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RESEARCH ARTICLE

Phytochemical characterisation of *Parthenium hysterophorus* L. extracts and their antioxidant and antimicrobial activities

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Abstract

Parthenium hysterophorus L. is a noxious and highly invasive weed known for its adverse effects on livestock and human health. Despite its negative impact, the plant possesses notable therapeutic potential. This study investigated the phytochemical composition, conducted quantitative analyses and evaluated the antioxidant and antibacterial activities of various *P. hysterophorus* extracts. The crude extract of methanol and ethyl acetate of different plant parts was prepared using maceration techniques and antibacterial activity of its crude extract through the agar well diffusion method. The methanol leaves extract exhibited the highest phenolic content (20.38 ± 0.02 mg/g GAE [gallic acid equivalent]), followed by the methanol flower extract (18.99 ± 0.02 mg/g GAE). The ethyl acetate flower extract showed the highest flavonoid content (123.98 ± 5.7 mg/g QE [quercetin equivalent]), while the methanol stems extract contained the most alkaloids (566 ± 6.14 mg/g AAE [ascorbic acid equivalent]). In terms of antioxidant activity, the methanol flower extract demonstrated greater DPPH radical scavenging capacity (IC_{50} = 482.99 µg/mL) compared to the ethyl acetate leaves extract (IC_{50} = 637.63 µg/mL). The strongest ABTS scavenging activity was observed in the ethyl acetate leaf extract (IC_{50} = 79.04 µg/mL) and the methanol flower extract exhibited superior ferric reducing antioxidant power. Antibacterial testing revealed the highest activity against *Microbacterium* sp. (26.33 ± 0.57 mm at 60 mg/mL) in the ethyl acetate leaf extract. The extract effectiveness against all tested bacterial strains ranked as follows: methanol flower > ethyl acetate leaf > methanol leaf > methanol root > ethyl acetate root > methanol stem. The Ultraviolet–Visible (UV-Vis) spectroscopy and Fourier transform infrared (FTIR) spectroscopy analyses further confirmed the presence of key bioactive compounds. These findings highlight the potential of *P. hysterophorus* as a source of natural antioxidant and antimicrobial agents, which may serve as alternatives to synthetic compounds.

Keywords: antibacterial agent; antioxidant; *Parthenium hysterophorus*; phytochemicals

Introduction

The plant kingdom harbours a vast array of bioactive compounds with significant therapeutic potential, including notable antimicrobial and antioxidant properties (1). Since ancient times, plants have been widely used in traditional medicine to treat a variety of ailments. According to the World Health Organization (WHO), approximately 25 % of modern pharmaceuticals are derived from traditional plant-based medicines (1). In India, this figure is even higher, with nearly 70 % of contemporary medicines tracing their origins to natural sources (2).

In recent decades, chronic diseases such as cancer, diabetes and cardiovascular disorders have been increasingly linked to oxidative stress a condition that arises when the production of free radicals in the body exceeds its ability to neutralise them. The WHO estimates that oxidative stress-related disorders contribute to nearly 70 % of deaths worldwide. Although synthetic antibiotics and antioxidants such as alkyl gallates, butylated hydroxy toluene (BHT) and tert-butylhydroquinone (TBHQ) are commonly used to combat oxidative and microbial threats, their overuse has led to antibiotic resistance and potential health risks (2). Additionally, synthetic drugs can be costly and are often accompanied by side effects, prompting

a shift towards phytochemicals derived from medicinal plants. Studies have shown that plant-based antioxidants, which are abundant in fruits and vegetables, can offer protective effects against aging, cancer, stroke and other chronic illnesses (3). However, dietary intake alone may be insufficient, highlighting the growing importance of natural antioxidants in the form of dietary supplements and therapeutic agents (4, 5).

One such plant of interest is *Parthenium hysterophorus* L., a highly invasive species native to the Americas but now widely distributed across the globe due to its adaptability and aggressive growth. Its rapid spread disrupts native ecosystems and agricultural productivity, earning it a reputation as an ecologically and economically harmful weed (6); and also poses direct health risks, as its pollen, leaves and sap can cause allergic dermatitis, asthma, hay fever and other respiratory problems in humans and ingestion or contact can lead to dermatitis, mouth ulcers and even death in livestock (7). Despite its adverse impacts, *P. hysterophorus* has drawn attention for its complex phytochemistry and potential medicinal applications. Various parts of the plant leaves, flowers, stems and roots-contain a wide range of bioactive compounds including sesquiterpene lactones, flavonoids, phenolic acids (e.g., ferulic, caffeic, chlorogenic) and other aromatic compounds (8, 9).

These constituents are not only responsible for the plant's phytotoxic effects such as inducing allergic dermatitis and inhibiting crop growth but also contribute to its traditional use in treating multiple ailments. Historically, the whole plant has been employed as an anti-dysenteric, febrifuge, emmenagogue and tonic. Ethnomedical records suggest that *P. hysterophorus* was used to treat wounds, ulcers, anaemia, fever and heart conditions (10, 11). It has also demonstrated utility in managing neurological, inflammatory and dermatological conditions and is noted for its analgesic and vermifuge properties (12–14).

Despite extensive reports on the toxicity and invasiveness of *P. hysterophorus*, comparative studies evaluating the antioxidant potential of different plant parts using multiple assays and solvent systems are limited. The relationship between solvent polarity, plant part-specific phytochemical composition and antioxidant efficacy remains insufficiently explored. Given its rich phytochemical profile and traditional medicinal value, this study aims to conduct a detailed analysis of the phytochemical composition of different parts of *P. hysterophorus* and evaluate their antioxidant and antimicrobial activities. This integrated approach will help better understand the plant's therapeutic potential and contribute to the development of novel natural remedies.

Materials and Methods

The plant material (*P. hysterophorus*) was collected from non-protected areas within the campus, Indira Gandhi National Tribal University Amarkantak, Madhya Pradesh, at (22°48'18" N, 81°45'3" E) between June and July and does not involve any endangered or protected species. Proper identification and documentation were carried out and no specific permissions were required as per local regulations. Leaf, root, stem and flower parts were washed thoroughly with tap water to eliminate soil particles. A grinder was used to grind the plant material into a fine powder after it had been cut up into tiny pieces and air dried in the shade at room temperature. The powder was weighed, kept in air tight container to prevent contamination and stored in freezer at 20 °C for the remainder of the extraction procedure (Fig. 1). A specimen was submitted to the Botanical Survey of India in Allahabad in a herbarium for identification and the creation of a voucher specimen No. B.S.I./C.R.C/Admn./2022-23/283.

Fig. 1. Whole plant of *Parthenium hysterophorus* and dried plant parts. A- leaf; B- stem; C- root; D- flower.

Chemical and reagents

Many of the chemicals, which included quercetin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid, gallic acid, sodium bicarbonate, sodium phosphate, sodium phosphate monobasic, sodium phosphate dibasic, potassium ferricyanide, TCA (trichloroacetic acid), ABTS reagent 2,2'-azinobis-(3-ethylbenzothiaziline-6-sulphonate) and Folin-Ciocalteu were acquired from Hi- Media, India. Analytical grade chemicals were used throughout the experiment. In the working experiment, the stock solution (1 mg/mL) was diluted with polar and non-polar solvent, buffer and double-distilled water to obtain the final concentration for the standard and samples.

Plant extraction procedure

The phytochemical extraction was carried out by combining a fine powder in a 1:10 ratio with the extraction solvent (methanol and ethyl acetate). The selection of both solvent is based on their polarities, which allows efficient extraction of phytochemicals, including alkaloids, phenolics, flavonoids and terpenoids, thereby enabling a broader phytochemical profiling. The extraction was done in a magnetic stirrer for 24 hr at 1000 rpm, filtered through Whatman filter paper and concentrated in a water bath for 90 min at 35 °C. Prior to use, the leftover extracts were weighed and kept at 4 °C for further analysis (15).

Phytochemical analysis

The phytochemical assessment was carried out for major compounds such as carbohydrates, tannins, quinones, terpenoids, phenolics, alkaloids, flavonoids, glycoside following standard methods and protocol (16).

Determination of total phenolic content

The Folin-Ciocalteu method was used to determine the total phenolic content (TPC) of a plant extract. The extract was combined with a diluted folin-Ciocalteu reagent and sodium carbonate and the absorbance was measured using a UV-visible spectrophotometer at 766 nm. The calibration curve of gallic acid as standards: 20, 60, 100, 140, 180, 220 and260 µg/mL. The total phenolic component was quantified as GAE/g of plant extract (17).

Determination of total flavonoid content

The aluminium chloride colorimetric method determined the total flavonoid content (TFC) of *P. hysterophorus* root, leaf, stem and flower extract. The extract was vortexed and UV-visible spectrophotometers detected absorbance at 415 nm. Quercetin calibration curves were created 20, 60, 100, 140, 180, 220 and 260 µg/mL and total flavonoid concentration was expressed using QE/g (17).

Determination of total alkaloids content

Total alkaloid content (TAC) was determined by dissolving in dimethyl sulfoxide (DMSO), 2 N HCl and filtered, then mixed with citrate buffer and bromocreasol green. Vibrant shaking and chloroform washing were used to collect the yellow precipitate. The calibration curves for the reference standard atropine solution (20, 40, 60, 80 and100 µg/mL) were generated and the alkaloid content was measured using a UV-visible spectrophotometer (18).

Determination of total tannins content

The total tannin content (TTC) was determined using modified Folin-Ciocaltue procedures. A mixture of 35 % Na_2CO_3, 0.5 mL Folin-Ciocaltue reagent and sample extract was mixed and left to sit at 37 °C for 30 min. Calibration curves were generated for tannic acid concentrations (20, 40, 60, 80 and 100 µg/mL). The tannin concentration was measured using a UV-visible spectrophotometer at 725 nm (19).

Antioxidant analysis

In this study, three different radical scavenging assays (DPPH, ABTS and FRAP) were performed to evaluate the antioxidant activity of *P. hysterophorus* L., using the same plant parts (leaf, stem, flower and root). Methanolic and ethyl acetate crude extracts, were analysed and compared with ascorbic acid (AA) as the standard.

DPPH scavenging activity assay

The extract's capacity to scavenge DPPH radicals was evaluated using 2,2-Diphenyl-1-picrylhydrazyl (DPPH). The extract was mixed with DPPH solution and absorbance was measured at 517 nm. The DPPH activity was estimated and reported as a percentage of inhibition using Equation (20).

RSA % = (Absorbance of control - Absorbance of sample) x 100/ Absorbance of control

ABTS scavenging activity assay

The capacity of plant extracts to scavenge free radicals was assessed using the modified ABTS radical cation decolorisation test. The radical's solution was created by combining stock solutions and a 7 mM ABTS solution. The absorbance was measured after adding 300 µL of plant extract to the ABTS solution. The ABTS assay was conducted 3 times with ascorbic acid as the standard and the amount of ABTS inhibition was estimated using the standard curve equation (20).

RSA % = (Absorbance of control-Absorbance of sample) x 100/ Absorbance of control

Ferric reducing antioxidant power assay

The potassium ferricyanide-ferric chloride method was modified to determine the ferric reducing antioxidant power (FRAP) of fig latex. The mixture was mixed with *P. hysterophorus* extracts, incubated for 20 min and then mixed with trichloroacetic acid, $FeCl_3$ and water. The absorbance was measured at 700 nm and the ascorbic acid equivalent (AAE) was determined by creating an ascorbic acid curve from 20 µg/mL to 260 µg/mL of standard solution (21).

Ultraviolet–Visible spectroscopy analysis

Using a Ultraviolet–Visible (UV-Vis) spectrophotometer (Evolution Series UV-1800, Shimadzu), the presence of major classes of phytochemicals including phenolic acids, terpenoids, alkaloids, flavonoids, carotenoids, tannins, saponins and carbohydrates was monitored in the 200–800 nm wavelength range. The UV-Vis spectra were interpreted based on characteristic absorption bands corresponding to electronic transitions such as $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$, which are indicative of conjugated systems and chromophoric groups commonly associated with these secondary metabolites. The characteristic absorption peaks and their corresponding wavelengths were recorded (Tables 1 , 2).

Fourier-transform infrared spectral analysis

Fourier-transform infrared (FTIR) spectroscopy was employed to identify the functional groups present in the dried extracts of *P. hysterophorus* samples. The analysis was conducted using a Bruker ALPHA II Compact FTIR spectrometer equipped with QuickSnap™ sample modules, which allow for flexible configuration based on specific analytical needs. The measurements were performed under ambient laboratory conditions. The instrument was operated in conjunction with OPUS and OPUS Touch software, enabling precise acquisition and analysis of FTIR spectra. Spectra

Table 1. FTIR spectral peak values (cm^{-1}) and corresponding functional groups identified in methanol and ethyl acetate extracts of *Parthenium hysterophorus* leaf and flower

Methanol leaf	Methanol flower	Ethyl acetate leaf	Ethyl acetate flower	Functional group	References
	3286.94			N-H	15
3255.03, 2920.00, 2852.20	2917.60,	29.18.15,	3286.22,2922.09	O-H	17
1730		1731.59	1729.47	C=O	16
	1606.28	1607.50	1636.77	C=C	17
	1410.71			C-C	17
1394.07		1373.10	1373.29,1325.19	N-O	17
1243.70	1238.29	1236.93	1237.62	C-H	17
1022.32	1017.48	1025.67		C-N	17
620.27, 592.03, 560.96, 518.25,	621.50,585.45, 562.11, 539.23, 529.44	639.57,611.88, 591.17, 550.85, 534.62	517.26, 523.15, 548.46,566.57, 604.10,628.13	C-Br	17

Table 2. Antibacterial activity of extracts (ethyl acetate and methanol) of *Parthenium hysterophorus* (mean values of zone of inhibition (in mm) including the diameter of disc 8 mm) against *Escherichia coli*, *Xanthomonas oryzae*, *Microbacterium* sp. and *Bacillus cereus*

Sample Name	Diameter of Zone of Inhibition (in mm)							
	Escherichia coli		*Xanthomonas oryzae*		*Microbacterium Sp.*		*Bacillus cereus*	
	30 mg/mL	60 mg/mL	30 mg/mL	60 mg/mL	30 mg/mL	60 mg/mL	30 mg/mL	60 mg/mL
Methanol Leaf	0	0	14.33±0.57	18.33±0.57	10±0	14±0	9±0	17±0
Methanol Flower	0	13.5±0.707	18.33±0.57	21.5±0.707	16±0	18.33±0.57	20±0	23.33±0.57
Methanol Stem	0	0	0	0	0	0	9±0	10±0
Methanol Root	0	0	12.5±0.707	16.33±0.57	11±0	13±0	11±0	13±0
Ethyl acetate Leaf	8.5±0.707	18.33±0.57	9.5±0.707	21±0.707	16±0	26.33±0.57	22±0	25.5±0.707
Ethyl acetate Flower	8.5±0.707	20.5±0.707	12.33±0.57	15±0.707	13±0.707	17±0	18±0	23±0
Ethyl acetate Stem	0	0	0	9±0	12±0	13±0	11±0	15±0
Ethyl acetate Root	0	0	10.33±0.57	12.33±0.57	9±0	12±0	11±0	14.33±0.57
DMSO	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Streptomycin	-	-	23.5±0.707	23.5±0.707	25.33±0.57	25.33±0.57	24±0	24±0
Ampicillin	13.5±0.707	13.5±0.707	-	-	-	-	-	-

were recorded in the range of 4000–650 cm^{-1} and background correction was applied by subtracting ambient air spectra to enhance spectral clarity and accuracy. The resulting spectra were used to infer the presence of key functional groups associated with bioactive phytoconstituents.

Test organism

Four bacterial strains *Bacillus cereus* (GMS4 Lab collection), *Microbacterium* sp. (GAS3 Lab collection). *Xanthomonas oryzae* (RR24) and *Escherichia coli* (BL21) were used to evaluate the antibacterial activity. All bacterial strains used in this study were cultured from recognised microbiological laboratories and handled following standard biosafety protocols. No human or animal subjects were involved.

Antimicrobial activity

The antibacterial activity of various solvent extracts was evaluated using the agar well diffusion method (22). Briefly, 1 mL of a fresh bacterial broth culture was aseptically transferred into a sterile Petri plate. Molten Mueller–Hinton agar, cooled to approximately 45–50 °C, was then poured into the plate containing the inoculum and gently mixed to ensure uniform distribution of the bacterial suspension. After solidification of the agar, wells of 5 mm diameter were aseptically prepared using a sterile cork borer. Subsequently, 100 µL of the crude extract was dispensed into each well. Based on preliminary experiments and previous reports, extract concentrations of 30 mg/mL and 60 mg/mL were selected for evaluation. The plates were incubated at 37 °C for 18 hr. Antibacterial activity was assessed by measuring the diameter of the zones of inhibition formed around the wells. Dimethyl sulfoxide (DMSO) was used as the negative control.

Statistical analysis

Results were expressed as mean ± SD from triplicate experiments. Linear regression analysis and graph plotting were performed using Microsoft Excel 2007 and GraphPad Prism (9.3.1). Data were analysed using one-way ANOVA followed by Tukey's post-hoc test. Significance was set at $p \leq 0.05$.

The methods validation was performed by evaluating the linearity range, limit of detection (LOD), limit of quantification (LOQ) and repeatability. Linearity was assessed using calibration curves prepared at different concentrations. Limit of detection and LOQ were calculated based on the standard deviation and slop of the calibration curve while repeatability was determined by analysing samples in triplicate.

Results and Discussion

The global pharmaceutical and healthcare industries place a lot of attention because of their higher biological activity and lower adverse effects. Vitamins, dyes, antioxidants, oils and other bioactive compounds with micro and macro-nutrients from plants are used in cosmetic formulation and medicine industries (23). These compounds are extracted from the root leaves, flower and stem of plants.

The percentage yields of extracts obtained using methanol leaf, methanol flower, methanol stem, methanol root, ethyl acetate leaf, ethyl acetate flower, ethyl acetate stem and ethyl acetate root (extracted with stirring at room temperature for 24 hr with a dried powder to solvent ratio of 1:10) were 14 %, 9 %, 11 %, 10 %, 3.7 %, 4.9 %, 8 % and 2 %, respectively (Fig. 2). Since the focus is on extracting bioactive phytoconstituents such as polyphenolics and antioxidants, the selection of the extraction solvent was based on previous study and various testing parameters, with percent yield serving as a comparative measure of extraction efficiency within the same solvent system.

Phytochemical screening

Results of phytochemical screening are shown in Table 3, Methanol extract (ME) of leaf and flower found to contain more quantity of phenol, flavonoids, alkaloids, carbohydrates, but these are low quantity in extract of root and stem. Ethyl acetate extract (EE) of stem and root was reported to have more quantity of flavonoids, glycosides, quinines but alkaloids in leaf are compare to leaf or flower.

Every test sample for ME and EE had its chemical composition examined using phytochemical screening. Pervasive distribution of alkaloids, phenol, flavonoids and terpenoids was present in all the test samples of both extract ME and EE but tannins were present in ME. Other phytoconstituents in the polar and non-polar extracts exhibited a different distribution pattern.

Total phenol content

The total phenolic content (TPC) in the *P. hysterophorus* L. extracts was quantified using the calibration curve of gallic acid, represented by the regression equation y = 0.0067x+0.0825 with a high correlation coefficient R2 = 0.9976, where *x* denotes the TPC and *y* is the absorbance. This strong linearity indicates the reliability of the method for accurate phenolic estimation. As shown in Fig. 2, the methanolic extract of the leaf exhibited the highest TPC (20.38 ± 0.02 mg GAE/g), followed by the flower extract (18.99 ± 0.02 mg GAE/g). The stem and root extracts showed comparatively lower values. In contrast, the ethyl acetate extracts presented consistently lower phenolic contents across all plant parts.

This pattern highlights the superior efficacy of methanol over ethyl acetate for extracting phenolic compounds, likely due to its higher polarity and better solubility for polar phytochemicals (23). Polyphenols, the dominant phytochemicals detected, are structurally characterised by hydroxylated aromatic rings and are known for their potent antioxidant properties. The presence of a high phenolic concentration in methanolic extracts correlates strongly with antioxidant capacity, supporting previous findings that link TPC with enhanced radical scavenging activity (24). The antioxidant mechanism of phenolics is largely attributed to their ability to donate hydrogen atoms or electrons to neutralise free radicals, as well as their ability to chelate pro-oxidant metal ions and quench singlet oxygen (25).

Moreover, phenolic compounds are recognised for a wide spectrum of pharmacological activities, including antiviral, anti-inflammatory, anti-ulcer, anti-spasmodic, anti-diarrheal and anticancer effects (26). The results of this study support earlier reports that suggest plant extracts with higher phenolic content generally exhibit better antibacterial and antioxidant activities (9, 10).

Total flavonoids content

Total flavonoid content (TFC) in *P. hysterophorus* extracts was determined using a quercetin calibration curve y = 0.0067x + 0.0825 with a high correlation coefficient R^2 = 0.9976, with absorbance measured at 415 nm. The results, expressed in mg/g quercetin equivalent (QE), revealed that the EE of the flower had the highest flavonoid concentration (41.32 ± 1.92 mg/g), followed by the stem (39.69 ± 1.39 mg/g) (Fig. 3). These values highlight the solvent-specific efficiency of ethyl acetate in extracting flavonoids from specific plant

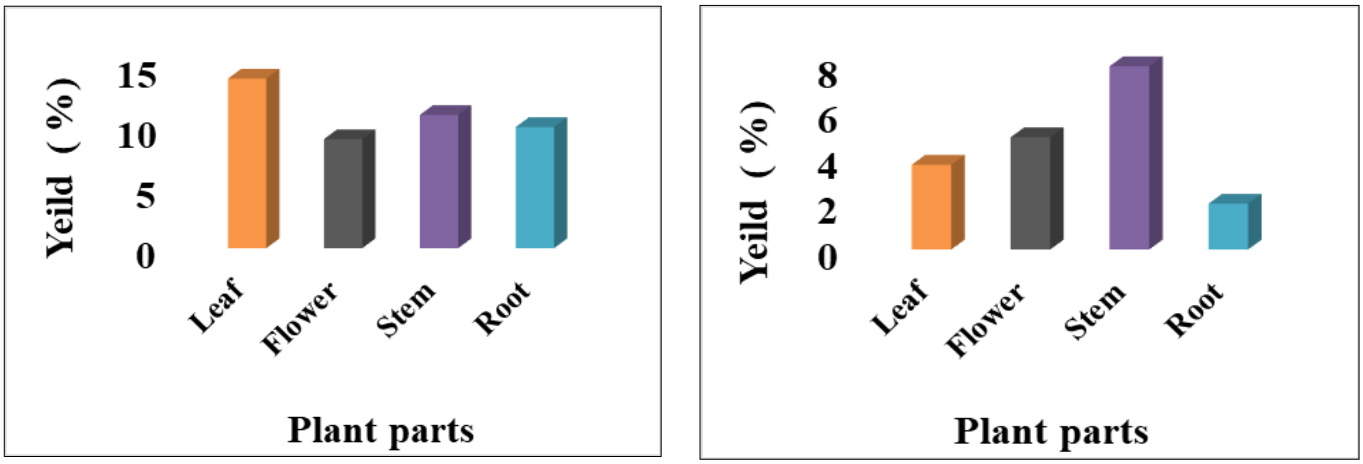

Fig. 2. Yield % of extracts obtained from different parts of *Parthenium hysterophorus* (leaf, flower, stem androot). (A) Methanol extract; (B) Ethyl acetate.

Table 3. Qualitative phytochemical analysis of methanol and ethyl acetate extract of *Parthenium hysterophorus* plant parts

Sl.No.	Test	Leaf		Flower		Stem		Root	
		ME	EE	ME	EE	ME	EE	ME	EE
1	Phenol	++	++	++	+	+	+	+	-
2	Flavonoid	++	+	++	++	+	++	+	+
3	Tannin	+	-	+	-	+	-	+	-
4	Alkaloid	+++	+++	+	+	+++	++	+	++
5	Terpenoid	+	-	+	+	+	-	+	++
6	Glycoside	+	++	-	+	-	++	-	+
7	Quinones	-	-	+	-	-	+	-	++
8	Carbohydrate	++	++	++	+	+	+	+	+

Note: **+++**present in more quantity,**++**present in moderate quantity, **+**present in low quantity,**-**absent. ME: Methanol extract; EE: Ethyl acetate

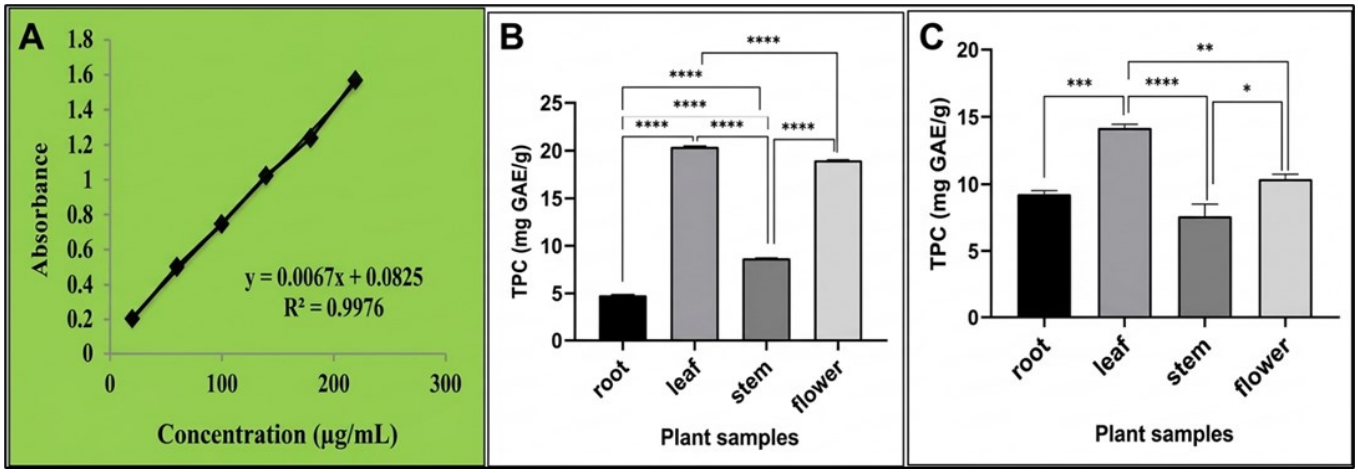

Fig. 3. Total phenolic content (mg GAE/g) of the *Parthenium hysterophorus* plant parts. A- Calibration curve; B- Methanol extract of plant parts; C- Ethyl acetate extract. Mean values which do not share a letter indicate significant differences (Tukey's test, p<0.05). Asterisk *, **, *** and **** representing the significance difference between samples at p< 0.033, 0.0021, 0.0002 and 0.0001, respectively.

parts.

Previous reserachers also confirmed the presence of flavonoids in *P. hysterophorus* using general phytochemical tests, although higher values (up to 320.17 mg pyrogallol equivalent (PGE)/ g) were reported, emphasising the influence of factors such as extraction method, environmental conditions and analytical protocols on phytochemical yield (27).

Flavonoids are a diverse group of polyphenolic compounds, with over 6000 types known to modulate plant metabolism and human health (22, 27). Their structural features-such as multiple hydroxyl groups and a C2-C3 double bond underlie their ability to scavenge free radicals, chelate pro-oxidant metals and inhibit oxidative enzymes (28). These biochemical properties contribute to their antioxidant, anti-inflammatory and antimicrobial activities (10). Moreover, a strong correlation between flavonoid and total phenolic content has been associated with enhanced antioxidant capacity, as seen in DPPH radical scavenging assays, reinforcing their therapeutic potential (24).

Total alkaloids content

The TAC in *P. hysterophorus* extracts was quantified using the atropine calibration curve (y = 0.0017x – 0.0022, R^2 = 0.9939), with results expressed as mg/g atropine equivalents. As shown in Fig. 4, the ME of the stem exhibited the highest alkaloid concentration (566 ± 6.14 mg/g), followed by the leaf and flower extracts. Ethyl acetate extracts generally showed lower alkaloid content across all plant parts.

The significant antioxidant activity in MEs, particularly in stem and leaf, may be attributed to the higher solubility of phenolic and flavonoid compounds in polar solvents like methanol. These compounds are well-known for their redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers (28), together high alkaloid levels in the methanol stem extract suggest that polar solvents like methanol are more effective in extracting these bioactive compounds, consistent with prior findings (29). Alkaloids are known to exhibit a wide range of pharmacological effects, including antioxidant, antimicrobial and neuroprotective properties (30). Studies have also reported that in some plant species, such as *Lepidium meyenii*, alkaloids can contribute more significantly to antioxidant activity than phenolics due to their lipid peroxidation inhibition and hydroxyl radical scavenging capacity (29).

These findings align with previous studies that have demonstrated the influence of solvent polarity on antioxidant extraction efficiency (31). The observed variation in TAC among different plant parts may also reflect tissue-specific differences in phytochemical accumulation (27). Furthermore, the high TAC in methanol stem and leaf extracts corresponds well with earlier reports on *P. hysterophorus* indicating its strong antioxidant

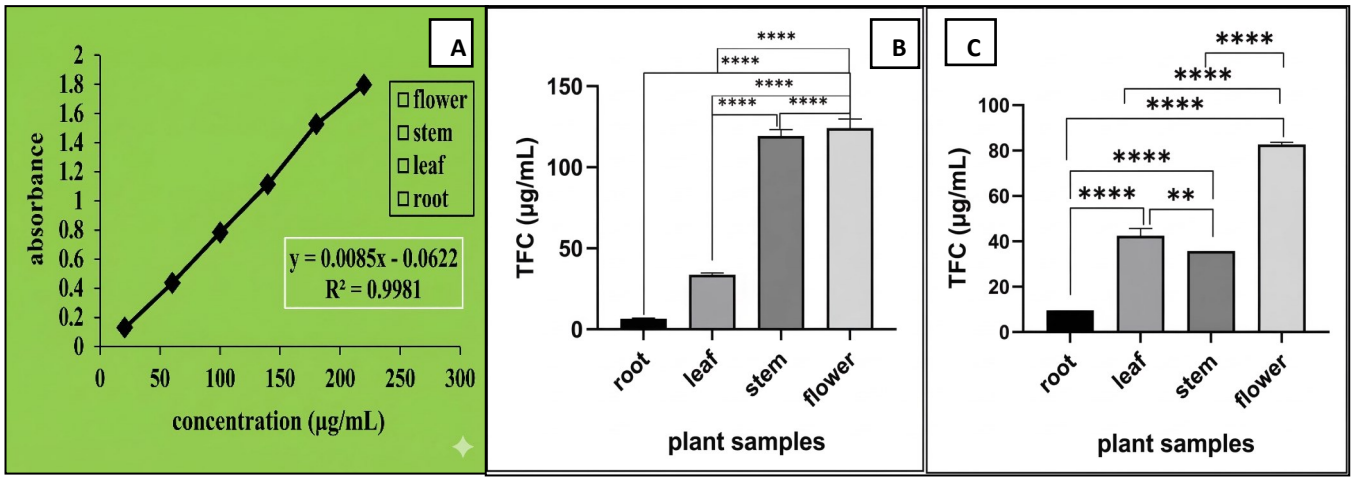

Fig. 4. Total flavonoid content (mg QAE/g) of the *Parthenium hysterophorus* plant parts. (A) Calibration curve; (B) Methanol extract; (C) ethyl acetate extract. Mean values which do not share a letter indicate significant differences (Tukey's test, p<0.05). Asterisk ** and ****representing the significance difference between samples at p<0.0021 and 0.0001, respectively.

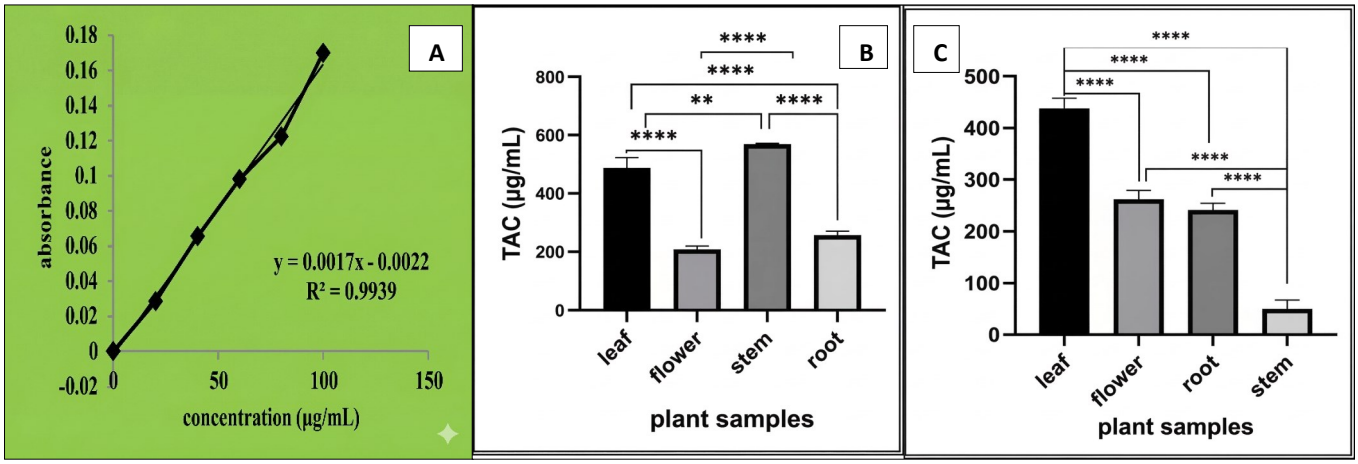

Fig. 5. Total alkaloid content (mg AE/g) of the *Parthenium hysterophorus* plant parts. A- calibration curve; B- methanol extract; C- ethyl acetate extract. Mean values which do not share a letter indicate significant differences (Tukey's test, $p< 0.05$). Asterisk **, & **** representing the significance difference between samples at $p< 0.0021$ & 0.0001, respectively.

potential (24).

Total tannin content (TTC)

The TTC in *Parthenium hysterophorus* was determined using the tannic acid calibration curve (y = 0.2699x + 0.1029, R^2 = 0.9999), with results expressed as mg/g tannic acid equivalents (TAE). As shown in the bar graph, the methanol extract of the leaf exhibited the highest tannin content (0.086 mg/g), followed by the flower extract. Ethyl acetate extracts displayed generally lower TTC across all plant parts, with the root showing the least concentration in both solvents (Fig. 5).

These findings indicate that tannins are more efficiently extracted using polar solvents like methanol, consistent with previous studies reporting the affinity of hydrolysable and condensed tannins for polar solvents (32). Tannins are a class of phenolic compounds known for their antioxidant, antimicrobial and anti-inflammatory properties (33). Their ability to complex with proteins and metals allows them to play a crucial role in free radical scavenging and enzyme inhibition (34), together the previous research suggesting that methanol, a polar solvent, efficiently extracts tannins and other polyphenolic compounds due to its ability to disrupt plant cell walls and solubilize phenolics (32). Tannins are known for a wide array of bioactivities, including wound healing, antimicrobial, antioxidant and anti-inflammatory effects (35). Their antioxidant activity is often linked to their capacity to inhibit lipid peroxidation, contributing to their anticarcinogenic and antimutagenic properties (36).

The elevated tannin content in the methanolic leaf extract suggests potential for biological applications, particularly as natural antioxidants or antimicrobial agents. Similar patterns have been observed in other medicinal plants, where leaf tissues typically accumulate more tannins than stems or roots (37), possibly as a defensive adaptation.

The phytochemical content and bioactivities observed in the present study were comparable to, but not identical with, those reported in earlier studies on *P. hysterophorus*. Variation of TPC, TFC, TAC, TTC, antioxidant assay and antibacterial activity may be associated to differences in geographical origin, seasonal collection time and extraction solvent as well as methodological differences, including extraction protocols, solvent polarity. Environmental factors such as soil types, temperature and rainfall are known to influence the accumulation of secondary metabolites in plants.

Antioxidant assay

DPPHS scavenging activity assay

Fig. 6 and 7 show the DPPH scavenging activity of *P. hysterophorus* extracts demonstrated a clear concentration-dependent pattern,

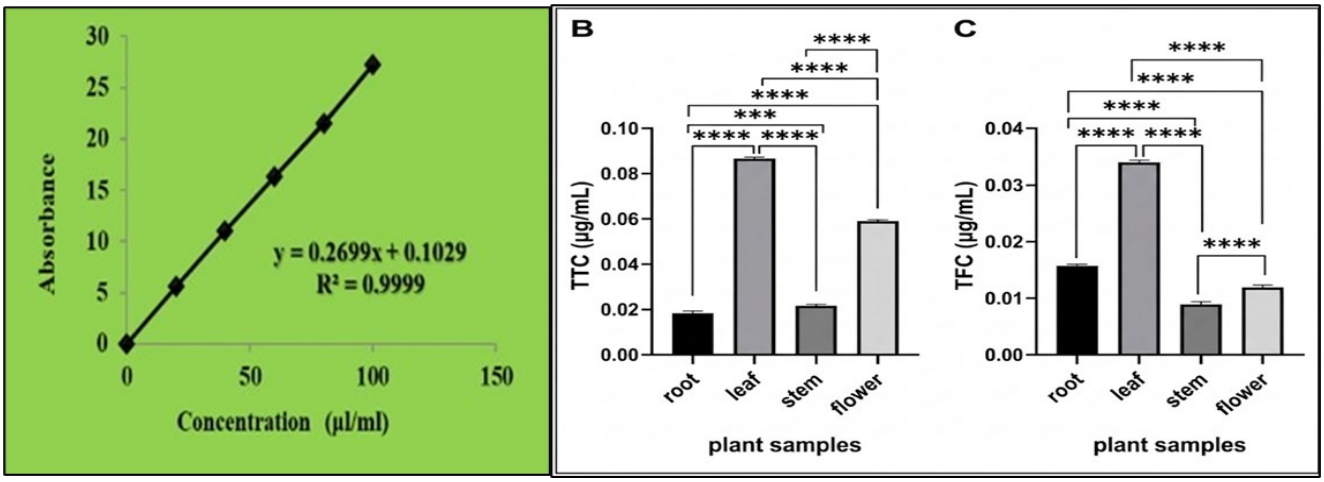

Fig. 6. Total tannin content (mg TAE/g) of the *Parthenium hysterophorus* plant parts. (A) Calibration curve; (B) Methanol extract; (C) Ethyl acetate extract. Mean values which do not share a letter indicate significant differences (Tukey's test, $p<0.05$). Asterisk *** and **** representing the significance difference between samples at $p<0.0002$ and 0.0001, respectively.

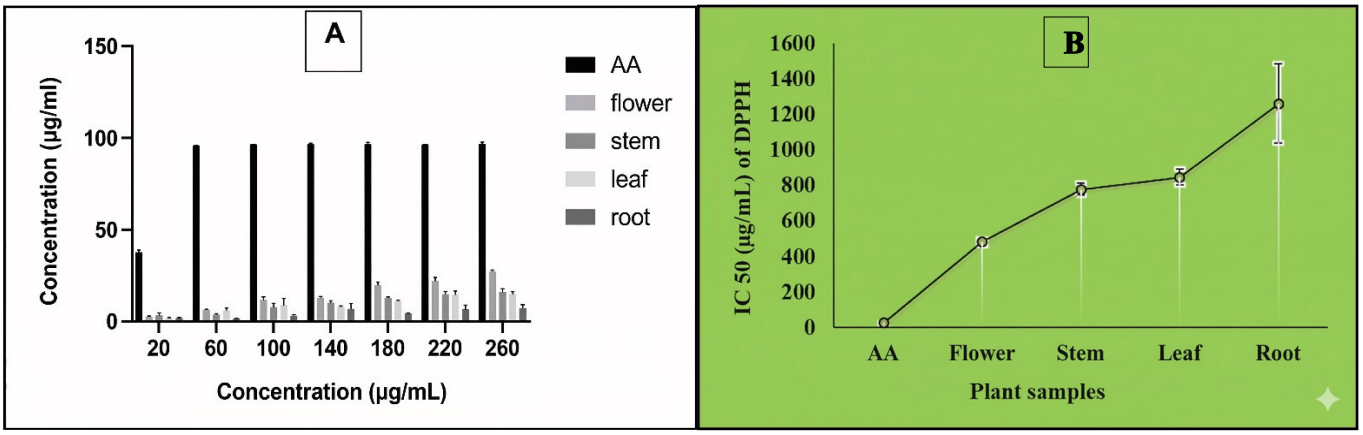

Fig. 7. DPPH scavenging activity of *Parthenium hysterophorus* L. plant parts. A- Concentration dependent activity methanol extracts; B- IC_{50} values dose response curve of plant extracts and ascorbic acid (standard) against DPPH radicals. Bars represent the mean of three replicates and error bars indicate the standard deviation.

with higher concentrations yielding increased radical scavenging potential. Among all tested samples, the methanol flower extract exhibited the highest activity, achieving 27.22 % inhibition at 260 µg/mL, followed by the methanol leaf extract. The EE, while slightly less effective, still showed notable activity, with the leaf extract reaching 25.97 % inhibition at the same concentration. In contrast, root extracts in both solvents exhibited minimal activity, ranging from 1.59 % to 1.67 % inhibition at lower concentrations (20 µg/mL).

These findings are in line with prior reports that highlight the solvent-dependent efficiency of antioxidant extraction. Methanol, due to its polarity, effectively extracts phenolic and flavonoid compounds, which are recognised for their hydrogen-donating ability and redox properties (24). The DPPH assay, based on the reduction of the stable free radical 2,2-diphenyl-1-picrylhydrazyl, is commonly used to measure such activities. A decrease in absorbance upon reaction with antioxidants reflects the compound's ability to donate electrons or hydrogen atoms (38). Our results also corroborate earlier findings (39), observed a 54.5 % DPPH inhibition at 100 µg/mL using methanolic leaf extracts of *P. hysterophorus*. Similarly, another study reported around 40 % inhibition with acetonic leaf extract of the same plant at the same concentration. These values underscore the variability of antioxidant potential depending on solvent polarity and extraction technique (40). The methanolic flower extract showed 59.73 % scavenging activity at 80 µg/mL concentration with IC_{50} values, 54.27 µg/mL (59). Similarly in aqueous and ethyl acetate extract reported 60 % DPPH inhibition with 300 µg/mL IC_{50} value (60).

Additionally, comparative IC_{50} analysis further supports methanol's superiority. In this study, methanol extracts showed lower IC_{50} values, indicating stronger antioxidant efficacy. Comparable studies reported 72.82 % DPPH inhibition with an IC_{50} of 168 µg/mL for *P. hysterophorus*, aligning closely with our results (41). For context, previous researchers observed much higher IC_{50} values (1114.17 µg/mL and 1832.25 µg/mL) in green and red bell pepper methanol extracts, respectively, emphasizing the relative potency of *P. hysterophorus* as an antioxidant source (42).

The variation in antioxidant activity among different plant parts and solvents can be attributed to differences in phytochemical composition, species genotype, plant maturity and environmental factors, all of which influence secondary metabolite accumulation (29, 40). In particular, the high concentration of flavonoids and phenolics in methanol flower and leaf extracts contributes significantly to the observed antioxidant activity, as these compounds neutralise free radicals by electron donation (28).

ABTS scavenging activity assay

The ABTS scavenging activity of *P. hysterophorus* extracts showed a clear concentration-dependent relationship, with both methanol and ethyl acetate leaf extracts exhibiting high antioxidant potential-

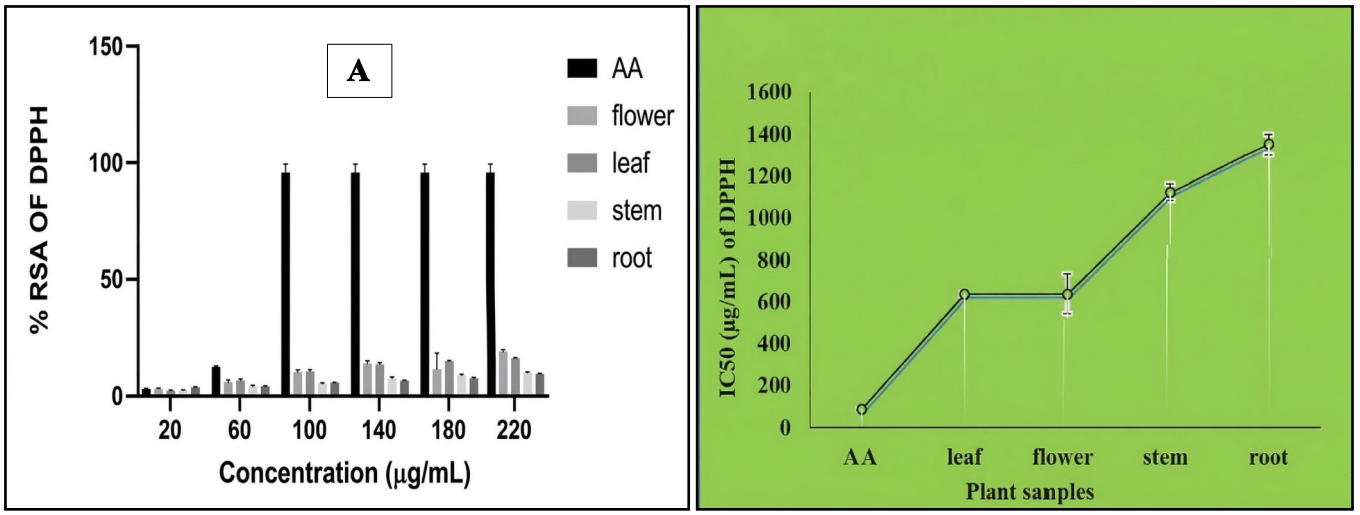

Fig. 8. DPPH scavenging activity of *Parthenium hysterophorus* plant parts. (A) Concentration-dependent activity of ethyl acetate extracts; (B) IC_{50} dose –response curves of plant extracts and ascorbic acid (standard) against DPPH radicals. Bars represent the mean of 3 replicates and error bars indicate the standard deviation.

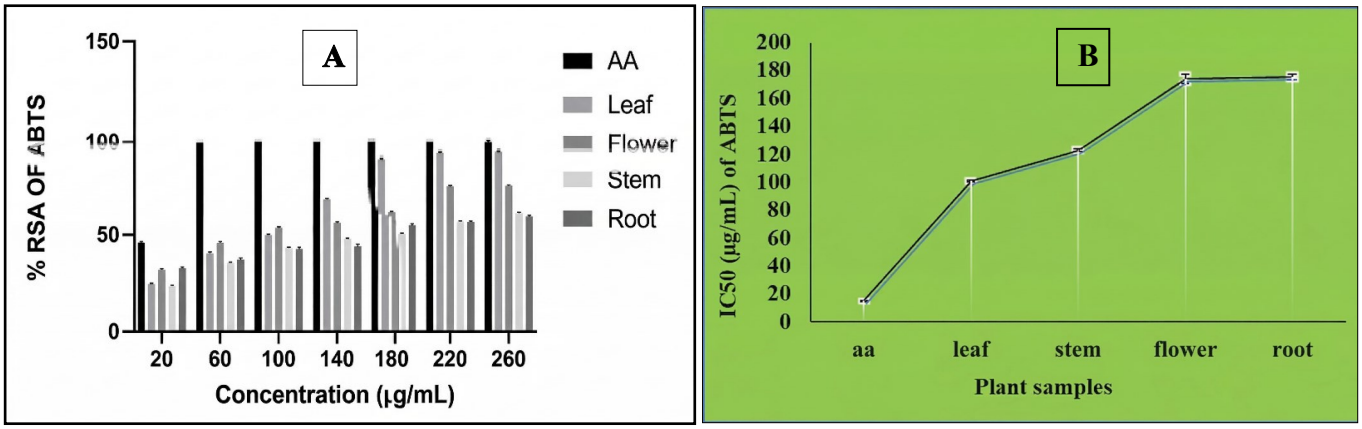

Fig. 9. ABTS scavenging activity of *Parthenium hysterophorus* L. plant parts. A- Concentration-dependent activity of methanol extracts; B- IC_{50} dose–response curves of plant extracts and ascorbic acid (standard) against ABTS radicals. Bars represent the mean of 3 replicates and error bars indicate the standard deviation.

94.94 % and 95.18 % inhibition, respectively, at 260µg/mL. These results reflect the strong hydrogen-donating capacity of phytochemicals, particularly phenolics and flavonoids, present in the extracts. The lower IC_{50} values of the leaf extracts (methanol: 100.67µg/mL; ethyl acetate: 79.04 µg/mL) compared to other parts confirm their superior efficacy (Fig. 8 and 9).

This aligns with findings by previous researchers , who reported 93.2 % ABTS inhibition at 100µg/mL in methanolic extracts of *P. hysterophorus* (39, 42). Similar high ABTS activity has also been observed in other medicinal plants, such as *Carissa opaca* (40) and *Cinnamomum camphora* (43), indicating that phenolic-rich extracts consistently demonstrate strong ABTS radical scavenging. Antioxidant assays like ABTS often produce higher inhibition values than DPPH, due to differences in solubility and radical reactivity was reported earlier (44).

Ferric reducing/antioxidant power (FRAP) assay

The FRAP assay revealed that *Partheniumhysterophorus* extracts exhibit a concentration-dependent ferric reducing ability (Fig. 10), with the methanol flower extract showing the highest antioxidant potential (728.69 ± 24.63 mg AAE/g), followed by the methanol leaf extract (665.97 ± 27.03 mg AAE/g) at 220µg/mL. Among ethyl acetate extracts, the root showed the highest FRAP value (655.97 ± 27.03 mg AAE/g).

These findings align with previous studies, observed a direct correlation between phytoconstituent levels and reducing capacity in *P. hysterophorus*, particularly in methanolic leaf extracts (40). The higher reducing power in methanol extracts likely reflects better solubilization of redox-active compounds like phenolics and flavonoids. Contrastingly, another study emphasized that FRAP values can vary significantly depending on assay conditions and plant species, cautioning against broad generalizations (45). Additionally, the antioxidant coefficient (PAC), which compares FRAP to total phenolic content, has been used to evaluate the efficiency of antioxidants across plant species. In some cases, low phenolic content has been associated with weak FRAP performance, limiting the plant's classification as a significant antioxidant source. However, in this study, the high FRAP values observed suggest *P. hysterophorus* may serve as a potent natural antioxidant, particularly when extracted with polar solvents like methanol.

UV-Vis analysis

To assess the phytochemical content in the extracts, UV-VIS spectroscopy was conducted over the 200-800 nm range. This technique helps detect compounds with aromatic rings, chromophores, σ-bonds and lone electron pairs. Distinct absorption peaks and a stable baseline supported the selection of this range for both methanolic leaf and flower extracts (Table 4).

A prominent absorption band at 265 nm in the standard gallic acid confirmed the presence of phenolics. Similar peaks were observed in the solvent extracts. Absorption in the 400–450 nm range, often linked to carotenoids and bands between 270–280 nm and 350–500 nm, indicative of flavonoids and tannins, were also detected (46). Additionally, peaks between 234–676 nm were

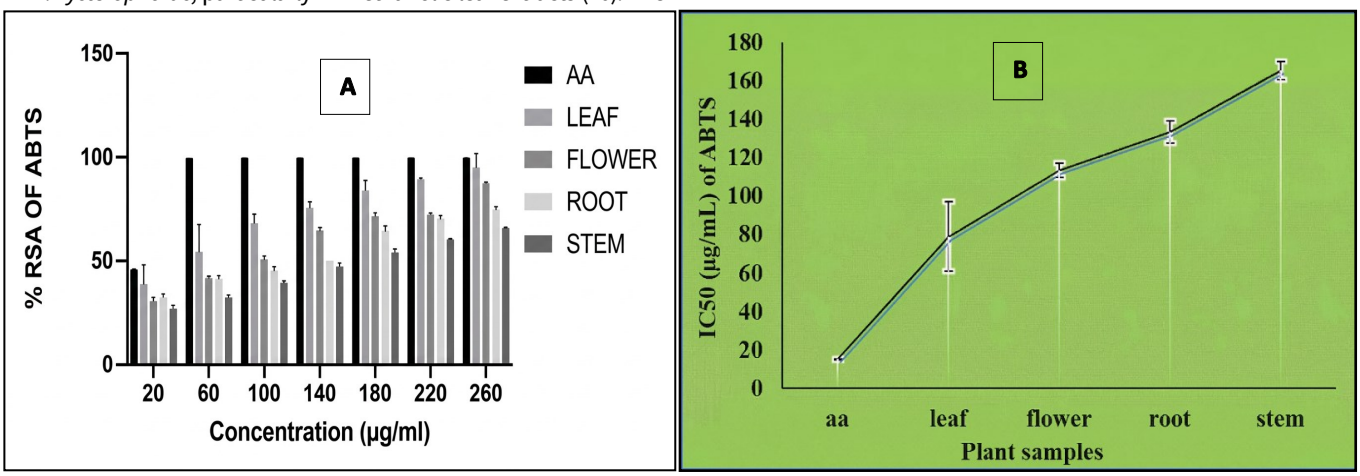

Fig. 10. DPPH scavenging activity of *Parthenium hysterophorus* plant parts. (A) Concentration-dependent activity of ethyl acetate extracts; (B) IC_{50} dose–response curves of plant extracts and ascorbic acid (standard) against DPPH radicals. Bars represent the mean of three replicates and error bars indicate the standard deviation.

Table 4. UV-Vis absorption peak wavelengths (nm) and corresponding functional groups identified in methanol and ethyl acetate extracts of *Parthenium hysterophorus* leaf and flower

Sl. No.	Methanol extract (ME)				Ethyl acetate extract (EE)			
	Leaf		Flower		Leaf		Flower	
	Wavelength (nm)	Absorption peak	Wavelength (nm)	Absorption peak	Wavelength (nm)	Absorption peak	Wavelength (nm)	Absorption peak
1	400	2.167	206	2.487	265	4.000	313	0.767
2	409	2.186	231	4.000	304	2.951	319	0.771
3	463	0.554	273	3.790	336	3.882	344	0.716
4	468	0.559	281	4.000	348	3.030	410	1.219
5	523	0.157	490	0.040	392	3.832	522	0.105
6	536	0.214	504	0.044	467	0.783	534	0.144
7	579	0.079	524	0.028	472	0.788	550	0.076
8	607	0.195	535	0.034	490	0.584	558	0.078
9	627	0.127	581	0.009	505	0.657	579	0.061
10	664	0.776	607	0.024	522	0.416	608	0.114
11			628	0.013	534	0.591	627	0.080
12			664	0.112	550	0.262	666	0.457
13					557	0.272		
14					579	0.162		
15					607	0.433		
16					628	0.259		
17					666	2.007		

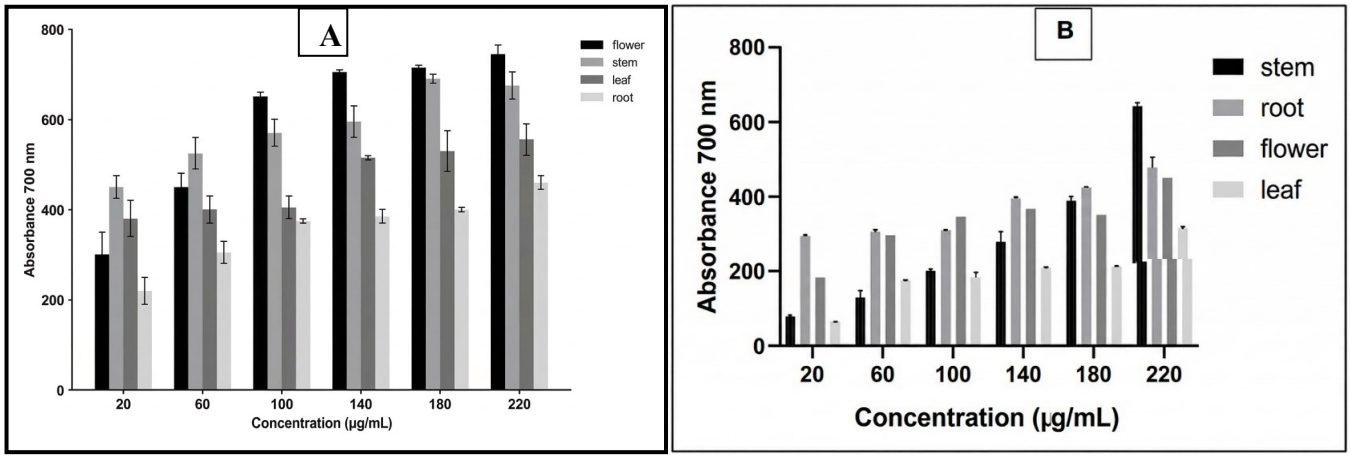

Fig. 11. Ferric reducing antioxidant power of *Parthenium hysterophorus* plant parts. (A) Methanol extracts; (B) Ethyl acetate extracts.

associated with alkaloids, flavonoids and phenolic compounds. The bands at 280 nm and 300–600 nm suggest π-π* transitions in aromatic systems and possible ligand-to-metal charge transfer (LMCT) overlaps (46, 47). These spectral features confirm the presence of key bioactive constituents in the plant extracts.

FTIR spectral analysis

The functional groups of the bioactive substances found in *P. hysterophorus* were identified using FTIR spectroscopy (Table 1). The presence of hydroxyl groups (O-H), aldehydes (C-H), alkenes (C=C), carboxyl groups (C=O), nitrogen containing groups (N-O), alkanes (C-C), aromatic primary amines (N-H), alkynes (C-C), aliphatic bromo compounds (C-Br), phenol, carboxylic acid, glycogen, alkyl halides, halogen, aliphatic amines, primary and secondary amines, esters, ethers, aromatics, lipids, triglycerides and nitro compounds were confirmed by FTIR spectra. These functional groups are an essential component of a variety of secondary metabolites, including alkaloids, flavonoids, terpenoids, polyphenol and tannin(29).

The broad absorption band around 3200–3600 cm^{-1} is O-H stretching band, which are associated with phenolic and flavonoid compounds known for antioxidant. Peak observed 2900 cm^{-1} correspond to C-H stretching, 1600–1650 attributed to C=O/C=C stretching, 1000–1300 correspond to C-O stretching indicating the presence of terpenoids, phenolic acid and alkaloids, alcohols, ether and phenolic esters, respectively (2). The absorption band around 3300–3500 were attributed to N-H stretching band showing the presence of amine and amides groups, which are typical of alkaloids or another nitrogenous phytochemical. Similarly peaks around 1020 –1050 correspond to C-N stretching band supporting the presence of aliphatic and aromatic amine-containing compounds such as alkaloids (2).

Various pharmaceutical products with anti-inflammatory, antioxidant, anticancer, antitumor and antibacterial properties often contain these functional groups (10, 11). These functional groups in bioactive compounds contribute to their diverse therapeutic potential. The FTIR spectroscopy serves as a reliable and precise method for identifying such components. In our study, FTIR analysis effectively distinguished between different plant parts, revealing notable variations. It also facilitated the identification of key bioactive compounds that could potentially be developed into herbal treatments for various ailments.

Antimicrobial activity

The antibacterial activity of methanolic and EE of *P. hysterophorus* was assessed at concentrations of 30 mg/mL and 60 mg/mL against 4 bacterial strains: *E. coli*, *X. oryzae*, *Microbacterium* sp. and *B. cereus*. The inhibitory effects were expressed in terms of the diameter of the inhibition zones (IZ), with results summarized in Table 2. Streptomycin and ampicillin served as positive controls, while DMSO was used as a negative control.

Across all extracts, the zones of inhibition ranged from

8.5 ± 0.707 mm to 26.33 ± 0.57 mm, indicating varying degrees of antibacterial potency. The ethyl acetate leaf extract at 60 mg/mL showed the highest inhibitory effect against *Microbacterium* sp. (26.33 ± 0.57 mm), whereas the lowest inhibition was observed for *E. coli* with both ME and EE at 30 mg/mL. The methanol flower extract and ethyl acetate leaf extract demonstrated the most potent antibacterial activities, with inhibition zones ranging from 13.5 ± 0.707 to 23.33 ± 0.57 mm and 8.5 ± 0.707 to 26.33 ± 0.57 mm, respectively. In contrast, extracts from stems (both solvents) exhibited the least antibacterial activity, indicating a lower concentration or absence of bioactive constituents in these parts. The effectiveness of the extracts against tested bacterial strains followed the order: methanol flower > ethyl acetate leaf > ethyl acetate flower > methanol leaf > methanol root > ethyl acetate root > ethyl acetate stem > methanol stem. The methanolic and chloroform leaf extract of *P. hysterophorus* showed zones of inhibition 26 mm against *Bacillus subtilis, 9* mm *Vibrio parahaemolyticus* and 23 mm against *Sarcina lutea,* 10 mm against *V. parahaemolyticus* respectively at tested concentration (44, 48), similarly 17.00 ± 0.12 mm against *Enterococcus* spp., 16.00 ± 0.03 mm against *Bacillus subtilis*, 14.00 ± 0.06 mm against *Pseudomonas aeruginosa* and13.00 ± 0.12 mm against *E. coli*. No activity was reported against *Klebsiella pneumoniae* in methanolic extract at 50 µL concentration of 15 mg/mL stock solution (49).

This pattern suggests that floral and foliar extracts possess higher concentrations of bioactive antibacterial compounds. Notably, both Gram-positive and Gram-negative bacteria were inhibited, though Gram-positive bacteria such as *B. cereus* exhibited greater susceptibility. This can be attributed to the structural differences in bacterial cell walls; Gram-negative bacteria possess an additional outer membrane composed of lipopolysaccharides, making them less permeable to plant-derived compounds (48). The antibacterial activity of *P. hysterophorus* extracts may be attributed to the presence of secondary metabolites such as flavonoids, tannins, alkaloids and terpenoids, which have well-documented antimicrobial properties (49). Supporting this, earlier studies reported significant antibacterial efficacy of methanolic extracts from *P. hysterophorus* leaves and flowers against a range of pathogens (50). Similarly, previous researchers confirm the effectiveness of *P. hysterophorus* extracts, particularly against *E. coli* and other phytopathogens (39, 42). Moreover, the activity of dichloromethane extract was 17.00±0.20, 19.00±0.12 and 20.00±0.05mm *P. aeruginosa, K. pneumoniae* and *Enterococcus spp*., respectively (50). Likewise, the aqueous leaf extract showed potent antibacterial activity, 20 mm against *Ralstonia solenocearum* and 1 8 mm against *Eriwinia carotovora* zone of inhibition (48–50).

Furthermore, comprehensive studies on solvent extracts of *P. hysterophorus* (including aqueous, methanolic, ethyl acetate and chloroform) have revealed MIC values ranging from 0.25 to 4.0 mg/mL, underscoring their strong bactericidal potential (27, 50). These findings support the growing consensus on the utility of *P. hysterophorus* as a promising source of natural antimicrobial agents and a potential biocontrol tool for managing plant diseases.

Conclusion

This study highlights the dual significance of *P. hysterophorus* as both invasive weed and a promising source of valuable natural antioxidants and antimicrobial agents. The results revealed the presence of diverse bioactive compounds, including phenolics, flavonoids, alkaloids and tannins, with significant variation across plant parts and solvents. Methanol flower and leaf extracts exhibited strong antioxidant activities in DPPH, ABTS and FRAP assays, while ethyl acetate leaf extract demonstrated the most potent antibacterial effect, particularly against Microbacterium sp. These findings highlight the potential of *P. hysterophorus* as a source of natural antioxidant and antibacterial agents, which may serve as alternatives to synthetic compounds. However, the study and its limitations include its *in vitro* assay, should focus on the isolation and characterization of individual bioactive compounds, toxicity assessment and *in vivo* validation address these gaps through *in vivo* studies and compound isolation to validate safety and efficacy for pharmaceutical or nutraceutical applications. Such efforts may also support sustainable management strategies by converting this noxious weed into a beneficial bioresource.

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Authors' contributions

AS[1], AS[2], HC, SS and NS contributed to conceptualization and methodology. MD, PP, PS and SP were responsible for instrumentation and data analysis. RG, AS[3], SD and PPB contributed to moderation, validation and proofreading. TS, NS and AKS were involved in review and final approval of the manuscript. All authors read and approved the final manuscript [AS[1]- Aman Saket; AS[2]- Amrita Singh; AS[3]- Ajay Singh].

Compliance with ethical standards

Conflict of interest: The authors declare no conflict of interest.

Ethical issues: None

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