4. Abstract

Plant derived bioactive compound(s) show various beneficial pharmacological actions. Thus, the present study is aimed to evaluate the phytochemical profile of aquatic fern *Azolla microphylla*. The 50gm of dried and powdered entire parts of *Azolla microphylla* were successively extracted with 250ml of petroleum ether, methanol, chloroform, benzene and water by using Soxhlet extractor for 8 h at a temperature not exceeding the boiling point of the solvent. Phytochemical screening was carried out according to the standard methods. From the results, it can be concluded that the aquatic fern *Azolla microphylla* shows the presence of many bioactive compounds after extensive investigation. We recommend further research on this plant to quantify the concentration of these compounds. Further work will accentuate the isolation and characterization of bioactive principles responsible for bio-efficacy and bioactivity.

4.1. Introduction

Fern plants are well-known traditional Chinese medicinal herbs and extensively used to treat skin tumefaction, to protect the liver and to treat hepatitis, being also used as antipyretics [1]. *Azolla microphylla* is an aquatic fern that freely floats on the surface of the water body. It is a pteridophyte plantae belongs to the salvinacea family. It has been traditionally used as a bio fertilizer for wetland paddy fields due to fix atmospheric-nitrogen (N₂) with the help of azolla-anabaena of the cyanobacterium [2]. *Azolla microphylla* is also rich in protein, vitamin and minerals and is used as food supplements for dairy cattle, pigs, ducks and chickens resulting in increased milk production, enhancement of weight of cattle, pigs, ducks, broiler chickens and production of eggs with layered yolk, as compared to conventional ones [3, 4].

It is concerned with enormous variety or organic substances namely alkaloids, caratenoids, steroids, flavonoids, terphenoids, etc. are elaborated and accumulated by plants and deals with the chemical structures of these substances the biosynthesis turn and metabolism natural distribution and their biological function. Flavonoids constitute one of the most characteristic classes of compounds in higher plants. Flavonoids have been recognized as having a protective effect in plants against microbial invasion by
plant pathogens [5]. Plant phenolics seem to be one of the important factors that evoke host plant alternation [6]. Flavonoids are involved in many enzymatic reactions, involving cyclooxygenase and lipooxygenase, resulting in a decrease of platelet activation and aggregation, against cardiovascular diseases, cancer chemoprevention and anti-inflammatory activity [7-11]. The traditional folklore medicine in India has exploited many plants as potential sources of drugs because of the presence of several secondary metabolites which are biologically active [12]. The identification and development of phenolic compounds or extracts from different plants has become a major area of health- and medical-related research [13]. Research on the biopotential of Azolla in India is limited despite the antimicrobial potential of these plants and scant information is available on useful compounds. Herein, we report the phytochemical profile of the organism A. microphylla.

4.2. Experimental Section

4.2.1. Plant material
Healthy and disease free entire plants of Azolla microphylla was purchased from Vivekananda Kendra-NARDEP (Natural Resources Development Project), Vivekanandapuram, Kanyakumari, Tamil Nadu, India.

4.2.2. Extraction of the plant and preliminary phytochemical screening
Fresh material was collected from the tanks and were brought to the laboratory and cleaned of all the debris. The fresh material was then washed several times in tap water. Subsequently the plants were washed using double distilled water and were air dried in shade for three weeks. The dried material was then made to a powder and the dried and powdered material (50 g) was extracted successively with 250ml of petroleum ether, methanol, chloroform, benzene and water by using Soxhlet extractor for 8 h at a temperature not exceeding the boiling point of the solvent. The aqueous extracts were filtered using Wattman No: 1 filter paper. The filtrates were then concentrated in vacuum at 45⁰C using rotary evaporator. The residues were then stored at -20⁰C till further use. Preliminary phytochemical screening was conducted using the standard methods.
4.3. Results

The crude extracts of *Azolla microphylla* showed diverse phytochemical profiles with response to solvents. The methanol extracts of *Azolla microphylla* demonstrated maximum occurrence of phyto-constituents, followed by petroleum ether, benzene chloroform and water. The extracts of the plant did not show the presence of any alkaloids, triterpenoids and amino acids. However, the extracts showed the presence of phenolics, flavonoids, proteins, saponins, tannins, steroids, anthraquinone glycosides and carbohydrates (Table 4.1).

Table 4.1. Preliminary phytochemical evaluation of *Azolla microphylla*

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Solvent Extracts</th>
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<tbody>
<tr>
<td></td>
<td>Petroleum Ether</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
</tr>
<tr>
<td>Phenols</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>Proteins</td>
<td>+</td>
</tr>
<tr>
<td>Amino acids</td>
<td>-</td>
</tr>
<tr>
<td>Triterpenoids</td>
<td>-</td>
</tr>
<tr>
<td>Anthraquinone Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>-</td>
</tr>
<tr>
<td>Xanthoproteins</td>
<td>-</td>
</tr>
<tr>
<td>Coumarins</td>
<td>-</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
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</tbody>
</table>
4.4. Discussions

The present study has screened the phytochemical profile of the aquatic fern *Azolla microphylla* with different solvent extracts. Isolation and screening of novel compounds and their bioactivity in traditional medicine has been attempted and extraction procedure is one of the crucial steps for research and development of plant secondary metabolites [14, 15]. There are some studies on phytochemistry and pharmacology on pteridophytes from Western Ghats of Tirunelveli Hills, but there is no report on Western Ghats of Kerala [16]. The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as phenols, flavonoids, anthraquinone glycosides, saponins, tannins etc. Thus the preliminary screening tests may be useful and lead to the detection bioactive principles and drug discovery.

Preliminary phytochemical screening revealed the presence of phenolics, tannins, anthraquinone glycosides and sugars confirming the findings of Mithraja et al [17]. Phenolic compounds of high value in the plant parts of *T. montanum* was observed [18]. These compounds are reported to have known bioactivity against a number of pathogens. The phytochemical studies performed on *Azolla* show a great variety of metabolites which include phenolic compounds, phenylpropanoids, flavonoids, tannins and others [19]. Phenolic compounds are known to have antioxidant properties for plants including *Azolla* ferns, established under stress conditions [20, 21]. There have been many reports on the antibacterial activity due to plant phenolics [22, 23]. Polyphenols have been reported to inhibit the growth of microorganisms by forming complexes with either microbial enzymes or proteins and one of the known inhibition mechanisms consists of iron depletion [24]. Presence of condensed tannins was reported in *Azolla* species [25, 26]. Therefore our preliminary screening provides an important lead regarding the bioactive molecules which could further be exploited in the formulation for antimicrobial preparations. Antimicrobial properties of plants have also been exploited by several workers in relation to medicinal importance. Inhibition of bacterial growth by flavonoids has been reported earlier [27]. The flavonoids are able to chelate some metals and consequently inhibit Fenton and Haber-Weiss reactions, which
are important sources of active oxygen radicals [28, 29]. However, the increase in the content of flavonoids in *A. microphylla* in relation to due to lower temperature could be exploited to induce enhanced production of this constituent by exposing the organism to low temperatures under artificial conditions. Environmental conditions play an important role in relation to the content of secondary metabolites and introducing a stress factor to standardize the level and production of these compounds has been reported [30]. The presence of tannins also indicates the importance of the organism as tannins have also been known to be involved in a variety of responses including antibacterial activities [31]. Potential antimicrobial activity of tannins and flavonoids has been established in the extracts of *Pistacia* and *Schinus* spp [32].

The present study suggests the importance of *Azolla microphylla* as an important reservoir of several compounds with considerable antimicrobial as well as pharmacological properties. However, we do not have much information on the phytochemical composition of *Azolla* plants. It is in this context that the present study gains its importance.

**References**


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