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RESEARCH ARTICLE

Phytochemical Analysis and Antimicrobial Efficiency of *Marsilea quadrifolia* linn (Aquatic Fern)

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ABSTRACT

The present investigation was carried out to screen the phytochemistry and antimicrobial efficiency of the aquatic fern *Marsilea quadrifolia*. The qualitative Phytochemical analysis carried out in Benzene, Ethanol and Aqueous extracts revealed the presence of Reducing sugar, amino acids, phenolic compounds, flavonoids, phytosterols, tannins, alkaloids, proteins and saponins. Quantitative analysis revealed the presence of 200mg of carbohydrates, 51mg of proteins, 28mg of amino acids, 3mg of flavonoids and 2.8mg of saponins per gram of plant powder. CHN analysis revealed the presence of 40.51% carbon, 5.47% hydrogen and 3.80% of nitrogen. The EDS Analysis revealed the presence of minerals like carbon $1.46x10^{-16}$, oxygen $3.48x10^{-17}$, potassium $1.20x10^{-17}$ and chlorine $4.5x10^{-18}$ micrograms. The chromatogram of HPTLC revealed the presence of about 11 compounds with different Rf values. Antimicrobial activity for all the three extracts was carried out against five bacterial strains (*Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, P.aeruginosa and Aeromonas hydrophila*) and three fungal strains (*Aspergillus niger, Candida albicans* and *Pencillium notatum*). Pronounced anti-bacterial potential was observed in Benzene extract followed by ethanolic extract, however better zone of inhibition in resisting the growth of fungus was observed with ethanolic extracts and no significant antimicrobial activity was observed in aqueous extract and control. Thus preliminary screening of *Marsilea quadrifolia* revealed its potential as a potent antimicrobial agent due to the presence of variety of bioactive compounds.

KEYWORDS

Marsilea quadrifolia, Phytochemical analysis, Antimicrobial activity, EDS analysis, CHN Analysis, HPTLC Analysis

INTRODUCTION

India is a mega biodiversity country not only with rich source of medicinal plants, but also with valuable information on traditional medical practices[1]. The history of herbal medicine starts from the ancient human civilization.

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The wealth of India is stored in the enormous natural flora which has been gifted to Indians [2-3]. Traditional healers and their plant medicines provide the only health care to majority of people in a curative rather than a preventive approach in the developing countries for common ailments[4]. Plant products and related drugs are used to treat 87% of all categorized diseases[5]. The ready availability and economy of plants as direct therapeutic agents make

plants more attractive when compared to modern medicine[6]. As a result, vast literature now exist on the use of traditional medicine with botanist reporting description of plants used for disease treatments, the phytochemist on the chemical constituents and the pharmacologist on the effectiveness of particular plant compound or extracts[4]. According to WHO[7], medicinal plants are plants, which when administered to man or animal exert a sort of pharmacological action on them. Herbs make up most of the plant sources for the production of useful drugs that are being utilized by people world wide[8]. Most existing plants have medicinal values, of which steps are being taken by scientific research to properly test and utilize these plants for therapeutic purposes.

Plants are the storehouses and sources of safer and cheaper chemicals which are pharmacologically active, as they have limitless ability to synthesize aromatic substances, mainly secondary metabolites such as alkaloids, tannins, saponins, flavonoids and phenolics which play defensive role in plants and therefore they protect the plants from their invaders like fungi, bacteria, viruses, nematodes etc[9]. The secondary metabolites from natural products show more drug likeness and biologically friendliness than total synthetic molecules[10]. Herbal preparations are known to have an important role in disease control due to their antioxidant, antimicrobial activities, and also they exhibit antistress, growth promotion, appetite stimulation, tonic, immune stimulation and aphrodisiac properties due to the presence of active principles such as alkaloids, flavonoids, pigments, phenolics, terpenoids, steroids and essential oils. Thus screening of plants for their Phytochemicals is the first step in the discovery of a new drug.

Ethno pharmacological information can be used to provide three levels of resolution in the search for new drugs: (1) as a general indicator of non-specific bioactivity suitable for a panel of broad screens; (2) as an indicator of specific bioactivity suitable for particular high – resolution bioassays; (3) as an indicator of pharmacological activity for which mechanism-based bioassays have yet to be developed[11].

Pteridophytes (ferns and fern allies) are called as reptile group of plants and are one of the earliest groups of vascular plants. Most of the indigenous people are not well aware of the use of pteridophytes since it is not easily available like flowering plants. Pteridophytes have an important role in the earth's biodiversity[12].

Marsilea quadrifolia Linn is an aquatic fern which belongs to the family (Marsileaceae) commonly named as Aaraikeerai in Tamil, Neeraral in Malayalam and Cauptiya, Sunsuniya in Hindi. It is an aquatic fern bearing 4 parted leaf resembling '4-leaf clover', and the leaves float in deep water or erect in shallow water or on land. It possesses long stalked petiole with 4 clover like lobes and are either held above the water or submerged. Juice extracted from the leaves is diuretic and febrifuge and also used to treat snake bite and applied to abscesses etc. The plant is anti-inflammatory, diuretic, depurative, febrifuge and refrigerant[13]. The plant is also useful to treat psychopathy, leprosy, haemorrhoids, skin diseases, fever, insominia and febrifuge[14]. The present work is carried out to screen the presence of secondary metabolites and also to analyse the antimicrobial efficiency of *Marsilea quadrifolia.*

MATERIALS AND METHODS

The plant selected for the present study is an aquatic fern ***Marsilea quadrifolia***. The plant was collected from different places at the foot of Western Ghats. The plant was identified with the help of Prof. Dr. Saravana Gandhi, P.G Department of Botany, Rani Anna Government College for Women, Tirunelveli. The plant was well washed with distilled water repeatedly to remove the adhered mud and other impurities, shade dried for about 10 days, powdered, packed air tightly and stored in refrigerator. Plant extracts were prepared by dissolving 5gram of the plant powder dissolved in 100ml of the solvent. Separate extracts were prepared with Ethanol, Benzene and distilled water (Aqueous).

Qualitative Phytochemical analyses for all the three extracts were performed following standard procedures described by Sofowra and Horbone[15,16].

Quantitative Phytochemical analyses were performed using following methods. The total Carbohydrates were estimated by Anthrone method, Total Proteins by Lowry's Method, Total Flavonoids by Aluminium Chloride method, Total Amino acids by Ninhydrin Method and Saponins by the method described by Obadoni and Ochuko[17].

CHN Analysis (Carbon, Hydrogen and Nitrogen Analysis) was performed using CHN Analyzer (Model Elementar Vario EL III) and EDS (Energy Dispersive Spectrum) was studied using Energy Dispersive Spectrometer (Joel Model JED-2300).

Antimicrobial efficiency of the different extracts (Ethanol, Benzene and Aqueous) was studied against five bacterial strains and three fungal strains. Distilled water was used as control. Well diffusion method[18] was followed for the study. The media used for antibacterial test were Nutrient Broth. The test bacterial strains were inoculated into nutrient broth and incubated at 37^0C for 24hrs. After the incubation period, the culture tubes were compared with the turbidity standard. Fungal inoculums were prepared by suspending the spores of fungus (as previously cultured) in saline water mixed thoroughly, made turbidity standard and used. Fresh bacterial culture of 0.1ml having 10^8 CFU was spread on nutrient agar (NA) plate using swab. The fungal strains also the same but the medium was Potato dextrose agar (PDA). Wells of 6 mm diameter were punched off into medium with sterile cork borer and filled with 50µl of plant extracts by using micro pipette in each well in aseptic condition. Plates were then kept in a refrigerator to allow pre-diffusion of extract for 30minutes. Further the plates were incubated in an incubator at 37^0C for 24hours and 28-30 ^{0}C for 3-4 days for bacterial and fungal cultures respectively. The antimicrobial activity was evaluated by measuring the zone of inhibition.

RESULTS AND DISCUSSION

Qualitative Phytochemical Analysis

In the benzene extract of *Marsilea quadrifolia* the results were positive for five compounds (Reducing sugar, amino acid, Phenolic compounds, Flavonoids and phytosterols). Whereas 8 compounds showed positive results in ethanolic extract and 7 compounds in aqueous extract. The common compounds present in both ethanolic and aqueous extracts were reducing sugars, tannins, phenolics compounds, flavonoids, alkaloids and phytosterols. Proteins and saponins were identified only in ethanolic extract (Table-1).

Table 1: Qualitative Phytochemical analysis of *Marsilea quadrifolia*

Name of the tests	Benzene extract	Ethanol extract	Aqueous extract
Reducing sugar	+	+	+
Amino acid	+	-	+
Vitamin- C	-	-	-
Protein	-	+	-
Iron	-	-	-
Tannin	-	+	+
Phenolic Compound	+	+	+
Flavonoid	+	+	+
Alkaloid	-	+	+
Phytosterols	+	+	+
Steroids	-	-	-
Saponins	-	+	-
Total Compounds	**5**	**8**	**7**

'+' indicates presence of compounds; '-' indicates absence of compounds

Quantitative Phytochemical Analysis

The quantitative analysis revealed the presence of 200mg of carbohydrates, 51mg of proteins, 28mg of amino acids, 3mg of flavonoids and 2.8mg of saponins per gram of plant powder (Table-2).

Table 2: Quantitative Analysis of Phytochemicalsof *Marsilea quadrifolia*

Contents	mg/g
Total carbohydrate	200±10.2
Total protein	51±3.4
Total amino acid	28±3.2
Total flavonoids	3±0.4
Total Saponin	2.8±0.5

CHN Analysis

CHN Analysis revealed the presence of 40.51% carbon, 5.47% hydrogen and 3.80% of nitrogen.

EDS Analysis

The EDS Analysis revealed the presence of minerals like carbon, oxygen, potassium and chlorine in mass percentage 69.03, 20.97, 7.28 and 2.71 which are converted into micrograms as $1.46x10^{-16}$, $3.48x10^{-17}$, $1.20x10^{-17}$ and $4.5x10^{-18}$ respectively (Table-3).

Table 3: EDS Analysis

Element	Mass (%)	Micrograms
Carbon	69.03	1.46×10^{-16}
Oxygen	20.97	3.48×10^{-17}
Potassium	7.28	1.20×10^{-17}
Chlorine	2.71	4.5×10^{-28}

HPTLC Analysis

The chromatogram of HPTLC revealed about 11 compounds with different Rf values. The list was enclosed in Table-4.

Table 4: HPTLC Analysis

Substance	Rf Value
1	0.03
2	0.05
3	0.09
4	0.35
5	0.47
6	0.61
7	0.63
8	0.74
9	0.81
10	0.87
11	0.93

Antimicrobial Activity

Antimicrobial activity for all the three extracts was studied against five bacterial strains (*Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, P.aeruginosa and Aeromonas hydrophila*) and three fungal strains (*Aspergillus niger, Candida albicans* and *Pencillium notatum*). Pronounced anti-bacterial potential was observed in Benzene extract followed by ethanolic extract, however better zone of inhibition in resisting the growth of fungus was observed with ethanolic extracts and no visible antimicrobial activity was observed in aqueous extract and control (Table-5).

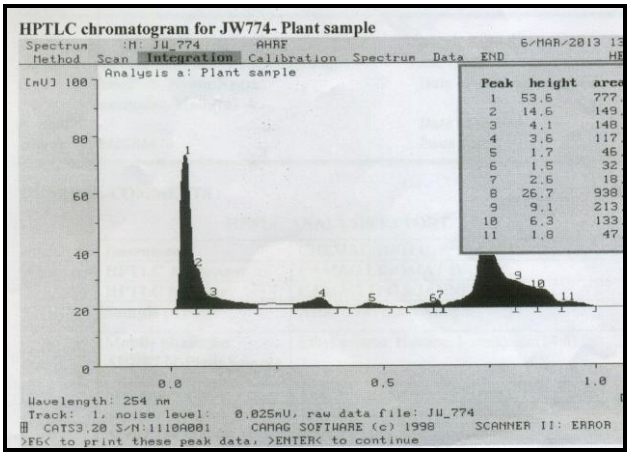

Table 5: *In vitro* antimicrobial activities (zone of inhibition in 'mm')

Test organisms	Benzene extract	Ethanol extract	Aqueous extract
Bacteria			
Escherichia coli	27±2.3	10±1.8	-
Klebsiellapneumoniae	32±4.1	15±2.1	-
Staphylococcus aureus	17±2.4	8±1.9	-
Pseudomonas aeruginosa	19±3.2	15±3.4	-
Aeromonashydrophila	38±4.3	16±3.1	
Fungi			
Aspergillusniger	12±2.5	13±3.1	-
Candida albicans	14±2.6	21±3.4	-
Penicilliumnotatum	11±2.4	13±3.8	-

CONCLUSION

The presence of antimicrobial activity in a particular part of a particular species may be due to the presence of one or more bioactive compounds such as alkaloids, glycosides, flavonoids, steroids, saponins etc[19]. The Phytochemical analysis of *Marsilea quadrifolia* revealed the presence of secondary metabolites like tannin, phenolic compound, flavonoid, alkaloid, phytosterols, and saponins.

Many tannin-containing drugs are used in medicine as astringent. They are used in the treatment of burns as they precipitate the proteins of exposed tissues to form a protective covering, and also medically used as healing agents in inflammation, leucorrhoea, gonorrhea, burns and piles. Besides anti-inflammatory they also have antiviral, antibacterial, antiparasitic[20-22], anti-diarrheal, haemostatic and antihemorrhoidal properties[23]. Saponin is used as mild detergent in intracellular histochemical staining. It is also used to allow antibody access in intracellular proteins.

In medicine, it is used in the human diet for controlling cholesterol and for weight loss[23], and also in treating hypercholesterolemia and hyperglycemia, It also has antioxidant, anticancer, antifungal and anti-inflammatory properties. Plant steroids are important for their cardiotonic activities and also possess insecticidal and antimicrobial properties. Flavonoids have been referred to as nature's biological response modifiers, because of their inherent ability to modify the body's reaction to allergies and virus further they also have anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activities[24], anti-oxidant activity and provide protection to the plants from attack by microbes and insects[23]. Phenolics' beneficial effects are related to their antioxidant activity[25] as they scavenge free radicals, they provide protection for plants against pathogens and predators they also exhibit anti-microbial, anti-inflammatory, anti-feedent, anti-viral, anti-cancer and vasodilatory actions[26]. Each plant is like factory capable of synthesizing unlimited

number of highly complex and unusual chemical substances whose structures could otherwise escape the imagination for ever[27]. There are at least 120 distinct chemical substances derived from plants that are considered as important drugs currently in use in the world, while several other drugs are simple synthetic modifications of the natural products[28]. From time immemorial herbal products were used for curing diverse type of bacterial, fungal and viral diseases. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for discovery of new drug because of the unmatched availability of chemical diversity. Plant with antimicrobial compounds have enormous therapeutic potential as they can act without any side effect as often found with synthetic antimicrobial products.

The selected aquatic fern *Marsilea quadrifolia* with many bioactive compounds may be useful as a new drug in the field of drug discovery.

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