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Antimicrobial activity of leaf extracts of the medicinal plant *Eclipta prostrata* from Manipur

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For centuries, indigenous people have relied on ethnomedicine to treat various ailments. In the last few years, there has been a rise of diseases and infections caused by microbes that are resistant to conventional fungicides and antibiotics.The present study investigates the antifungal and antibacterial activities of *Eclipta prostrata* leaf extracts. *E. prostrata* plants were collected from the Imphal-East district of Manipur. The fresh leaves were dried, powdered, macerated, and the secondary metabolite was extracted using methanol and ethyl acetate solvents. Antifungal activity was assessed against the phytopathogens *Nigrospora oryzae*, *Fusarium oxysporum*, and *Colletotrichum capsici* using the petriplate culture assay. Both extracts inhibited fungal growth, with the ethyl acetate extract showing superior efficacy, especially against *Nigrospora oryzae* (75.89%). The antibacterial activity was evaluated against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella typhi* using the agar well diffusion method and the 96-well microplate method. The ethyl acetate extract exhibited significant antibacterial activity, particularly against *B.subtilis*(19.50 mm) with an MIC of 6.25 µg/ml.The results suggest that the leaves of *E. prostrata* possess significant potential as a natural source of antifungal and antibacterial agents, and solvent selection greatly influences the extraction of bioactive compounds. Further investigation into its active constituents using analytical tools and mechanism of action is needed.

Keywords : Antibacterial, antifungal, biocontrol,*Eclipta prostrata*, ethyl acetate.

INTRODUCTION

Ethnomedicine is the study of traditional healing techniques and healthcare practices across various cultures, utilizing indigenous flora and fauna (Sadeghi *et al.* 2014). Ethnomedicinal plants constitute the foundation of traditional medicine systems worldwide and continue to serve as an important resource for modern drug discovery.

Many medications derived from ethnomedicinal plants contain bioactive compounds with potential therapeutic properties, such as antioxidant, anti-inflammatory, antimicrobial, antiviral, and anticancer. The study of ethnomedicinal plants has led to the discovery of numerous drugs, including aspirin, morphine, and taxol (Belete and Beyna, 2021). India offers a rich and diverse array of medicinal plants, with an estimated 8,000 species used by various communities across different ecosystems, accounting for a significant portion of the world's medicinal plant diversity (Pelletier *et al.* 2018). This vast biodiversity is deeply intertwined with the country's traditional systems of medicine, such as ayurveda, siddha, unani, and homeopathy, which have relied on these plants for centuries. Traditionally, around 2,000 plant species are used in India as medicine, demonstrating the deep-rooted connection between these plants and the country's cultural heritage (Shankar and Rawat, 2010). While India possesses a remarkable wealth of medicinal plants, several challenges threaten this biodiversity, like unsustainable harvesting practices, particularly in wild populations, which can lead to the depletion of valuable medicinal

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plant species. Manipur has a rich diversity of over 1,200 plant species with medicinal properties, making it a treasure trove of natural remedies (Maibam*et al.*, 2022). Medicinal plants are closely associated with the cultural and spiritual practices of Manipur's indigenous communities (Singh *et al.* 2023). Plants synthesize a diverse array of natural compounds, which include alkaloids, terpenoids, flavonoids, tannins, saponins, phenols, and lipids (Ahmadu and Ahmad, 2020). Medicinal herbs are used by 80% of the world's rural population for primary health care.

The Asteraceae family is one of the largest plant families, with a rich history of medicinal use and therapeutic applications (Rolnik and Olas, 2021). *Eclipta prostrata* (L.) L. (common name: false daisy) is an annual herbaceous medicinal plant locally known as "Uchi sumbal" in the Manipuri dialect. Herbal plants play a pivotal role in traditional medicinal practice and are primarily used to treat several infections in developing countries (Parham *et al*. 2020). This plant has been traditionally used in folk medicine, as well as in Ayurveda and Siddha for its curative properties (Molligoda *et al.*, 2021). It has been reported to possess anticancer, analgesic, anti-venom, antifungal, antibacterial, antioxidant, anti-hyperglycemic, and immunomodulatory properties (Rana, 2020). *E. prostrata* has broad medicinal value and is used for skin diseases, burns, wounds,hepatitis, gastritis, respiratory disorders, jaundice, diabetes, hair fall, fatigue, and fever (Sanyal *et al.*2022, Chung *et al.* 2017).

The antifungal and antibacterial properties of plant-derived secondary metabolites have gained attention as a potential solution to combat pathogenic fungi and bacteria, which are responsible for both agricultural losses and human infection. Chemical fungicides, while effective in controlling fungal diseases, have several drawbacks, such as contaminating water bodies and degrading soil quality. Also, excessive use can lead to the development of resistant pathogen strains, requiring stronger and potentially more harmful chemicals. Additionally, they pose risks to human health, causing acute and chronic health issues (Brauer *et al.* 2019). These chemicals can also reduce biodiversity by harming beneficial insects and microorganisms. To minimize these negative impacts, it's crucial to adopt biological management strategies, which are more advantageous than traditional chemical fungicides and antibiotics. In this study, the secondary metabolites of the leaves of *E. prostrata* were extracted using methanol and ethyl acetate and evaluated for their antifungal and antibacterial activities.

MATERIAL AND METHODS

Collection of plant samples

Healthy plants of *Eclipta prostrata*(L.) L. were collected in the month of December (2023) from Yairipok(Latitude, 24.689781^0 and Longitude, 94.9068642^0), Imphal East, Manipur, India. The plant was identified at the Manipur University Museum of Plants (MUMP), Department of Life Sciences (Botany), Manipur University, Manipur. The collected leaves were thoroughly washed with tap water to remove dust particles.

Drying and Preparing leaf powder

The clean leaves were dried at room temperature for three weeks by regularly checking at an interval of 4 days. The weight of the leaves was recorded before and after drying. With the help of a mortar and pestle, dried leaves were ground, making them into powder and packed in a plastic bag for further investigation.

Extraction of secondary metabolites

The maceration process was performed according to the protocol outlined by Hidayat and Wulandari (2020). Methanol (CH_3OH) (polar) and Ethyl acetate (CH_2COOC_2,H_2) (semi-polar) solvents were used during the extract preparation. 10 g each of the sample was kept in two different conical flasks (250 ml) and,100ml each of chosen solvents (methanol and ethyl acetate) was added to the sample, mixed and shaken for about 2 hours and kept at room temperature for 3 hrs.The mixtures were then filtered using a centrifuge at 1000RPM for 5 minutes at 4^0C. The mixtures were then kept in the oven for about 7days at low temperature (35^0C) to allow evaporation and form a precipitate.Once evaporated, the precipitate is then taken out with the help of a needle and kept

in a small eppendorf tube and put inside the fridge for further observation.

Evaluation of Antimicrobial activity of leaf extract

Antifungal activity

The plant pathogens *Nigrospora oryzae* (ITCC 6318), *Fusarium oxysporum* (ITCC 4998), and *Colletotrichum capsici* (ITCC 8451)were procured from the Indian Type Culture Collection (ITCC), New Delhi,and used to evaluate antifungal activity. The fungal pathogen cultures were incubated for 5 dayat 28±1°C in Potato Dextrose Agar (PDA) medium. The antifungal activity was evaluated using the modified petriplate culture method described by Salhi *et al.* (2017).The leaf extracts of methanol and ethyl acetate were mixed with 10% DMSO (dimethyl sulfoxide) (mg/ml) separately in different test tubes (test tube A containing the mixture of methanol extract and DMSO, and test tube B containing the mixture of ethyl acetate extract and DMSO).100µl (100 µg/ml) and 200µl (200 µg/ml) from test tubes A and B were put in different petridishes (90 mm) using a micropipette. 30ml each of the prepared PDA was mixed with the extract sample in the petridishes and allowed to cool down inside the laminar flow cabinet. After solidification, a 5mm size ofeach pathogen culture wasgently placedat the centerwith the help of an inoculation loop and then incubated at 28±1°C. Pathogens grown on PDA plates without any *E. prostrata* extract were used as a control plate.The diameters of the pathogen cultures were recorded on the 5th and 10th days after incubation. The inhibition percentage was determined using the formula I% = (C – T)/ C × 100, where C represents the diameter of the control colony and T denotes the diameter of the treatment colony. All the experiments were conducted in triplicate.

Antibacterial activity

Antibacterial activity of methanol and ethyl acetate extracts was determined using the agar well diffusion method and the 96-well microplate method (Nongthombam and Mutum, 2024). Theleaf extracts were tested for antibacterial activity against the pathogenic bacterial strains of *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 121),*Escherichia coli* (MTCC 443), and*Salmonella typhi* (MTCC 531) obtained from Microbial Type Culture Collection, Chandigarh, India. Inoculum was prepared by transferring a loopful of overnight-grown fresh bacterial culture to a test tube which containing 10ml of nutrient broth and incubated at 37±1°C for 24 hrs, which served as a fresh suspension inoculum. Petridishes were prepared by pouring 45mL Mueller-hinton (MH) agar into sterile petridishes (90 mm diameter) and allowed to solidify. Inoculum containing test bacteria was spread on plates with the help of a sterile swab moistened with the bacterial suspension. Four wells were made on an MH agar plate using a cork borer (6mm).The extracts were dissolved in 10% dimethyl sulfoxide (DMSO) at1 mg/ml concentration. Holes were filled with 100 µl of samples at different doses of 40 µg/ml and 80 µg/ml.Tetracycline hydrochloride (10 µg/ml) served as the positive control, while DMSO was employed as the negative control. Antibacterial activity was expressed by measurement of the inhibition zone around the well formed by the leaf extracts after 24hrs incubation at 37±1°C. Minimum inhibitory concentrations (MICs) were evaluated using the 96-well microtitre plate method. Different concentrations of the extract from 100-0.78µg/ml were put into the wells of columns 1–8, respectively. Column 9 was taken as the positive control (tetracycline hydrochloride) and column 10 as negative control (nutrient broth). 100 µl of the test bacterial inoculum was then added to all the wells. After 24 hrs of incubation, iodonitrotetrazolium chloride solution was added to all wells, incubated for 1 hr, and the lowest concentration well that did not show pink colour was observed. All the observations were taken in triplicate.

RESULTS AND DISCUSSION

The collected fresh leaves weigh 228g, and the dried leaves weigh 48g. The higher secondary metabolite yield was obtained from the methanol extract.The pH of the rhizosphere soil of *Eclipta prostrata* was 6.9(slightly acidic).

Antifungal activity ofleaf extract of Eclipta prostrata

Methanol and ethyl acetate extracts of *E. prostrata* leaves showed notable antifungal activity against

Table 1: Antifungal activity of methanol and ethyl acetate extract of *E. prostrata* leaf at different concentrations against the selected fungal pathogens after 4 and 9 days of incubation.

Pathogen	Concentration (µg/ml)	Inhibition percentage (%) Methanol extract		Inhibition percentage (%) Ethyl acetate extract	
		5th Day	10th Day	5th Day	10th Day
Colletotrichum capsici	100	29.63	46.78	26.31	46.55
	200	47.47	61.78	48.5	69.11
Fusarium oxysporum	100	34.14	41.55	28.57	44.67
	200	41.86	59.11	34.86	62.33
Nigrospora Oryzae	100	10.34	26.67	39.72	52.22
	200	18.24	34.44	58.79	75.89

all the tested fungal pathogens. As the concentration of both the methanol and ethyl acetate extracts increases, inhibition also increases. In case of methanol extract, after 9 days of incubation, the highest inhibition percentage was displayed against *Fusarium oxysporum* (59.11%) and lowest against *Nigrospora oryzae* (34.44%); for ethyl acetate extract, the highest inhibition was shown against *Nigrospora oryzae* (75.89%) and the lowest against *Fusarium oxysporum* (62.33%) (Table 1, Fig. 1 & 2).Antifungal assay demonstrated that both methanol and ethyl acetate extracts possess measurable inhibitory effects on fungal growth. The pathogens used in the study, viz.,*C. capsici*, *F. oxysporum*, and *N. oryzae*,are economically important as they have a broad host range and cause mass destruction to cereals, pulses, and vegetables (Behera *et al.* 2022; Liu *et al.* 2021; Gupta *et al.* 2017).Guluma *et al*. (2020) reported antifungal activity of a methanolic extract of *Brucea antidysenterica* against *Candida albicans*, highlighting the potential of methanol as an effective solvent for extracting antifungal compounds.In a study conducted by Abkhoo and Jahani (2017), the methanolic extract of *Glycyrrhiza glabra* showed an MIC of 6.25 ppm against *Fusarium oxysporum*. In a recent study, Jan *et al.* (2025) reported 82% growth inhibition of *Fusarium oxysporum* f. sp. *vasinfectum* using an ethanolic extract of *Zataria multiflora* leaves,demonstratingan environment friendly method of managing Okra wilt disease. In another study, the methanolic extract of *Balanites aegyptiaca* fruit inhibited 54.3% of the growth of *Penicillium italicum*, which causes blue mold disease in citrus fruits (Ibrahim and Abo-Elyousr, 2023). Hernández-Ceja *et al.* (2021) have shown very strong antifungal activity against dieback disease of blueberry, which displayed 100% inhibition of *Pestalotiopsis clavispora* and *Colletotrichum gloeosporioides* using ethyl acetate extracts of *Lantan ahirta* and *Argemone ochroleuca*, and 100% inhibition against *P. clavispora*, *C. gloeosporioides*, and *Lasiodiplodia pseudotheobromae* using ethyl acetate extract of *Adenophyllum porophyllum*. In a similar study, the methanolic extract of *Eclipta alba* aerial parts demonstrated good antifungal activity against the sorghum pathogens *Fusarium thapsinum*,

Table 2 : Antibacterial Activity of leaf extracts of methanol and ethyl acetate extract of *E. prostrata* at different concentrations against *B. subtilis*, *S. aureus*, *E. coli*, and *S. typhi*

Sample		Inhibition clear zone diameter (mm)			
		Gram positive bacteria		Gram negative bacteria	
		B. subtilis	*S. aureus*	*E. coli*	*S. typhi*
Methanol extract	40 µg/ml	12.5±0.15	8.67±0.15	9.70±0.20	9.63±0.15
	80 µg/ml	17.50±0.30	15.50±0.30	12.50±0.30	12.73±0.21
	+ve control	33.50±0.30	35.50±0.30	28.50±0.30	29.83±18.22
	MIC (µg/ml)	12.50±0.00	12.50±0.00	12.50±0.00	12.50±0.00
Ethyl acetate extract	40 µg/ml	13.50±0.30	9.63±0.15	9.67±0.15	6.73±0.15
	80 µg/ml	19.50±0.30	16.17±0.25	13.50±0.30	9.30±0.72
	+ve control	31.63±0.15	36.73±0.21	29.50±0.30	30.80±0.61
	MIC (µg/ml)	6.25±0.00	12.50±0.00	12.50±0.00	25.00±0.00

Data are means of clear zone diameter (triplicate) ± standard deviation (SD); 10 µg/ml tetracycline hydrochloride was employed as positive control Agar-well diffusion method and MICs by 96-well method followed.

Alternaria alternata, *Epicoccum sorghinum*, and *Curvularia lunata* resulting MIC of 80 mg/ml (Boregowda*et al*., 2019). The need forbiocontrol agents, especially medicinal plants,has increased its demand due to environmentally friendly nature, specificity, abundance, and negative health hazards. Our study hints at a promising biocontrol agent that could reduce yield loss and increase food safety.

Antibacterial activity of leaf extract of Ecliptaprostrata

Both the plant extracts have shown good antibacterial activity against all the pathogenic bacterial strains used in the study. The highest antibacterial inhibition zone was displayed bythe ethyl acetate extract against *B. subtilis*(19.50 mm) at 80 µg/ml, with MIC of 6.25 µg/ml and, the lowest zone was also shown by the ethyl acetate extract against*S. typhi* (9.30 mm), having a MIC of 25 µg/ml (Table 2, Fig. 3& 4). These results demonstrated that the extract of *E. prostrata* can serve as a broad-spectrum antibacterial agent. Also,methanol and ethyl acetate extracts showvaried sensitivity towards the pathogen tested.In a previous study, Valle *et al.* (2015) reported strong antibacterial activity of the ethanolic leaf extracts of *Psidium guajava*, *Phyllanthus niruri*, *Ehretia microphylla*, and *Piper betle* against methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus* spp.,with MICs of 19–156 µg/ml and MBCs of 312 µg/ml. In another study, the ethanolic extract of *Piper betle* has shown prominent antibacterial activity against the oral pathogens *Enterobacter faecalis*, *Lactobacillus fermentum*, *Lactobacillus salivarius*, *Streptococcus sobrinus*, *Streptococcus mutans*, *Aggregatibacter actinomycetemcomitans*, and *Fusobacterium nucleatum* with MIC and MBC of 1.04–5.21 mg/ml and 2.08–8.33 mg/ml, respectively (Teanpaisan *et al.*, 2017). Mudzengi *et al.* (2017) claimed the methanolic and ethanolic extract of

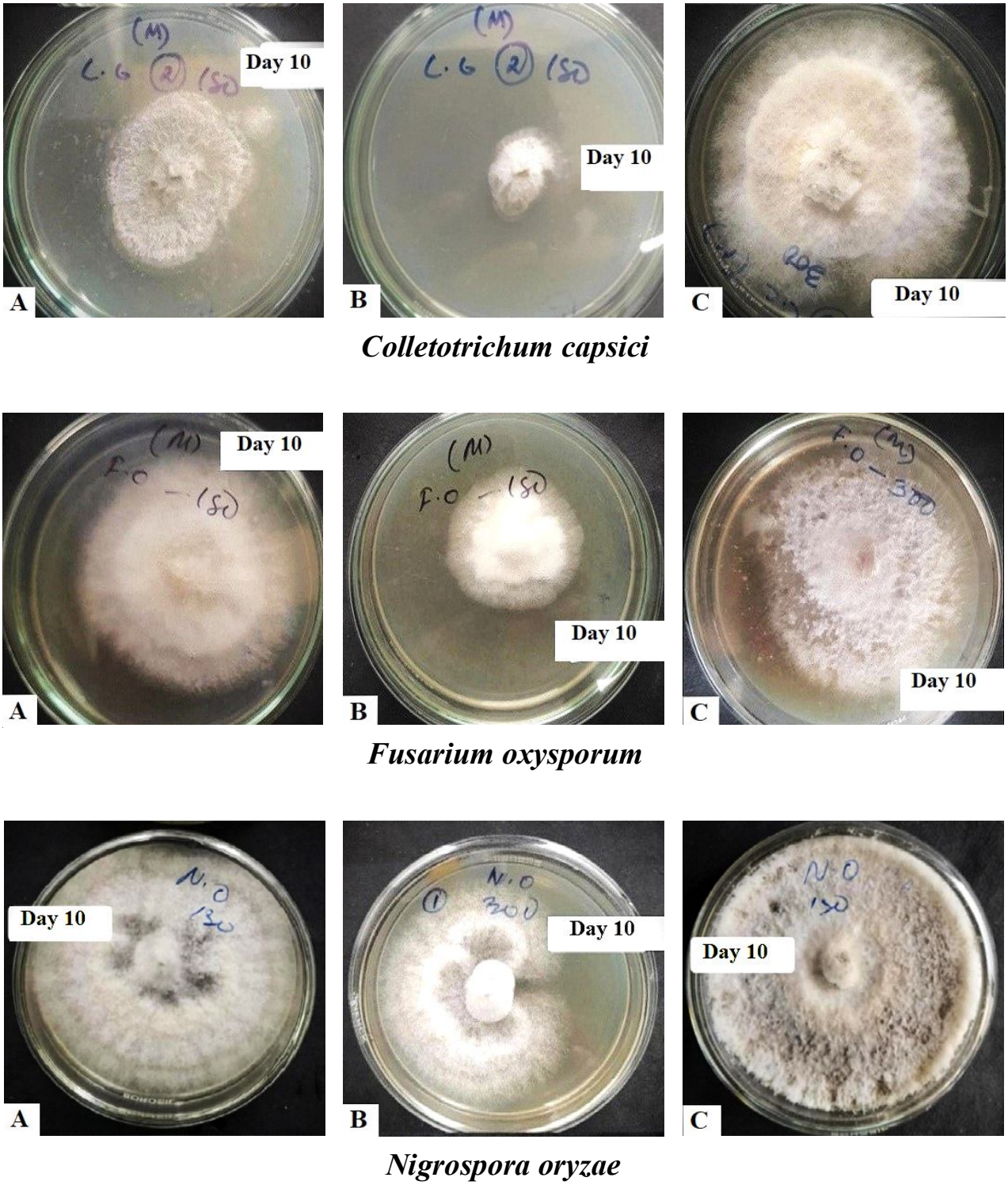
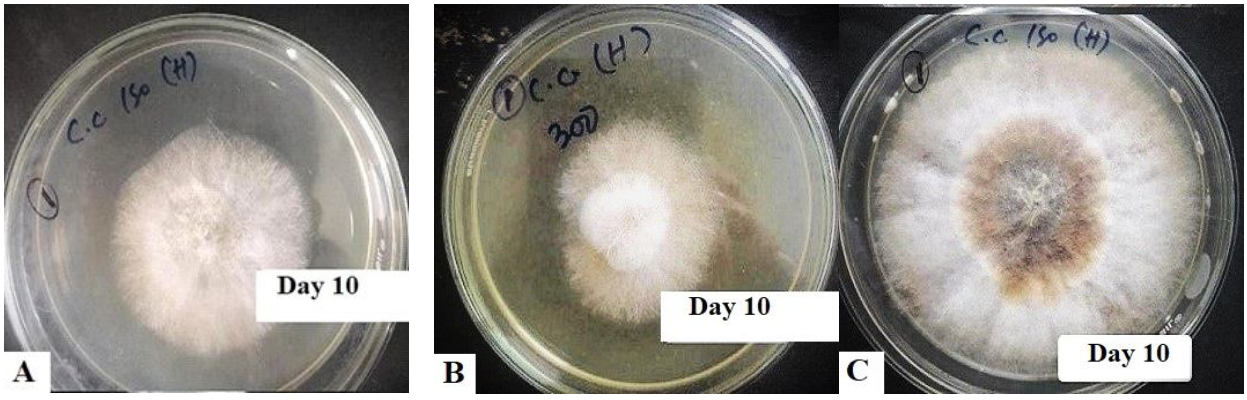
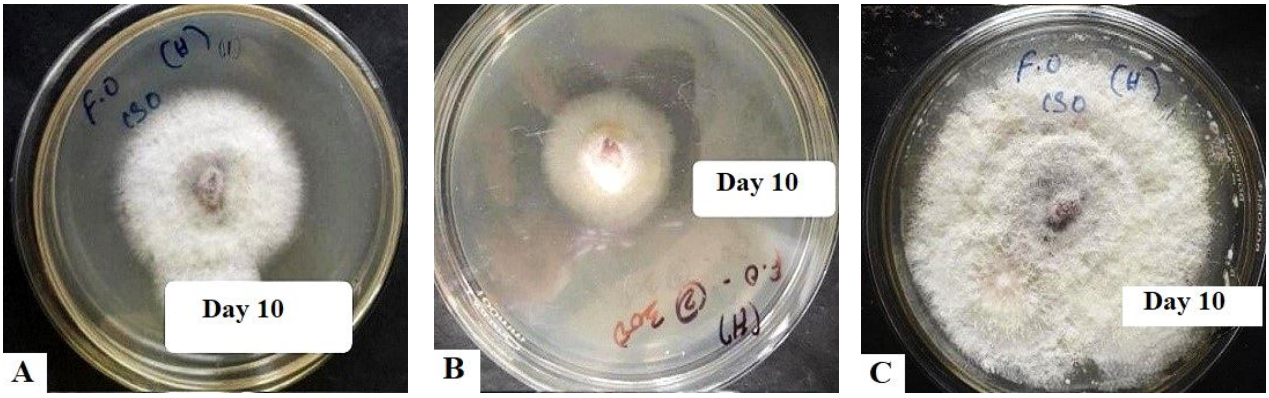


Fig.1: Antifungal activity of methanol extract of *E. prostrata* after 9 days of incubation. Plate 'A' represents 100 µg/ml concentration, 'B' represents 200 µg/ml concentration, and 'C' represents control plate.

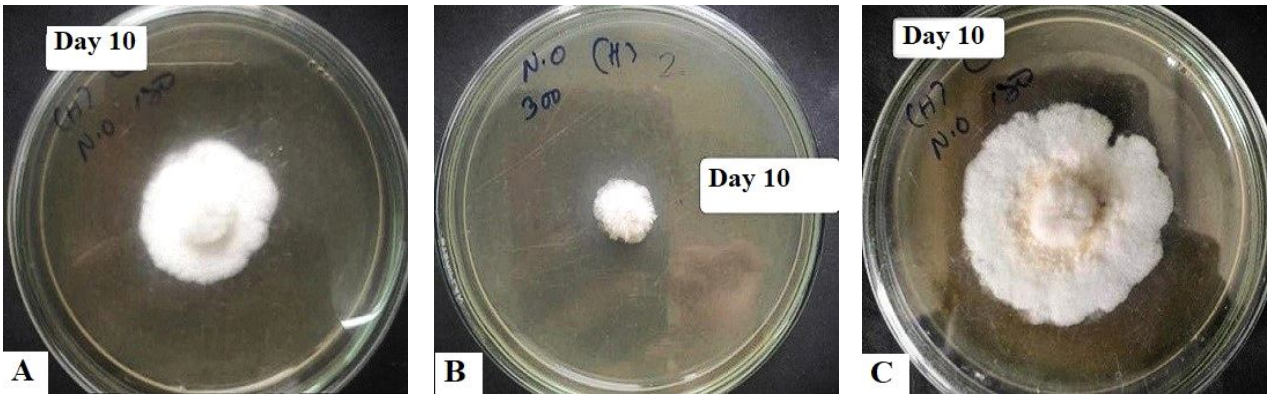
Colophospermum mopane (bark) exhibits strong antibacterial activity against livestock pathogens *Staphylococcus aureus* and *Escherichia coli* with MICs of 9.64 mg/ml and 7.86 mg/ml, respectively.In another investigation, Mostafa *et al.* (2018) reported that the ethanolic extracts of *Punica granatum* and *Syzygium aromaticum* were effective against the food-borne pathogens



Colletotrichum capsici



Fusarium oxysporum



Nigrospora oryzae

Fig.2: Antifungal activity of ethyl acetate extract of *E. prostrata* after 9 days of incubation. Plate 'A' represents 100 µg/ml concentration, 'B' represents 200 µg/ml concentration, and 'C' represents control plate.

Staphylococcus aureus and *Pseudomonas aeruginosa* with MICs ranging from 2.5 to 5.0 mg/ml and MBCs of 5.0 and 10 mg/ml.While conducting an experiment on pathogens found in the poultry gastrointestinal tract, McMurray *et al.* (2020) found the most potent antibacterial activity against *Escherichia coli* with the aqueous extract of *Agrimoniapilosa* with an MIC of 7.81 mg/l. Rising of antibiotic-resistant bacterial strains has been a major issue in health care, animal husbandry, and the poultry industry, and our findings could be utilized in combating these strains.

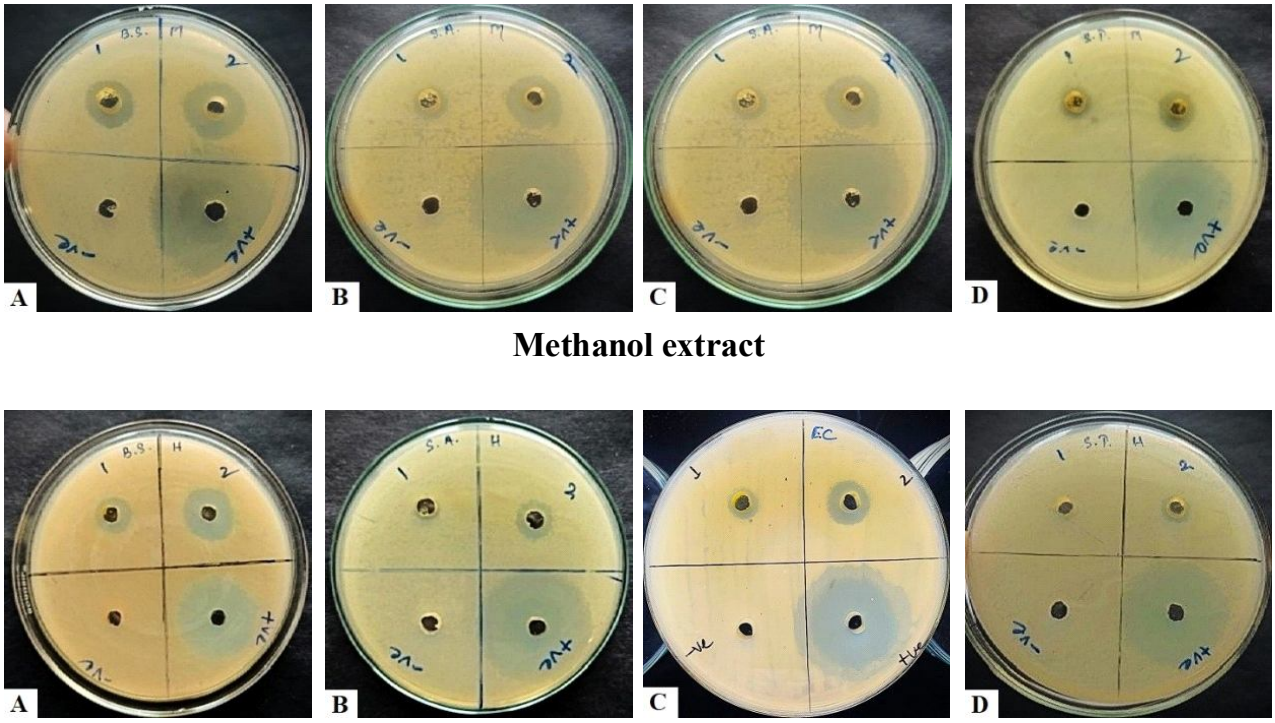


Fig 3 : Antibacterial activity of methanol and ethyl acetate extracts of *E. prostrata* leaves against the pathogenic bacteria (A) *B. subtilis*, (B) *S. aureus*, (C) *E. coli*, and (D) *S. typhi*, using agar well diffusion method.

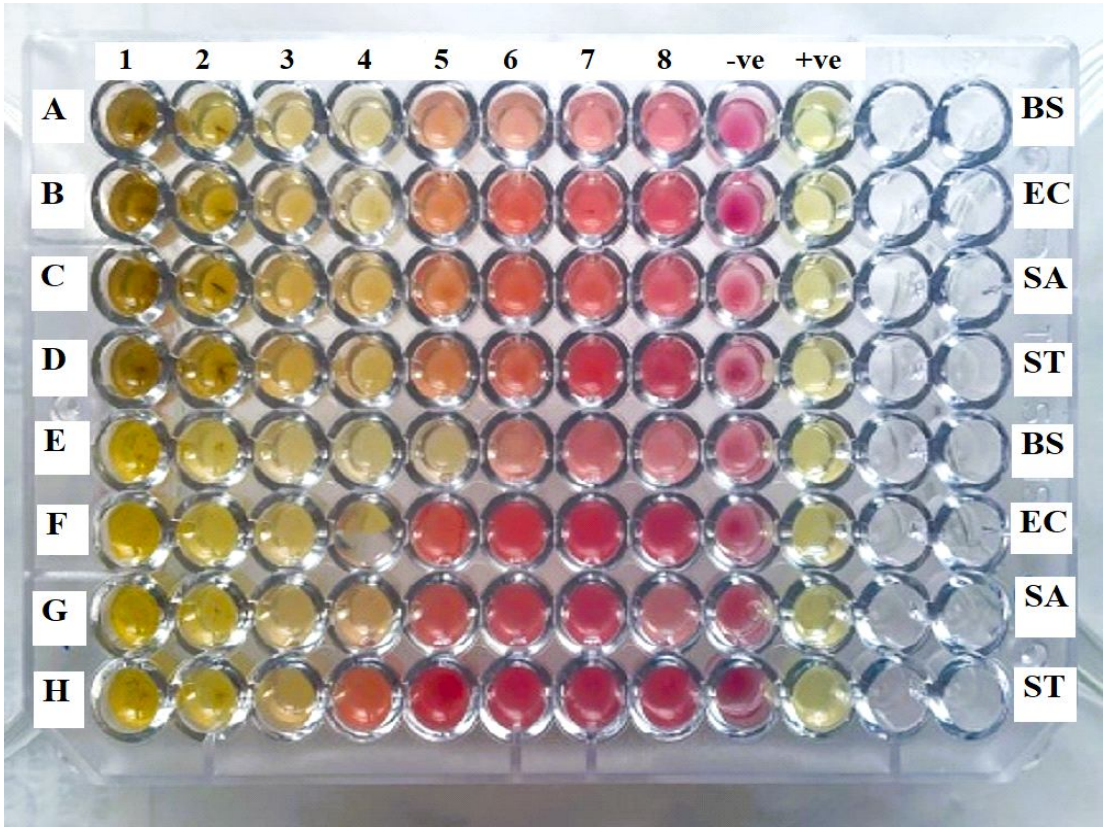


Fig 4 : Minimum inhibitory concentrations (MICs) of the methanol and ethyl acetate extracts of *E. prostrata* against *B. subtilis* (BS), *E. coli* (EC), *S. aureus* (SA), and *S. typhi* (ST) using 96-well microtitre plate method. Methanol extract was inoculated on row A, B, C, and D; and ethyl acetate extract on E, F, G, and H.

CONCLUSION

This study demonstrates broad-spectrum antifungal and antibacterial activity of *E. prostrata* leaf extracts against pathogenic strains of *C. capsici*, *F. oxysporum*, *N. oryzae*, *B. subtilis*, *S. aureus*,*E. coli*, and *S. typhi*.Comparatively, the ethyl acetate extract exhibit stronger antimicrobial properties. This study justifies the usage of *E. prostrata* in ayurvedic medicinal practice for treating several ailments. Both methanol and ethyl acetate extracts demonstrated inhibitory effects against the pathogens used in the study, with ethyl acetate extract generally showing broader and more consistent antibacterial activity. The findings of our study show that the leaves of *E. prostrata* possessed bioactive compounds that can be isolated, purified, and applied in the fields of agriculture, biomedical, and pharmaceutical sectors. Further studies using analytical tools such as GC-MS, LC-MS, FTIR, and NMR would provide in-depth information on the compounds responsible for antimicrobial activity.

DECLARATION

Conflict of Interest.Authors declare no conflict of interest.

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