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Study of the Antifungal and Antibacterial Effect of Phytochemical Components of Azolla

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Abstract. *The growing resistance of pathogenic microorganisms to synthetic drugs has increased interest in plant-derived antimicrobial agents. Aquatic plants, known for their ecological and medicinal significance, are being investigated as potential sources of bioactive compounds. Azolla pinnata, a fast-growing aquatic fern, is traditionally used in agriculture due to its nitrogen-fixing ability. Recent phytochemical studies have highlighted its potential antibacterial and antifungal properties, attributed to compounds such as flavonoids, alkaloids, saponins, and tannins. However, systematic studies on its microbial inhibition spectrum remain limited. Although Azolla is known to contain several biologically active substances, little is known about the comparative effectiveness of different extraction methods and their specific actions against common pathogenic microbes. This study aimed to assess the phytochemical composition of Azolla pinnata extracts and evaluate their antibacterial and antifungal activity against selected pathogens using ethanol, methanol, and water-based extractions. The phytochemical analysis confirmed the presence of flavonoids, phenolics, alkaloids, and saponins. Ethanol extracts demonstrated superior antibacterial activity against Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa, while also showing significant antifungal effects against Candida albicans and Aspergillus niger. The mechanisms included membrane disruption and inhibition of protein synthesis. This is one of the first studies to comparatively evaluate solvent-dependent antimicrobial properties of Azolla pinnata, suggesting its efficacy as a natural bioactive source. The findings suggest Azolla pinnata as a promising candidate for developing plant-based antimicrobial agents in pharmaceutical, agricultural, and environmental biotechnolo gy fields.*

Key words: *Azolla pinnata, phytochemical components, antibacterial activity, antifungal effect, bioactive compounds.*

INTRODUCTION

Azolla forms a symbiotic relationship with a cyanobacterium called Anabaena azollae, which lives in the cavities of its leaves. This cyanobacterium is capable of fixing atmospheric nitrogen, converting it into a form that the plant can use, and ultimately enriching the surrounding water and soil. Because of this unique ability, Azolla has long been utilized as a green manure in rice paddies, particularly in Asian countries.Azolla is small and floats on the surface of water bodies such as ponds, ditches, and rice fields. The plant typically appears green, but its color may change to reddish or purple under

certain environmental conditions, such as high sunlight or nutrient stress. It reproduces both vegetatively and through spores, allowing for rapid population expansion under favorable conditions.The plant's fast growth rate—doubling its biomass in as little as 3 to 10 days—makes it an efficient biomass producer. Its ability to absorb heavy metals and pollutants from water has led to increased interest in using Azolla for phytoremediation, a method of environmental cleanup using plants[1].

METHODOLOGY

The methodology employed in this study involved a systematic and comparative analysis of the antifungal and antibacterial properties of Azolla pinnata through a series of laboratory-based experimental procedures. Initially, Azolla specimens were collected and shade-dried to preserve their bioactive compounds. The dried material was finely ground and subjected to solvent-based extraction using ethanol, methanol, and water to determine the impact of extraction medium on the efficacy of phytochemical constituents. Each extract was then filtered and concentrated to isolate the active compounds for further testing. To identify the phytochemical makeup of the extracts, standard qualitative tests were conducted, confirming the presence of flavonoids, phenolics, alkaloids, saponins, and tannins—compounds known for their antimicrobial properties. The antimicrobial activity was assessed using both agar diffusion and minimum inhibitory concentration (MIC) methods for antibacterial evaluation, while disk diffusion and microplate techniques were utilized for antifungal assays. Test pathogens included common bacteria such as *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, and fungi such as *Candida albicans* and *Aspergillus niger[2]*.

These methods enabled the quantitative and qualitative measurement of microbial inhibition by Azolla extracts. Results from these procedures provided comparative insights into the spectrum and strength of antimicrobial effects, with ethanol-based extracts consistently demonstrating higher efficacy. This comprehensive methodological framework not only facilitated the validation of Azolla's antimicrobial potential but also laid the foundation for evaluating its use in pharmaceutical and agricultural applications as a natural alternative to synthetic agents[3].

RESULTS AND DISCUSSION. Distribution and ecological significance Azolla grows in natural water bodies, artificial lakes and ditches. It increases soil fertility by accumulating atmospheric nitrogen. In addition, it forms a thick coating on the water surface, reducing water evaporation and helping to improve water quality in fish farms.Biochemical compositionAzolla contains the following bioactive substances:Flavonoids - have antioxidant and antibacterial properties.Alkaloids - have an effect against microorganisms.Tannins - have an effect against fungi and bacteria.Saponins - are substances that strengthen immunity and have an anti-inflammatory effect.Antifungal and antibacterial effect of Azolla The biologically active substances contained in Azolla can be an effective remedy against various fungi and bacteria[4].

The present study aimed to explore the antibacterial and antifungal efficacy of Azolla pinnata extracts, a plant traditionally known for its ecological and agricultural significance. The findings confirmed that Azolla pinnata possesses significant antimicrobial activity, with ethanol-based extracts exhibiting stronger effects compared to methanol and water extracts. This observation aligns with earlier studies indicating that ethanol is more effective at extracting non-polar phytochemicals such as flavonoids and alkaloids, which are largely responsible for antimicrobial properties[5].

The potent antibacterial activity observed against Staphylococcus aureus and Escherichia coli suggests that the bioactive compounds in Azolla pinnata target both Gram-positive and Gram-negative bacteria, albeit with a higher efficacy against the former[5].

The mechanisms underlying this activity—namely disruption of bacterial membranes, inhibition of protein synthesis, and interference with cell wall integrity—are consistent with the antimicrobial actions described for flavonoids, tannins, and alkaloids in earlier phytochemical research. Moreover, the antifungal effect of the extracts, especially against Candida albicans and Aspergillus niger, reveals another important aspect of Azolla's medicinal potential, supported by previous findings that highlight ergosterol synthesis inhibition and oxidative stress induction as key antifungal pathways[6].

The results contribute to a growing body of literature advocating the use of plant-derived compounds as alternatives to synthetic antimicrobials, particularly in the face of increasing antibiotic resistance. Unlike synthetic drugs, plant-based agents like Azolla offer a multifaceted approach, combining multiple bioactive molecules with diverse modes of action, potentially reducing the risk of microbial resistance[7].

From a practical standpoint, the implications of this study are significant. The ability to harness Azolla pinnata as a source of natural antimicrobial compounds opens opportunities for sustainable pharmaceutical and agricultural applications[8].

In agriculture, Azolla extracts could be developed into bio-fungicides or bactericides to protect crops without contributing to soil toxicity. In pharmaceuticals, the development of topical or systemic antimicrobial agents from Azolla could address infections resistant to conventional drugs. Environmentally, its use supports eco-friendly practices, particularly as the plant is fast-growing, widely available, and already used in agroecological systems[9].

Nevertheless, the study is not without limitations. The antimicrobial assays were conducted under controlled in vitro conditions, which may not fully replicate in vivo biological systems. Furthermore, the study did not isolate or quantify individual bioactive compounds, making it difficult to attribute observed effects to specific molecules. Solvent residue, plant part variation, and environmental factors affecting phytochemical concentration were also not analyzed in detail[10].

Future research should focus on isolating and characterizing individual antimicrobial agents within Azolla pinnata, determining their mechanisms of action in greater molecular detail, and testing efficacy in animal models or clinical settings. Additionally, exploring synergistic effects between Azolla extracts and conventional antibiotics could open a new frontier in antimicrobial therapy. Expanding the range of test organisms, especially multidrug-resistant strains, will also be vital in assessing the full potential of Azolla in combating global health threats[11].

As a result of studies, Azolla extracts have been shown to be effective against the following pathogens:Antibacterial effect Azolla extracts are highly effective against bacteria such as Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa. Studies show that Azolla extract has the property of suppressing bacterial growth, especially against gram-positive bacteria.The antibacterial effect is carried out through the following mechanisms:[12]

Action on the cell wall - the protective layer of bacteria is destroyed. Inhibition of protein synthesis - prevents the reproduction of bacteria. Membrane disruption – bacterial cells lose their viability. Antifungal effect Azolla extract is effective against fungi such as Candida albicans, Aspergillus niger, Penicillium spp. These extracts affect the membrane of fungal cells and stop their development.Mechanisms of antifungal action: Cell membrane damage – fungal cell growth stops. Inhibition of ergosterol synthesis – fungi lose membrane stability[13]. Formation of hydroxyl radicals – fungal cells die due to oxidative stress.Research methods: Several research methods were used in the laboratory to evaluate the antibacterial and antifungal properties of Azolla extracts: Extract preparation: Azolla plant was extracted in the following steps: Collection and drying – The plant was dried in the shade and ground. Solution preparation – The dried material was dissolved in ethanol or water. Filtration and concentration – The beneficial substances were isolated in the form of an extract[14].

Antibacterial tests: Agar diffusion and minimum inhibitory concentration (MIC) methods were used to evaluate antibacterial properties.Antifungal tests Disk diffusion and microplate methods were used to measure antifungal activity.According to the results of the study: Azolla extract showed a strong antibacterial effect against Staphylococcus aureus and Escherichia coli bacteria. High antifungal efficacy was noted against Candida albicans and Aspergillus niger fungi. The effect of the extract depended on the extraction method, and ethanol extract gave stronger results than water extract[15].

CONCLUSION

Azolla plant is of great importance as a natural antifungal and antibacterial agent due to the phytochemical components contained in it. It can be widely used in the pharmaceutical, agricultural

and biotechnology fields.Recommendations for future research:In-depth study of the effect of Azolla on other pathogens.

Development of extracts as drugs.Development of environmentally safe and effective extraction methods.The bioactive properties of Azolla can play an important role in the creation of natural antibacterial and antifungal drugs in the future.

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