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RESEARCH ARTICLE

Investigation of phytochemical constituents in *Azolla microphylla* for antibacterial activity

Sathammaipriya N, Thamilmaraiselvi B, Steffi P F, Sangeetha K

Department of Microbiology, Cauvery College for Women, Trichy, Tamil Nadu, India

Correspondence to: Steffi P F, E-mail: steffi.titu@gmail.com

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ABSTRACT

Background: Modern day food and lifestyle have resulted in an increasing number of diseases and disorders. A major percentage of the population depends on herbal medicines due to various needs. **Aim and objective:** The aim of the study was to investigate phytochemical and antibacterial activity in *Azolla microphylla*. **Materials and methods**: The present studies will reveal the activity of *A. microphylla* as an antimicrobial agent. Preliminary phytochemical studies were carried out to screen the components present in the species, and their antimicrobial activity was performed. **Results:** *Azolla* extract found to possess enhanced antimicrobial action on bacteria. **Conclusion:** The methanol, ethanol, and water extracts showed good antibacterial activity when compared to chloroform and petroleum ether and it may be due to the presence of more phytochemical constituents, and hence, it can be used in therapeutic preparations.

KEY WORDS: *Azolla microphylla*; Phytochemical; Antimicrobial; Blue-green Algae

INTRODUCTION

Azolla is a pteridophyte having agronomic significance in developing as well as developed countries.[1-6] It produces maximum biomass in a relatively shorter period of time.[3] *Azolla* acts as a nitrogen biofertilizer, and it increases the productivity of rice.[4] The oceanic plant *Azolla* turned out to be progressively prominent as bioenergy feedstock on account of its high development rate, generation of biomass with elevated amounts of biofuel-creating capacity. Besides, *Azolla* has been appeared to be fit for hyper aggregating an incredible assortment of overwhelming metal toxins and additionally purifying ammonium and phosphorous in wastewater.[5] *Azolla* is utilized as a feed for animals, food for humans, water purifier, green fertilizer, hydrogen fuel, biogas, and weed and bug controller.[7] *Azolla* enhances the water quality by expelling nitrates and phosphorous.[8] In the present study, we have encapsulated about *Azolla's* attributes such as antimicrobial and phytochemical activities, but still, there are many fields in which *Azolla* can act as fern hero in many more areas which can be future enhanced.[9]

Objectives

- Current study was done to investigate phytochemical and antibacterial action in *Azolla microphylla*.
- This study was conducted to assess whether *Azolla* extract found to possess better antimicrobial action on bacteria.

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MATERIALS AND METHODS

Phytochemical Activity

Leaves of diverse extracts of the *A. mycrophylla* were analyzed for the presence of alkaloids, saponins, tannins, phenols, amino acids, and monosaccharides.

Screening for Alkaloids

Alcoholic extract was heated with 5 ml of 2N HCL. To the filtrate, Mayer's reagent was added and observed for cream color precipitate which indicated the nearness of alkaloids.

Screening of Tannins

To 5 ml of concentrate, a couple of drops of 1% lead acetate were included. Yellow color shows the nearness of tannins.

Screening of Phenols

Around 2 ml of the concentrate was added to 2 ml of ferric chloride ($Fecl_3$), a deep bluish green solution is formed which indicated the nearness of phenols.

Screening of Steroids

One ml of the concentrate was disintegrated in 10 ml of chloroform, and an equivalent volume of concentrated sulfuric acid (H_2SO_4) was included in the test tube. The upper layer turns red, and H_2SO_4 layer showed yellow with green fluorescence. This demonstrated the nearness of steroids.

Screening of Cardiac Glycosides

To the concentrate of glacial acetic acid, few drops of $Fecl_3$ and concentrated H_2SO_4 were included and watched for the reddish-brown coloration at the intersection of two layers, and the bluish green color in the upper layer demonstrates the nearness of cardiac glycosides.

Screening for Anthraquinones

To 5 ml of the concentrate, 10 ml of H_2SO_4 was added and filtered. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was pipette out, and 1 ml of dilute ammonia was included. The solution was observed for color changes.

Screening for Flavonoids

To 1 ml of the concentrate, a couple of dilute sodium hydroxide was included. Yellow color was developed in the plant extract, which became colorless when few drops of dilute acid were added. This indicated the nearness of flavonoids.

Screening for Terpenoids

The concentrate was dispersed in 1 ml of chloroform; 1 ml of acidic anhydride was included after the inclusion of 2 ml of conc. H_2SO_4. Formation of reddish-violet color demonstrated the nearness of triterpenoids. 1 ml of the concentrate was treated with few drops of Ninhydrin reagent. Appearance of purple color demonstrates the nearness of amino acids.

Screening for Reducing Sugars

To 1 ml of the concentrate, few drops of Fehling's solution were included and observed for the brick red precipitate.

Screening for Monosaccharides

To 1 ml of the concentrate, 1 ml of Barfoed's reagent was included and heated which lead to the development of red cupric oxide indicated the nearness of monosaccharide.

Test Organisms

The test organisms used in this study include *Bacillus sp., Staphylococcus sp*, *Escherichia coli, Klebsiella sp.,* and *Proteus sp.*

Antibacterial Assay

The crude extracts (methanol, ethanol, chloroform, petroleum ether, and hot water) obtained from the leaves of *Azolla microphylla* were studied for its antibacterial activity using agar well diffusion and filter paper disc diffusion methods.

Antifungal Assay

Ethanol and methanol extracts of the leaves alone were tested for its antifungal activity. The media used were Potato Dextrose Agar (HiMedia).

RESULTS

The current study on *A. microphylla* explains the presence of medically active components. The phytochemical constituents of the *Azolla* were investigated, and the results were tabulated.

Phytochemical Activity

Table 1 showed phytochemical constituents of various extracts of the leaves. Aqueous extracts of leaves showed the incidence of alkaloids and the ethanolic extract contained cardiac glycosides. All the extracts showed the absence of anthraquinones, Monosaccharide's, and cardiac glycosides. Among the various extracts, aqueous extracts showed the presence of significant amount of phytochemicals followed by methanol, ethanol, water, chloroform, and petroleum ether extract and this may contribute to the better antibacterial activity. Hence, in our study, leaves extracts were investigated for its antimicrobial activity.

Antibacterial Activity

Table 2 showed the antibacterial action of leaves extracts of *A. microphylla* against the test organisms using agar well

Table 1: Phytochemical screening of leaf extract of *A. microphyla*

Phytochemicals	Methanol	Ethanol	Chloroform	Petroleum ether	Aqueous
Tannins	+++	++	+	–	–
Phenols	+++	+++	–	–	–
Saponins	++	++	+	–	–
Alkaloids	+	+	+	–	–
Flavonoids	++	+++	++	–	–
Anthraquinones	–	–	–	–	–
Amino acids	++	++	+++	–	–
Monosaccarides	+	–	–	–	–
Reducing sugars	+	+	+	++	+
Terpenoids	++	++	+++	–	–
Cardiac glycosides	–	+	–	–	–
Steroids	+++	+++	++	+	–

+++: Highly present, +: Weakly present, ++: Fairly present, -: *Nil. A. microphyla: Azolla* microphyla

Table 2: Antibacterial action of leaf extracts of *A. microphylla*

Extracts	Conc. (µl)	*Bacillus*	*Staphylococcus*	*Klebsiella*	*E. coli*	Proteus
Methanol	10	1.0	1.0	1.0	1.3	0.2
	20	1.5	1.5	1.4	1.8	0.5
	30	2.0	2.0	1.6	2.4	1.5
	40	2.5	2.3	2.4	2.7	1.7
Ethanol	10	1.0	0.5	1.6	0.7	-
	20	1.4	0.9	1.8	1.9	-
	30	1.6	1.2	2.0	2.0	-
	40	2.0	1.5	2.5	2.7	-
Water	10	0.4	-	-	-	-
	20	0.6	-	-	-	-
	30	1.3	-	-	1.0	-
	40	1.7	0.5	0.3	1.5	-
Chloroform	10	1.0	0.5	0.5	0.5	-
	20	1.6	1.0	1.2	1.8	-
	30	2.0	1.2	2.0	2.3	-
	40	2.5	1.6	2.5	2.7	-
Pet Ether	10	-	-	-	0.5	-
	20	-	-	-	0.7	-
	30	1.0	-	1.0	1.5	-
	40	2.0	0.5	1.4	2.0	-

The values on the table are±mean value. *A. microphyla: Azolla microphyla*

diffusion method. Among the test organisms used in the study, Gram-positive bacteria showed more inhibitory effect than Gram-negative bacteria, and especially, *Bacillus* species showed a higher zone of inhibition than *Staphylococcus* species. Among the Gram-negative bacteria, *E.coli* showed better activity than *Klebsiella species*. All the extracts did not exhibit any inhibitory effect on Proteus species [Figures 1-4].

Antifungal Activity

Methanol and ethanol extracts of the leaves alone were tested for its antifungal activity. The media used were Potato Dextrose Agar. Various concentration of extract was mixed into 20 ml molten PDA media and the content was allowed to solidify in plates [Figures 5 and 6].

DISCUSSION

The summary of this work revealed that the *Azolla sp.* contained bioactive agents such as alkaloids, saponins, tannins, flavonoids, steroids, and cardiac glycosides and these agents could be responsible for its antimicrobial activity. In many cases, alkaloids, flavonoids, and terpenoids prove to showed good antimicrobial action.[10]

Prasad *et al.* investigated the phytochemicals present in *Azolla*.[11] Temmink *et al.* discussed that saponins are a special class of glycosides which have a soapy characteristic and facilitate the absorption of foods and medicine.[12] Thagela *et al.* discussed that saponins and steroids are main source for normal activities of the CNS (central nervous system).[13] Vannini *et al.* observed that tannins act as an antimicrobial agent by inhibiting extracellular enzymes, deprecating the substrates required for microbial growth or by inhibiting oxidative phosphorylation of microbial metabolism.[14] From the above result, the *Azolla* extract found to possess good antimicrobial action on bacteria. Similar reports were shown by some other investigators.[15]

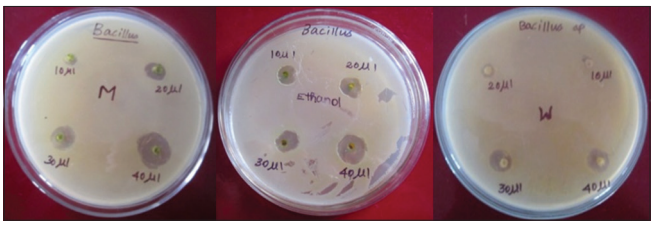

Figure 1: Antibacterial action of leaves extracts against *Bacillus* species using well diffusion method

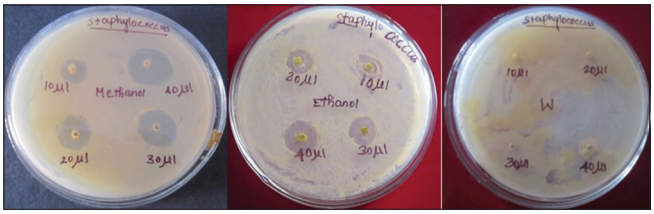

Figure 2: Antibacterial action of leaves extracts against *Staphylococcus* species using well diffusion method

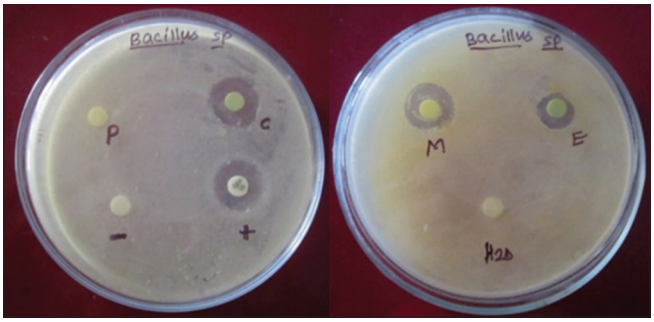

Figure 3: Antibacterial action of leaves extracts using Filter paper disc diffusion method *Bacillus* sp

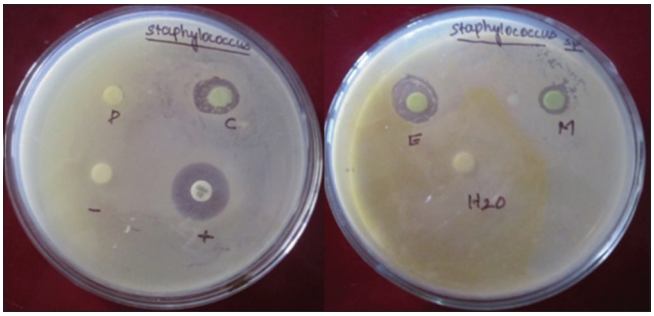

Figure 4: Antibacterial action of leaves extracts using *Staphylococcus* sp

Strengths and Limitations of the Study

Strength of this study includes it is easy to find and establish the vegetative material which helps in quick recovery. *Azolla* requires no extra fertilization beyond P applied to crop and aids in nitrogen fixation which, in turn, increases crop yields. Limitations of this study include it can be used only in wet fields. It may require a nursery pond and difficult to establish in winter.

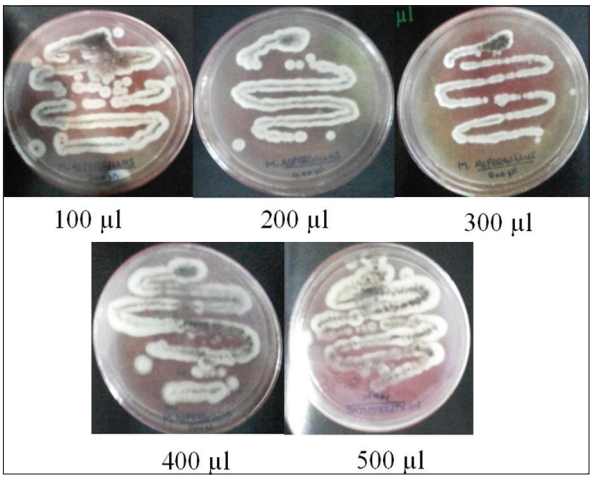

Figure 5: Antifungal Activity of methanol extracts of leaves against Aspergillus species

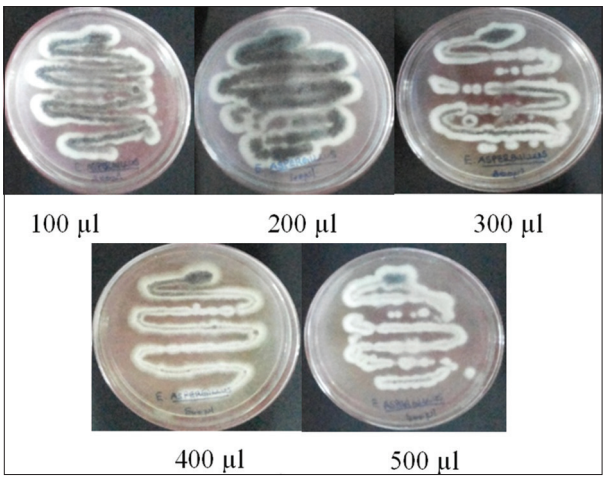

Figure 6: Antifungal activity of ethanol extracts of leaves against *Aspergillus species*

CONCLUSION

The organic solvent extracts of *A. mycrophylla* leaves were analyzed separately for its phytochemical constituents. The species was found to contain alkaloid, tannin, saponin, steroid, terpenoid, flavonoid, and phenols. Almost all the phytochemical components were present in ethanol, methanol, and water. All the extracts of the leaves were screened for its antimicrobial activity against bacterial species such as *Bacillus species, Staphylococcus species, Klebsiella species, E. coli,* and *Proteus species* and the maximum antibacterial activity was shown against like *Bacillus species* followed by *Staphylococcus species.* The result of the current study suggested that the extract of *A. mycrophylla* possesses phytochemical compounds with significant antimicrobial activity.

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