In vitro Antimicrobial and pharmacological potential of Parthenium hysterophorus

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Abstract
Ethanomedicinal-based plant medicine plays a vital role in health care around 80% of the world's population depends on herbal medicine. The annual or ephemeral herb Parthenium hysterophorus is an upright, heavily branched member of the family Asteraceae. It occurs by various names, including Congress Weed, Carrot Weed, and Wild Feverfew. The Scourge of India is one of the most dreaded noxious weeds. It was unintentionally introduced to India in 1956 through imported food grains.P. hysterophorus is used to treat fever, anemia, heart problems, ulcerated sores, and wounds. It is thought to be the root cause of a variety of clinical patterns, including allergic respiratory issues, contact dermatitis, and mutagenicity in both humans and animals. Additionally, P. hysterophorus has a number of health advantages, including relief from neuralgia, tinnitus, nausea, vomiting, diarrhea, urinary tract infections, malaria, psoriasis, and skin inflammation. The first steps in isolating a new biologically active molecule, which leads to the discovery of new medications, are phytochemical and biological activity screening. P. hysterophorus is frequently used to treat a variety of ailments in traditional medicine. The crude extract of the plant was examined phytochemically to provide a rationale for the traditional applications of P. hysterophorus. Terpenoids, flavonoids, saponins, and steroids are only a few of the different kinds of bioactive secondary metabolites found in the plant. The fresh plant extract's antimicrobial capacity was examined. The current study demonstrates the ability of these plants to treat a variety of diseases and may aid in the future development of novel medications derived from these plants.

Keywords: Parthenium hysterophorus, Sesquiterpene lactone, Asthma, Parthenium.

Introduction

In the earliest times of recorded history, plants have been utilized as medicines. In non-industrialized nations, herbs are almost always utilized to treat illnesses and are frequently less expensive than modern pharmaceuticals. According to the World Health Organization, 80 percent of people in various Asian and African countries utilize herbal remedies for some aspect of basic healthcare. (Sofowora et al., 2013). Weeds are frequently considered to be unwanted in a particular circumstance and to be toxic, dangerous, or economically detrimental, posing a serious threat to primary production and biodiversity. In the wild, they have grown into self-replicating groups and have caused significant changes in nearby biological and simulated systems. The most prevalent foreign weed that displaces native plants is Parthenium hysterophorus. Parthenium is derived from the Latin term parthenice, which means "to utilize for therapeutic purposes"(Krishnavignesh, et al., 2013). The invasive weed plant Parthenium hysterophorus belongs to Asteraceae family. This short-lived, erect plant is recognized for its prolific growth and profusion, especially in hot areas. North-east Mexican native parthenium was originally thought to be native to America but is now also found in Asia and Europe (Meena et al., 2017). Parthenium (Parthenium hysterophorus L.) is known by a variety of names in many countries, including carrot weed, star weed, congress grass, wild feverfew, ragweed, bitter weed, white top, and the "Scourge of India." This weed, which produces a lot of seeds, has a lot of allelopathic effects on nearby plants and is competitive with economically significant crops (Gupta, S., & Chanda, S. (1991).Parthenium hysterophorus has shown promise as an antibacterial agent, successfully eradicating a variety of Gram-positive and Gram-negative bacteria. Additionally, this plant has showed antifungal action against a variety of fungal strains and probable antiviral activity by preventing the development of particular viruses. The Parthenium hysterophorus plant possesses impressive pharmacological properties in addition to its antibacterial abilities (Kaur et al., 2016). Its potential for pain relief and a reduction in inflammation has been suggested by studies on its anti-inflammatory and analgesic characteristics. Its antioxidant properties have also been discovered by researchers, which are essential for preventing damage brought on by oxidative stress (Kumar et al., 2013, Kumar et al., 2023). The spread of Parthenium hysterophorus has been reported to cause massive biodiversity loss by displacing native species in

natural habitats, resulting in habitat change in some cases. In agriculture, understanding Parthenium hysterophorus impacts, habitat, and biology is critical. Only by having a thorough understanding of this noxious weed will it be feasible to control and manage it in various ways (Rauf et al., 2022).

A hydroalcoholic extract of P. hysterophorus was reported to be efficient in vitro against Plasmodium falciparum by Valdes et al. in 2010. This herb had equivalent in vitro antiamoebic activity to that of conventional medicine. Entamoeba histolytica, the amoebic parasite that causes amoebiasis, has been demonstrated to be resistant to both axenic and polygenic cultures when treated with metronidazole. (Talakal et al., 1995; Zaheer et al.,2012). Fusarium wilt, an economically significant fungal disease in potatoes caused by Fusarium solani, was significantly reduced by aqueous, methanol, and n-hexane extracts. (Zaheer et al.,2012). Aqueous extracts of this plant's inflorescence were found to be effective against Penicilliusm chrysogenum, Microsporum gypseum, and Rhizopus stolonifer at greater doses of 1000 g/ml and 500 g/ml, respectively, while various organic preparations showed no activity. Aqueous extracts from the leaves also had antifungal action against Alternaria alternative (Ramanujam et al., 2011).Plant secondary metabolites known to have a variety of pharmacological and biochemical effects on living creatures are known as phytochemicals. Flavonoids 6-hydroxykaempferol-3,7-dimethyl ether, parthenin, and stigmasterol are found throughout the plant. Essential oil, flavonoid, parthenin, acids, campesterol, and stigmasterol are all found in the leaves. Ambrosanoli is found in flowers. Parthenin and its derivatives have been discovered to be largely insoluble in water, but soluble in alcohol, chloroform, ether, acetone, and ethyl acetate [Jain and Kulkarni, 1999]. The boiling points of all elements of Parthenium Hysterophorus extract are between 165 and 220 degrees Celsius, while Methanol is 64.7 degrees Celsius, indicating that they do not form an azeotropic combination and can thus be separated using basic distillation techniques (Pandey et al., 2012). It was proposed to separate Sesquiterpene Lactones from Parthenium Hysterophorus using Chemical Engineering procedures (Soxhlet Extraction and Packed Bed Extraction), which might be employed as pesticides or insecticides. (Makheja and Bailey, 1982).

Materials and methods

Collection and Characterization of plant leaves

In Mullana Ambala, on the MMDU campus, Parthenium hysterophorus leaves were gathered. To eliminate soil, dust, and other impurities, the collected plant leaves were first washed with DDW and then cleaned with tap water. The pH, moisture content, ash content, bulk density, and other properties of plant leaves were measured.

Determination of pH of Plant leaves

5g Biomass was taken into a beaker of 1000ml. To this beaker 150ml of deionized water. The mixture (water and biomass) was allowed to stand for 10 minutes before being filtered. Utilizing buffer solution with pH values of 4.0, 7.0, and 9.2, a pH meter was calibrated.

Determination of Electrical Conductivity (EC)

Digital conductivity meter pH-COND-TDS-SAL-TEMP meter(51-15C-364-04) was used to measure EC of sample at room temperature. After calibrating EC meter with 0.01 M KCl standard solution cm^{-1} having the conductivity of 1413μScm^{-1}.

Determination of Moisture Content of Plant leaves

5 g of leaves was taken in glass beaker and oven dried at 60ºC till the constant weight of the sample was achieved. However, drying time mainly depends on the quantity, nature/type and condition of the sample. The moisture content of biomass was calculated as.

Weight of moisture in biomass = Wet weight – Dry weight

$$\% \text{ moisture content} = \frac{\text{Weight of sample} \times 100}{\text{Weight of oven dry sample}}$$

Extraction Preparation by Soxhlet Method

Parthenium leaves were washed, air dried in the shade, then powdered using a grinder. Weighed powdered leaves were put in soxhlet. Ethanol was utilised as the soxhletion solvent. Extraction was continued at 35°C until a clear solvent was visible in the thimble. At 40°C, the extract was concentrated in a water bath. An airtight container was used to store the concentrated extract (Redfern et al., 2014)

Phytochemical studies of Parthenium hysterophorus Extract preparation

In a rotator shaker, the powdered plant leaves were soaked overnight with (10g/100ml) in various solvents (aqueous, methanol, and ethanol).\.

Qualitative analysis of phytochemicals (Shah et al., 2021)

1. Test for alkaloids:

Dragendroff's test- To 2-3 ml of sample, a few drops of Dragendroff's reagents were applied. Precipitation that was orange-brown in color indicated the presence of alkaloid chemicals.

Wagner's test –Wagner's reagent was added sparingly to 3 ml of the sample. Alkaloid was seen in the precipitation's reddish brown color.

2.Test for carbohydrates:

Molish's test – A few drops of an alpha naphthol solution in methanol were added to a 2-3 ml sample. Concentrated H2SO4 was added to the solution after it had been shaken and added from the test tube's sides. At

the intersection of two layers, a violet ring formed, indicating the presence of carbohydrates molecules.

Fehling's test – 1ml Fehling's A and 1ml Fehling's B solutions was mixed. 2 ml od sample was added and boiled for 1min. Brick red colour precipitation was observed, showed the presence of carbohydrate compounds.

3.Test for terpenoids

Noller's test: The sample was warmed with tin and thionyl chloride. Pink coloration indicated the presence of triterpenoids.

4. Test for phenols

Ferric chloride test: A solution of ferric chloride in 3–4 drops was used to treat the sample. The creation of a bluish-black color suggested the presence of phenols.

5. Test for protein and amino acid

Million's test: To 1ml of million's reagent, 1-2 ml of test solution was added. Observe the pink to brick red precipitate observed which indicated positive test.

6.Test for phytosterols

Libermann-burchard's test: Sample was filtered after chloroform treatment. A few drops of acetic anhydride were added to the filtrate before it was heated and chilled. Sulphuric acid concentration was then added. At the intersection, the development of a brown ring suggested the presence of phytosterols.

7. Test for tannins

Braymer's test ($FeCl_3$): 1ml of the sample was treated with 1ml of the ferric chloride solution made up of 10% alcohol. Tannins were present when blue or greenish color formation occurred.

Antimicrobial Assay

By using the well diffusion method, the antibacterial and disc diffusion method for antifungal activity was determined. The medium was autoclaved, sterilised and allowed to cool at room temperature. The medium was poured in a sterile petri plate. Using a well cutter, the infected plates were set aside for a few minutes. To the well, Parthenium hysterophorus extract was applied. The plates were incubated overnight at 37°C. The zone of inhibition was measured to determine microbial growth. Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumoniae (Gram negative), Bacillus subtilis, Staphylococcus aureus and Micrococcus luteus (Gram positive) were used for antibacterial activity and Candida albicians, Candida kefyr and Aspergillus niger were used for antifungal activity (Zanna et al 2021).

Results and Observation

Collection of characterization of plant leaves

Parthenium hysterophorus leaves were collected from MMDU campus, Mullana Ambala, the collected plant leaves were prepared for further processing. Plant leaves were collected and cleaned with DDW before being dried. Finally, the leaves of the plants were pulverized separately using a pestle and mortar to produce a homogenous powder known as biomass or adsorbent. To protect the biomass from moisture, it was placed in an airtight container or a zippered polythene bag. Figure 1 indicates the processing of plant leaves for further study.

Figure 1:Preparation of P.hysteropherous leaves

Physicochemical characterization: The sample were analysed by physicochemical characterization of P.hysteropherous such as pH, Electrical conductivity and moisture content Table 1. The pH of P.hysteropherous leaf extract are 5.2, EC 6.83 and the moisture content is 10.6 %.

Table 1. Physicochemical characterization of P.hysteropherous

Characterization	P. hysteropherous
pH	5.2
Electrical Conductivity	6.83
Moisture content	10.6%

Phytochemical Analysis

Using the Harborne method, the samples were screened for the presence of alkaloids, carbohydrates, terpenoids, phenolic compounds, proteins, amino acids, phytosterols, and tannins. The + symbol denotes the presence of these compounds, while the - symbol denotes their absence with respect to extract solvents (Table 2).

Table 2: Phytochemical analysis of methanol and ethanol

Extracts and tests	P. hysterophorus leaves	
	Methanol	Ethanol
Alkaloids	+	+
Carbohydrate	+	+
Glycoside	+	+
Terpenoids	+	+
Amino acid	+	+
Phenolic	+	+
Phytosterol	+	+
Tannin	+	-

Antimicrobial activity of Parthenium hysterophorus

By using the agar well diffusion method, which involves spreading a volume of the bacterial culture over the entire surface of the agar plate, the antibacterial activity of plants was examined. Most of a plant's antibacterial activity is attributed to secondary metabolites. Terpenoids, alkaloids, phenolic and polyphenolic phytochemicals, lectins, and polypeptides are important phytochemical groups with antimicrobial effects. The anti-microbial effect of Parthenium hysterophorus plant extract was investigated against Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Bacillus subtilis, Staphylococcus aureus and Micrococcus luteus. Antimicrobial activity was observed against Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae and Bacillus subtilis (Fig.2) but no activity was observed against Staphylococcus aureus and Micrococcus luteus. The size of zone of inhibition was summarized in table 3.

Table 3: Antibacterial activity of Parthenium hysterophorus (Zone of inhibition)

Bacterial pathogens	Zone of Inhibition (in mm) 50mg/ml		
	Control	Methanol	Ethanol
Escherichia coli	0	6.90	5.76
Pseudomonas aeruginosa	0	18.65	17.00
Klebsiella pneumonia	0	13.91	18.85
Bacillus subtilis	0	19.45	15.00

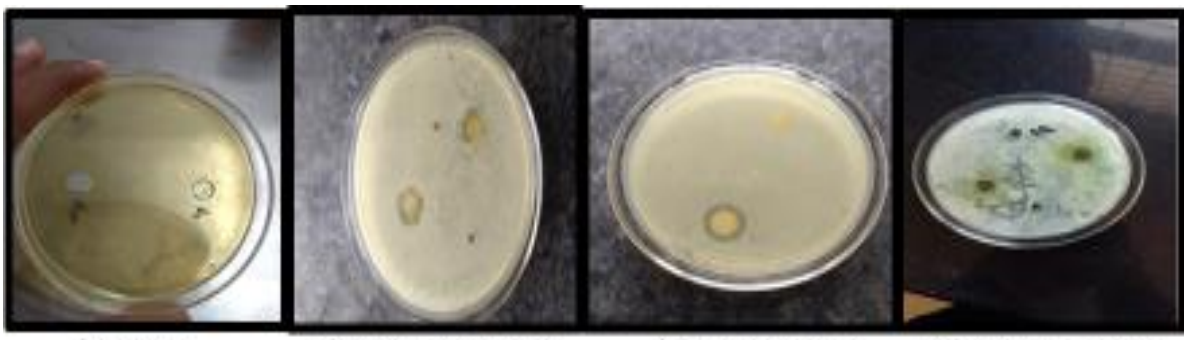

Fig. 2: Antibacterial activity of Parthenium hysterophorus extracts against bacterial pathogens.

Antifungal properties of Parthenium hysterophorus against pathogenic fungus

In eight different solvent systems, including ethanol, chloroform, methanol, acetone, ethyl acetate, hexane, petroleum ether, and aqueous extracts, Parthenium hysterophorus crude solvent extracts' antifungal efficacy was demonstrated in Table 4. Candida albicians, Candida kefyr and Aspergillus niger, a fungus, was utilised as a test organism. Leaf extract made with a mixture of methanol was shown to be more effective than the other solvent system in preventing Candida albicans growth on Sabourad dextrose agar plates in this early study. The highest activity of ethanol against the test organism is just 80%. While the leaf extracts of ethyl acetate, acetone, petroleum ether, hexane, and chloroform had only 20% to 75% maximal effectiveness against the test organism Candida albicans growth appears to be hampered by the aquas extract. Based on these findings, methanol extracts were used to conduct additional tests on the antifungal properties of Parthenium hysterophorus.As noted earlier, the disc diffusion method was used to test a methanol extract of Parthenium hysterophorus against the pathogenic fungal species Aspergillus niger, Candidia albicans, and Candida kefyriae at a concentration of 700 g/disc. The leaf extract residues exhibited the best anti-Candida albicans performance, with a Parthenium hysterophorus inhibitory zone of 17 mm. Parthenium hysterophorus extract residue has 70.5 to 58.8% activity against Candida kefyr and Aspergillus niger. (Table 5) summarises the findings.

Table 4: Antifungal activity of crude solvent extracts of the Parthenium hysterophours

Sr.No	Solvent used for extraction	Antifungal activity (%maximum activity)
1.	**Methanol**	**100%**
2.	**Ethanol**	**80%**
3.	**Ethyl acetate**	**75%**
4.	**Acetone**	**68%**
5.	**Chloroform**	**55%**
6.	**Petroleum Ether**	**32%**
7.	**Hexane**	**20%**
8.	**Aquas Extract**	**12%**

Table 5: Antifungal activity of the crude methanol extracts residue of the Parthenium hysterophorus

Sr.No.	Test fungal	Zone of inhibition	
		P. hysteropherous	Ketoconazole
1.	Candida albicans	17 ± 0.022 (100)	15 ± 0.110
2.	Candida kefyr	12 ± 0.125 (70.5)	10 ± 0.043
3.	Aspergillus niger	10 ± 0.005 (58.8)	8± 0.150

Discussion

The current study varies from the previous study in that the antifungal activity was assessed using Parthenium hysterophorus methanol extract leftovers. Parthenium hysterophorus was shown to be a powerful antifungal agent when its antifungal properties were examined. The results of this study also pave the door for more investigation to pinpoint the precise bioactive substances that are accountable for its purported antifungal activity.For Parthenium hysterophorus extract, Candidia albicans had maximum activity (zone of inhibition) of 17 mm. The antifungal activity of Parthenium hysterophorus methanol extract residues against three pathogenic fungal strains was compared to that of conventional drugs.The aqueous extract showed no efficacy against the fungus that was tested. Our findings are consistent with Ravindra et al observations (Rao, 1956). The varied zones of inhibition found in our experiments against microbes could be due to different components diffusing at different rates. We might be able to use each component separately as an antibacterial agent to fight antimicrobial infection if the components could be separated and examined further. (Jayachandra, 1971). Parthenium hysterophorus, a widely distributed weed, has garnered significant scientific interest due to its promising in vitro antimicrobial and pharmacological potential. Numerous studies have highlighted the plant's antibacterial characteristics by demonstrating its effectiveness against germs like Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa. (Ullah et al., 2017). Additionally, Parthenium hysterophorus exhibits notable antifungal activity against Candida albicans and Aspergillus species, underlining its relevance in combating fungal infections (Waghmode et al., 2012). Beyond its antimicrobial prowess, the plant showcases pharmacological significance with its anti-inflammatory, antioxidant, and analgesic properties (Rao et al., 2017). These qualities make it a possible candidate for the creation of brand-new medications and therapeutic agents. To extract and identify the active ingredients responsible for these effects, however, as well as to demonstrate the plant's safety and effectiveness for therapeutic uses, more study is required.

Conclusion

The Parthenium plant is well-known for its ecological characteristics. The Parthenium plant can be utilized as an herbicide, pesticide, insecticide, and industrial raw material. The plant also has anticancer, antidiabetic, anthelminthic, anti-diarrheal, anti-microbial, and other properties. More investigation is therefore needed to separate the active component from the plant extract and carry out pharmacological trials. Numerous illnesses, such as diarrhoea, dysentery, malaria, rheumatoid arthritis, and neuralgia are all treated with it. It makes alkaloids like Parthenin. Medicinal plants have been used to create a variety of unique pharmaceutical compounds that have been shown to have a strong pharmacological effect on humans. It has also been shown to have antioxidant and antimutagenic properties. The extract clearly contains antimicrobial elements that could be used as antimicrobial agents in novel treatments for harmful bacteria. The most active extract could be subjected to therapeutic antimicrobial isolation and additional pharmacological testing. Parthenium leaf extract has a lot of anti-cancer activity. The results of the current investigation showed that the phytochemicals in P. hysterophorus extracts have biological effects. Antibacterial and antifungal properties are seen in plant extracts. Parthenium leaf extract was found to be efficient against Candida and Aspergillus in this investigation, with a significant zone of inhibition in all extraction media. As a result, P. hysterophorus, which has a wide range of medicinal uses, may help avoid various diseases and be useful for the future development of novel medications derived from this plant.

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