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COMPARATIVE ANTIOXIDANT POTENTIAL IN VARIOUS ANATOMICAL STRUCTURES OF *PARTHENIUM HYSTEROPHORUS* BEFORE AND AFTER FLOWERING

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ABSTRACT

Parthenium hysterophorus, commonly known as invasive Parthenium weed, is a native species of the Americas but is now found in many parts of the world. Recently, the plant has gained notoriety for causing allergic reactions and allelopathic responses. The current study was designed to test the different parts, particularly the roots, for their antioxidant potential. The finding will help in the eradication of the plants if the roots possess higher antioxidant activity than other parts. Recent research has highlighted its potential application in the context of antioxidant activity. For antioxidant activities, the different parts of *P. hysterophorus* (flowering and non-flowering) plants were collected from the University of Swat. The extract of these parts was subjected to a spectrophotometer using DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical to investigate its antioxidant potential. The extract showed a higher antioxidant activity in all parts of both flowering and non-flowering plants of *P. hysterophorus*. Still, the highest result was displayed and observed in the stem of both flowering and non-flowering plants of *P. hysterophorus*. These findings support the potential use of *P. hysterophorus* as a natural source of antioxidants, considering the toxicity of the species as a promising source of functional food or supplements. The study observed differential antioxidant potential in various parts, which is closely related to the season, as compared to previous studies. Further research is needed to identify the specific antioxidant compounds and evaluate their clinical relevance.

***Keywords*:** *Parthenium hysterophorus*; different parts; antioxidant activity; natural product.

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1. INTRODUCTION

Parthenium hysterophorus is an herbaceous plant belonging to the family *Asteraceae*, also commonly known as Congress grass and carrot grass. Plants of *P. hysterophorus* spread rapidly throughout subtropical and tropical regions, thereby becoming a globally recognized invasive species of concern worldwide. The American origin of *P. hysterophorus* supported its widespread distribution across India, Australia, and particular African regions (Shabbir and Bajwa 2006). The plant develops a deep taproot system, making it efficient in multiple ecological scenarios, arid environments. Plants achieve dominance over local vegetation because they demonstrate both effective drought resistance and strong adaptive abilities in their environment. *P. hysterophorus* achieves 2 meters vertical growth through pale green leaflets that develop deep lobed structures. Individual plants of *P. hysterophorus* produce off-white flowers in clusters at the tips and spread extensively by generating 25,000 seeds (Mao et al. 2021; Naz et al., 2024). *P. hysterophorus* demonstrates its top competitive abilities when soil pH ranges from 5 to 6, allowing it to invade agricultural land successfully (Mao et al. 2021).

The introduction of *P. hysterophorus* to India occurred through wheat import shipments brought in during the 1950s (Bezuneh 2015). *P. hysterophorus* possesses phytochemical elements that include alkaloids, saponins, flavonoids, and terpenoids with essential oils alongside essential compounds. *P. hysterophorus* demonstrates antimicrobial properties due to two main active components: parthenin, ambrosin and hysterine (Bezuneh 2015; Ahmad et al. 2021). Bacterial culture elimination, in addition to antidiabetic and anticancer capabilities makes *P. hysterophorus* effective as an anti-inflammatory compound. The plant achieves strength through potassium and magnesium minerals, which assist calcium to helping adaptation to environmental changes (Bezuneh, 2015). The

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plant *P. hysterophorus* contains two antioxidant phenolic compounds, including the dual chemistries of protocatechuic acid and vanillic acid (Bezuneh et al. 2015). Current scientific evidence demonstrates that *P. hysterophorus* essential oil fractions, containing substantial concentrations of sesquiterpenes, exhibit clear biochemical properties. Studies within scientific fields indicate that plant-derived oils eliminate dermatophytes and other fungal infections making them appropriate for medical treatment (Ghazal et al., 2024; Bagchi et al. 2016; Ahmad et al. 2021; Ahmad et al. 2022).

P. hysterophorus holds elevated value for medical and natural remedy applications due to all its bioactive compounds. The Caribbean and Central American regions have used *P. hysterophorus* as traditional medicine for many years. Multiple therapeutic properties have been identified in *P. hysterophorus* according to scientific research. Studies with diabetic rats revealed that *P. hysterophorus* effectively reduces blood glucose levels, suggesting potential future medical applications as antidiabetic medication (Bagchi et al., 2016). In studies, Scientists discovered that lymphocytic leukemia-transplantable mice had increased survival rates after receiving treatment with *P. hysterophorus* extract (Sajid et al., 2020; Khan et al., 2024). Medical scientists also use *P. hysterophorus*, combined with different treatments, for treating hepatic amoebiasis that targets the liver (Sharma et al., 2001). The plant functions through allelopathy to limit fungal development between three species, including *Fusarium moniliforme* and *Alternaria alternata (Fr.) Keissl* and *Drechslera hawaiiensis* (*M.B. Ellis*) (Sharma et al. 2007). *P. hysterophorus* possesses active biological components, making it suitable for agricultural use as a natural fungicide.

A wide assortment of antimicrobial action characterizes *Parthenium hysterophorus*, making it one of its central biological functions. According to (Kaur et al. 2021; Rashid et al. 2024; Ali et al. 2024). antimicrobial functions result from sesquiterpene lactones and other bioactive compounds in the plant, which additionally deliver antifungal, alongside antibacterial, antiviral, and anti-inflammatory effects. Studies have investigated various therapeutic aspects of the plant in the treatment of skin rashes, wounds, and fevers (Ahmad et al., 2025). Physical investigations of *P. hysterophorus* are essential, as the rise of antibiotic-resistant pathogens justifies the need for new antibiotic systems. The plant shows potential for natural medical treatment of antimicrobial infections, given the growing resistance to antibiotics (Bezuneh, 2015).

The combination of aqueous solution and solvent-derived extracts from *P. hysterophorus* leaves eliminates survival rates of different bacterial strains. The antibacterial strength of these extracts was evaluated through well-diffusion testing to determine inhibition zones (Atta et al., 2017; Ashraf et al., 2023; Rashid et al., 2024; Abbas et al., 2024). Laboratory research suggests that P. hysterophorus has the potential to serve as a natural antimicrobial treatment for bacterial infections, including those caused by resistant strain isolates. Experimental tests with *P. hysterophorus* have demonstrated an adequate anti-inflammatory capacity in both laboratory and animal model results. The research confirms that plant extract components regulate inflammatory substances, including TNF-α, IL-1β, and IL-6, due to their involvement in cancer development (Gul et al., 2022). The scientific community identifies *P. hysterophorus* as a prospective therapeutic agent for inflammatory diseases, along with an approach to limit their health consequences in society (Gul et al. 2022; Ashraf et al. 2023; Abbas et al. 2024). *P. hysterophorus* protects cells through its antioxidant mechanism against ROS, as well as in diabetes, neurodegenerative diseases, and cancer development (Hossain et al. 1985). *P. Hysterophorus* contains antioxidant compounds that include phenolic compounds and flavonoids, which protect cells against oxidative damage while securing overall health (Atta et al. 2017).

The present research aimed to determine the antioxidant properties of *P. hysterophorus* weed using the DPPH method as its measurement approach. The study examined the antioxidant properties of *P. hysterophorus* in both leaves, stems, and flower segments. The research monitored changes in antioxidant behavior between plant sections and assessed the alterations before and after flowering to gain insights into the evolution of antioxidants throughout the plant's lifetime. The flowchart for the current study is shown in Fig. 1.

2. MATERIALS AND METHODS

2.1. Experimentation

The DPPH assay was used to evaluate the antioxidant potential of the various *P. hysterophorus* components. The analysis was conducted using a 96-well plate reader at the University of Swat (UOS) Center for Biotechnology and Microbiology (CBM) laboratory.

2.2. Selection and Collection of Plant Materials

The present study aimed to evaluate the antioxidant activities of several parts of *Parthenium hysterophorus*, namely the roots, stem, leaves, and flowers. Collections of *P. hysterophorus* specimens were made from a variety of sites, including at the University of SWAT (UOS). The plant was identified by expert plant taxonomist at University of Swat and the specimen was submitted to the herbarium.

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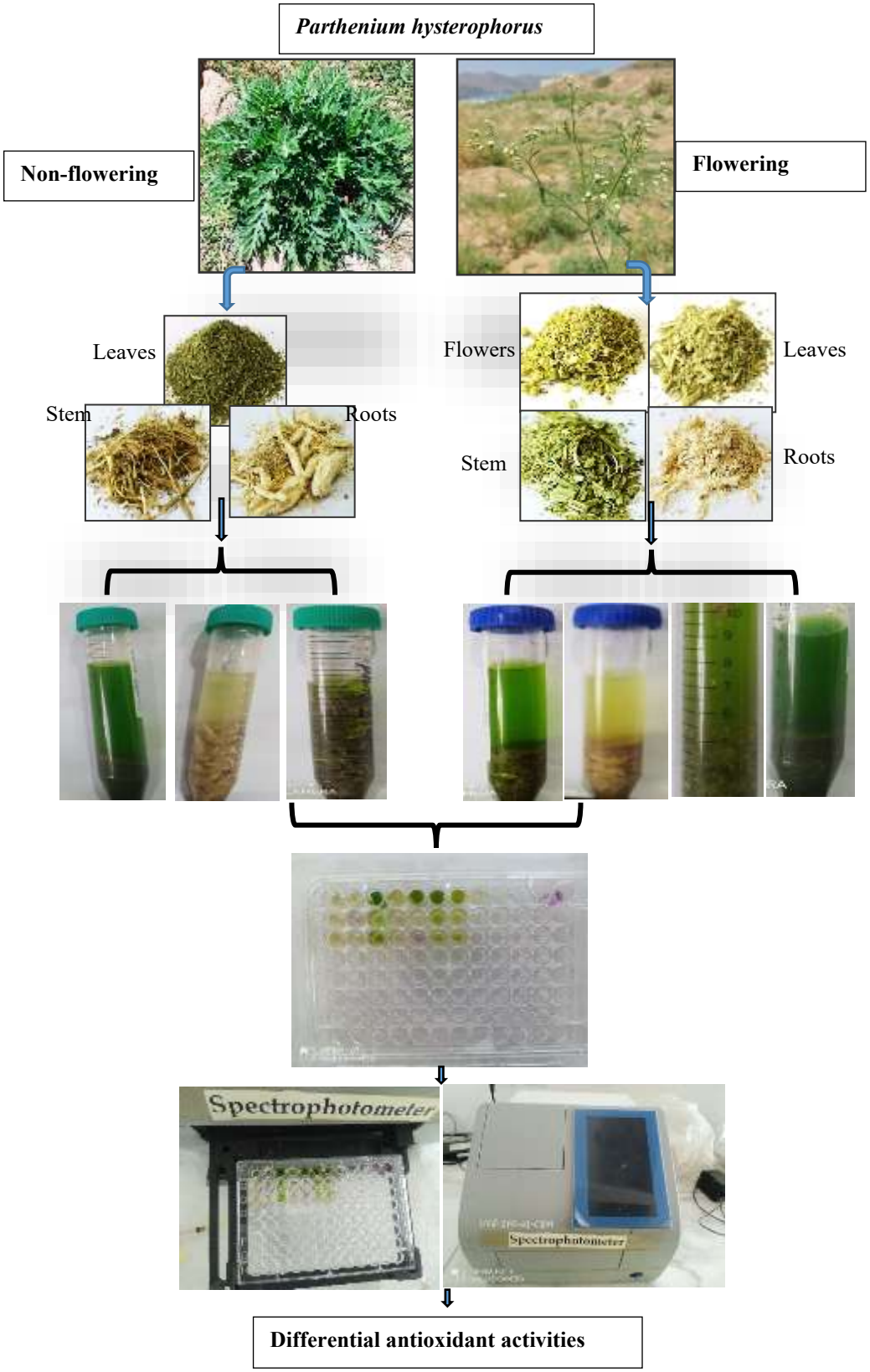

Fig. 1: Pictorial overview of the overall experiment.

2.3. Identification of *P. hysterophorus* Plant

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Taxonomists with expertise in *P. hysterophorus* confirmed its authenticity, and the plant samples were stored in the herbarium of the Medicinal Botanic Center (MBC) at the PCSIR Labs complex in Peshawar. Plant scientists at the University of Swat's Department of Plant Sciences also verified the authenticity of the *P. hysterophorus* plant.

2.4. Extraction preparation

With extreme caution, the *P. hysterophorus* plant was brought to the lab. The plant's leaves, stem, roots, and flowers were carefully separated, and each part was dried for 26 hours at 45°C. Once the sample had dried sufficiently, it was powdered into a powder. Initially, 10 mL of ethanol was added to one gram of each portion of *P. hysterophorus* to create the solution. After two hours, test tubes holding one gram of the material with ethanol were shaken to dilute it. Plant material was filtered out of the solution the next day. Centrifugation was used to purify the solution from each component. Before centrifugation, the solution from each component was moved from the test tubes to the Eppendorf tubes. After the mixture was allowed to incubate, it was centrifuged for 10 minutes at 10,000 rpm to produce a clear solution. This purification procedure was completed all at once. Before the extract was used in more operations, it was kept at room temperature.

2.5. Template designing for antioxidant activity using 96 well plate

First, a mixture of 1 gram of powder and 10 mL of ethanol was prepared. After that, differential concentrations were created in the 96-well plates using serial dilution in both ascending and descending orders. The differential activity was assessed using this approach, which yielded a result between 3 and 10µg/1mL. It is significant to note that the concentration must be adjusted to match the well size, as only 200 µL of the solution can fill each well of the microtiter plate reader. For the activity concentration, a further decreasing order was used (3 to 10 µg/2 mL). A detailed investigation was conducted to determine the optimal antioxidant potential in both ascending and descending concentrations.

2.6. DPPH Solution preparation and activity procedure

As per the methodology of Ahmad et al. (2022), 1.0mg of powder was mixed with 100mL of ethanol to create the 2,2 diphenyl-1-picrylhydrazyl (DPPH) stock solution. When determining the optical density (OD) for antioxidant activity tests, 200 µL of the DPPH solution was first diluted with ethanol to a concentration of 0.2. In order to prevent oxidation, the absorbance at 0.8nm was measured in a microtiter plate after an hour at room temperature in the dark. Then, using the formula, the percentage of scavenging activity was determined:

DPPH scavenging activity (%) = [(Abs Control-Abs Sample) Abs Control] ×100

Here, Abs Control represents the absorbance of the control reaction solution (DPPH) without the polyphenolic extract (PE), and Abs Sample represents the absorbance of the test compound**s.**

2.7. Statistical Analysis

An Excel spreadsheet was used to record the data, which was gathered in triplicate. After that, Excel was used to calculate the means for each sample.

3. RESULTS

3.1. Antioxidant potential in various parts of flowering and non-flowering *P. hysterophorus*

Antioxidant potential was investigated in different parts of flowering (Fig. 2a) and (Fig. 2b) non-flowering *P. hysterophorus* using different concentrations. Initially, the lower concentration (10 mg/10 mg) of each part extract was tested for its antioxidant potential (Fig. 2).

Before flowering, the antioxidant activity of 57.4% was recoded, was observed in the leaves of *P. hysterophorus* as shown in Fig. 3. The antioxidant activities of stem, and roots, of non- flowering plants was also investigated, while the different parts (stem, roots, leaves, flowers) of flowering plants was also tested for antioxidant potential using lower concentration (10 mg/10 mg). We used 3 treatments to find the antioxidant activity of *P. hysterophorus* in percentage (%) with three concentrations such as 10mg/10mL, 50mg/10mL, and 100mg/10mL which was shown in the below figures, in the first Fig. 3 we measured and recorded the highest antioxidant activity of parthenium plant parts is non- flowering stem (73.22%). The second-highest antioxidant activity was recorded in the flowering plant part, specifically the stem (66.22%).

3.2 Antioxidant potential in various parts of flowering and non-flowering *P. hysterophorus*

In this study, the antioxidant activity of different parts of *P. hysterophorus* was recorded after incubating for approximately 30 minutes at room temperature. After the reaction, the result was recorded in different parts of the

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parthenium, such as stem, roots, and leaves of non-flowering plants, while the antioxidant potential was also investigated in different parts of the flowering plant (stem, roots, leaves, and flowers). As the concentration of the different parts of flowering and non-flowering was increased from 10 mg/10 mL to 50 mg/10 mL, an increase in overall antioxidant potential was observed.

Fig. 2: Pictorial presentation of a flowering (A) and a non-flowering plant (B) of *P. hysterophorus*.

After the incremental increase in plant extracts, the extract displayed significantly higher activity than lower concentrations of extract obtained from different parts of \parthenium plant before and after flowering. Among the non-flowering plant parts, the stem extract displayed the highest activity at 81.58%, followed by roots (80%) and Leaves (70%). While the flowering plant parts such as stem displayed a comparatively higher activity of 74.33% as compared to 10mg/10mL, the roots exhibited 50.33% activity, leaves (67.2%), and flowers (32.5%) as shown in Fig. 3. Here, the maximum activity was observed in stem of parthenium plants extracted before (81.58%) and after flowering (74.33%) upon the application of higher concentration (50mg/10mL) as shown in the Fig. 4.

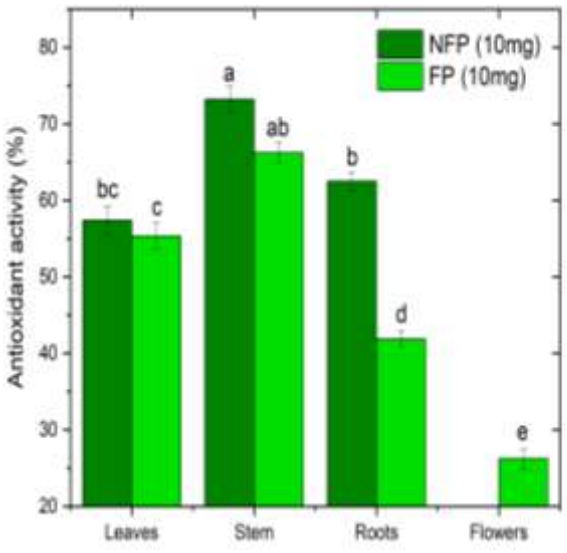

Fig. 3: Comparative antioxidant potential in various parts of flowering and non-flowering plants of *P. hysterophorus* using lower concentration (10mg/10mL). Bars bearing different letters differ from each other at P<0.05.

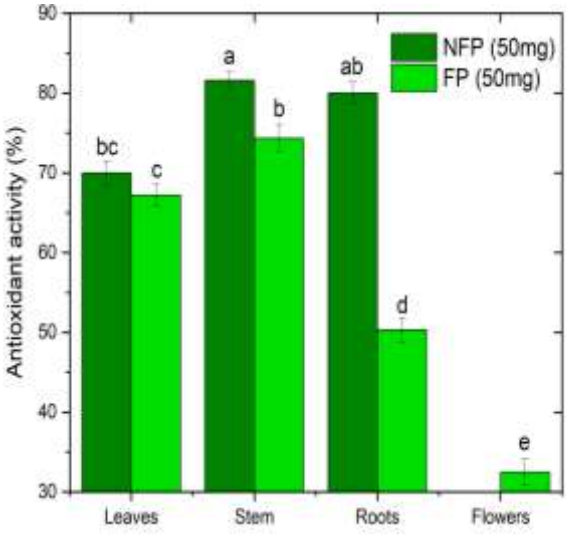

Fig. 4: Comparative antioxidant potential in various parts of flowering and non-flowering plants of *P. hysterophorus* using moderate concentration (50mg/10mL). Bars bearing different letters differ from each other at P<0.05.

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3.3. Antioxidant potential in various parts of flowering and non-flowering *P. hysterophorus*

In this study, a higher concentration of 10mL/100mg was applied to investigate the antioxidant activity of different parts of the *P. hysterophorus* plant. Giving the concentration of 10mL/100mg the results displayed highest activity as compared to the concentration of 50mg/10mL. the antioxidant activity of different parts of *P. hysterophorus* was observed. The maximum concentration is displayed in percentage (%). The following results were investigated and recorded after the reaction of flowering plants such as stems (78.33%), Roots (57.5%), Leaves (74.37%), flowers (55.41%), and non-flowering plants such as stems (86.87%), Roots (85%), Leaves (78.5%). This experiment demonstrates that increasing the concentration will enhance the antioxidant activity of the different parts of *P. hysterophorus*. In Fig. 5, the highest antioxidant activity of the parthenium plant parts was also recorded in the stem before and after flowering.

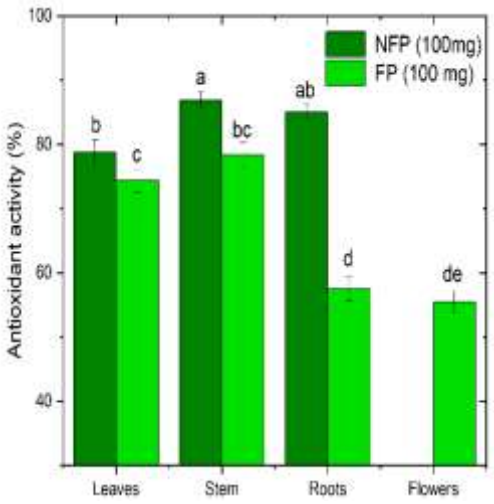

Fig. 5: Comparative antioxidant potential in various parts of flowering and non-flowering plants of *P. hysterophorus* using the highest concentration (100mg/10mL). Bars bearing different letters differ from each other at P<0.05.

4. DISCUSSION

The emergence of medication resistance in human pathogenic organisms has sparked a hunt for novel antibacterial compounds from non-traditional sources. The antibacterial effect of strong medications has been explained by several different mechanisms, including those involving plant extracts (Mishra et al. 2011). Because they have a complex blend of components, phytochemicals can sometimes be more effective than artificially manufactured pure molecules. Because of their complexity, which allows them to interact with a variety of molecular targets, target microorganisms find it more difficult to acquire resistance due to the abundance of response sites (Acamovic and Pennycott et al. 2004; Acamovic et al. 2004; Khan et al. 2024). The antibacterial properties of higher plants have been linked to secondary metabolites such flavonoids, tannins, alkaloids, and other phytochemicals, according to the literature. Flavonoids from *P. hysterophorus* extracts and other phytochemicals that we reported in our earlier study (Kumar et al. 2013). might be useful as antibacterial agents in the future. In a human population, the incidence of many malignancies rises exponentially with age between the fourth and eighth decades of life. Cancer is the leading cause of mortality for both men and women globally, taking the lives of almost 6 million people annually (Kaufmann et al 2000). In India, there would be over 250,000 new instances of breast cancer by 2015, according to a study conducted by the WHO's International Agency for Research on Cancer (IARC) (Kaufmann et al. 2000). Roughly 60% of anticancer medications come from plants; examples include camptothecin from *Cuscuta reflexa* and taxol from Taxus *brevifolia* (Verma et al. 2008). The preferred anticancer medication is one that induces apoptosis, targets particular cytotoxicity to cancer cells, and has minimal adverse effects (Verma et al. 2008).

P. hysterophorus is known to contain a wide variety of allelochemicals/compounds that are primarily responsible for both its beneficial and harmful properties. Parthenin, a major sesquiterpene lactone, is primarily responsible for its allelopathic properties; allelochemicals are also present in the plant's trichomes and pollens. *P. hysterophorus* properties associated with health benefits are primarily due to the presence of certain oils, histamines, terpenes, polyphenols, alkaloids, and pseudo guaianolides (Kamal et al. 1991). The polyphenols in *P. hysterophorus* extracts give them antioxidant activity; qualitative analysis of the plant extract confirmed the presence of alkaloids, terpenoids, carbohydrates, and cardiac glycosides; Rf values obtained from thin-layer chromatography indicated the presence of multiple phytochemical compounds in the *P. hysterophorus* plant extract; the extracts were found to have inhibitory potential against HIV-1 RT and some bacterial strains responsible for several human diseases, including S. *epidermis, Salmonella typhi*, *gonococci*, *Citrobacter*, and *Flexner* species.

The biological activity seen in different plants are caused by their phytochemical makeup. Consequently, two crucial elements for biological activities, such as antioxidant potential, are their phenolic and flavonoid content (Balamurugan et al. 2019; Khan and Javed 2021; Iqbal et al. 2024). Moreover, FCe has substantial concentrations of phenolics and flavonoids at 80µg (65.022g QE/g and 89.364g GAE/g). At 80µg of FCe, the antioxidant activity was also high, confirming the intriguing correlation between the percentage of DPPH scavenging and the phenolic and flavonoid levels as described by (Nadkarni et al. 1982; Yadav et al. 2010). Numerous investigations discovered a connection between antioxidant activity and phenolic concentration (Mau et al. 2002; Ayaz et al. 2014). The antioxidant properties of FCe can be attributed to its high phenolic and flavonoid content. DPPH is a synthetic

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chemical used in in vitro tests to determine biological materials' antioxidant potential. They create free radicals once their solutions are incubated. Their colors are different when they are oxidized. The color of the solution will change if the substance being tested has antioxidant qualities, as it will donate electrons to these free radicals. A UV-visible spectrophotometer is used to compute the percent inhibition, which measures the color shift or reduction in intensity (Sharma et al. 2001; Abbasi et al. 2011)

The same protocol is used of DPPH in the recent study for finding the antioxidant activity of different parts *P. hysterophorus*. And we get accurate result of *P. hysterophorus* different parts like stem, roots, leaves and flowers due to good condition season. Because the different parts of *P. hysterophorus* displayed highest antioxidant activity.

5. CONCLUSION

Parthenium hysterophorus is a species of flowering plant in the family *Asteraceae*. It is native to the Americans tropics. This plant has both harmful and beneficial ability and also this is used for traditional medicines including anti-inflammatory, antibacterial, antimicrobial, antifungal, and anti-cancerous phytochemical analysis reveal that species contain phenome compounds and flavonoids and phenolic acids and its organic extract the essential oil of *P. hysterophorus* primarily consist of antimicrobial, antibacterial, anti-inflammatory and antioxidant activities. Recent studies in the UK and US have focused on *P. hysterophorus* phytochemical properties leading to this investigation of its antioxidant potential of plant parts. In the recent experiment we did to find out the antioxidant potential activities of different parts of *P. hysterophorus*. for finding the antioxidant activity of *P. hysterophorus* taking parts of this plant like stem, roots, leaves, and flowers of flowering plant and also took parts of non-flowering plant like stem, roots, and leaves. The results were displayed in the statistical graph and recorded. This result aimed to evaluate the antioxidant activity of different parts of *P. hysterophorus* using the DDPH scavenging method, revealing its potential as a natural source. The study found that the stem of *P. hysterophorus* exhibits the highest antioxidant activity, with a maximum scavenging of 86.87% in the stem. The study concludes that *P. hysterophorus* stem, roots, leaves, and flowers are rich in antioxidants, making it a valuable ingredient for pharmaceutical formulation due to its high antioxidant potential.

DECLARATIONS

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Conflicts of Interest: All the authors declare that they have no conflict of interest regarding this manuscript.

Data Availability: Data will be available from the corresponding author upon request.

Ethics Statement: No animals or human study is involved in this experimentation; thus, no ethical approval is needed.

Author's Contributions: Sher Ali performed the experimentation and wrote the initial manuscript, Noor Muhammad helped in data interpretation, Rahman Ali Khan helped in methodology, Liaqat Ali helped in graphs preparation, Salman Khan performed statistical analysis, Sajjad Ahmad helped in pictures collection, Sidra Amin helped in results preparation, Hayat Khan reviewed the manuscript for mistakes, Hina Fazal provided DPPH for the experimentation and Nisar Ahmad finalized the manuscript for submission, revision and supervision.

Generative AI Statements: The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

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