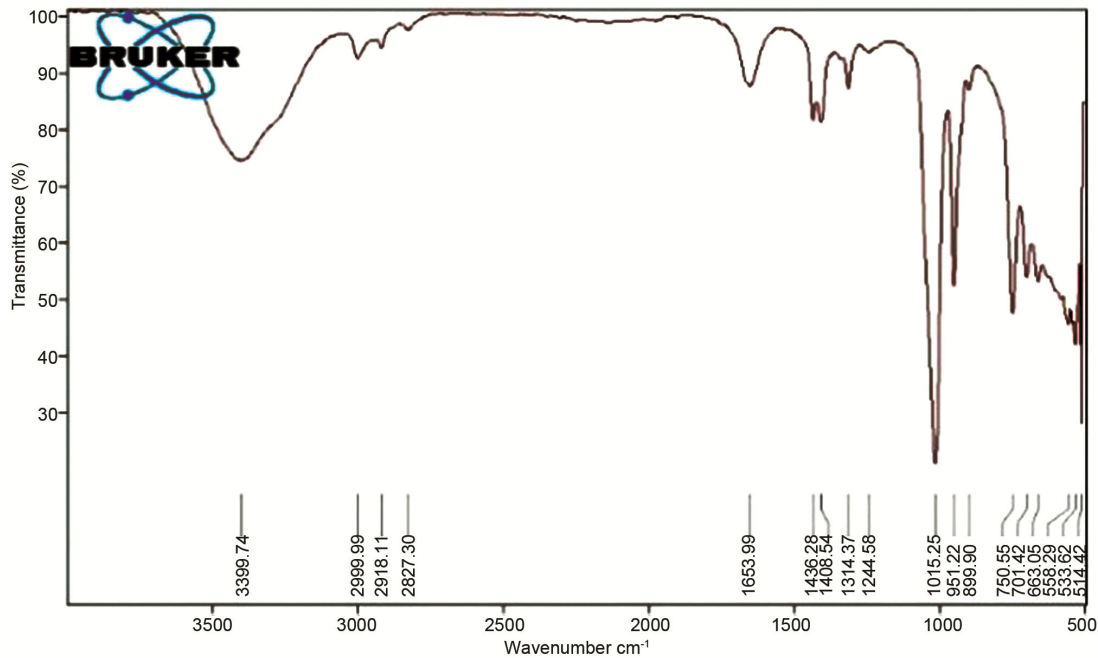


Fig. 3 — FTIR spectrum of *P. hysterophorus* leaf extract. The characteristic absorption bands represent functional group.

phytochemicals. The leaf extract exhibited 29 peaks, whereas the flower extract contained 27 peaks (Fig. 5), indicating a slightly higher chemical diversity in the leaves. Among the compounds identified, dimethyl sulfone emerged as the predominant constituent in both extracts, accounting for approximately 24.31% in the leaf extract and 32.83% in the flower extract. Other major components included dimethylsulfoxonium formylmethylide, benzaldehyde derivatives such as benzaldehyde, 4-propyl-, and long-chain fatty acid, notably n-hexadecanoic acid (palmitic acid) and octadecanoic acid (stearic acid), along with their methyl esters (Table 4 & 5). These compounds have been extensively associated with biological activities.

Antibacterial assay of crude extracts of *P. hysterophorus*

P. hysterophorus extracts exhibited antimicrobial properties. *In vitro* antibacterial activity of the leaf

and flower extracts was evaluated by measuring the zone of inhibition (ZOI) (Table 2). The leaf extract inhibited *P. carotovorum* at 60 mg/mL and 30 mg/mL concentrations, with ZOI of 18mm and 13mm, respectively. The flower extract showed ZOI of 16 mm and 13 mm at the same concentrations. Both leaf and flower extracts inhibited *R. solanacearum* at 60 mg/mL and 30 mg/mL concentrations, with 11 mm and 6 mm zoi, respectively, while the flower extract showed 6 mm and no inhibition. Nalidixic acid (NA) was used as a positive control, and distilled water (D/W) served as a negative control (Fig. 6).

Discussion

The present study focused on the phytochemical analysis of *P. hysterophorus* extracts and on their antioxidant and antibacterial activities against plant pathogens. Phytochemical analysis indicated that

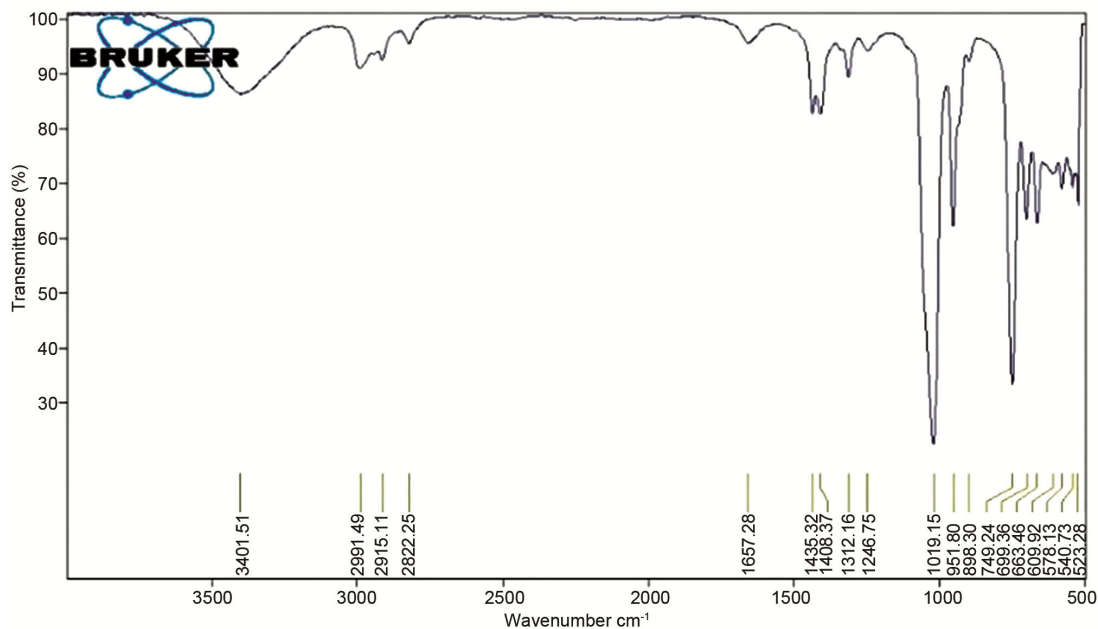


Fig. 4 — FTIR spectrum of *P. hysterophorus* flower extract. The characteristic absorption bands represent functional group.

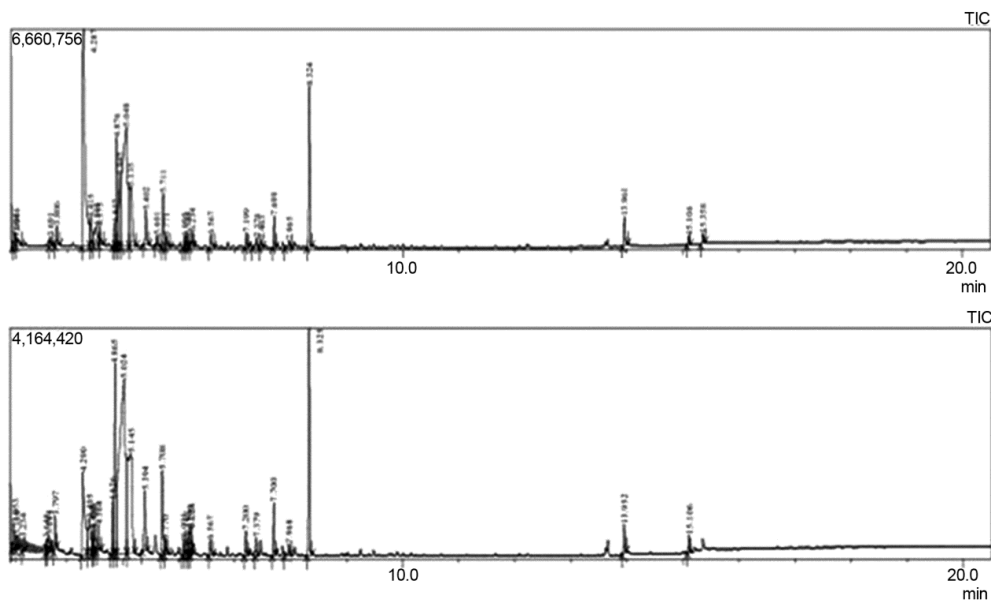


Fig. 5 — GC-MS chromatograms of *P. hysterophorus* leaf and flower extracts, respectively. The peaks correspond to bioactive phytoconstituents identified based on their retention time and spectral fragmentation patterns.

Table 4 — Major compounds identified in the leaf extracts of *P. hysterophorus* by GC-MS analysis.

Retention Time	Area (%)	Name of the compound
5.048	24.31	Dimethyl sulfone
4.287	22.68	Dimethylsulfoxoniumformylmethylide
4.876	6.18	Ethane, 1,1,2,2-tetrachloro-

Table 5 — Major compounds identified in the flower extracts of *Parthenium hysterophorus* by GC-MS analysis.

Retention Time	Area %	Name of the compound
5.024	24.31	Dimethyl sulfone
4.865	8.07	Ethane, 1,1,2,2-tetrachloro-
4.290	6.25	Dimethylsulfoxoniumformylmethylide

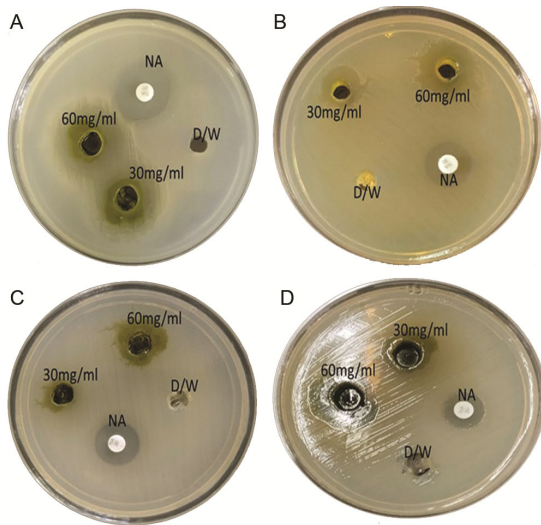


Fig. 6 — Antibacterial extract of *P. hysterophorus* extracts against phytopathogenic bacteria (A) *P. carotovorum* (leaf extract), (B) *P. carotovorum* (flower extract), (C) *R. solanacearum* (leaf extract) (D) *R. solanacearum* (flower extract) at concentrations of 30 and 60mg/mL, with positive (NA) and negative (D/W) control.

water-soluble compounds are known for their various biological activities. Phenols and tannins are reported to have antioxidant and antimicrobial properties, making them valuable in therapeutic applications¹⁷. Terpenoids and alkaloids are recognized for anti-inflammatory and antitumor properties¹⁸. The presence of glycosides suggests their widespread distribution throughout the plant, contributing to its overall bioactivity. Among the phytochemicals analyzed, the leaf extract showed a particularly abundant amount of all tested compounds, suggesting that the leaf of *P. hysterophorus* is a rich source of bioactive compounds when extracted with water, consistent with earlier studies emphasizing the medicinal potential of leaf extracts¹⁹. Compared with the flower, leaves contained approximately 20% more total alkaloids, 30% more tannins, and 22% more flavonoids, whereas phenolic content showed only a marginal increase of 1.8%. In summary, the content of total alkaloid, total phenols, total flavonoid, and total tannin was all elevated in leaf extract compared to flower extract. Comprehensive, the content of total alkaloids, total phenols, total flavonoids, and total tannins was all elevated in the leaf extract compared to the flower extract. Contrary to the present results, in an earlier investigation, the flower extract of *P. hysterophorus* exhibited more flavonoid concentration than the leaf, but less than the present findings¹⁶. Iqbal *et al.* (2022) reported higher phenolic content in the methanolic extract than in the current

study²⁰. The variation in the quantitative data from previous studies on leaves relative to flowers can be ascribed to genetic variation and micro-environmental influences. The antioxidant effect on DPPH (2,2-diphenyl-1-picrylhydrazyl) is due to its electron-donating capacity²¹. Radical scavenging is essential for reducing the harmful effects of free radicals, and the DPPH method is widely used to evaluate the antioxidant activity²², indicating the presence of phenolics and flavonoids. Key phenolic compounds in *P. hysterophorus* includes caffeic acid, ferulic acid, p-coumaric acid, quercetin, kaempferol, and ellagic acid were identified in *P. hysterophorus*; these compounds play key role as antioxidants by targeting bacterial cell walls and inhibiting oxidative damage²³. Antioxidants in plant extracts can donate hydrogen atoms to lipid peroxides, forming non-radical species and interrupting lipid peroxidation²⁴. The reaction between ABTS and potassium persulfate ($K_2S_2O_8$) produces ABTS cation radicals, which indicate the hydrogen/electron-donating or reducing capacities of plant extracts, thus neutralizing their harmful effects. The higher the inhibition percentage, the greater the antioxidant activity. The greater scavenging power correlates with their elevated levels of flavonoids, polyphenols, and proanthocyanidins. These findings supported the potential of *P. hysterophorus*, particularly its capability to neutralize free radicals and oxidative damage and inhibit the propagation of lipid peroxidation reactions. This protective mechanism could have significant implications for reducing the risk of diseases linked to oxidative stress, including heart disease, tumors, diabetes, and neurodegenerative conditions²⁵, and could serve as a potential nutraceutical. Compared with highly active antioxidant herbs such as rosemary (DPPH IC_{50} 13.4 $\mu\text{g/mL}$)²⁶, the crude *P. hysterophorus* leaf and flower extracts (IC_{50} 605 and 1,029 $\mu\text{g/mL}$) are relatively weak, however they are stronger than some nonpolar crude fractions like *Psidium* chloroform fraction, IC_{50} 1,103 $\mu\text{g/mL}$ ¹⁶, suggesting that solvent choice and fractionation could substantially improve apparent activity. The reducing power of the *P. hysterophorus* leaf extract was assessed through the ferric reducing power assay. The capacity of antioxidants to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) is the basis for reducing power²⁷. The transition from extracts (leaf and flower) to the blue color indicated its reducing activity, assessed by measuring absorbance at 700 nm²⁸. The values found in this

study suggested that the leaf extract had more potent antioxidant activity than flower because it includes bioactive components that can donate electrons and neutralize free radicals. The leaf extracts phenolic components, flavonoids, which are known for their antioxidant qualities and may be the cause of the observed reducing power. Phenolic chemicals are essential for scavenging free radicals and halting oxidative damage. In the flower extract, a broad O-H stretching band at 3401 cm^{-1} suggests the presence of hydroxyl-rich compounds such as phenols or alcohols, C-H stretching vibration at $2991\text{-}2921\text{ cm}^{-1}$ and 2822 cm^{-1} are characteristics of aliphatic chains, alkanes, and aldehydes²⁹, respectively, a pronounced peak at 1677 cm^{-1} corresponds to C=O stretching of carbonyl moieties (ketones, esters), bands in the $1453\text{-}1240\text{ cm}^{-1}$ region are attributable to C-H bending and C-O stretching, commonly associated with esters and phenolic compounds and absorptions within the fingerprint region ($1019\text{-}532\text{ cm}^{-1}$) point to C-N stretching and complex skeletal vibration, suggesting the presence of amines, aromatic, and alkyl halide structures³⁰. The leaf extract mirrored this profile, exhibiting an O-H band at 3399 cm^{-1} , C-H stretching at $2999\text{-}2927\text{ cm}^{-1}$, a sharp C=O peak at 1653 cm^{-1} , and corresponding bending and fingerprint region vibrations in the $1453\text{-}1244\text{ cm}^{-1}$ and $1015\text{-}531\text{ cm}^{-1}$ ranges³¹. Together, these spectra confirmed the existence of phenolics, alcohols, esters, alkanes, aldehydes, and amines in both extracts, though FTIR alone cannot definitively identify compound classes, and further analytical profiling is recommended for comprehensive phytochemical characterization³². In the current investigations, GC-MS analysis revealed that dimethyl sulfone was the major component in both leaf and flower extracts; the percentage of compound content varies in both plant extracts. Dimethyl sulfone is known for its antioxidant and anti-inflammatory potential³³, whereas benzaldehyde derivatives possess antimicrobial and antibiofilm properties³⁴. Fatty acids such as palmitic and stearic acids contribute to antimicrobial activity by disrupting microbial membrane, in addition to supporting oxidative stability, thus indicating antioxidant relevance. The detection of halogenated hydrocarbons, including ethane, 1,1,2,2-tetrachloro-, is likely due to analytical artifacts or environmental contamination rather than plant metabolism²⁴. Similarly, 1(2H)-Naphthalenone, 6-(1,1-Dimethylethyl) Octahydro-2,8A-Dimethyl- was found to be major in

the amount in the leaf extract³⁵, and Histidine, 1, N-dimethyl-4-nitro was found to be in major quantity in flower extract¹⁹. However, the chemical composition observed in this study supports the pharmacological importance of *P. hysterophorus*, particularly its antimicrobial and antioxidant potential, consistent with previous phytochemical investigations. Leaf extracts exhibited the maximum inhibition zone compared to flower extracts. Various phytochemical constituents isolated from *P. hysterophorus* have exhibited significant bactericidal and antioxidant properties. Some of the key compounds identified include Parthenin and hysterin¹⁹, which are major sesquiterpene lactones isolated from *P. hysterophorus*, showing antimicrobial activity against multiple bacterial strains, such as *Staphylococcus aureus* and *Escherichia coli*³⁵. These compounds are known to disrupt bacterial cell membranes, increase permeability and cause leakage of essential intracellular contents. In addition, they bind to key bacterial enzymes, inhibit nucleic acid synthesis and interfere with energy metabolism, ultimately leading to cell death, similar mechanism have been documented for phenolic-rich plant extract where hydroxyl group in phenolic compounds interact with bacterial proteins, resulting in structural destabilization, enzyme inactivation, and oxidative stress induction³⁶, in the present study, several important phenolic and flavonoid compounds were detected, including ferulic acid, caffeic acid, ellagic acid, quercetin, and luteolin²⁴. These compounds play a dual role as antioxidant and antibacterial agents by targeting the bacterial cell wall and inhibiting oxidative damage³⁷. Furthermore, the higher abundance of alkaloids, flavonoids, phenols, tannins, and glycosides in leaf extract compared to flower likely explains their greater antibacterial potency, as these metabolites collectively contribute to cell wall disruption, oxidative stress induction, and metabolic inhibition in pathogens³⁸.

Conclusion

The present study successfully demonstrated that aqueous extracts of *P. hysterophorus* leaves and flowers possess significant antioxidant and antibacterial activities, aligning with the objective of exploring eco-friendly alternatives for crop protection. The leaf extract exhibited higher levels of flavonoids, tannins, and alkaloids compared to the flower extract, contributing to its superior radical scavenging capacity in DPPH and ABTS assays, as

well as greater reducing power. FTIR and GC-MS analyses further confirmed the presence of bioactive functional groups and phytochemicals, including dimethyl sulfone, fatty acids, and benzaldehyde derivatives. The leaf extract also exhibited notable antibacterial activity against *P. carotovorum* and *R. solanacearum*, achieving inhibition zones of 18 mm and 11 mm, respectively, at a concentration of 60 mg/mL. These findings underscore the potential of *P. hysterophorus* as a natural source of antioxidant and antibacterial compounds, supporting its application as a sustainable biocontrol agent in agricultural settings. Further research, including purification and structural characterization of antioxidant and antibacterial compounds, as well as *in vivo* evaluation, is necessary to validate its efficacy and safety for practical use.

Ethics declaration

Not applicable

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Conflict of interest

The authors declare that they have no conflict of interest.

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Supplementary information

Supplementary data to this article can be found.

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