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Phytochemical analysis and antibacterial properties of *Parthenium hysterophorus*

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Abstract

The development of antibiotic-resistant pathogens poses a significant challenge to modern medicine, necessitating the investigation of alternative antimicrobial agents from natural sources. *Parthenium hysterophorus*, an invasive plant species, has been traditionally used in folk medicine for its antimicrobial properties. This study aims to investigate the phytochemical composition of *P. hysterophorus* extracts and evaluate their antimicrobial activity against pathogenic bacterial strains. Aqueous and methanol extract of *Parthenium hysterophorus*L were screened for their phytochemical constituents and its antimicrobial activity by Agar well diffusion method against pathogenic bacteria including Gram negative *Escherichia coli, Escherichia coli devis*, *Salmonella typhi, Salmonella paratyphi, Pseudomonas aeruginosa* and Gram positive organism- *Bacillus megaterium, Staphylococcus aureus.* Phytochemical screening of the plant extracts was conducted to detect the presence of alkaloids, flavonoids, tannins, saponins, and other bioactive compounds. Results revealed the presence of potent bioactive compounds, with significant antibacterial activity observed in methanolic extract. Aqueous extract only inhibit growth of *E.coli*. The inhibition zones of *P. hysterophorus* extracts were comparable to standard antibiotic tetracycline. The findings highlight the importance of plant-based compounds in combating drug-resistant infections and pave the way for further studies on the isolation and characterization of active constituents.

Keywords: *Parthenium hysterophorus*; Antimicrobial activity; Soxhlet; Methanolic extract

1. Introduction

Development of new drugs from natural sources is highly influenced by its ethno botanical uses. Though *Parthenium hysterophorus* L. is a weed and causes severe allergies to human being, It is reported with therapeutic applications. Decoction prepared its roots has been used by American Indians in medicine to treat amoeboitic dysentery. Its applications in treating neurologic disorders, fever, urinary infections, dysentery and malaria. This is very much resistant to plant pathogenic microorganisms; this could be attributed to presence of antimicrobial metabolites in plant parts. In present investigation an attempt was made to ascertain the effectiveness of leaves extract which imparts antimicrobial effect to the whole plant extracts[1].The prevalence of infectious diseases caused by bacterial pathogens presents a significant public health challenge today. Antibiotics are commonly used to treat these bacterial infections; however, the extensive and frequent use of these medications has led to the emergence of multi-drug resistant strains, which are increasingly difficult to manage with currently available treatments. In this context, the use of plant-based products emerges as a promising and safer alternative for addressing such multi-drug resistant bacterial pathogens. Plants are known to contain a diverse array of bioactive compounds, including alkaloids, tannins, flavonoids, chlorophyll pigments, linalool, eugenol, and caffeine, which have demonstrated the potential to inhibit the growth of bacterial pathogens effectively.[2]To combat AMR, scientists are actively investigating both naturally occurring and synthetically produced antimicrobial agents as possible therapeutic alternatives.[3] Currently there is a wide variety of plant species known to be used in the traditional medicine and which could subject to extraction procedures to recover bioactive compounds with antioxidant activity. One of them is *Parthenium hysterophorus*, also known as

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Partheniumweed,altamisa ,carrot grass ,bitterweed and wild feverfew ,which is an annual herbaceous shrub belonged to the Asteraceaefsmily.[4]

The phytochemical profile, antioxidant activity, total phenolic contents (TPC), total flavonoid contents (TFC), and cytotoxicity of *Parthenium hysterophorus* L. flower extract were evaluated, and the toxic effects were assessed in rabbits. The HPLC-DAD system was used for phytochemical analysis. The hemolytic and DPPH assays were performed. The findings also confirmed the presence of phenolic and flavonoid content in the flower extract, both of which contribute to the plant's antioxidant potential.[5]*Parthnium hysterophorus*Linn (Asteraceae) is an aggressive and exotic weed plant reported to be used as remedy for various diseases.Aqueous and ethanolic extracts obtained from various parts of *P.hysterophoruLinn*.were evaluated for antimicrobial activity against three bacterial strain. (*Staphylococcus* aureus MTCC306, Bacillus cereus MTCC12172, *E. coli* 277 and fungal strain *Saccharomyces cerevisiae* MTCC36, candida MTCC3017, *Aspergillus flavus* MTCC277.[6] Phytochemical composition was determined by colorimetric tests and RP-HPLC-MS analysis andantioxidantactivity was evaluated with the Trolox equivalent antioxidant capacity (TEAE),2-2-diphenyl -1-picrylhydrazyl (DPPH)and hydroxyl radical (-OH) scavengingassay.The phytochemical screening of the AE and EE by colorimetric test showed the presence of flavonoids, steroids, terpenes, saponins ,coumarins, tannis.[4]*Parthenium hysterophorus*is a plant used in traditional medicine to treat health issues andwhich could be a source of phytochemicals with possible antioxidant activity without causing cytotoxic effects.[4]This is very much resistant to plant pathogenic microorganism, this could be attributed to presence of antimicrobial metabolites in plant parts. Most of this studies regarding evaluation of antimicrobial potency used the whole plant in present investigation an attempt was made to ascertain the effective plant part which imparts antimicrobial effect to the whole plant extract.[6]The phytochemical screening of the Aqueous extract and ethanolic extract by colorimetric test showed the presence of flavonowids ,steroids ,triterpene, lactones ,tannins and carbohydrates[4] and qualitative analysis of aqueous *Parthenium hysterophorus* leaf extract was studied for the presence of carbohydrates ,alkaloids, steroid ,glycosides,tannins ,phenolic compound, saponins, flavonoid, oil,carbohydrate, protein, amino acid were determined spectrophotometrically. The aqueous extract was studied for its fluorescence and yield.[7]

Beyond its status as a weed, *P. hysterophorus* has been the subject of research due to its potential medicinal properties. The plant contains a variety of phytochemicals, including flavonoids, alkaloids, tannins, and essential oils, which contribute to its biological activities.

Referring all the data, this work was designed to evaluate its phytochemical profile, and antimicrobial activity.

2. Materials and Methods

2.1. Chemicals and reagents

All chemicals used for the study were of AR grade and purchased locally.

2.2. Plant materials

Authentication of the plant
The leaves of *Parthenium hysterophorusLinn*(Asteraceae) were collected from premises nearby Sangli city. The samples were authenticated by the Botany Department Kasturbai Walchand Arts, Science College Sangli.

2.3. Extract preparation

*Parthenium hysterophorus*L.leaves was collected from well grown plant. Collected leaves were washed thoroughly with running then with distilled water and dried at room shade for a week. Then it was homogenized to a fine powder and stored in an airtight glass container, protected from sunlight, stored at 4°C until the further process. Water and methanol used for the extraction. The aqueous extract was prepared by maceration process in the ratio 1:10(w/v)[8]. The methanol extraction of the plant leaves was carried out using a Soxhlet extractor in the ratio 1:10(w/v). Both the extract filtered through whatman filter No.1 and allows evaporating to obtain residue. The semisolid extract obtained was stored in an airtight container at 4º C in freezer for further use. For antimicrobial activity, a volume of 5mg of the extract was dissolved in 1ml of 5 per cent dimethyl sulphoxide (DMSO).[9]

2.4. Phytochemical analysis

Qualitative test of both the aqueous and methanolic extracts were performed to confirm the presence of bioactive compounds having pharmacological importance through following test-

2.4.1. Test for Alkaloids

Wagners test

To about an ml of extract few drops of Wagners reagent were added. Reddish –brown precipitate indicates presence of alkaloids.

Hangers test

The test solution treated with Hangers reagent (saturated picric acid) gives yellow precipitate.

2.4.2. Test for Flavonoids

Lead acetate test: 1ml of extract few was taken and 10% of lead acetate was added. The yellow precipitate is positive inference for the flavonoids.

Concentration of Hydrochloric test: The extract is treated with conc.hydrochloric resulting in the formation of orange color indicates the positive result for flavonoids.

2.4.3. Test for steroids

2ml of extract with 2ml chloroform and 2ml of conc. H_2SO_4 are added, the appearance of red color and yellowish green fluorescence indicates the presence of steroid.

2.4.4. Test for tannin

To 5ml of extract few drops of neutral 5%ferric chloride solution was added, the production of dark green color indicates the presence of tannin.

2.4.5. Test for protein (Biuret reagent)

2ml of filtrate was taken to which 1 drop of 2% copper sulphate solution was added.1ml of 95% ethanol was added. Then it was followed by excess addition of KOH. The appearance of pink color indicates the presence of protein.

2.4.6. Test for carbohydrate (Benedict reagent)

About 0.5 ml of the filtrate was taken to which 0.5 of Benedict reagent is added .This mixture was heated for about 2 minutes in a boiling water bath. The appearance of red precipitate indicates the presence of sugar.

2.4.7. Test for Amino acid

To 1 ml of the extract, few drops of ninhydrin reagent (10 mg of ninhydrin in 200ml of acetone) were added. The appearance of purple color indicates the presence of amino acid.[10]

2.5. Methods for Assessing Antimicrobial activity

2.5.1. Collection and maintenance of pathogens

Pathogenic bacteria used for the study were Gram negative organism- *Escherichia coli, Escherichia coli devis*, *Salmonella typhi, Salmonella paratyphi, Pseudomonas aeruginosa* and Gram positive organism- *Bacillus megaterium, and Staphylococcus aureus.* ,

The organisms were obtained from MTCC Chandigarh .All the strains maintained on nutrient agar slants in cold room at 4°Cand retrieved when required. Sub culturing was done at the interval of 15 days.

2.6. Antibacterial assay

Both the extract screened for antibacterial activity by following method. The antibacterial activity of the both the extract of *Parthenium hysterophorus, were* determined using agar well diffusion method. The Nutrient agar plate surface is inoculated by spreading a volume of the microbial inoculum over the entire agar surface. Then, a hole with a diameter of 6 to 8 mm is punched aseptically with a sterile cork borer or a tip, and a volume (50and100 µL) of the antimicrobial agent or extract solution at desired concentration (5mg/ml) is introduced into the well. Then, agar plates are incubated under suitable conditions depending upon the test microorganism. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain will be tested. Tetracycline (1mg/ml) was served as positive reference

standard to determine the sensitivity of the tested microbial strains. The Antibacterial activity was determined by measuring the diameter of Zones of inhibition (mm) produced after incubation and results were expressed in millimetres (mm) and DMSO used as a negative control[11, 9, 10].

The following formula was used to calculate [12] the activity index:

$$\text{Activity index} = \frac{\text{Zone of inhibition of extract}}{\text{Zone of inhibition of tetracyclin}}$$

3. Results

3.1. Phytochemical Analysis

The results of the phytochemical screening likely revealed the presence of various bioactive compounds such as alkaloids, flavonoids, carbohydrate, steroids, amino acids, and tannins in Parthenium *hysterophorus*, as summarized in Table 1.Methanolic extract showing presence of alkaloids, flavonoids, steroids and tannins while, aqueous extract gives presence of alkaloids, flavonoids, carbohydrates, Steroids, tannins and amino acids.

Table 1 Qualitative Phytochemical Screening of Methanol and Aqueous Extract of *Parthenium hysterophorus*

Solvent used for extraction	Alkaloids	Flavonoids	Carbohydrates	Steroids	Tannins	Amino acids	Protein
Methanol	+	+	-	+	+	-	-
Aqueous	+	+	+	+	+	+	-

3.2. Antibacterial Activity

Antibacterial activity of *Parthenium hysterophorus*was seen against several bacteria namely *Bacillus megaterium*, *and* gram negative *E.coli ,Pseudomonas aurogenosa, S. typhyi, E.ColiDevis, and S.Paratyphishown in* Table 2& 3.The Antibacterial studies revealed that, highest activity was against *E.coli*(37 mm) and *E.coli*davis(25mm) with 100µl dilution of methanolic extract as shown in table no.2 .The lowest activity was observed against salmonella typhi(9mm) and salmonella paratyphi(10mm)with 100µl dilution of methanolic extract as shown in table 3.But aqueous extract only positive for *E.coli*(4mm)at *50*µl and 7 mm at 100µl dilution. When results compare with standard teracyclin (Table 4.) methanolic extract most potent for antibacterial activity.

Table 2 Antibacterial activity of methanolic Extract of *Parthenium hysterophorus*

	Antibacterial activity Zone of inhibition (mm)	
Extract	50µl	100µl
Escherichia coli	17	37
Escherichia coli devis	15	25
Bacillus meagaterium	7	11
Salmonella typhi	4.5	9
Salmonella paratyphi	4	10
Pseudomonas aerogenosa	20	32

Table 3 Antibacterial activity of Aqueous Extract of *Parthenium hysterophorus*

	Zone of inhibition in mm	
Extract	**50µl**	**100µl**
Escherichia coli	4	7
Escherichia coli devis	-	
Bacillus meagaterium	-	-
Salmonella typhi	-	-
Salmonella paratyphi	-	-
Pseudomonas aerogenosa	-	-

Table 4 Antibacterial activity of Standard Tetracyclin**.**

Organism	**Antibacterial activity Zone of inhibition (mm)**
Tetracycline	100nµl
Escherichia coli	50
Escherichia coli devis	38
Bacillus meagaterium	41
Salmonella typhi	41
Pseudomonasaerogena	31
Salmonella paratyphi	34

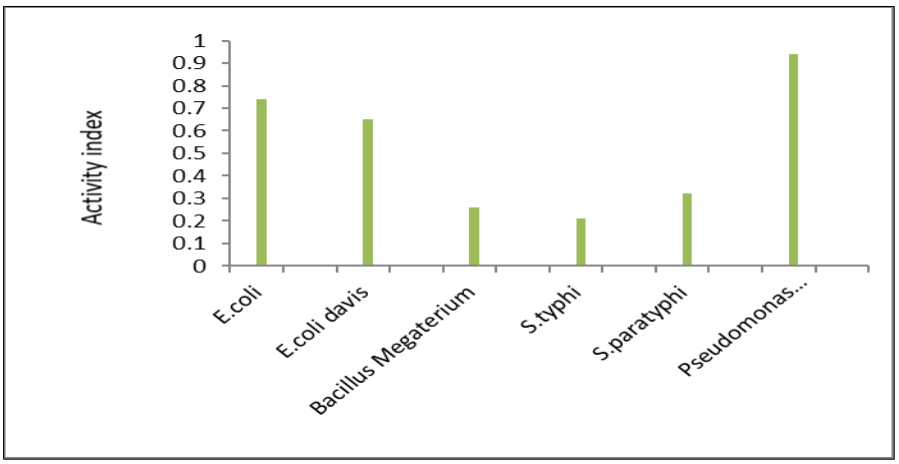

Figure 1 Activity index of methanol extracts

4. Discussion

The plant contains a variety of phytochemicals, including flavonoids, alkaloids, tannins, which contribute to its biological activities.

The presence of phytoconstitutents in methanolic and aqueous extract as shown in table no.1.

Several studies showed that medicinal plants have bioactive components, like flavonoids and phenols, with such various functions as bacteriostatic, bactericidal, chemotherapeutic, and antimicrobial functions.[13]Alkaloids, flavonoids, tannins, phenols known for their medicinal and antimicrobial properties. Traditional medicine systems utilize various plants known to contain biologically active compounds with medicinal properties. Research has demonstrated that plants generate substantial amounts of antioxidants to combat oxidative stress caused by reactive oxygen species and free radicals formed during metabolism. Antioxidants, including vitamins, phenolics, and other phytochemicals from plants and natural sources, have gained significant interest due to their superior biological compatibility. These secondary metabolites play a crucial role as chemo preventive agents, protecting against oxidative damage[14]Flavonoids are group of phenolic compounds having antioxidant, antiviral, antibacterial and anticancer properties.[15]

The antibacterial properties of *P. hysterophorus* are of considerable interest, especially in the context of increasing antibiotic resistance. Studies have shown that extracts from various parts of the plant—leaves, stems, and flowers—exhibit significant antibacterial activity against a range of pathogenic bacteria, including both Gram-positive and Gram-negative strains. 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 extract were tested against selected bacterial strain by applying disc diffusion process .The most prominent inhibition zones appeared in the case of n-hexane extract had the lowest potential to inhibit the bacterial function[16.]

The Antibacterial studies revealed that, highest activity was against *E.coli* and *E.colidavis* with 100µl dilution of methanolic extract .The lowest activity was observed against *salmonella typhi* and *salmonella paratyphi* with100µl dilution of methanolic extract as shown in table 2 and 3.When results compare with standard teracyclin (Table 4.) methanolic extract most potent for antibacterial activity. Therapeutic activity of plants is because of their biologically active polyphenolic compounds, typically flavonoids and phenolic acids which possess antioxidant, anti-lipoxygenase and anticancer activities. They have been vital sources of both preventive and curative traditional medicine preparations for human beings and livestock since ancient times . The phenolics and flavonoids of medicinal plants contribute to the antioxidant.[12]

The antibacterial effects may be attributed to the disruption of the bacterial cell wall, inhibition of protein synthesis, or interference with metabolic pathways due to the active phytochemicals. For instance, flavonoids and tannins can disrupt membrane integrity, while alkaloids may interfere with nucleic acid synthesis.

Alkaloids are naturally occurring chemical compounds containing basic nitrogen atoms. They often have pharmacological effects and are used as medications and recreational drugs.[10]

The phytochemical test of plant extract confirmed the presence of Alkaloids, Flavonoids, tannins, steroids, protein, carbohydrate, amino acid. Additional research is also necessary to isolate and characterize their active compounds for pharmacological testing.

Several metabolites known in the plant world, several families and sub-families are often speculated to be antimicrobial. These include terpenes, flavonoids, tannins, Quinone set alkaloids.[17]

Soxhlet extraction is a simple method, making it suitable for small-scale plant extraction research. However, to extract fruit bioactive compounds more effectively, alternative modified extraction techniques could be explored.[12]Subsequently, High-Performance Liquid Chromatography (HPLC) could be employed to identify and isolate the specific bioactive compounds within the *Parthenium hysterophorus* responsible for their antimicrobial properties. These identified compounds could then be studied further for amplification and potential use in combating pathogens.

While the preliminary findings regarding the antibacterial properties and phytochemical composition of *P. hysterophorus* are promising, some limitations remain:

- Standardization of Extracts: There is a need for standardized extraction methods to maintain consistency in active ingredient concentration and enhance reproducibility of results.
- Toxicity Assessment: While some studies suggest medicinal benefits, other reports indicate the plant can have adverse effects on health when consumed or inhaled, necessitating thorough toxicity studies.
- Synergistic Effects: Future research could explore the synergistic effects of P. hysterophorus with other medicinal plants or pharmaceutical compounds, potentially leading to enhanced antibacterial activity.

Abbreviations

- AMR: Antimicrobial resistance
- TPC: Total phenolic content
- TFC: Total flavonoid content
- DMSO: Dimethyl sulfoxide

5. Conclusion

Research into *Parthenium hysterophorus* has demonstrated that it is rich in various bioactive phytochemicals such as alkaloids, flavonoids, carbohydrates, steroids, tannins, and amino acids, which are believed to enhance its antibacterial properties. Notably, the methanolic extract exhibits significant antibacterial activity, outperforming tetracycline, which is used as a reference drug. In contrast, the aqueous extract shows comparatively lower efficacy against bacteria. These findings indicate that *Parthenium hysterophorus* may be a valuable source of natural antibacterial agents. The presence of secondary metabolites also supports the plant's longstanding use in traditional medicine. Nevertheless, further research is essential, including the isolation of active compounds and clinical trials, to fully understand its potential in pharmaceutical applications. It is crucial to proceed with caution, however, due to the plant's established toxicity when considering therapeutic developments.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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