

Preliminary phytochemical analysis and antimicrobial activity of rhizome extract of *Marsilea minuta*, L



Botany

KEYWORDS : Marsilea minuta, rhizome, acetone, ethanol, chloroform, phytochemicals, antimicrobial activity.

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ABSTRACT

The use of *Marsilea minuta* L. as sedative has been referred in many text of Ayurveda for the treatment of insomnia and other mental disorders. The five organic solvent extracts (acetone, DMSO, ethanol, chloroform and petroleum ether) of rhizome of *M. minuta* were tested for phytochemical screening for the major derivative of terpenoids, flavonoids, sugar, quinone, coumarin, tannin, saponin, phenols and anthroquinone. The same five organic solvent extracts were tested for their potential antimicrobial activity against chemically important standard reference bacterial strains and fungal strains. The bacterial strains studied were *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus pyrogenes* and *Escherichia coli* and the fungal strains were *Aspergillus niger*, *Aspergillus flavus* and *Trichophyton rubrum*. The phytochemical screening of extracts answered for the major derivatives of flavonoids, quinine, Tannins, Saponins and anthroquinone. The antibacterial activity of the rhizome extracts has better inhibition at high concentration. The DMSO, ethanolic, chloroform and petroleum ether extracts showed good inhibition against *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus pyrogenes* respectively. Similarly antifungal activity of the extracts showed better inhibition at high concentration. The DMSO and ethanolic extracts showed good inhibition against *Aspergillus niger*, *Aspergillus flavus* and *Trichophyton rubrum*.

Introduction:

The Pteridophytes are long known for their medicinal and therapeutic utility. Recently enormous efforts have been made to determine the potentiality of Pteridophytes in relation to their chemical composition and other aspects. These plants are distinct in having glycosides, flavonoids, terpenoids, alkaloids and many primary as well as secondary metabolites which are used for preparation of expectorant. Tabulation of these plants is also advised as supplement of aphrodisiac, appetizer, stimulants; however, certain species are used for the ailment of diuretic, ulcer as well as stomachic. These Pteridophytes are mostly distributed in the Himalayas. More than 300sps of ferns and fern allies are reported from the Western Ghats, South India¹

Other reported activities include antifertility² and hypocholesterolemia activity³. The neuropsychological properties of *Marsilea minuta* have been strongly suggested in Ayurveda, keeping these facts in mind the present investigation was undertaken to investigate the phytochemical and antimicrobial activity of *M. minuta*, rhizome extracts.

Materials and Methods:

Plant collection:

The underground part of the sporophytic plants of *M. minuta* is the rhizome. These rhizomes were collected from the sporophytic plants from the rice fields of Mannachanallur. The rhizomes were cleaned with running tap water and dried at shade for 10 days. Blotted rhizomes were ground and sieved using four layers of gauze cloth. The rhizome powder was stored in air tight container and maintained at 4°C until use.

Preparation of extract:

Shade dried powder of the rhizome was passed through sieve No.40 and 100g of powder was extracted by Soxhlet apparatus using the solvents like acetone, DMSO, ethanol, chloroform and petroleum ether for 48h. The extract obtained was filtered and solvent was evaporated at 50°C under reduced pressure and then lyophilized.

Phytochemical screening of the plant rhizome extract:

Phytochemical tests were carried out on all the extracts using standard procedure to identify the constituents as described by Sofawara (1993)⁴, Trease and Evans (1989)⁵ and Harborne (1973)⁶.

Test microorganisms:

The bacterial cultures of different strains like *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus*

pyrogenes and *Escherichia coli* and fungal strains like *Aspergillus niger*, *Aspergillus flavus* and *Trichophyton rubrum* were obtained from the department of Microbiology, Institute of Basic Medical science, Chennai, India.

Growth medium and inoculum preparation:

The media used for antibacterial test was nutrient Agar/Broth of High Media Pvt Ltd Mumbai, India. The bacterial and fungal strains were inoculated in liquid medium (nutrient broth) and incubated at 37°C for 8hrs and further used for the test (10⁵-10⁶ CFU/ml). These suspensions were prepared immediately before the test was carried out.

Antimicrobial testing:

The disc diffusion assay⁷ was performed to determine the growth inhibition of bacteria by rhizome extract. A diluted culture (0.2ml) was spread over nutrient agar plates using sterile glass L-rod. Around 0.3ml of the each extract was applied per filter paper disc and were allowed to dry before placed on the top layer of the agar plates. Each extract was tested in triplicate and the plates were incubated at 37°C for 24 hours and the zones of inhibition were noted.

Sensitivity of the microbes to standard antibiotics:

The antibiotics such as Amphotericin B, Ampicillin, Gentamycin and Ofloxacin were selected as standard for determining the antibacterial activity of the experimental plant samples. The microbes sensitivity to the antibiotics were analysed.

Statistical analysis:

Random sampling was made for the entire test in triplicates. Calculations were carried out in triplicate with their values and standard deviation by using the formula⁸.

Results and Discussion:

Phytochemical analysis:

The crude extracts of rhizome showed diverse phytoprofiles with reference to solvents. The results of phytochemical analysis are presented in Table-1. Among the five solvents used the chloroform and ethanolic rhizome extract showed the presence of flavonoids, sugar, coumarin, tannin, saponins. The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites. Thus the preliminary screening tests may be useful and lead to the detection of bioactive principles⁹. Many naturally occurring compounds found in plants have been shown to possess antimicrobial functions and could thus serve as a source of both traditional and orthodox medicine¹⁰.

Antimicrobial activity:

The antimicrobial activity of the extracts is presented in Table-2. The results of antibacterial activity showed that the rhizome extracts have better inhibition at high concentration (Fig. -1). The ethanolic & DMSO rhizome extract showed better inhibition against *Pseudomonas aeruginosa*. Similarly DMSO, ethanolic, chloroform and petroleum ether extracts showed good inhibition against *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus pyrogenes* respectively.

The results of antifungal activity showed that all the rhizome extracts showed better inhibition at high concentration (Fig. - 1). The DMSO and ethanolic rhizome extracts showed good inhibition against *Aspergillus niger*. DMSO also showed better inhibition against *Aspergillus flavus*. Similarly ethanolic and DMSO extracts showed good inhibition against *Trichophyton rubrum*.

Very less work has been done on the antimicrobial activity of Pteridophytes, yet ethanobotanical importance of these plants have been investigated and studied by various authors. They have been found for their biological activity¹¹. The phytochemical composition of *Adiantum radiata* has been studied and found that the isolated phytochemicals were effective against the growth of microorganisms¹². Antibiotic activity of Pteridophytes have been studied¹³, while the antiviral activity of crude extracts of some Pteridophytes have also been analyzed¹⁴. The antibacterial activity of *Adiantum capillus-veneris* was also been studied and found that nearly all the extracts were effective against the selected microorganisms^{15&16}.

In general, gram-negative bacteria were more resistant to an-

tibiotics than gram-positive bacteria. The resistance is due to the differences in their cell wall composition. In gram-negative bacteria the outer membrane acts as a barrier to many environmental substances including antibiotics¹⁷. Presence of thick murine layer in the cell wall prevents the entry of the inhibitors¹⁸. But in the present study gram-negative bacteria were more susceptible to the crude extracts than gram-positive bacteria. It may be due to the presence of broad spectrum of antibiotic compounds present in the selected ferns.

Sensitivity of the microbes to standard antibiotics:

Table-3 reveals the antimicrobial activity of the rhizome extracts and was compared with commercially available antibiotics. Amphoterin B shows 18 mm., Ampicillin and Gentamycin shows 16 and 15 respectively whereas ofloxacin shows the highest inhibiting capacity up to 35.. Most of the samples in 75 and 100 Microgram/ml is beyond 15 mm inhibition. Maximum inhibition area was seen in Ethanol (upto 26mm) and DMSO (upto 21mm) at 100 microgram/ml.

Conclusion:

This study has confirmed the antibacterial potentials of ferns, thus supporting their application as a biocontrol herbal remedy. With these, there is need for the preparation of different formulations towards ensuring acceptable dosing to field trials. It is hoped that this study would lead to the establishment of some compounds that could be used to formulate new and more potent antimicrobial agents of natural origin for the treatment of bacterial and fungal infections.

Table-1 Phytochemical analysis of rhizome extract of *Marsilea minuta*,L.

S.NO	Compounds	Ethanol	Acetone	Chloroform	Petroleum ether	DMSO
1	Terpenoids	-	-	-	-	-
2	Flavonoids	+	-	+	-	+
3	Sugars	+	-	+	-	-
4	Quinones	-	+	-	-	+
5	Coumarin	+	-	+	-	-
6	Tannins	+	-	+	-	-
7	Saponins	+	-	-	-	+
8	Phenols	-	-	-	-	-
9	Anthroquinones	-	-	-	+	-
(*) Present;		(-)absent				

Table -2 Antimicrobial activity of five solvent extracts at different concentrations on rhizome of *Marsilea minuta*.L.

Ec	Sp	Pa	Sa	Bs	Ns	Ethanolic (µg/ml)				Acetylene (µg/ml)				Chloroform (µg/ml)				Petroleum ether (µg/ml)				DMSO (µg/ml)							
						12±1.4	13±0.4	16±1.6	14±0.8	—	—	13±1.2	15±1.6	11±0.2	13±0.8	14±0.8	15±1.2	10±0.2	11±1.2	12±0.8	12±0.4	14±0.9	10±0.6	13±1.2	15±1.6	16±0.2	16±1.2		
						12±0.8	14±0.4	15±0.6	17±0.3	9±0.2	10±1.2	11±0.6	12±0.4	11±0.3	12±0.4	13±1.2	14±0.8	10±0.7	11±0.8	12±0.6	14±0.9	12±0.8	14±0.4	10±0.6	13±1.2	15±1.6	16±0.2	16±1.2	
						25	50	75	100	25	50	75	100	25	50	75	100	25	50	75	100	25	50	75	100	25	50	75	100
						10±0.9	11±1.2	15±1.2	17±0.9	12±1.7	15±0.8	16±0.8	18±1.7	12±1.2	14±0.8	16±0.6	18±0.4	—	9±0.2	11±0.4	12±0.9	13±0.6	15±1.6	17±0.8	18±0.4	13±0.6	15±1.6	17±0.8	18±0.4
						10±0.6	11±1.4	13±1.2	19±1.2	9±0.4	10±0.8	11±1.6	13±0.4	10±0.8	12±1.4	15±0.9	18±0.4	10±0.3	11±0.6	13±1.7	15±1.2	10±1.6	12±1.8	15±1.4	17±0.2	13±0.6	15±1.6	17±0.2	18±0.4
						10±1.2	13±0.8	16±0.4	26±0.4	—	—	11±0.4	13±1.6	—	—	10±0.2	13±1.2	—	—	9±0.4	10±0.3	12±0.2	15±0.6	16±0.3	20±0.6	13±0.6	15±1.6	17±0.2	18±0.4
						25	50	75	100	25	50	75	100	25	50	75	100	25	50	75	100	25	50	75	100	25	50	75	100

An	10±0.2	13±0.6	17±0.8	20±0.4	—	11±0.8	14±1.2	16±1.4	—	—	11±1.6	14±1.2	—	—	14±0.2	11±0.2	12±0.6	18±0.9	21±1.2
Af	—	—	—	—	9±1.2	10±1.6	12±0.8	13±0.2	—	—	10±1.2	12±1.6	—	—	—	10±0.2	13±1.2	16±1.6	20±0.8
Tr	11±1.2	13±1.6	15±0.8	17±0.2	10±0.2	11±0.4	12±0.3	14±0.8	9±1.2	10±1.6	11±0.8	13±0.4	—	—	10±0.8	11±0.2	12±1.2	14±0.8	16±0.4

Data given are mean of three replicates ± standard error

Ns - Number of samples. Bs - *Bacillus subtilis*, Sa- *Staphylococcus aureus*, Pa - *Pseudomonas aeruginosa*, Sp - *Streptococcus pyrogenes*

Ec - *Escherichia coli*.

An - *Aspergillus niger*, Af - *Aspergillus flavus* and Tr - *Trichophyton rubrum*.

Table-3 Sensitivity of microbes to standard antibiotics

Antibiotics	Inhibition zone (mm)
Amphotericin B	18
Amplicilin	16
Gentamycin	15
Oflacin	35
Amphotericin B	18



Figure - 1 Showing antibacterial and antifungal activity of the Rhizome extract of *Marsilea minuta* Linn.

Fig. 1a - Culture of *Bacillus subtilis* treated with DMSO Rhizome extract and its inhibition zone

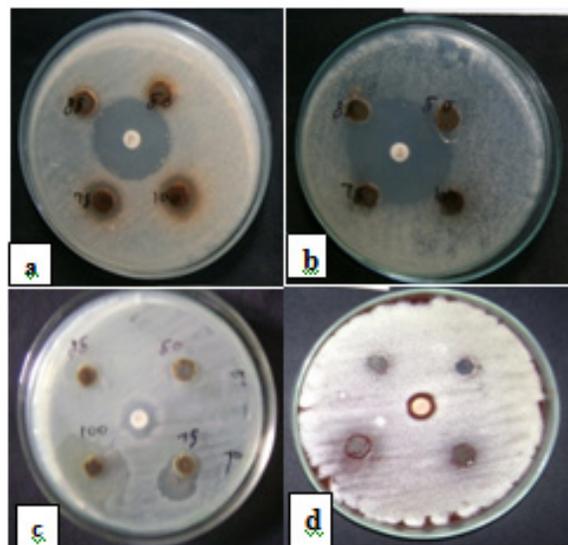
Fig. 1b - Culture of *Bacillus subtilis* treated with Chloroform Rhizome extract and its inhibition zone

Fig. 1c - Culture of *E.coli* treated with Ethanol Rhizome extract and its inhibition zone

Fig. 1d - Culture of Fungi *Aspergillus niger* treated with DMSO Rhizome extract and its inhibition zone

Fig. 1e - Culture of Fungi *Aspergillus flavus* - treated with Chloroform - rhizome extract and its inhibition zone

Fig. 1f - Culture of Fungi *Trichophyton rubrum* - treated with Chloroform - rhizome extract and its inhibition zones



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