ELUCIDATION AND COMPARATIVE ASSESSMENT OF THE PHYTOCHEMICAL CONTENT AND ANTIBACTERIAL ACTIVITY OF *PARTHENIUM HYSTEROPHORUS* EXTRACT IN DIFFERENT SOLVENTS

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Abstract. The current study presents a comparative assessment of the phytochemical contents and antibacterial activity of *Parthenium hysterophorus* in different solvents of varying polarities (n-hexane, acetone, and water). Extraction was carried out using orbital shaker and extracts were tested against selected bacterial strains by applying disc diffusion process. The antioxidant potential of the Parthenium weeds extract was measured in terms of total phenolic contents (TPC), total flavonoid contents (TFC) and diphenyl picrylhydrazyl (DPPH) free radical scavenging activity. The results revealed that aqueous extracts had higher TPC values (151 µg GAE/g) relative to acetone (127 µg GAE/g) and n-hexane (118 µg GAE/g) extracts. Whereas in the case of TFC, the acetone extract exhibited higher values (31 µg QE/g) followed by n-Hexane (26 µg QE/g) and aqueous extract (5 µg QE/g). DPPH scavenging activity was higher in the case of n-Hexane extract followed by acetone and aqueous extracts. The antibacterial potential of the extracts prepared using three different solvents demonstrated that these extracts are capable of resisting bacterial activity and hence cease their function. The most prominent inhibition zones appeared in the case of n-hexane extract, whereas the aqueous extract had the lowest potential to inhibit the bacterial function. The results obtained in this current study support that Parthenium weed extracts have significant antioxidant and antibacterial functions and hence can be used as an ingredient in pharmaceuticals.
Keywords: *Parthenium weed, antioxidant potential, DPPH, antibacterial activity, total phenolic content, total flavonoid content*

Introduction

Parthenium (*Parthenium hysterophorus* L.), belonging to the Asteraceae family, is an invasive, and herbaceous weed that produce throughout the year. Although it is native to the Gulf of Mexico and the southern USA, it is widely spread in different countries all over the world such as Brazil, Africa and Australia (Dhileepan and Strathie, 2009; Fite et al., 2017; Hundessa et al., 2016). In Asia, it also grows in most of the countries including India, Bangladesh, and Pakistan (Weyl et al., 2021). It is locally known by different names as Gaajar ghas, star weed, congress grass and also as chatak chandni. This weed usually grows on roads, empty places, mountains, railway lines and construction tracks (Singh et al., 2004). Two different varieties of this weed have been

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known so far such as North American which is the most common and South American. Substantial variations in terms of biochemistry and morphology have been observed between these two varieties of Parthenium (Javaid et al., 2010).

From the perspective of its morphology, this weed is 1.5 to 2.5 m tall and has narrow leaves and white flowers (Nguyen, 2011). Although spring season is the most favorable for its germination it can produce almost every time in the year and starts producing flowers just after one month that can last for 6-8 months. Besides its quick growing ability and spreading in nearby places, the aerial weed part does not bear cold weather and thus mostly dies in the winter season. This weed can tolerate different soil conditions and thus can grow easily in different environments ranging from alkaline, black, and cracking clay soil to sandy/clay loams (Strathie et al., 2011). Parthenium weed is considered one of the seven most feared weeds of the world (Bagchi et al., 2016). Pertaining to its physiological behavior, morphological structure, rapid spread, and high germination efficacy, it can grow easily in different areas throughout the year (Verma et al., 2020). It possesses a huge seed reservoir that enables the spread of seeds in nearby places and thus leads to an increase in the growth of the weed (Dhileepan, 2012). Moreover, *P. hysterophorus* weed is also reported to release volatile/non-volatile entities that can disrupt the growth and biodiversity of the nearby flora (Batish et al., 2002).

Parthenium has been alarmingly dispersing to other areas and countries across the world, and hence posed severe effects on the bio-diversities of the species in neighboring areas/countries due to its quick dispersal, toxicity and noxious behavior (Boja et al., 2022). This weed brings a large seed reservoir and is also capable of growing accidentally via the dispersal of its seeds in different areas. The literature reports have revealed the impact of Parthenium weed on human health, nearby habitats, and soil composition. Due to invasive efficacy on nearby habitats, Parthenium weed might also be affecting the activities and structure of soil enzymes (Gioria and Pyšek, 2016), however, the extent of disturbance on the ground community and its biological behavior is still ambiguous (Khaliq et al., 2015). Earlier data presents an inconsistency in the soil composition such as phosphorus, carbon, potassium and nitrogen, and pH due to Parthenium weed invasion. In this context, it might be inferred that biological factors of the nearby areas and communities could also be responsible for the varied soil response (Stefanowicz et al., 2017). Hence, there is a dire need for time to control the growth/dispersal of this noxious weed to other nearby areas.

Due to the lack of environmental safety, inefficiency and higher cost, no single method is better adapted for the control of Parthenium, and a cohesive approach might be adopted with multiple options (Tabe Ojong et al., 2022). In this regard, crops and plants are planted as interfering plants in different countries but this method is not being applied in all countries. *Cassia uniflora*, *Abutilon indica* and *Cassia sericea* are considered effective plants in suppressing the growth of parthenium weed up to 70%. Other plants such as *Croton sparciflorus, Sida latifolia, Hyptis savolensis,* and *Tephrosea purfurea* can also be used but these need to be investigated first for the allergens present in them (Kumar, 2023).

Parthenium can also be a source of pathogens and insects that can attack other nearby plants or crops. In India, Sri Lanka, Ethiopia and South Africa, the Parthenium weed is being controlled by different imported biological agents for plant pathogens/pests (Dhileepan et al., 2018). In Queensland, this weed was announced as noxious and a scheme was launched to control its spread in neighboring areas in 1976 (Retief et al., 2013). Nevertheless, Parthenium weeds are recognized as noxious in different areas of

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the world, however it possesses some medicinal characteristics like anti-inflammatory, antifungal/viral/bacterial, and anthelmintic potential (Durai et al., 2016) and are therefore employed in the cure of several pathologies such as constipation, malarial infection, diarrheal issue, urinary tract infections, cardiovascular/neurological disorders, and even treat gynae problems. These properties might be linked to the presence of secondary metabolites in this weed (Kushwaha et al., 2012).

Based on the multiple biological activities and uses against different diseases, the Parthenium weed was studied in this current study for its phytochemical contents and antibacterial potential. Moreover, the study will also establish the scientific basis for its use in different nutraceuticals and pharmaceuticals.

Experimental section

Collection of plant

The Parthenium weed in fully mature stage and fresh form was collected from District Swat, Khyber Pakhtunkhwa, Pakistan and further authenticated by Dr. Muhammad Nisar, Professor, Department of Botany, University of Malakand. The voucher number allocated was H.UOM.BG.774 dated 7th October 2021 and the specimen was placed in the Herbarium, University of Malakand. The plant debris was removed and washed with water until all the debris was removed and then placed under shade at room temperature for a couple of weeks. The dried plant was converted into a fine powder by using a grinder and the powder was refrigerated till further analysis.

Extraction

Solvent extraction was carried out using three solvents such as acetone, n-hexane, and water. The powdered weed plant (10 g) was mixed into 100 mL of each solvent and stirred on an orbital shaker for 3-4 hours. After this, the dissolute was filtered using sterilized Whatmann Filter paper (2.5 m) and the residue was separated from the filtrate. The extracts obtained from each solvent were further dried by using the rotary evaporator and stored at 4°C until further analyses.

Phytochemical analysis

Total phenolic content

The total phenolic content of the solvent extracts was measured using the Folin-Ciocalteu (FC reagent) method as reported by Iqbal et al. (2022) and Hussain et al. (2022) with little modifications. In brief, 20 µL of each extract and 90 µL of FC reagent were mixed into the wells. After that, sodium carbonate (90 µL) was also added in each well to obtain a final volume of up to 200 µL. The whole mixture was kept in incubation at room temperature for 4-5 min. The standard gallic acid and methanol were employed as positive and negative controls, respectively. After incubation, the absorbance of samples was noted at 630 nm using a plate reader.

Total flavonoid content

Total flavonoid contents of the solvent extracts were measured following the method reported earlier by Iqbal et al. (2022) and Hussain et al. (2022) with little modifications. A mixture of 10 mg/mL was prepared for each extract and this sample (20 µL) was

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agitated with aluminum chloride and potassium acetate (10 µL each) in a well plate. To make a final volume up to 200 µL, distilled water was added in each well plate. After that, the whole mixture was kept under incubation for 30 minutes. A 1mg/mL quercetin and 20 µL of methanol were used as positive control and negative control, respectively. The absorbance was calculated by a plate reader at 450 nm.

DPPH radical scavenging assay

The scavenging potential of the solvent extracts was measured using the DPPH assay as stated earlier by Iqbal et al. (2022) and Hussain et al. (2022). Briefly, each extract (20 µL) was mixed thoroughly with DPPH reagent (180 µL) and ascorbic acid was used as a standard. The whole mixture was placed in incubator for a period of 30 minutes. The absorbance was recorded at 517 nm by a microplate reader.

Minimum inhibitory concentration (MIC)

Minimum inhibitory concentrations (MIC) of each solvent extract were measured using the microdilution broth assay. At first, the nutrient broth was placed in each well and selected bacterial strains were placed into the wells in the first row and then again the same process was carried out for the next three rows. Each solvent extract of 5 µL (2 mg/ml) was poured into these wells and continually kept on diluting up to the 11^{th} column well. The 12^{th} column having a standard solution of broth media and bacterial culture, having no plant extract was used as positive control (Iqbal et al., 2022; Hussain et al., 2022). The reader plate was visually observed for MIC.

Antibacterial activity

Bacterial strains

The bacterial strains employed in this study were procured from the Department of Biotechnology, University of Malakand. *Staphylococcus aureus, Klebsiella pneumoniae, Staphylococcus aureus*, and *Enterococcus faecalis.* Among these bacteria *Pseudomonas aeruginosa* and *Enterococcus faecalis* are Gram + while *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* are Gram-. Furthermore, these strains were placed on Mannitol Salt agar, Bile Esculin agar and MacConkey agar to get refreshed.

Disc diffusion method

Disc diffusion method was employed to explore the antibacterial potential of solvent extract of Parthenium weed. Bacterial colonies (4-5) were inoculated into freshly prepared nutrient broth by using a wire loop. After that, the broth temperature was optimized to attain the turbidity of MacFarland (standard). Sterilized cotton swabs were employed to spread the bacterial suspensions on the surface of agar plates. The above procedure was repeated thrice to obtain the even distribution of inoculum, and plates were kept in drying for 3-5 minutes. In the next step, the sterilized forceps were used to place the Whatman filter paper discs in these agar plates at accurate position. Varying concentrations of extracts (5, 10, 20 mg/mL) were added on these discs on each plate by keeping the equal amount (5 µL). The Cefepime was used as a standard (control) for the antibacterial activity. At the end, the plates were incubated at 37°C for 24 h. The inhibition zones were measured in mm by using a vernier caliper.

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Statistical analysis

The current study data was analyzed statistically using one-way ANOVA and Tukey test to compare the mean values of different parameters. The values obtained were stated as mean standard deviation. The *p*-value (<0.05) was considered significant, and GraphPad Prism (version 05) was also employed.

Results and discussion

In present study, antimicrobial potency of *P. hysterophorus* weed was analyzed in term of zone inhibition and minimum inhibitory concentration from the extracts obtained by using three different solvents against four selected bacterial strains. In this study, total phenolic contents, total flavonoid contents, and DPPH assay were used to determine antioxidant potential of Parthenium weed extracts of different solvents.

Antibacterial activity

Currently, worldwide interest has been developed to explore new sources of antimicrobial drugs due to drug resistance in pathogenic organisms Among these novel sources natural antimicrobial compounds from plant extracts have been gaining attention as those are safe to use with no side effects and they are considered to be more effective in relation to synthetic molecules (Ammara et al., 2023; Monisa et al., 2023; Aziz et al., 2023; Ahmad et al., 2023; .Gul et al., 2023; Syed et al., 2023; Khurshaid et al., 2023; Riaz et al., 2023; Sana et al., 2022; Saleem et al., 2023). About their complicated behavior, and capability to intermingle with a variety of molecular entities, the targeted could not produce resistance against them. Using the i*n-vitro* protocol, three different extracts of *P. hysterophorus* based on the solvents used, were assessed for their antimicrobial efficacy and the results obtained in this study exhibited that Parthenium weed is good antimicrobial plant and therefore might be consumed as a ingredient to cure various diseases (Aziz et al., 2023; Hayat et al., 2023; Zawar et al., 2023; Hussain et al., 2023b).

Antibacterial activities of *Parthenium hysterophorus* extracts of acetone, n-Hexane and water with selected concentrations (5, 10 and 20 mg/mL) were analyzed against 4 bacterial strains (*S. aureus, E. faecalis, P. aeruginosa* and *K. pneumonia*) while Cefepime drug was used as positive control as shown in *Table 4.* and *Figure 4*. All results were measured in terms of zone inhibition in mm. The zone inhibition value of all solvent extracts (acetone, n-Hexane, water) of different concentration (5, 10 and 20 mg/mL) ranged as 7.33 ± 0.577 - 12.67 ± 0.577 for acetone, 9 ± 1 - 13.67 ± 1.155 for n-Hexane and 7.67 ± 0.57 - 10.7 ± 5.77 for water. The highest zone inhibition was shown against *Staphylococcus aureus* by 5 mg/mL n-hexane while the lowest for 20 mg/mL acetone extract against *Pseudomonas aeruginosa.*

Results showed the effectiveness of extracts against bacterial strains were in order: n-Hexane > acetone > water. All concentrations of acetone and n-hexane extracts were more effective against *Staphylococcus aureus* while in the case of water extracts all concentrations showed more zone inhibition against *Klebsiella pneumonia.* Results also revealed that there is no significant difference among different concentrations of extracts but all extracts have significantly different values against selected bacterial strains.

Earlier literature reports revealed significant antibacterial activities of various solvent extracts of *P. hysterophorus* against selected bacterial strains such as *S. mutans*, *P. vulgaris*, and *S. typhi* analyzed (Kumar et al., 2014). Likewise, potent antimicrobial

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activity was presented by different solvent extracts of fresh/dry parts of this weed against *S. paratyphi A.*, *P. aeruginosa*, *K. pneumoniae*, *E. coli*, and *P. mirabilis* using the agar well diffusion method (Sharif et al., 2021). In another study, the extract from aerial parts of Parthenium weed was evaluated for its antibacterial potency against the selected microbial agents and results revealed that aerial parts of plant weed were also antimicrobial (Madan et al., 2011). Ramteke et al. (2021) prepared the nanoparticles of Parthenium weed extract using magnesium oxide and tested them for their antimicrobial efficacy and the results showed better antimicrobial potential against the selected microbial pathogens (Ramteke et al., 2021).

Tukey Pairwise Comparisons for the antibacterial activity determination have been mentioned in *Table 1* while the comparative graph has been shown in *Figures 1* and *2*.

The findings of the current study and the previous data reveal that the better antimicrobial potential of Parthenium weed can be linked to the presence of potent secondary metabolites in it that are biologically active and possess therapeutic properties. Among the solvents used, the extracts based on acetone and n-hexane solvent had better antibacterial activities than aqueous extract (*Table 2; Figure 3*).

Table 1. *Tukey pairwise comparisons*

Test	Acetone	Water	Hexane	P-Value	F-Value
TPC	127.4 ± 4.9^{B}	151.0 ± 4.5^{A}	118.7 ± 7.5^{B}	0.001	24.87
TFC	32.0 ± 1.8^{A}	5.1 ± 2.2^{B}	26.6 ± 5.3^{A}	0.00	50.43
DPPH	57.6 ± 7.0^{B}	32.7 ± 6.5^{C}	79.9 ± 0.5^{A}	0.001	34.20

P-Value is ≤ 0.05 so the results are significant
Null hypothesis: All means are equal, Alternative hypothesis: At least one mean is different, Significance level, α = 0.05
Means that do not share a letter are significantly different

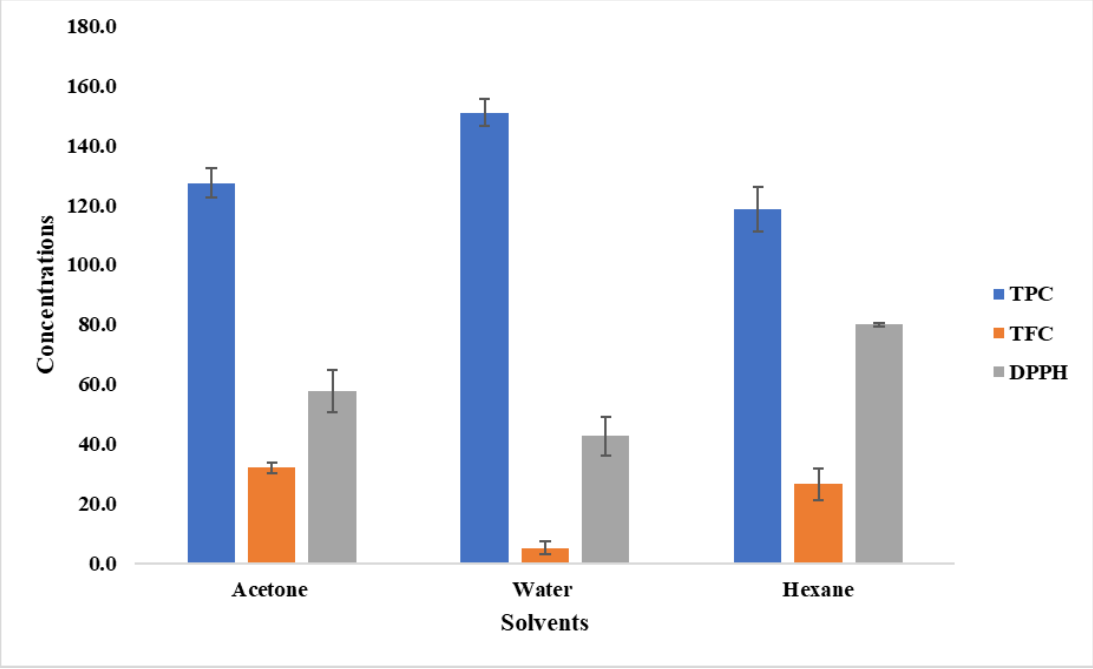

Figure 1. *Comparative graph of the antibacterial activities in different solvents*

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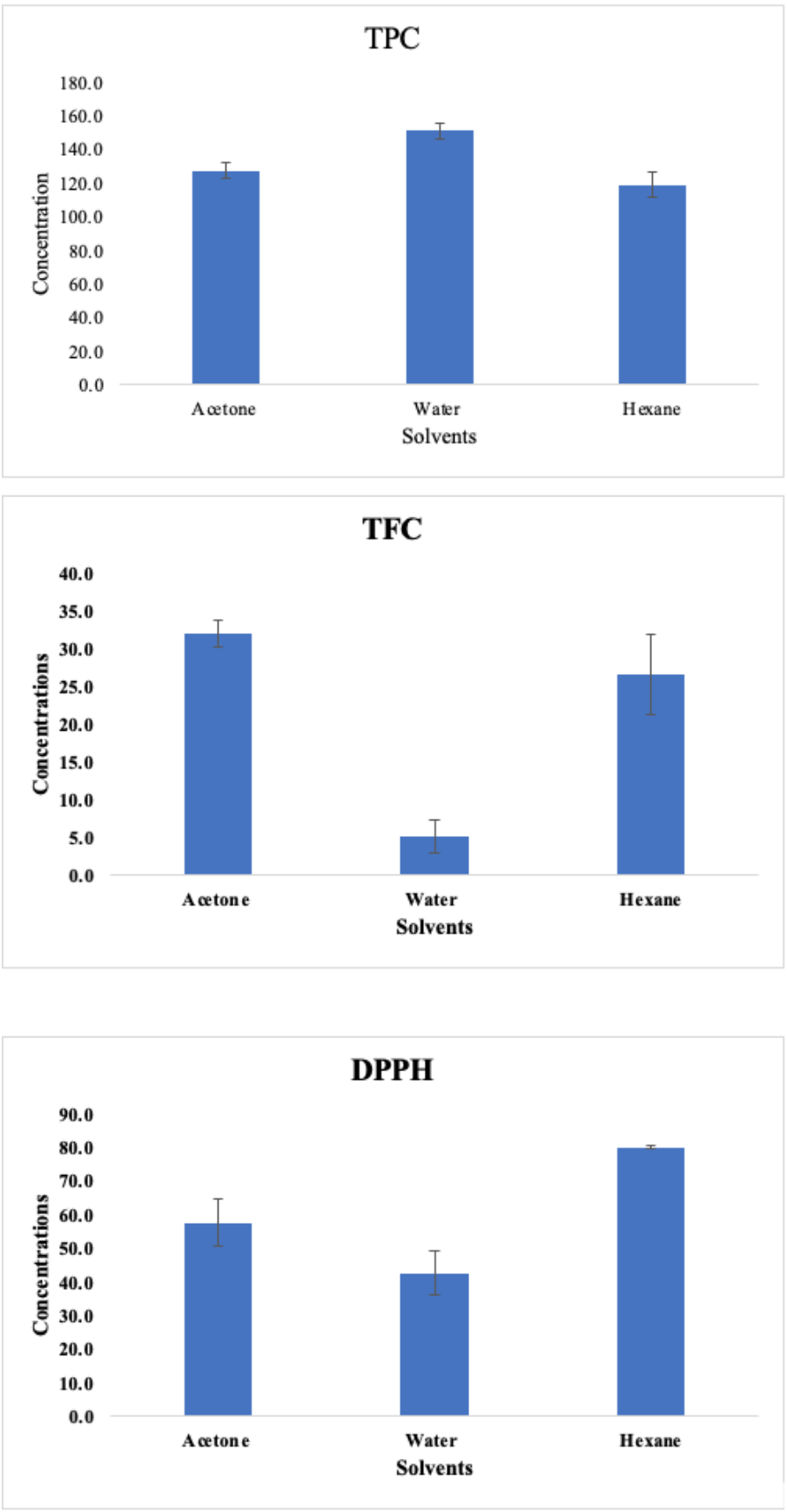

Figure 2. *Different activities in different concentrations*

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Table 2. *Antibacterial activity of water, acetone, and n-Hexane extracts of Parthenium weed against selected bacterial strains*

Solvents	Concentration (mg/mL)	Bacterial strains zone inhibition (mm)			
		E. faecalis	***S. aureus***	***K. pneumoniae***	***P. aeruginosa***
Acetone	20	13.00 ± 1.00	11.67 ± 0.58	9.00 ± 1.00	10.33 ± 0.57
	10	12.67 ± 0.58	13.67 ± 1.55	10.67 ± 0.57	9.33 ± 0.58
	5	12 ± 1.00	13.33 ± 1.53	12.33 ± 1.16	10.33 ± 0.57
Cefepime	-	14.00 ± 1.00	15.00 ± 1.00	16.00 ± 1.00	21.67 ± 1.52
n-Hexane	20	13.00 ± 1.00	11.67 ± 0.58	9.00 ± 1.00	10.33 ± 0.58
	10	12.67 ± 0.577	13.67 ± 1.16	10.67 ± 0.58	9.33 ± 0.58
	5	12.00 ± 1.00	13.33 ± 1.53	12.33 ± 1.16	10.33 ± 0.57
Cefepime	-	14 ± 1.00	15 ± 1.00	16 ± 1.00	21.67 ± 1.52
Water	20	7.67 ± 1.15	7.67 ± 0.57	8.00 ± 1.00	7.67 ± 5.77
	10	7.67 ± 0.57	8.00 ± 1.00	10.70 ± 5.77	7.67 ± 1.15
	5	8.00 ± 5.77	8.00 ± 1.00	9.67 ± 5.77	8.33 ± 0.57
Cefepime	-	21.70 ± 1.15	14.00 ± 1.00	13.00 ± 1.00	20.30 ± 0.57

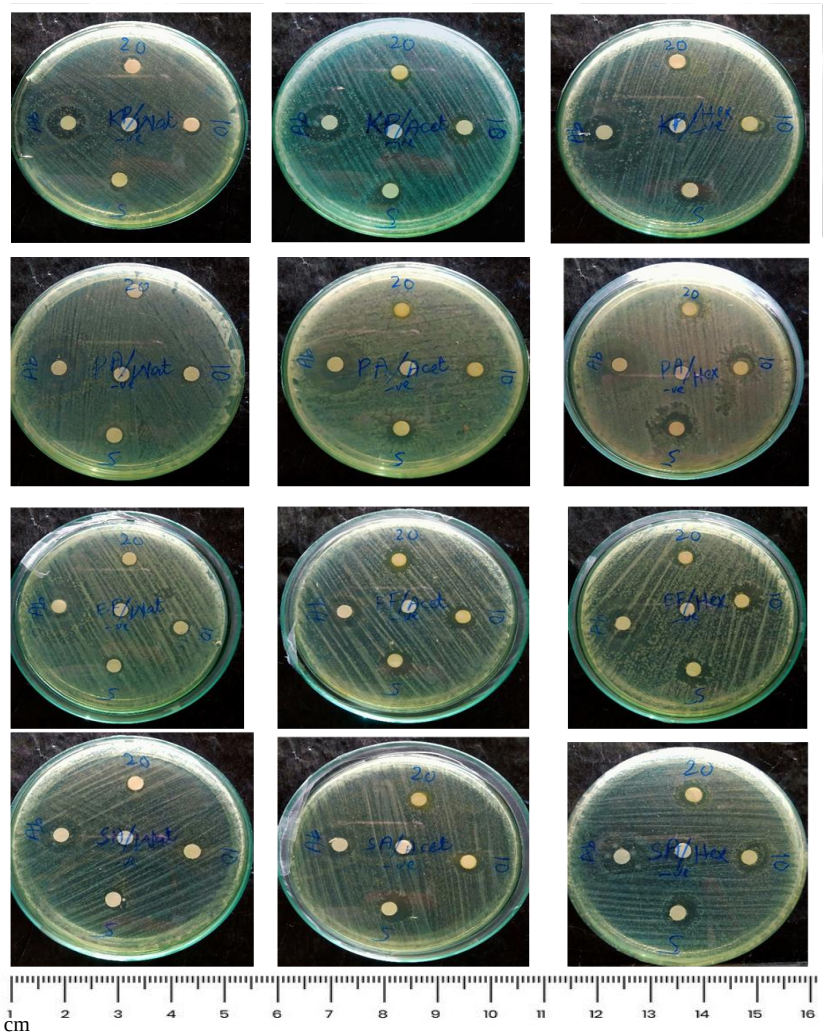

Figure 3. *Inhibition zones of different solvent extracts of Parthenium weed against the selected bacterial strains*

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Minimum inhibition concentration (MIC)

The MIC of different solvent extracts against four pathogenic bacteria is presented in *Table 3*. The results obtained in this study showed that the MIC value of acetone extract (125 µg/mL) was lowest against *P. aeruginosa*. Whereas in the case of *K. pneumoniae* and *E. faecalis*, a much higher MIC value (250 µg/mL) was offered by the acetone extract, and against *S. aureus*, the highest MIC value (1000 µg/ml) was seen. The MIC results obtained for n-Hexane extract revealed the MIC value against *K. pneumonia* was 125 µg/mL, while it was 250 µg/mL for *E. faecalis*, *P. aeruginosa*, and *S. aureus*. Whereas in case of water extract, the MIC values for *E. faecalis* and *P. aeruginosa* was 125 and 250 µg/mL for *S. aureus* and *K. pneumoniae*.

The MIC values of aq. methanolic extracts of selected plants against the microbial strains were also reported by Hussain et al. (2022), by using the agar diffusion/agar dilution assays. Wherein the results reported by Joshi et al. are quite similar to the presented in this current study. In another study by the same group Joshi et al. (2020) % zone inhibition for the under-studied microbial strains was noted to be higher for ash extract (water-soluble) of *P. hysterophorus*. Similarly, the antimicrobial potential of *P. hysterophorus* extracts based on different solvents (Ether, Benzene and Chloroform) was analyzed against rice pathogens, and different microbial strains, and the MIC values ranged between 0.25 and 4.0 mg/mL (Ashfaq et al., 2013) which is quite in line with our results.

Table 3. *Minimum inhibition concentration of water, acetone, and n-Hexane extracts of Parthenium weed against different bacterial strains*

Solvents used	**Minimum inhibition concentration (µg/ml)**			
	P. aeruginosa	***K. pneumoniae***	***E. faecalis***	***S. aureus***
Acetone	125	250	250	1000
n-Hexane	250	125	250	250
Water	125	250	125	250

Total phenolic content (TPC)

Phenolic compounds belong to a major class of the phytochemicals that may restrain the radical scavenging. TPC of Parthenium weed extracts were measured by Folin-Ciocalteu reagent (FCR) assay. Nevertheless, functioning and chemical configuration of this reagent are ambiguous but depending upon its reproducing ability, this reagent is used by majority of the scientists for estimating the total phenols. The results exposed as good correlation between the TPC and antioxidant activity (Zahoor et al. 2018).

In the present study, TPC of *Parthenium hysterophorus* extracts from three solvents (acetone, n-Hexane, water) were analyzed. TPC values ranged 151.0 ± 4.503 - 118.7 ± 7.535 µg GAE/ml. the highest TPC value was showed by aqueous extract while lower was shown by n-Hexane (*Fig. 2 and 4*). TPC values obtained from three solvent extracts were analyzed and means were compared using Tukey's multiple comparison test as shown in *Table 4*. Results showed substantial differences ($P < 0.05$, 95% CI of difference: -38.11 to -9.0) between the TPC values of water and acetone extracts as well as between aqueous extract and n-Hexane extract ($P < 0.05$, 95% CI of difference: 17.78 to 46.87) while TPC value of acetone and water extracts showed no significant differences (($P < 0.05$, 95% CI of difference: -35779 to 23.31).

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Present results are in good agreement with previous research of methanolic flower extract of *P. hysterophorus* (89.364 ± 4.715 g GAE/g) (Iqbal et al., 2022) and methanolic leaves extract of *P. hysterophorus* (57.35 ± 4.12 µg GAE/µg) (Tariq et al., 2022). Contrary to current study findings, Panwar et al. (2015) reported different range of values for TPC (20.82 ± 0.82 mg GAE/g dry sample) in *P. hysterophorus* (Panwar et al., 2015). It might be attributed to the several factors including geographical/climate, soil conditions and varying polarity of solvents.

Table 4. *Comparison test of mean TPC values of water, acetone, and n-Hexane extracts of Parthenium weed*

Tukey's multiple comparison test	Mean difference	Q	Significance ($p < 0.05$)	Summary	95% CI of difference
Acetone & water	-23.56	7.02	Yes	**	-38.11 to -9.01
Acetone & n-Hexane	8.76	2.61	No	ns	-5.779 to 23.31
Water & n-Hexane	32.33	9.64	Yes	**	17.78 to 46.87

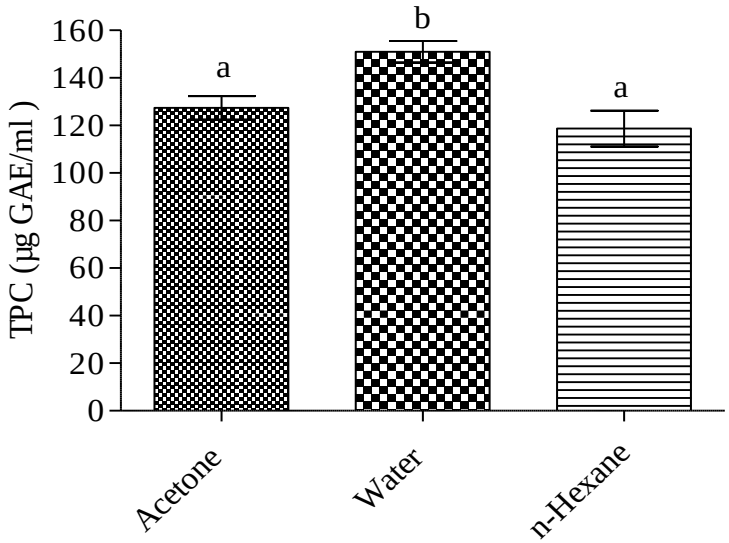

Figure 4. *Mean TPC values of water, acetone, and n-Hexane extracts of Parthenium weed*

Total flavonoid content (TFC)

Total flavonoid contents of *P. hysterophorus* extracted by three different solvents (acetone, n-Hexane, water) is elaborated and compared in *Table 5* and *Figure 5*. Results showed values ranged between 31.96 ± 1.803 and 5.115 ± 2.158 µg QE/ml with highest value was obtained from acetone and lowest by water while n-Hexane extract showed TFC value in between of these two (26.56 ± 5.299 µg QE/ml). As like TPC values, the mean TFC values of these three extracts were analyzed and compared to illustrate significant differences between extracts of selected solvents by using Tukey's multiple comparison test (*Table 5*). Results showed that there was significant difference ($P < 0.05$, 95% CI of difference: 18.17 to 35.52) in mean TFC values of Acetone and water extract as well between water and n-Hexane extracts ($P < 0.05$, 95% CI of difference: -30.12 to -12.76) while acetone and n-Hexane extracts were not significantly related to each other's (($P < 0.05$, 95% CI of difference: -3.267 to 14.08).

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The TFC values of flower extract of *P. hysterophorus* based on methanolic solvent was assessed employing HPLC and the results (65.02 ± 2.69 g QE/g) exhibited in that study were opposite to the finding reported in our study (Iqbal et al., 2022). Likewise, Tariq et al. (2022) worked on the measurement of TFC values for leaf extracts of *P. hysterophorus* using the HPLC technique and the results (39.44 ± 0.41 µg QE/µg) offered were contrary to ours (Tariq et al., 2022).

Table 5. *Comparison test of mean TFC values of water, acetone, and n-Hexane extracts of Parthenium weed.*

Tukey's multiple comparison test	Mean difference	Q	Significance ($p < 0.05$)	Summary	95% CI of difference
Acetone & water	26.85	13.43	Yes	***	18.17 to 35.52
Acetone & n-Hexane	5.40	2.70	No	ns	-3.26 to 14.08
Water & n-Hexane	-21.44	10.72	Yes	***	-30.12 to -12.76

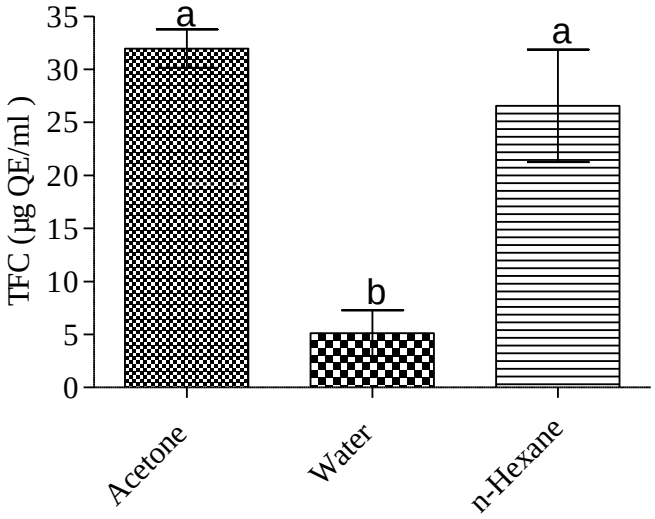

Figure 5. *Mean TFC values of of water, acetone, and n-Hexane extracts of Parthenium weed.*

Antioxidant activity

Antioxidant activity of extracts is usually measured in terms of DPPH radical scavenging potential. Basically, the DPPH is violet colored radical that can accept electrons/hydrogen radicals as well. However, upon accepting the radical/electron from any phenolic moiety, its color turns yellowish and loses its chromophoric property. Discoloration of the radical depends upon the antioxidant potential of the compound being tested. It has also been documented and reported in the literature as with the increase of the amount of phenolic moieties, the degree of DPPH radical scavenging also increases at the same rate.

Antioxidant activity in term of DPPH radical scavenging activity of *P. hysterophorus* extracted by three different solvents (acetone, n-Hexane, water) is elaborated and compared in *Table 6* and *Figure 6* and the values ranged between 79.93 ± 0.4678% and 45.33 ± 2.456% with order of n-Hexane extract > acetone extract > aqueous extract. All extracts were analyzed statistically, and results showed scientifically differences between them as shown in *Table 6*. In a study conducted by Tariq et al. (2022), the

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scavenging activity of methanolic extract from the leaves of Parthenium weeds was 72.82%, that is in close agreement with current study results. Similar results were also presented by another researcher group about the antioxidant activity (76.90%) of methanolic extract from flowers of Parthenium weeds.

Table 6. *Antioxidant activity of water, acetone, and n-Hexane extracts of Parthenium weed*

Tukey's multiple comparison test	Mean difference	Q	Significance ($p < 0.05$)	Summary	95% CI of difference
Acetone & water	12.31	4.94	Yes	*	1.518 to 23.11
Acetone & n-Hexane	-22.28	8.95	Yes	**	-33.08 to -11.49
Water & n-Hexane	-34.60	13.90	Yes	***	-45.40 to -23.80

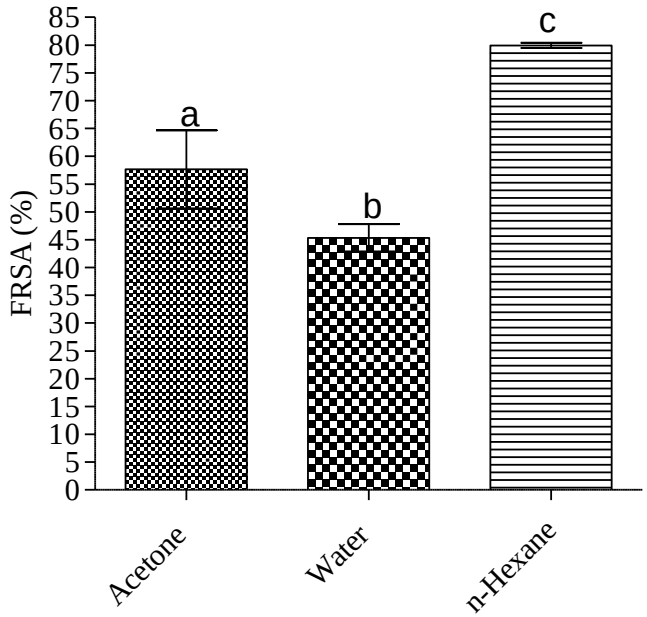

Figure 6. *Free radical scavenging activity (%) of water, acetone, and n-Hexane extracts of Parthenium weed*

Conclusions

It was concluded that the antibacterial potential of the extracts prepared using three different solvents demonstrated that these are capable of resisting bacterial activity and hence cease their function. The most prominent inhibition zones appeared in the case of n-hexane extract, whereas the aqueous extract had the lowest potential in inhibiting bacterial function. The results obtained in this current study support that Parthenium weed extracts have significant antioxidant and antibacterial functions and hence can be analyzed further to be used after clinical trials as an ingredient in pharmaceuticals.

Conflict of interest. The authors declare that they have no conflict of interest.

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