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EXPLORING THE UNREPORTED ANTIMICROBIAL AND ANTIFUNGAL PROPERTIES OF *Vigna vexillata* AGAINST CLINICALLY SIGNIFICANT PATHOGENS

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ABSTRACT: *Vigna vexillata*, a lesser-known wild legume, has recently gained increasing attention for its potential therapeutic properties. Recent studies suggest that the plant holds promise as a novel source of treatment against various pathogens not previously reported. Research has demonstrated that *Vigna vexillata* extract exhibits significant natural antimicrobial activity against several microorganisms, including *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), *Pseudomonas aeruginosa* (*P. aeruginosa*), and *Candida albicans*. The leaf extract was obtained using the maceration method, employing water and ethanol as solvents. The antimicrobial activity of the extract was evaluated at five different concentrations using the agar cup method and compared to standard antibiotics such as gentamycin (for bacterial strains) and amphotericin B (for fungal strains). The results revealed a concentration-dependent inhibition of microbial growth, with higher concentrations showing more pronounced antimicrobial effects. These findings offer a new perspective on the potential of *Vigna vexillata* as a natural source of antimicrobial agents.

KEYWORDS: Antibacterial activity, Antifungal activity, *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Candida albicans* (*C. albicans*)

1. Introduction

Antimicrobial resistance has emerged as a major worldwide health problem, prompting researchers to investigate alternate medicines derived from natural sources. [5] Medicinal herbs, noted for their abundance of bioactive substances, have been used for ages in traditional medicine to treat a variety of infectious diseases. Among the many medicinal plants, legumes have received attention for their potential therapeutic effects due to the presence of secondary metabolites such as alkaloids, flavonoids, saponins, and tannins, which have been shown to have antibacterial and antifungal characteristics. [11]

Vigna vexillata, sometimes known as the wild cowpea, is a lesser-known wild legume species that has lately gained attention for its undiscovered therapeutic qualities. While other wild legumes, such as *Vigna unguiculata* (cowpea), have been extensively investigated for their nutritional and therapeutic properties. [4] Preliminary phytochemical screenings of various legumes indicated the existence of bioactive chemicals that can suppress microbial development. These findings indicate that *Vigna vexillata* may be a source of new antibacterial compounds. [2]

Several bacterial and fungal infections represent significant health hazards, especially when drug-resistant forms emerge. *Escherichia coli*, a Gram-negative bacterium, causes a variety of illnesses, including urinary tract infections and gastrointestinal disorders.

Similarly, *Staphylococcus aureus* (*S. aureus*), a Gram-positive bacterium, can cause skin infections, pneumonia, and food poisoning. *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Candida albicans* (*C. albicans*) [8] are opportunistic infections that have developed resistance to standard therapies. *P. aeruginosa* is known for causing hospital-acquired infections, while *C. albicans* is the major source of fungal infections in immunocompromised people. As a result, there is an increasing demand for alternative antibacterial agents, particularly those derived from natural sources. [9]

Recent research on additional plant species has shown that plant extracts are effective in inhibiting microbial growth via a variety of ways, including disrupting microbial cell walls, inhibiting protein synthesis, and interfering with important metabolic activities. In this perspective, the utilization of *Vigna vexillata* leaf extract as a possible antibacterial agent is very intriguing. Bioactive substances are often extracted from plants using solvents like ethanol and water, which may dissolve a wide spectrum of phytochemicals. [13].

2. Materials and method

Selection of plant

The plant *Vigna vexillata* was selected for study. Its leaves were collected from Lonavala. The collected leaves were identified and authenticated Xavier's Blatter Herbarium.

Leaf extract

The completely shade dried material was coarsely powdered and then the leaves extracts of 30g in 100 ml concentration were prepared in Water: Ethanol (1: 1 v/v) solvent. The samples were macerated for 72 hrs. The filtered extract was then used for analysis.

Microorganism

The Pathogenic strains of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans* were used. These strains were procured from Microbial Type Culture Collection and Gene Bank (MTCC)- Chandigarh.

Antimicrobial activity assay

Culture Preparation: The Gram-positive, Gram-negative bacteria and fungal strain were pre-cultured in Mueller Hinton broth (MHB) for bacterial and Sabouraud dextrose broth for fungal culture kept overnight in a rotary shaker at 37°C. Afterward, each strain was adjusted at a concentration of 10⁸ cells/ml using 0.5 McFarland standard.

Medium: The fresh bacterial was pipetted in the centre of sterile Petri dish. Molten cooled Muller Hinton agar for bacteria was then poured into the Petri dish containing the inoculum and mixed well. Upon solidification, wells were made using a sterile cork borer (6 mm in diameter) into agar plates containing inoculums.

Method:

The Antibacterial activity was checked by following Zone Inhibition Method (Kirby-Bauer method).

Sample preparation for assay: Leaf extract was prepared using Dimethyl sulfoxide solvent to dissolve the plant extract and then placed on the inoculated agar.

Standard antibiotic solution for bacterial: Ciprofloxacin (2mg/ml) for *P.aeruginosa* and Gentamycin (1mg/ml) for *S.aureus* and *E.coli* , were used as positive control and the solvent used for preparing extract was used as negative or vehicle control.

Standard antibiotic solution for Fungal: Amphotericin B (5mg/ml) was taken as positive control

For bacterial assay: The MHA plates were inoculated by spreading with 100 µl of Bacterial cultures, and followed by making the wells. Each well was filled with 30µl of different concentration (0 – 300 mg/ml) of leaves extracts. The plates were incubated overnight at 37° C for 18-24 hours. Antimicrobial activity was evaluated by measuring zone of inhibition.

For fungal assay: The Sabouraud dextrose agar –SDA plates were inoculated by spreading with 100 µl of Fungal culture, *C. albicans* and followed by making the wells containing 30µl of different concentration (0 to 300 mg/ml). One well in each plate was loaded with solvent alone which served as vehicle control and Amphotericin B well was taken as positive control. The plates of *C. albicans* were incubated at 37 °C for 24 hrs. The clear zones created around the well were measured and recorded.

3. Results & discussion

Table 1. Invitro activity of leaves extract against pathogens.

Amount (µg/well)	<i>P. aeruginosa</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>C. albicans</i>
PC	22	14	15	22
0	0	0	0	0
187.5	0	0	0	0
375	3.0±0.03	2.4±0.2	0.0	6.0±0.1
760	4.7±0.3	4.0±0.3	1.9±0.1	6.0±0.1
1500	8.8±0.6	5.1±0.1	3.3±0.3	6.3±0.4
3000	11.3±0.3	9.2±0.2	6.5±0.2	10.3±1.1

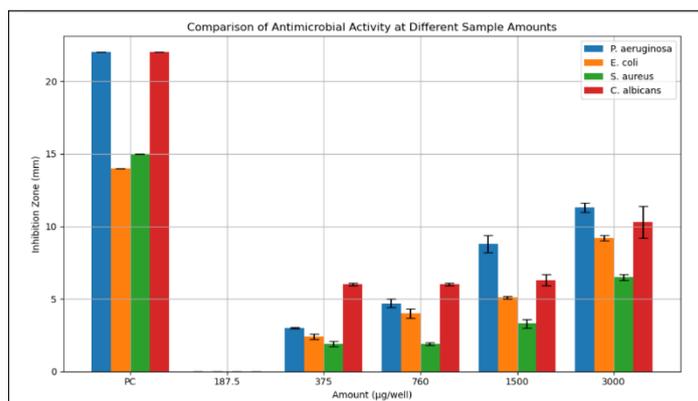


Figure 1. Comparison of antimicrobial activity

The MIC is generally considered to be the lowest concentration where no visible growth is observed. The MIC for each pathogen where the zone of inhibition first appears (for bacterial and fungal growth):

- *Pseudomonas aeruginosa* shows inhibition starting at 375 µg/well with the highest zone at 3000 µg/well.
- *Escherichia coli* also shows inhibition starting at 375 µg/well with increasing zones up to 9.2 mm at 3000 µg/well.
- *Staphylococcus aureus* demonstrates resistance up to 375 µg/well, with inhibition starting at 760 µg/well and increasing to 6.5 mm at 3000 µg/well.
- *Candida albicans* shows inhibition starting at 375 µg/well, with a steady increase in the zone of inhibition, peaking at 10.3 mm at 3000 µg/well.

4. Conclusion

This study evaluated the antimicrobial activity of *Vigna vexillata* leaf extract against *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans* using the agar cup method. The results demonstrated a concentration-dependent inhibition of microbial growth, with higher concentrations showing more pronounced effects.

The Minimum Inhibitory Concentration (MIC) for *P. aeruginosa*, *E. coli*, and *C. albicans* was identified at 375 µg/well, whereas *S. aureus* displayed resistance up to 375 µg/well, with inhibition starting at 760 µg/well. These findings highlight that *Vigna vexillata* has significant antimicrobial potential, particularly against Gram-negative bacteria and fungal strains. The plant extract was less effective against *S. aureus*, indicating that higher concentrations are required for Gram-positive bacteria.

These results support the potential use of *Vigna vexillata* as a natural antimicrobial agent, especially in addressing infections caused by *Pseudomonas aeruginosa* and *Candida albicans*. Further research is recommended to isolate and characterize the specific bioactive compounds responsible for the observed antimicrobial effects and to evaluate their efficacy in *in vivo* studies. Additionally, the potential synergistic effects of combining *Vigna vexillata* extract with conventional antibiotics should be explored, particularly in the context of combating antibiotic-resistant pathogens.

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