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Antimicrobial Potential of *Utricularia stellaris* Extracts: A promising natural therapeutics to curb infectious pathogen

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ABSTRACT

In managing microbial infections, health departments are facing a slowdown in curbing the antibiotic menace. This concurs with the scenario that in the last decade antibiotic-resistant bacteria are emerging around the globe, necessitating the need to find alternative treatments from natural sources. Carnivorous plants are known for their unique ability to thrive in nutrient-poor environments by capturing and digesting prey. Recent research has focused on their potential as sources of bioactive compounds with antibacterial properties. The present study investigates the antibacterial activity of methanolic, ethanolic, and aqueous extracts of *Utricularia stellaris*, an aquatic carnivorous plant, against six bacterial strains: *Staphylococcus aureus, Enterococcus faecalis, Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae,* and *Pseudomonas aeruginosa*. The result noted that the methanolic extracts exhibited the highest antibacterial activity, particularly against *Bacillus subtilis* and *Staphylococcus aureus*, with moderate effects on *Escherichia coli*, whereas ethanolic extracts showed similar results, demonstrating strong inhibition of gram-positive bacteria, while aqueous extracts displayed limited activity, primarily due to their inability to dissolve non-polar bioactive compounds effectively. Overall, methanolic extract emerged as the most effective solvent across studies, underscoring its superiority in extracting potent bioactive compounds. Notably, *Pseudomonas aeruginosa* exhibited resistance across all extracts, consistent with its known efflux pump mechanisms and impermeable outer membrane. The findings suggest that *Utricularia stellaris* is a promising natural source of antibacterial agents, particularly for combating gram-positive pathogens. This study provides a foundation for further research on isolating specific compounds and exploring their potential applications in natural therapeutics.

INTRODUCTION

Microbial diseases pose a global health threat, with antibiotics as the main treatment. However, increase in antibiotic resistance makes infections harder to treat, increasing the risks of disease spread and severe outcomes. This challenge has driven researchers to explore new treatments from natural sources, where herbal products show promise due to their specificity and fewer side effects. Plants are vital to global health, serving as sources of medicinal compounds essential for traditional and modern treatments (Oladeji 2016; Dar *et al.*, 2016). Rich in phytochemicals like saponins, tannins, and phenolic compounds, certain plants—especially insectivorous ones—exhibit therapeutic effects against various ailments. They also have antibacterial and antifungal properties, benefiting conditions such as sunburn, toothache, and heart disease.

Carnivorous plants grow in nutrient-poor soils and compensate by trapping insects and small organisms for nutrition (Mishra *et al.*, 2021; Mishra and Kumar, 2019). These unique plants have fascinated humans. Since ages, 46 carnivorous plants have been used to treat many health problems, but still, they are unexplored. Keeping this in view, in present work an attempt has been made to document the antimicrobial properties of common *Utricularia* species. *Utricularia,* a small herb of the family *Lentibulariaceae* and order Lamiales, is the largest genus of carnivorous plants found in both aquatic and terrestrial habitats. These rootless plants have rhizoids, stolons, and bladder-like trapping organs, earning them the name bladderworts. The traps are hollow, ovoid structures on tiny stalks with an opening for capturing prey. They produce terminal flowers on long, upright shoots, which are vibrant and vary in color (Mishra and Kumar 2021; Saxena and Brahmam 1995).

Thus, the present study focused on ***Utricularia stellaris*** of genus *Utricularia*, which might be a pharmacologically important plant

for its antimicrobial activity. The aim of this study to evaluate the antimicrobial activity of ***Utricularia stellaris*** plant extracts.

Material and Methods:

Collection of Plant: *Utricularia stellaris* was collected from the local area of Gondwana University, Gadchiroli (Kasavi pond/lake taluka Armori district Gadchiroli). The collected plant was cleaned, shade-dried to remove moisture and ground into a fine powder for efficient extraction.

Plant Extract Preparation : Ethanol, methanol and aqueous extracts were prepared using a Soxhlet apparatus. Ten grams of plant powder were extracted with 100 mL of each solvent at optimal boiling temperatures: 80°C for ethanol and 60°C for methanol (Omar *et al.,* 2017).

Antimicrobial agent and Chemical: All antimicrobial agent and chemicals were purchased from Hi-Media Mumbai, India. The six bacteria species representing different strains were tested for antimicrobial activity. Among tested bacteria were Gram-positive - *Staphylococcus aureus*, *Bacillus subtilis* and *Enterococcus faecalis*, and Gram-negative- *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*. All tested strains were collected from the Department of Microbiology, Sardar Patel Mahavidyalaya, Chandrapur. For preparation of bacterial suspension bacteria were cultured in the nutrient broth at 37 °C for 24 hrs.

Antimicrobial Activity: The antimicrobial activity of the samples was tested using a modified agar well diffusion assay (Ginovyan *et al.,* 2015). MHA plates were prepared for bacteria with lawns of growth created using sterile swabs. Wells (8 mm) were cut into the agar, and 100 µL of the test samples or controls were added. Plates were incubated at 37°C for 24 hours, and inhibition zone diameters were measured. Gentamicin (10 µg/mL) served as positive controls, while DMSO was the negative control.

Result

The ethanol plant extract showed concentration-dependent antibacterial activity. It was most effective against ***Enterococcus faecalis*** **(19 mm)** and ***Bacillus subtilis*** **(18 mm)** at 100 µL, with moderate effects on ***Staphylococcus aureus*** and ***Escherichia coli***. ***Klebsiella pneumoniae*** and ***Pseudomonas aeruginosa*** were inhibited only at 100 µL, while lower concentrations showed no effect on these resistant strains, shown in Table no.1.

Table No.1: Antibacterial activity of Ethanolic Plant Extracts

Zone Of Inhibition in mm			
Organisms	**25µL**	**50µL**	**100µL**
SE1-SA01	12	13	14
SE1-EF02	NI	NI	19
SE1-BS03	16	17	18
SE1-EC04	12	14	15
SE1-KP05	NI	NI	14
SE1-PA06	NI	NI	14

Note: NI- No Inhibition, Strain no-SA01 (*Staphylococcus aureus*),Strain no - EF02 (*Enterococcus faecalis*) , Strain no - BS03 (*Bacillus subtilis*),Strain no - EC04 (*Escherichia coli*),Strain no - KP05 (*Klebsiella pneumoniae*),Strain no-PA06 (*Pseudomonas aeruginosa*),E - Ethanol extract

Whereas, the methanol extract exhibited strong antibacterial activity against ***Bacillus subtilis*** **(18 mm)** and ***Escherichia coli*** **(17 mm)** at 100 µL, with consistent inhibition of ***Klebsiella pneumoniae*** **(14-15 mm)** at all concentrations. Moderate activity was observed against ***Staphylococcus aureus*** **(12 mm)** and ***Enterococcus faecalis*** **(14 mm)** at higher concentrations. ***Pseudomonas aeruginosa*** **(12 mm)** was inhibited only at 100 µL, while no inhibition was observed at lower concentrations for certain strains, shown in Table no.2.

Table No.2: Antibacterial activity of Methanolic Plant Extracts

Zone Of Inhibition in mm			
Organisms	**25µL**	**50µL**	**100µL**
SM1-SA01	NI	11	12
SM1-EF02	NI	NI	14
SM1-BS03	16	17	18
SM1-EC04	15	16	17
SM1-KP05	14	15	15
SM1-PA06	NI	NI	12

Note: NI- No Inhibition, Strain no-SA01 (*Staphylococcus aureus*),Strain no - EF02 (*Enterococcus faecalis*) , Strain no - BS03 (*Bacillus subtilis*),Strain no - EC04 (*Escherichia coli*),Strain no - KP05 (*Klebsiella pneumoniae*),Strain no-PA06 (*Pseudomonas aeruginosa*),M- Methanol extract

The aqueous extract showed moderate antibacterial activity, with the strongest effect against ***Bacillus subtilis*** **(18 mm)** at 100 µL. ***Klebsiella pneumoniae*** showed consistent inhibition (**13-15 mm**) across concentrations. ***Escherichia coli*** exhibited moderate activity (**10-14 mm**), increasing with concentration. ***Staphylococcus aureus*** showed limited activity (**13 mm)** at 100 µL. ***Enterococcus faecalis*** was inhibited only at 100 µL (**16 mm**), while ***Pseudomonas aeruginosa*** showed no inhibition at any concentration, shown in Table no.3.

Table No.3: Antibacterial activity of Aqueous Plant Extracts

Zone Of Inhibition in mm			
Organisms	**25µL**	**50µL**	**100µL**
SW1-SA01	NI	12	13
SW1-EF02	NI	NI	16
SW1-BS03	16	17	18
SW1-EC04	10	12	14
SW1-KP05	13	14	15
SW1-PA06	NI	NI	NI

Note: NI- No Inhibition, Strain no-SA01 (*Staphylococcus aureus*),Strain no - EF02 (*Enterococcus faecalis*) , Strain no - BS03 (*Bacillus subtilis*),Strain no - EC04 (*Escherichia coli*),Strain no - KP05 (*Klebsiella pneumoniae*),Strain no-PA06 (*Pseudomonas aeruginosa*),W- Aqueous extract

DISCUSSION

The genus *Utricularia* is a group of carnivorous plants known for their unique bladder traps and production of bioactive secondary metabolites. According to **Adamec *et al*. (2010)** and **Fang *et al*.**

(2021) study *Utricularia* species are rich in phenolic compounds, flavonoids, and iridoids, which are responsible for their antimicrobial properties and showing effective activity against both Gram-positive and Gram-negative bacteria. Thus, the present study studied the antibacterial activity of methanolic, ethanolic and aqueous extract of *Utricularia stellaris* which belongs to *Utricularia* species against the various strains of gram positive and gram negative bacteria.

The present study noted the strong activity of the ethanol extract of *Utricularia stellaris* particularly against ***Enterococcus faecalis*** **(19 mm)** and ***Bacillus subtilis*** **(18 mm)**, which aligns with the work of **Adamec et al. (2010)**, who found that ethanol extracts of *Drosera spp.* exhibited significant antibacterial activity against Gram-positive bacteria, owing to the presence of flavonoids and naphthoquinones. Similarly **Schnitzler et al. (2010)** demonstrated that *Dionaea muscipula* extracts exhibited potent activity against ***Staphylococcus aureus***, further supporting the effectiveness of ethanol as a solvent for extracting bioactive compounds from carnivorous plants.

Similarly, the methanol extracts of *Utricularia stellaris* showed strong inhibition of ***Bacillus subtilis*** **(18 mm)** and ***Escherichia coli*** **(17 mm)**. Present study more concerned with the findings of **Juniper *et al*. (1989)**, who reported that methanol extracts of *Nepenthes spp.* contain phenolic acids and alkaloids with broad-spectrum antibacterial activity. Also, **Cowan (1999)** observed that methanol extracts of *Sarracenia purpurea* showed consistent antibacterial effects against Gram-positive and Gram-negative bacteria, reinforcing the observations made in this study.

Whereas the aqueous extract of *Utricularia stellaris* showed the moderate activity particularly against ***Bacillus subtilis*** **(18 mm)**, which aligns with **Fang *et al*. (2021)** who reported that aqueous extracts of *Drosera rotundifolia* showed limited antibacterial effects compared to ethanol and methanol extracts. This is attributed to water's inability to extract lipophilic secondary metabolites, which are often the most active antimicrobial agents. Similarly, **Ellison and Gotelli (2001)** observed weak activity of aqueous extracts from *Nepenthes spp.* against Gram-negative bacteria like ***Pseudomonas aeruginosa***, which were resistant in both their study and this one.

While studying the strain specific sensitivity it was noted that ***Pseudomonas aeruginosa*** resistance to all extracts which was aligns with the findings of **Rischer *et al*. (2002)**, who noted that the antimicrobial compounds from carnivorous plants, such as plumbagin and droserone, are less effective against Gram-negative bacteria due to their impermeable outer membrane and efflux pumps. Conversely, the strong activity against ***Bacillus subtilis*** and ***Enterococcus faecalis*** is consistent with **Heslop-Harrison (1978)**, who suggested that the compounds produced by carnivorous plants are particularly effective against Gram-positive bacteria, which lack the protective outer membrane found in Gram-negative strains.

CONCLUSION

From the given study it concludes that methanolic, ethanolic, and aqueous extracts of *Utricularia stellaris* highlights its potential as a natural source of antimicrobial agents. , **ethanol** and **methanol** extracts showed superior antibacterial activity while **aqueous extract**, demonstrated limited activity, emphasizing the significance of solvent choice in extracting bioactive compounds. However, the resistance of ***Pseudomonas aeruginosa*** across all extracts indicates the need for further investigation into optimizing extraction methods or exploring synergistic effects with other antimicrobial agents. Overall, the findings support the promising potential of *Utricularia stellaris* as a natural antimicrobial agent. This study contributes to the growing interest in plant-based therapeutics and sets the stage for future research on isolating specific bioactive compounds and evaluating their clinical applications.

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