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Inhibition of Plant Pathogens by *Parthenium hysterophorus*: An Investigation into Antimicrobial Properties

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ABSTRACT

The goal of the present study was to assess the antimicrobial properties of leaf extracts of *Parthenium hysterophorus* using the agar well diffusion method against some plant pathogens. In the current investigation, the results illustrate that the leaf extracts of *Parthenium hysterophorus* exhibit a wide range of antimicrobial activity and can potentially be used as an alternative to chemical pesticides. This is the first complete study to report on antimicrobial effects of *Parthenium hysterophorus* against some selected plant pathogens with a view of searching a novel extracts to use as an alternative to chemical pesticides for sustainable agriculture production.

Keywords: *Parthenium hysterophorus*, Antimicrobial, Leaf Extract.

INTRODUCTION

The problem of invasive weeds has become a notable concern in recent years due to the ecological and socio-economic issues them pose [1]. *Parthenium hysterophorus* is a type of invasive weed from the Asteraceae family. This relatively short-lived, upright plant is known for its rapid growth and is particularly abundant in hot climates [2].

Commonly referred to as congress weed or gajar ghans (carrot weed) in India, it also goes by names like white top or feverfew (in the Caribbean) and ragweed parthenium (in the USA) [3-5]. It has gained recognition as the seventh most harmful weed globally, posing a significant threat to biodiversity.

Originally native to regions around the Gulf of Mexico, Southern North America, West Indies, and central South America, this weed has now spread to over 20 countries worldwide, spanning five continents and multiple islands [1]. Introduced to India as an ornamental plant in 1910, it initially failed to establish itself. However, in the 1950s, it reappeared in India and Australia due to contamination of wheat and pasture seeds imported from the United States. Its growth is promoted by nitrogen-rich waste from humans and livestock, explaining its proliferation near urban areas and settlements [6]. Despite being a weed causing allergies

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in humans, *Parthenium hysterophorus* L. has shown various potential medicinal applications [6]. Ramos et al. [7] noted its uses in treating neurological disorders, fever, urinary infections, dysentery, malaria, and as an emmenagogue. It has also shown promise as a remedy for hepatic amoebiasis [7].

Parthenium hysterophorus is a notable source of terpenoids, volatile oils, flavonoids, amino acids, sugars, and phenolic compounds. Parthenolide, the primary sesquiterpene lactone, along with parthenin and different solvent extracts, have demonstrated significant analgesic, anti-inflammatory, and antipyretic properties. These properties have led to its use in treating migraine headaches and fever. The application of crushed *P. hysterophorus* leaves externally has shown wound healing potential [7].

The plant exhibits strong resistance to plant-pathogenic microorganisms, possibly due to the presence of antimicrobial substances in its various parts. This study aimed to confirm the antimicrobial properties of *Parthenium hysterophorus* against specific plant pathogens, with the goal of finding an alternative extract for sustainable agricultural pest control, reducing reliance on chemical pesticides.

METHODS AND MATERIALS

The antimicrobial effectiveness was evaluated through a growth inhibition zone test utilizing the agar well diffusion method subsequent to the creation of the extracts.

Sample preparation

Roughly 500 grams of indigenous *Parthenium hysterophorus* plant leaves were gathered and subjected to oven drying for approximately 4 days at a temperature of 40-42°C, until a consistent weight was achieved. Subsequently, the dried leaves were pulverized into a fine powder. The resultant sample was stored at room temperature for subsequent utilization [8].

Preparation of Extracts

The extraction process was conducted employing the maceration technique. In this approach, 5 grams of leaf powder were combined with 50 ml each of ethyl acetate, methanol, ethanol, and 50% aqueous ethanol, separately. These mixtures were allowed to stand for duration of 3 days. Subsequent to this period, the resulting extracts were filtered and subjected to concentration on a water bath. The concentrated extract was then dissolved in Dimethyl Sulfoxide (DMSO) at a concentration of 1g/ml and preserved at a temperature of 4°C for subsequent utilization [9].

Bacterial and fungal strains

For the study, five distinct bacterial strains were chosen, encompassing 3 Gram-positive and 2 Gram-negative strains, primarily focusing on plant pathogens. The selected Gram-positive bacteria comprised *Staphylococcus epidermidis, Bacillus subtilis* (NCIM 2920), and *Staphylococcus aureus* (NCIM-5345), while the Gram-negative bacteria included *Escherichia coli* (NCIM 5346) and *Pseudomonas aeruginosa*. Additionally, a yeast strain, Candida albicans, was included. The fungal species utilized in this investigation consisted of *Fusarium oxysporum* (NCIM 1008), *Aspergillus flavus* (NCIM 1316), *Penicillium citrinum* (NCIM 1435), and *Rhizopus stolonifera* (NCIM 1139).

To facilitate the study, standard cultures of these bacteria and fungi were maintained at Amity AIHRS, Amity University UP, and Noida, India. Viability tests for each of the isolates were performed by reviving the organisms in nutrient agar medium and Sabouraud's dextrose agar (SDA) medium, respectively. The bacterial stocks on nutrient agar medium (provided by Hi Media, Mumbai, India) were incubated at 37 °C for 24 hours, while the fungal stock was incubated at 28 °C for 3 days. Following incubation, the cultures were stored at 4 °C until they were ready to be employed for sensitivity testing.

Antibacterial activity testing using agar well diffused technique

The antibacterial efficacy of the leaf extracts from *P. hysterophorus* was evaluated using the agar well-diffusion technique. Initially, a pure culture of each bacterial strain was sub-cultured in nutrient broth and incubated at 37°C for 24 hours. A standardized inoculum of (100µL, 106 CFU/mL; 0.5 Mac-Farland), was evenly spread using a sterile spreader onto sterile agar plates to achieve a uniform growth across the plate surface. Once the plates were air-dried, sterile cork borers with a diameter of 6.0 mm were employed to create wells in the agar medium.

Following this, 50 µL of each extract was introduced into the wells of the agar plates. The plates were allowed to stand for an hour to permit the diffusion of the extracts and were subsequently incubated at 37°C for 24 hours. After the incubation period, the diameter of the inhibition zone was observed and measured in millimeters as an indicator of antibacterial activity [10].

Antifungal assay

To assess the antifungal properties of the leaf extracts from *Parthenium hysterophorus*, sterilized Sabouraud's dextrose

agar (SDA) plates were utilized. Using a sterile cork-borer with a diameter of 6 mm, wells were created in the agar medium. In separate plates, 50 µL of ethanol, methanol, and ethyl acetate extracts were introduced into distinct peripheral wells. Additionally, a fungal disc was placed in the central well. To establish a baseline, a negative control (using DMSO) was included in one of the peripheral wells. The plates were then incubated at 28°C.

Following an incubation period of 3 to 5 days at 28°C, the inhibition zones around the wells were observed and measured in millimeters. These measurements were recorded to quantify the extent of antifungal activity exhibited by the extracts [10].

RESULTS AND DISCUSSION

Antimicrobial activity evaluation was conducted using a selection of five bacterial species, a yeast strain, and four fungal species. The method employed to gauge antimicrobial effectiveness was the agar well-diffusion technique, which targeted three Gram-positive bacterial strains and two Gram-negative bacterial strains. The outcomes revealed that the leaf extracts exhibited antimicrobial action against both Gram-positive and Gram-negative bacteria.

The extracts showcased their highest efficacy against *Bacillus subtilis*, followed by *Pseudomonas aeruginosa*, resulting in inhibition zone diameters (IZD) of 27.5 mm and 25 mm, respectively (as presented in Table 1).

Figure 1-6 Zone of antibacterial inhibition of *Parthenium hysterophorus* leaf extracts against (A) *Escherichia coli*, (B) *Bacillus subtilis*, (C) *Staphylocuccus aureus*, (D) *Staphylococcus epidermis*, (E) *Pseudomonas aeruginosa* (F) *Candida albicans*.

A perusal of the study reveals that highest zone of inhibition against *Escherichia coli* was showed by methanol extract (22.5 mm) followed by ethyl acetate extract (17.5 mm) while ethanol extract exhibited no inhibition at all.

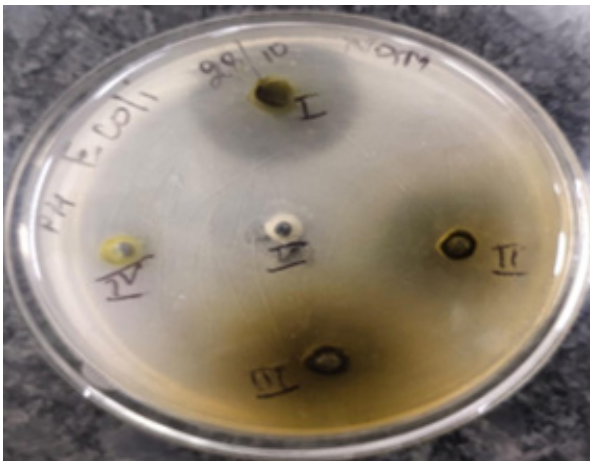

Figure 1. *Escherichia coli*

The methanolic leaf extract exhibited active inhibition with the highest zone against *Bacillus subtilis* of 27.5 mm followed by ethyl acetate (22.5 mm).

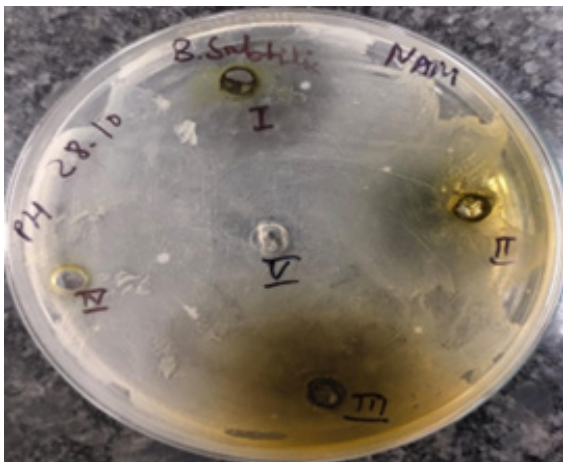

Figure 2. *Bacillus subtilis*

Among the other tested bacterial pathogens, highest zone of inhibition against Staphylocuccus aureus was exhibited by ethanol extract (20 mm) followed by methanol extract (15 mm) while against the *Pseudomonas aeruginosa* and Staphylococcus epidermis, maximum growth was inhibited by 50% ethanol extract (25 mm) and methanol extract (20 mm) followed by ethyl acetate extract (17.5 mm) and ethanol extract (18.5 mm) respectively.

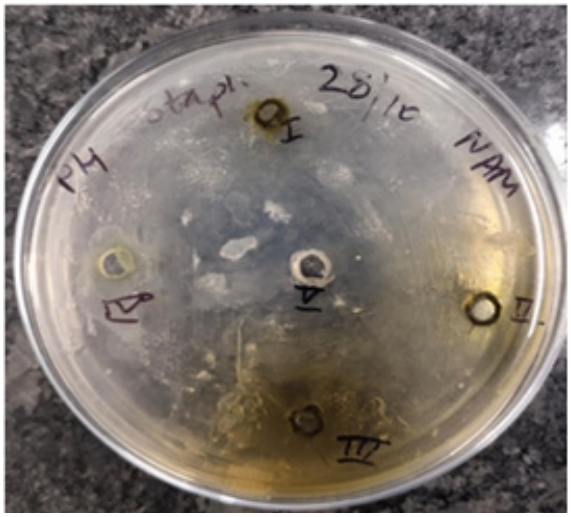

Figure 3. Staphylococcus aureus

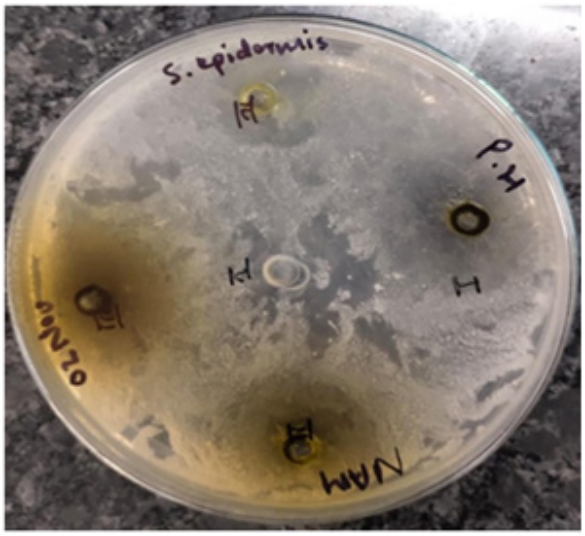

Figure 4. Staphylococcus epidermis

Figure 5. *Pseudomonas aeruginosa*

The only tested yeast, ethyl acetate extract exhibited maximum zone of inhibition against the Candida albicans (23 mm) followed by ethanol extract (17.5 mm).

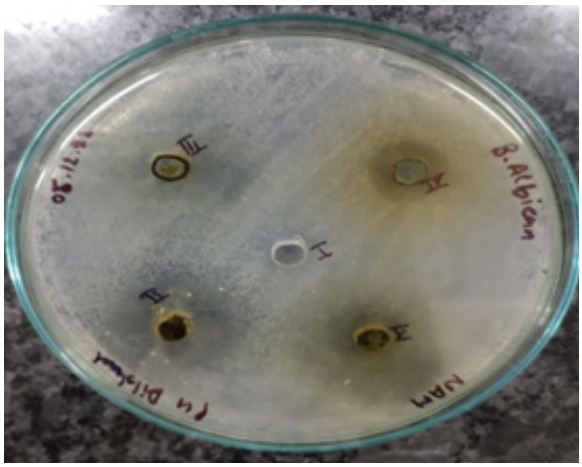

Figure 6. Candida albicans

Table 1. The mean value diameter of inhibitory zones (mm) with *Parthenium hysterophorus* Leaf extracts and one negative control (DMSO)

Bacterial species	Ethanol	Methanol	Ethyl acetate	50% Ethanol	DMSO
Escherichia coli (NCIM 5346)	_	22.5 mm	17.5 mm	5 mm	_
Bacillus subtilis (NCIM 2920)	12.5 mm	27.5 mm	22.5 mm	10 mm	_
Staphylocuccus aureus (NCIM 5345)	20 mm	15 mm	12.5 mm	10 mm	_
Pseudomonas aeruginosa	12.5 mm	15 mm	17.5 mm	25 mm	_
Staphylococcus epidermis	18.5 mm	20 mm	12.5 mm	17 mm	_
Candida albicans	17.5 mm	11 mm	23 mm	15 mm	-

The antifungal effects of extracts have also been investigated and a total of 4 test fungi were used. Ethanol and methanol extracts exhibited antagonistic activity against all the tested fungi while ethyl acetate extract exhibited inhibition against *Fusarium oxysporum* and Rhizopus stolonifera respectively. When evaluating the antifungal properties, we computed the Percentage of Inhibition (PI) index for each individual fungal species where:

Percentage of Inhibition (PI) = C-T/C*100

Where C= Radial growth of the pathogen in control

T= radial growth of the pathogen in treatment

Table 2. The mean value of PI (in %) with the three leaf extracts and one negative control

Fungal species	C = growth of pathogen in control (DMSO) in mm	T = growth of pathogen in treatment in mm			Percentage of inhibition (PI)		
		Ethanol extract	Methanol extract	Ethyl acetate extract	Ethanol extract	Methanol extract	Ethyl acetate extract
Fusarium oxysporum (NCIM 1008)	90	30	30	35	66.67%	66.67%	61.11%
Penicillium citrinum (NCIM 1435)	25	20	20	NI	20%	20%	-
Rhizopus stolonifera (NCIM 1139)	90	45	60	60	50%	33.34%	33.34%
Aspergillus flavus (NCIM 1316)	90	60	60	NI	33.34%	33.34%	-

*NI= No inhibition

From the above observation we can say that ethanol extract exhibited active inhibition against the fungi *Fusarium oxysporum*, Penicillium citrinum, Rhizopus stolonifera and Aspergillus flavus with the percentage of inhibition (PI) of 66.67%, 20%, 50% and 33.34% respectively. Methanol extract also showed active inhibition against the fungi *Fusarium oxysporum*, Penicillium citrinum, Rhizopus stolonifera and Aspergillus flavus with the percentage of inhibition (PI) of 66.67%, 20%, 33.34% and 33.34% respectively. While ethyl acetate extract exhibited active inhibition only against the fungi *Fusarium oxysporum* and Rhizopus stolonifera with percentage of inhibition (PI) 61.11% and 33.34% respectively.

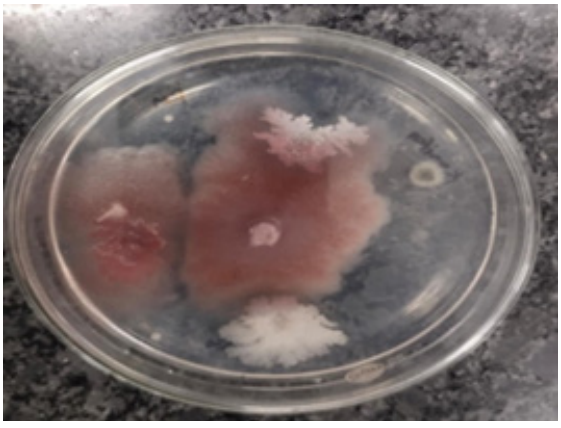

Figure 7A. Control- *Fusarium oxysporum*

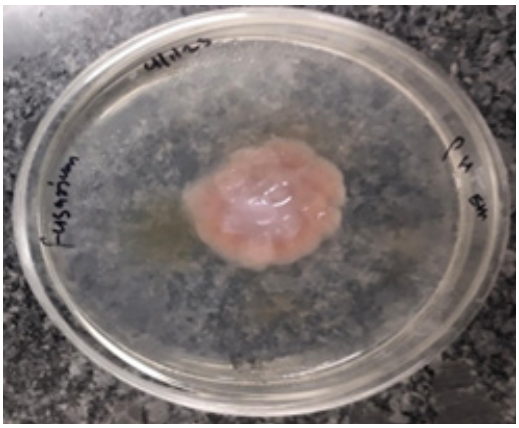

Figure 7B. *Fusarium oxysporum*- Ethanol

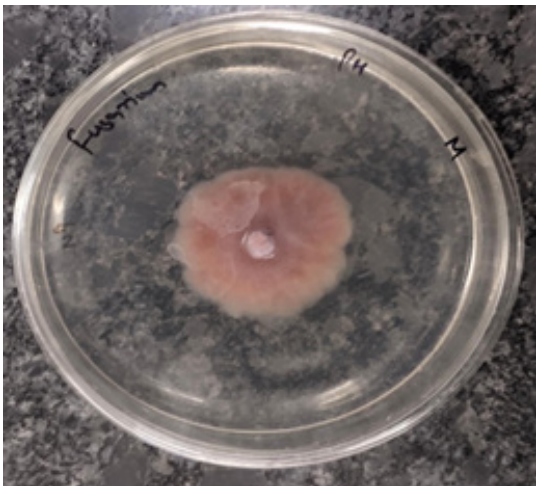

Figure 7C.*Fusarium oxysporum* Methanol

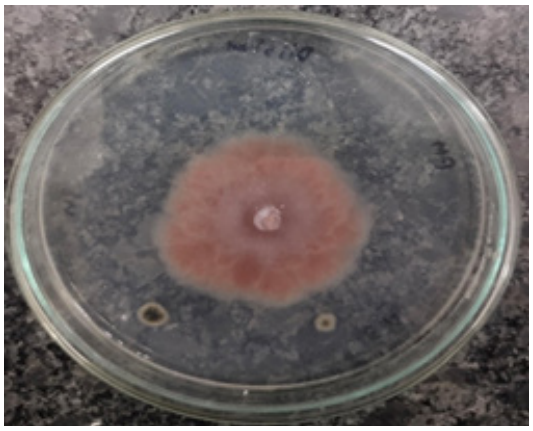

Figure 7D. *Fusarium oxysporum*- Ethyl acetate

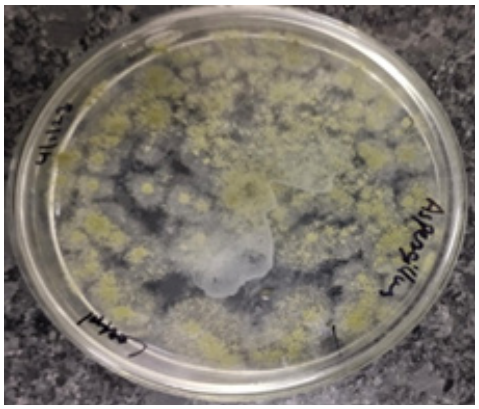

Figure 8A. Control–Aspergillus flavus

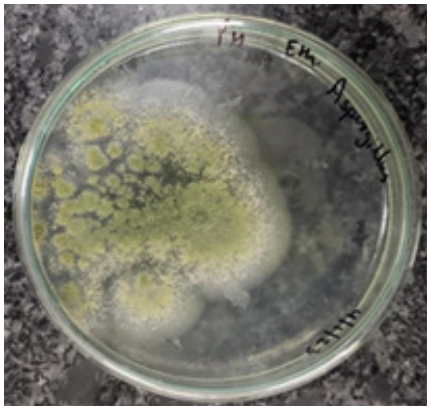

Figure 8B. Aspergillus flavus- Ethanol

Figure 8C. Aspergillus flavus Methanol

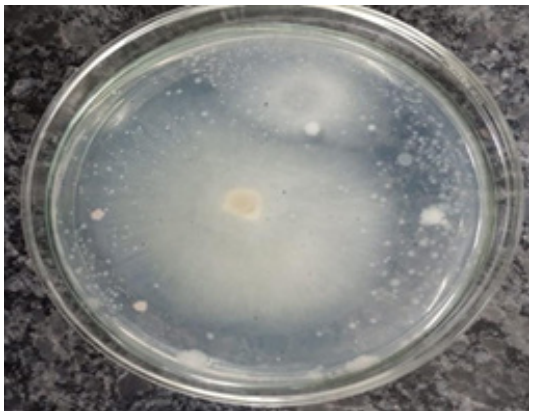

Figure 9A. Control-Rhizopus stolonifer

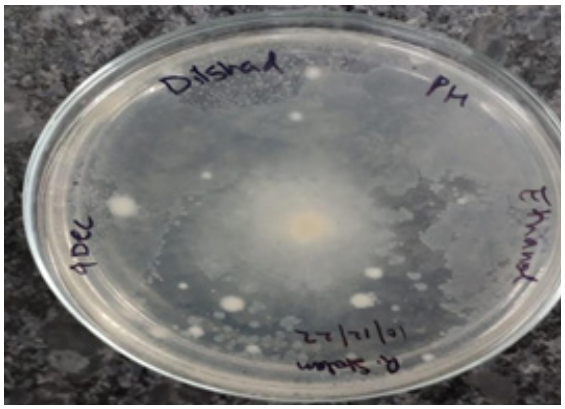

Figure 9B. Rhizopus stolonifera-Ethanol

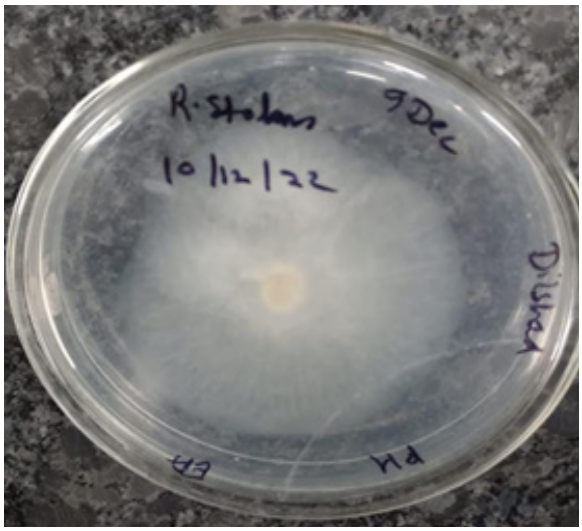

Figure 9C. Rhizopus stolonifera–Ethyl acetate

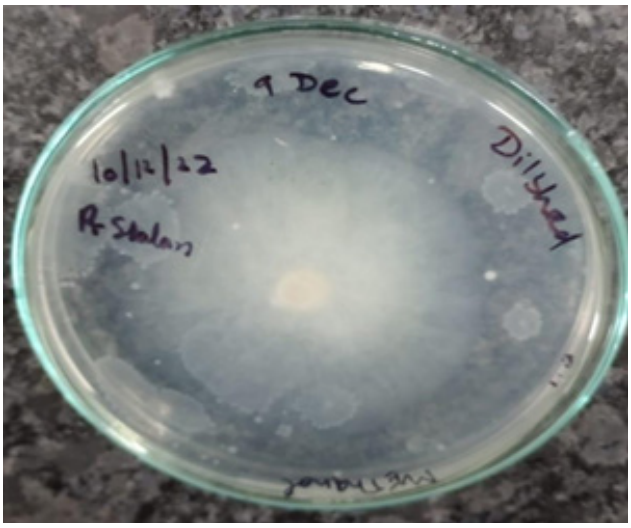

Figure 9D. Rhizopus stolonifera – Methanol

Figure 10A. Control- Penicillium citrinum

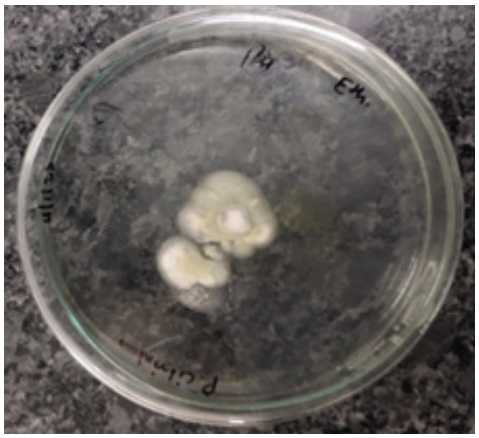

Figure 10B. Penicillium citrinum – Ethanol

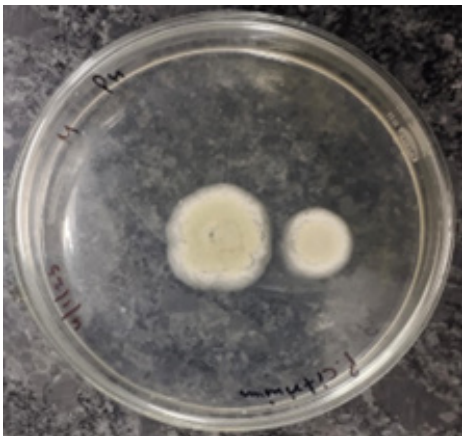

Figure 10C. Penicillium citrinum- Methanol

Fusarium oxysporum was found to be the most sensitive among the all tested fungi and it was also found that ethanol and methanol are the most effective solvents for the extraction of bioactive compounds from the *Parthenium hysterophorus*.

There are very few investigations reporting the antibacterial and antifungal properties of parthenium. The antimicrobial efficacy of *P. hysterophorus* has been reported by various scientists as: *Escherichia coli* [9], *Bacillus subtilis* [11], Staphylococcus aureus [12], *Pseudomonas aeruginosa*, Candida albicans [13], Aspergillus flavus [14].

The effectiveness of plants in combating microbes is commonly attributed to constituents such as tannins, saponins, phenolic compounds, essential oils, and flavonoids [6]. In the case of Parthenium, its antimicrobial prowess is potentially owed to the presence of five terpenoids, volatile oils, flavonoids, as well as amino acids, sugars, and phenolic derivatives [15].

The assessment of *Parthenium hysterophorus* leaf extracts for their antimicrobial potential revealed varying levels of activity against the tested bacterial, yeast, and fungal species (as detailed in Tables 1 & 2).

Plant-derived antimicrobials hold significant therapeutic promise, being both cost-effective and environmentally friendly. Moreover, they tend to lack the side effects often associated with synthetic antimicrobials. *Parthenium hysterophorus* stands as a noteworthy addition to the medicinal plants exhibiting potent antimicrobial properties [16,17].

This current study differentiates itself from prior research in that it gauged antimicrobial activity across four distinct organic extract residues of *Parthenium hysterophorus*. Furthermore, this study serves as a stepping stone for future investigations aimed at identifying specific bioactive compounds that could potentially offer an alternative to chemical pesticides, thereby contributing to sustainable agricultural production.

CONCLUSION

The main objective of the aforementioned study was to evaluate the effectiveness of *Parthenium hysterophorus* leaf extracts in combating selected plant pathogens through antibacterial and antifungal actions. The utilization of plant-derived biocides in agriculture has garnered increased attention historically, primarily due to their minimal health risks and practicality.

The findings of this study substantiate that the leaf extracts from the *Parthenium hysterophorus* plant possess noteworthy antibacterial and antifungal attributes against the range of tested bacterial and fungal strains. Thus, based on the outcomes of this investigation, it can be reasonably deduced that *Parthenium hysterophorus* demonstrates substantial antibacterial and antifungal efficacy. These findings provide a foundation for further exploration, suggesting the potential for utilizing *Parthenium hysterophorus* as an alternative to chemical pesticides, thereby promoting enhanced and sustainable agricultural production practices.

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CONFLICT OF INTERESTS

Authors declare no conflict of interests.

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