

Research Article



Antibacterial Activity and Phytochemical Screening of *Salvinia auriculata* Aubl. from Tirumala Hills, Tirupati

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ABSTRACT

The present paper deals with the antibacterial studies and Phytochemical screening of *Salvinia auriculata* Aubl. The different parts of *Salvinia auriculata* Aubl. was collected, gabbled, pulverized, air dried and subjected to gradient extraction with soxhlet apparatus with different solvents (acetone, ethanol, methanol, ethyl acetate and benzene) was screened phytochemically for its chemical components and antibacterial assays. Phytochemical studies showed the presence of Alkaloids, Flavonoids, Phenols, Tannins, and Saponins in higher to moderate concentrations and percentage of Alkaloid 0.25, Flavonoid 0.48, Saponin 0.36, Tannin 0.28 and Phenol 0.36. Antibacterial assay of the different extracts by the use of agar Disc-Diffusion method revealed that methanol and acetone extracts showed maximum activity against *P.aeruginosa*, *E.coli*, and *S.aureus*.

Keywords: *In vitro* Antibacterial assay, Phytochemical Screening, *Salvinia auriculata*

INTRODUCTION

The recent ethnobotanical, pharmacological and biological searches have revealed medicinal, pharmaceutical and phytochemical attributes of pteridophytes, which have valuable potential applications for health and industry, still many species of pteridophytes are yet to be explored for their potential applications for future use and to isolate new active principles from them. The ferns had an important role in folklore medicine. These plants have been successfully used in the different systems of medicines like Ayurvedic, Unani, Homeopathic, and other systems of medicines. Kirthikar (1935) have described 27 species of ferns having varied medicinal uses.¹ Chopra have included 44 species and Nadkarni (1954) recorded 11 species of Pteridophytes having medicinal importance.^{2,3} Nayar (1959) recorded 29 medicinal ferns.⁴ May (1978) published a detailed review of the various uses of ferns and listed 105 medicinal ferns.⁵ In a recent compilation, Singh (1999) reported 160 species of useful Pteridophytes in India on the basis of phytochemical, pharmacological and ethnobotanical studies.⁶ In general the fern *Salvinia auriculata* is a eared water moss, free floating aquatic fern up to 20 centimeters long. Horizontal rhizome is below the water surface. Frounds are of two types. Buoyant or submerged, light green to medium green with brownish edges when mature, with a distinctive fold is the centre floating leaves are boat shaped. The frounds has the number of medicinal properties like radical scavenging activity, reduces lipid peroxidation, reduces the levels of apoptic and inflammatory proteins.

The medicinal importance of a plant is due to the presence of some special compounds like Alkaloids, Flavonoids, Phenols, Tannins and Saponins. These active principles usually remain concentrated in the storage

organs of the plants viz., roots, leaves etc., considering all these facts, present investigation is designed to find out phytochemical analysis and antibacterial activities of *S.auriculata* which evokes various therapeutic effects.



Figure 1: *Salvinia auriculata* Aubl.

MATERIALS AND METHODS

Collection and Identification of Plants

The whole plant were collected from the Japalitheertham of Tirumala hills, Tirupati.⁷ The plant was identified using a dictionary of the pteridophytes of India and was authenticated by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, S.V. University, Tirupati. The plant was washed 2-3 times with tap water and distilled water to remove the soil and dirt particles then gabbled, pulverized, air dried and subjected to gradient extraction with soxhlet apparatus.

Preparation of Plant Extracts

Solvent Extraction

The total plant of *S.auriculata* was shade dried, pulverized and passed through a 40-mesh sieve. 20 gms of dried powder plant material was taken and subjected to



successive extraction with 100 ml of acetone, ethanol, methanol, ethyl acetate and benzene in Soxhlet apparatus. The extracts were concentrated to dry residue by distillation (temperature 60 °C without vacuum) and dried completely in desiccators and weighed. Prepared extracts were collected and stored in glass vials for further studies.⁸

Microorganisms Used

Bacillus subtilis (MTCC441), *Staphylococcus aureus* (MTCC 96), *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Salmonella typhi* which were collected from IMTECH, Chandigarh and Sri Venkateswara Institute of Medical Sciences, (SVIMS) Tirupati.

From stock culture 0.1ml bacterial culture was inoculated into the test tubes containing 3ml of nutrient broth each. After inoculation, the test tubes were kept in incubator at 37 °C for 24 hrs.

Antimicrobial Activity by Disc Diffusion Method

The antibacterial activity of organic extracts of *S. auriculata* was assayed by disc diffusion method. The test organisms were grown on nutrient agar medium. A sterile, non-toxic swab on an applicator stick was dipped into standardized suspension of inoculums and excess broth was removed by pressing and rotating the swab firmly against the inside of the tube above the fluid level. The swab was then streaked evenly in all directions over the entire surface of the agar plate to obtain a uniform lawn culture. A final sweep was made of the agar rim with the cotton swab. This plate was then allowed to dry for 10 minutes before the discs were applied. Small sterile discs of Whatman No.1 filter paper (6 mm diameter) were placed on seeded agar plates and standard antibiotic discs with known concentrations for which the test organism is sensitive had been set up.⁹

Qualitative Phytochemical Analysis

The extracts were analyzed for the presence of phytoconstituents such as Alkaloids, Flavonoids, Saponins, Tannins and Phenols. Following standard procedures were used.¹⁰⁻¹²

Mayer's Test for Alkaloids

To the acidic solution, Mayer's reagent (Potassium mercuric iodide solution) was added. Appearance of cream coloured precipitate indicates the presence of alkaloids.

Ferric Chloride Test for Tannins

Small quantity of extract was mixed with water and heated on water bath. The mixture was filtered and ferric chloride was added to the filtrate. A dark green solution indicates the presence of tannins.

Shinoda's Test for Flavonoids

To one ml each of alcoholic extract, a small piece of magnesium ribbon or magnesium foil was added and 3-4

drops of conc. HCl was added, change in colour from red to pink shows the presence of flavonoids.

Froth Test for Saponins

About 0.2 g of the extract was shaken with 5 ml of distilled water and then heated to boil. Frothing (appearance of creamy mass of small bubbles) shows the presence of saponins.

Ellagic Test for Phenols

One ml of each of the various extracts dissolved in alcohol and treated with 2-3 ml of 5% neutral ferric chloride solution. Colour change indicates the presence of phenols.

Quantitative Phytochemical Analysis

Alkaloid Determination

5 g of the sample were weighed into 200 ml of 20% acetic acid in ethanol was added and covered to stand for 4 h. This was filtered and the extract was concentrated using a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected by filtration and weighed.¹³

Flavonoid Determination

To estimate flavonoids quantitatively, 10 g powdered sample of each plant material was extracted twice with 10 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No.1, the filtrate was later transferred into crucibles, evaporated to dryness on a water bath to a constant weight.¹⁴⁻¹⁵

Saponin Determination

Twenty gram of each powdered sample was added to 100 ml of 20% aqueous ethanol and kept in a shaker for 30 min. The samples were heated over a water bath for 4 h at 55 °C. The mixture was then filtered and the residue re-extracted with another 200 ml of 20% aqueous ethanol. The combined extracts were reduced to approximately 40 ml over water bath at 90 °C. The concentrate was transferred into a 250 ml separatory funnel, extracted twice with 20 ml diethyl ether. Ether layer was discarded while aqueous layer was retained and 60 ml n-butanol was added to it. Then n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath and after evaporation the samples were dried in oven at (40 °C). The saponin content was calculated as percentage of the initial weight of sample taken.¹⁶

Tannin Determination

Distilled water (50 ml) was added to 500 mg of the sample taken in a 500 ml flask and kept in shaker for 1 h. It was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtrate was pipetted out



into a test tube and mixed with 2 ml (10 fold diluted) of 0.1 M FeCl₃ in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 605 nm within 10 min.¹⁷

Statistical Analysis

The experimental results were expressed as mean \pm standard deviation of three replicates.

RESULTS

In the present investigation the Flavonoids and other phytochemical constituents in *Salvinia auriculata* may be responsible for potent antibacterial activity.¹⁸ The antibacterial activities of different solvent extracts of *S.auriculata* are presented in (Table 1). *P.aeuroginosa*

showed maximum inhibition zone of 20 mm in methanol, moderate inhibition zone, 15 mm in acetone and least inhibition zone, 8 mm in ethanol extract. *E.coli* showed maximum inhibition zone 12 mm in acetone extract, moderate inhibition zone 10 mm in methanol extract and least activity 8 mm in benzene. *B.subtilis* and *P.vulgaris* showed maximum inhibition in acetone extract, moderate activity in methanol extract and least activity in Ethylacetate & benzene extract. *S.typhi* showed maximum inhibition zone of 10 mm in methanol extract and least activity of 5 mm in acetone and benzene extracts. *S.aureus* showed maximum inhibition zone of 12 mm in ethanol extract, moderate activity of 10 mm in methanol extract and least activity 8 mm in ethyl acetate extract.

Table 1: Result of Antibacterial Activity of *Salvinia auriculata* in Disc Diffusion Method

Plant material extracted with solvent	Zone of inhibition measured in (mm)30 μ g/disc					
	<i>E.coli</i>	<i>B.subtilis</i>	<i>P.vulgaris</i>	<i>S.typhi</i>	<i>P.aeuroginosa</i>	<i>S.aureus</i>
Acetone	11.33 \pm 1.15	9.33 \pm 1.15	9.00 \pm 1.00	6.33 \pm 1.52	12.33 \pm 2.51	6.66 \pm 2.0
Benzene	6.33 \pm 1.52	5.66 \pm 1.15	6.66 \pm 2.88	5.33 \pm 0.57	7.33 \pm 2.08	1.66 \pm 2.88
Ethanol	3.00 \pm 2.64	1.66 \pm 2.88	5.00 \pm 0.00	0.00 \pm 0.00	7.33 \pm 1.15	10.00 \pm 2.00
Methanol	9.33 \pm 1.15	5.00 \pm 0.00	7.33 \pm 1.15	9.33 \pm 1.15	19.33 \pm 1.15	9.33 \pm 1.15
Ethyl acetate	0.00 \pm 0.00	8.00 \pm 0.00	10.00 \pm 0.00	0.00 \pm 0.00	5.00 \pm 0.00	7.33 \pm 1.15
Standard (Gentamycin)	25.00 \pm 0.00	21.66 \pm 2.88	25.00 \pm 0.00	25.00 \pm 0.00	23.33 \pm 2.88	23.33 \pm 2.88

Values are mean \pm standard deviation of three replicates

Table 2: Results of Preliminary Phytochemical Evaluation of *Salvinia auriculata*

Test for Secondary Metabolites	Alkaloids Mayers Test	Flavonoids Shinodas Test	Tannins Fecl3 Test	Phenols Ellagic Test	Saponins Froth Test
Acetone	++	+++	+	++	-
Benzene	+++	++	+++	+++	++
Ethylacetate,	+	+	-	++	+
Methanol	-	-	++	++	+++
Ethanol	-	+	++	-	+++

+++ - High Concentration, ++ - Moderate Concentration, + - Low Concentration - Negative

In the present study, the phytochemical screening of *Salvinia auriculata* qualitatively shown the presence of Alkaloids, Flavonoids, Tannins, Saponins along with Phenols in all the extracts investigated. Pink colour was observed for Flavonoids (Shinodas test), cream coloured precipitate for Alkaloids, blue colour was observed for tannins and Saponins (Table 2).

Table 3: Quantitative Phytochemical Constituent of *Salvinia auriculata*

Phytochemical Study	Results (mg/g)
Alkaloids	0.25
Flavonoid	0.48
Saponin	0.36
Tannin	0.28

Quantitative analysis of phytochemical studies showed the presence of Alkaloids, Flavonoids, Phenols, Tannins, and Saponins in higher to moderate concentrations and percentage of Alkaloid 0.25, Flavonoid 0.48, Saponin 0.36,

Tannin 0.28 and Phenol 0.36 (Table 3).

DISCUSSION

In general gram-negative bacteria were more resistance to antibiotics than gram-positive bacteria.^{19,20} The resistance is due to the differences in their cell wall composition. In Gram-negative bacteria the outer membrane acts as a great barrier to many environmental substances including antibiotics.²¹ Presence of thick Murine layer in the cell wall to prevent the entry of the inhibitors.²²

The present study revealed that Gram negative and positive bacteria such as *E.coli*, *S.typhi*, *P.vulgaris*, *P.aeuroginosa*, *B.subtilis* & *S.aureus* were more susceptible to the solvent extracts.

Some of the flavonoids that favour polar solutes entry bind to the bacteria's structural membrane proteins called porines, causing changes in the tridimensional confirmation exposing the hydrophilic character of the pore, which lead to an easier passage of other polar

bioactive compounds via diffusion.²³ Saponins have the property of precipitating and coagulating human RBC.²⁴ Flavonoids on the other hand are water soluble antioxidants and free radical scavengers, which are capable of preventing oxidative cell damage and have strong anticancer activity.^{25,26} Tannins have astringent property, hasten healing of wounds and inflamed mucous membrane. A combination of these factors, together with the observed antimicrobial activity may explain some of the previous claims on the use of the plants in traditional medicine. Our results are in similar with reports given above, have been as a source of medicinal plant to cure urinary tract infections, gastrointestinal disorders, enhance healing of wounds and stop bleeding.²⁷

CONCLUSION

The present study confirmed the antibacterial activity and phytochemical analysis of *Salvinia auriculata*. The phytochemical screening revealed chemical constituents that form the foundation of their pharmacological activity. These data further support the view that the *Salvinia auriculata* is promising sources of natural antioxidants, and could be seen as potential sources of useful drugs. However isolation and purification of the active principles will be essential to give more insight into their mode of action.

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