Annexure
GC-MS Evaluation of Bioactive Compounds of Marsilea quadrifolia Linn (Aquatic Fern)
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ABSTRACT
Gas Chromatography-Mass Spectrometry (GC-MS) analysis of the aquatic fern Marsilea quadrifolia was carried. The thirteen bioactive compounds identified are Hexadecanoic acid, ethyl ester (26.88%), Phytol (16.97%), 9,12-Octadecadienoic acid (Z,Z) (12.46%), 1,2-Benzenedicarboxylic acid, dixooctyl ester (8.62%), 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (6.71%), 2(3H)-Furanone, dihydro-3-hydroxy-4,4-dimethyl (α) (6.56%), Octadecanoic acid, ethyl ester (5.69%), 2-Piperidinone, N-[4-bromo-n-butyl (4.53%), Spiro[androst-5-ene-17,1'-cyclobutan]-2'-one, 3-hydroxy-, (3α,17α) (4.16%), Z,Z,Z-1,4,6,9-Nonadecatetraene (3.86%), 2,6,10-Dodecaatrien-1-ol, 3,7,11-trimethyl-, (E,E) (1.82%), 10-Undecen-1-al, 2-methyl (1.20%), 5α-Androstan-16-one, cyclic ethylene mercaptole (0.53%).

KEYWORDS
Marsilea quadrifolia, GC-MS Analysis, Phytochemicals

INTRODUCTION
Traditional medical knowledge is important not only for its potential contribution to drug development and market values, but also for the people’s healthcare. According to the WHO, 80% of the world’s population primarily those of developing countries rely on plant-derived medicines for their healthcare needs. Phytochemicals are chemical compounds formed during the plants normal metabolic processes. These chemicals are often referred to as “Secondary metabolites” of which there are several classes including alkaloids, flavonoids, coumarins, glycosides, gums, polysaccharides, phenols, tannins, terpenes and terpenoids. In addition to these substances, plants contain other chemical compounds. These can act as agents to prevent undesirable side effects of the main active substances or to assist in the assimilation of the main substances. Plants have an almost limitless ability to synthesize aromatic substances, mainly secondary metabolites of which 12,000 have been isolated, a number estimated to be less than 10% of the total. These active components serve as molecules of plant defense against attack by microorganisms, insects and herbivores and at the same time also exhibit medicinal properties for treating several ailments. Scientific research has allowed us to discover a wide range of active components, of which the most important, as far as health is concerned, are essential oils, alkaloids, glycosides or heterosides, mucilage and gums and tannins.

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. It is the demand of present time to discover new alternative antimicrobial compounds with diverse chemical structure and novel mechanism of action for new and reemerging infectious diseases.6

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 uses of pteridophytes since it is not easily available like flowering plants. Pteridophytes have an important role in the earth’s biodiversity.7

*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.8 The plant is also useful to treat psychopathy, leprosy, haemorrhoids, skin diseases, fever, insomnia and febrifuge.9 However the literature lacks GC-MS analysis of *Marsilea quadrifolia* to identify the different bioactive compounds present in it. Hence the present work was carried out.

**MATERIALS AND METHOD**

The plant *Marsilea quadrifolia* was collected as whole plant from the shores of the pond and the banks of the fields of Manimuthaar (foot region of Western Ghats), Tirunelveli District, and Tamil Nadu. They were shade dried and pulverized to powder. 50gram of the plant powder is soaked in 500ml of ethanol in a stoppered flask, shaking intermittently for 48 hours. Then the extract was filtered through whatmann No.1 filter paper. The filtrate was evaporated to dryness by vacuum distillation unit and stored.

**GC-MS Analysis**

GC-MS Analysis of the extract was performed using Perkin-Elmer GC Clarus 500 system and Gas chromatograph interfaced to a Mass spectrometer (GC-MS) equipped with a Elite-5Ms, fused silica capillary column (30mm x 0.25mm X 0.25µM df, composed of 5% Diphenyl/95% Dimethyl poly siloxane). For GC-MS detection, an electron ionization system with ionizing energy of 70eV was used. Helium gas (99.99%) was used as the carrier gas at constant flow rate 1ml/min and an injection volume of 1µl was employed (split ratio of 10:1); Injector temperature 250°C; The oven temperature was programmed from 110°C (isothermal for 2 min.), with an increase of 10°C /min upto 200°C, then 5°C /min to 280°C, ending with a 9 minute isothermal at 280°C. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time was 36 minutes. The relative % amount of each component was calculated by comparing its average peak area to the total area. The software adopted to handle mass spectra and chromatogram was Turbomass 5.2. Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST)-version Year 2005. The spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test material were ascertained.

**RESULTS AND DISCUSSION**

GC-MS analysis of *Marsilea quadrifolia* whole plant extract revealed the presence of 13 compounds. The name of the compound with retention time, molecular formula, molecular weight, and concentration was listed in the table-1.

The thirteen compounds were Hexadecanoic acid, ethyl ester (26.88%), Phytol (16.97%), 9,12-Octadecadienoic acid (Z,Z) (12.46%), 1,2-Benzenedicarboxylic acid, diisooctyl ester.
Table-1. GC MS analysis of *Marsilea quadrifolia*. The components identified and their activity

<table>
<thead>
<tr>
<th>No.</th>
<th>RT</th>
<th>Name of the compound</th>
<th>Molecular formula</th>
<th>MW</th>
<th>Peak Area %</th>
<th>Compound nature</th>
<th><strong>Activity</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.68</td>
<td>2(3H)-Furanone, dihydro-3-hydroxy-4,4-dimethyl-, (ñ)-</td>
<td>C₆H₁₀O₃</td>
<td>130</td>
<td>6.56</td>
<td>Ketone compound</td>
<td>No activity reported</td>
</tr>
<tr>
<td>2</td>
<td>11.08</td>
<td>3,7,11,15-Tetramethyl-2-hexadecen-1-ol</td>
<td>C₂₀H₄₀O</td>
<td>296</td>
<td>6.71</td>
<td>Terpene alcohol</td>
<td>Antimicrobial Anti-inflammatory</td>
</tr>
<tr>
<td>3</td>
<td>12.82</td>
<td>Hexadecanoic acid, ethyl ester</td>
<td>C₁₈H₃₆O₂</td>
<td>284</td>
<td>26.88</td>
<td>Palmitic acid ester</td>
<td>Antioxidant Hypocholesterolemic Nematicide Pesticide Anti androgenic Flavor Hemolytic 5-Alpha reductase inhibitor</td>
</tr>
<tr>
<td>4</td>
<td>14.26</td>
<td>Phytol</td>
<td>C₂₀H₄₀O</td>
<td>296</td>
<td>16.97</td>
<td>Diterpene</td>
<td>Antimicrobial Anticancer Antioxidant Diuretic</td>
</tr>
<tr>
<td>5</td>
<td>14.96</td>
<td>9,12-Octadecadienoic acid (Z,Z)-</td>
<td>C₁₈H₃₂O₂</td>
<td>280</td>
<td>12.46</td>
<td>Linoleic acid ester</td>
<td>Hypcholesterolemic Nematicide Antiarthritic Hepatoprotective Anti androgenic Nematicide 5-Alpha reductase inhibitor Antihistaminic Anticoronal Insectifuge Antieczemic Antiacne Anticancer</td>
</tr>
<tr>
<td>No.</td>
<td>R.T.</td>
<td>Compound</td>
<td>Molecular formula</td>
<td>Molecular weight</td>
<td>% Area</td>
<td>Activity</td>
<td>Notes</td>
</tr>
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<td>----------</td>
<td>-------</td>
</tr>
<tr>
<td>6</td>
<td>15.29</td>
<td>Octadecanoic acid, ethyl ester</td>
<td>C&lt;sub&gt;20&lt;/sub&gt;H&lt;sub&gt;40&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>312</td>
<td>5.69</td>
<td>Stearic acid ester</td>
<td>No activity reported</td>
</tr>
<tr>
<td>7</td>
<td>17.10</td>
<td>Z,Z,Z-1,4,6,9-Nonadecatetraene</td>
<td>C&lt;sub&gt;19&lt;/sub&gt;H&lt;sub&gt;32&lt;/sub&gt;</td>
<td>260</td>
<td>3.86</td>
<td>Alkene compound</td>
<td>No activity reported</td>
</tr>
<tr>
<td>8</td>
<td>19.05</td>
<td>10-Undecen-1-al, 2-methyl-</td>
<td>C&lt;sub&gt;12&lt;/sub&gt;H&lt;sub&gt;22&lt;/sub&gt;O</td>
<td>182</td>
<td>1.20</td>
<td>Aldehyde compound</td>
<td>Antimicrobial Anti-inflammatory</td>
</tr>
<tr>
<td>9</td>
<td>19.97</td>
<td>1,2-Benzenedicarboxylic acid, diisooctyl ester</td>
<td>C&lt;sub&gt;24&lt;/sub&gt;H&lt;sub&gt;38&lt;/sub&gt;O&lt;sub&gt;4&lt;/sub&gt;</td>
<td>390</td>
<td>8.62</td>
<td>Plasticizer compound</td>
<td>Antimicrobial Antifouling</td>
</tr>
<tr>
<td>10</td>
<td>23.66</td>
<td>2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-, (E,E)-</td>
<td>C&lt;sub&gt;15&lt;/sub&gt;H&lt;sub&gt;26&lt;/sub&gt;O</td>
<td>222</td>
<td>1.82</td>
<td>Alcoholic compound</td>
<td>Antimicrobial</td>
</tr>
<tr>
<td>11</td>
<td>26.92</td>
<td>5â-Androstan-16-one, cyclic ethylene mercaptole</td>
<td>C&lt;sub&gt;21&lt;/sub&gt;H&lt;sub&gt;34&lt;/sub&gt;S&lt;sub&gt;2&lt;/sub&gt;</td>
<td>350</td>
<td>0.53</td>
<td>Steroid</td>
<td>Antimicrobial Anticancer Anti-inflammatory Antiarthritic Antiasthma Hepatoprotective</td>
</tr>
<tr>
<td>12</td>
<td>29.24</td>
<td>2-Piperidinone, N-[4-bromo-n-butyl]-</td>
<td>C&lt;sub&gt;9&lt;/sub&gt;H&lt;sub&gt;16&lt;/sub&gt;BrNO</td>
<td>233</td>
<td>4.53</td>
<td>Alkaloid</td>
<td>Antimicrobial Anti-inflammatory</td>
</tr>
<tr>
<td>13</td>
<td>30.70</td>
<td>Spiro[androst-5-ene-17,1'-cyclobutan]-2'-one, 3-hydroxy-, (3â,17â)-</td>
<td>C&lt;sub&gt;22&lt;/sub&gt;H&lt;sub&gt;32&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>328</td>
<td>4.16</td>
<td>Steroid</td>
<td>Antimicrobial Anticancer Anti-inflammatory Antiarthritic Antiasthma Hepatoprotective</td>
</tr>
</tbody>
</table>

**Activity source: Dr. Duke's Phytochemical and Ethnobotanical Database**
(8.62%), 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (6.71%), 2(3H)-Furanone, dihydro-3-hydroxy-4,4-dimethyl (n) (6.56%), Octadecanoic acid, ethyl ester (5.69%), 2-Piperidinone, N-[4-bromo-n-butyl (4.53%), Spiro[androst-5-ene-17,1’-cyclobutan]-2’-one, 3-hydroxy-, (3a,17a) (4.16%), Z,Z,Z-1,4,6,9-Nonadecatetraene (3.86%), 2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-, (E,E) (1.82%), 10-Undecen-1-al, 2-methyl (1.20%), 5α-Androstan-16-one, cyclic ethylene mercaptale (0.53%).

The identified compounds with more percentage like Hexadecanoic acid, ethyl ester (Palmitic acid ester) (26.88%), Phytol (Diterpene) (16.97%), 9,12-Octadecadienoic acid (Z,Z) (Linoleic acid ester) (12.46%) showed a wide range of potent bioactivity. Among the thirteen identified compounds 8 showed Anti-microbial activity, 6 showed Anti-inflammatory, 4 showed Anti-cancer and 2 other showed anti-oxidant and hypocholesterolemic activity. It was also observed that activity for 3 compounds have not been reported, a thrust area which has to be worked out.

Fatty acids always occur in plants. Fatty acids in plants (Hexadecanoic acid, Octodecadienoic acid etc.) react with alcohols in an esterification reaction to form esters. Unsaturated fatty acids are important to every cell in the body for normal growth, especially of the blood vessels and nerves and to keep the skin and other tissues youthful and supple through their lubricating quality. These are nutrients which are invaluable for the production and movement of energy throughout the body, regulation of transportation of oxygen and are vital in maintaining the integrity of cell structure as well as the unique ability to lower cholesterol levels of the blood.

Steroids are abundant in nature; many derivatives of steroids have physiological activity. Steroid hormones control sexual development and fertility in the human body. Many steroids are used in medicine in the treatment of cancer, arthritis or allergies and in birth control. Detection of steroids in

*Marsilea quadrifolia* may also be used in fertility therapy.
Phytols are the precursor for the manufacture of synthetic forms of Vitamin E and Vitamin K. Plants use phytol and its metabolites as chemical deterrents against predation. Phytol acts as effective adjuvants and also increases the titers of all major Immunoglobulin G (IgG) subclass and is also capable of inducing specific cytotoxic effector T-cell responses.

Alkaloids have marked physiological activity. Pure isolated plant alkaloids and their synthetic derivatives are used as basic medicinal agent for its analgesic, anti-spasmodic and anti-bacterial properties. For example Quinine (alkaloid) extracted from Cinchona is used to treat malaria.

Thus from the present GC MS study it is evident that *Marsilea quadrifolia* is a potent medicinal fern of pharmaceutical importance.
ACKNOWLEDGEMENT

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REFERENCES


Phytochemical Analysis and Antimicrobial Efficiency of *Marsilea quadrifolia* linna (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. The history of herbal medicine starts from the ancient human civilization.

The wealth of India is stored in the enormous natural flora which has been gifted to Indians. 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. Plant products and related drugs are used to treat 87% of all categorized diseases. The ready availability and economy of plants as direct therapeutic agents make...
plants more attractive when compared to modern medicine. 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. According to WHO, 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. 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. The secondary metabolites from natural products show more drug likeness and biologically friendliness than total synthetic molecules. 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.

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.

*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. The plant is also useful to treat psychopathy, leprosy, haemorrhoids, skin diseases, fever, insomnia and febrifuge. 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 5 gram of the plant powder dissolved in 100 ml 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 Horbòne\textsuperscript{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\textsuperscript{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\textsuperscript{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\textdegree C 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\textsuperscript{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 50\mu l 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\textdegree C for 24hours and 28-30 \textdegree C for 3-4 days for bacterial and fungal cultures respectively. The antimicrobial activity was evaluated by measuring the zone of inhibition.

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### 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*

<table>
<thead>
<tr>
<th>Name of the tests</th>
<th>Benzene extract</th>
<th>Ethanol extract</th>
<th>Aqueous extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reducing sugar</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Amino acid</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Vitamin- C</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Protein</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Iron</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tannin</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phenolic Compound</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloid</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phytosterols</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

\`+\` 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>
<thead>
<tr>
<th>Contents</th>
<th>mg/g</th>
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<tbody>
<tr>
<td>Total carbohydrate</td>
<td>200±10.2</td>
</tr>
<tr>
<td>Total protein</td>
<td>51±3.4</td>
</tr>
<tr>
<td>Total amino acid</td>
<td>28±3.2</td>
</tr>
<tr>
<td>Total flavonoids</td>
<td>3±0.4</td>
</tr>
<tr>
<td>Total Saponin</td>
<td>2.8±0.5</td>
</tr>
</tbody>
</table>

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).

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’)

<table>
<thead>
<tr>
<th>Test organisms</th>
<th>Benzene extract</th>
<th>Ethanol extract</th>
<th>Aqueous extract</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacteria</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>27±2.3</td>
<td>10±1.8</td>
<td>-</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>32±4.1</td>
<td>15±2.1</td>
<td>-</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>17±2.4</td>
<td>8±1.9</td>
<td>-</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>19±3.2</td>
<td>15±3.4</td>
<td>-</td>
</tr>
<tr>
<td><em>Aeromonas hydrophila</em></td>
<td>38±4.3</td>
<td>16±3.1</td>
<td></td>
</tr>
<tr>
<td><strong>Fungi</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aspergillus niger</em></td>
<td>12±2.5</td>
<td>13±3.1</td>
<td>-</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>14±2.6</td>
<td>21±3.4</td>
<td>-</td>
</tr>
<tr>
<td><em>Penicillium notatum</em></td>
<td>11±2.4</td>
<td>13±3.8</td>
<td>-</td>
</tr>
</tbody>
</table>

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. 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, anti-diarrheal, haemostatic and antimicrobial 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, anti-oxidant activity and provide protection to the plants from attack by microbes and insects. Phenolics’ beneficial effects are related to their antioxidant activity 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. 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. 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. 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.

**ACKNOWLEDGEMENTS**

We place our heart felt thanks to UGC for their financial help to carry out this work.

**REFERENCES**


LIST OF PUBLICATIONS


This is to certify that Mr. A. Sivagurunathan (Part time Research Scholar, Reg. No: 5668 dated 26.11.2010) has undertaken the following work for past four years for his Ph.D work and the biannual reports were given below.

<table>
<thead>
<tr>
<th>Date</th>
<th>Works Under Gone</th>
</tr>
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<tr>
<td>13.04.2011 - 12.10.2011</td>
<td>Collection of the plant, extract preparation, Qualitative and Quantitative phytochemical analysis,</td>
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<tr>
<td>13.10.2011 - 12.04.2012</td>
<td>Antimicrobial studies and GCMS Analysis</td>
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<tr>
<td>13.04.2012 - 12.10.2012</td>
<td>Standardization of haematological and serological techniques</td>
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<tr>
<td>13.10.2012 - 12.04.2013</td>
<td>Standardization of Immunological techniques and SDS-PAGE</td>
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<td>Challenge studies experiments</td>
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<tr>
<td>13.04.2014 - till date</td>
<td>Thesis writing</td>
</tr>
</tbody>
</table>

Guide and Supervisor
COURSE WORK CERTIFICATE

This is to certify that Mr. A. Sivagurunathan, (Part time – Internal Research Scholar, (MSU / Res / RI / Reg. No. 5668, dated : 26.11.2010) has registered for his Ph.D in Manonmaniam Sundaranar University, Tirunelveli. The title of the thesis is “IMMUNODIAGNOSTIC STUDIES IN A CHOSEN FRESH WATER FISH ADMINISTERED WITH A MEDICINAL AQUATIC FERN MARSILEA QUADRIFOLIA” and he has worked for the same under my guidance and supervision since 09.10.2010, in the research department of zoology, St. Xavier’s college, Palayamkottai.

Guide and Supervisor