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A preliminary examination of the phytochemical profile of *Azolla microphylla* with respect to Seasons

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ABSTRACT

Objective: To evaluate the role of seasons on the phytochemical properties of *Azolla microphylla*. **Methods:** Dried and powdered leaves (50g) were extracted with 300 ml of methanol using soxhlet extractor for 16 h at a temperature not exceeding the boiling point of the solvent. Standard methods have been used to screen the phytochemical constituents. **Conclusions:** From the observations it can be concluded that the plant extracts show the presence of several bioactive compounds which could be exploited further.

1. Introduction

Azolla is an aquatic pteridophyte widely distributed in the water bodies. It has been traditionally used as a biofertilizer for rice paddy fields owing to its potential to fix atmospheric nitrogen [1]. In addition to this it has several other uses and Wagner [2] referred it as "green gold mine". The plant system has the inherent capacity to synthesize several biologically active constituents which in turn protect them against the attack of insects and other plant pathogens such as bacteria and fungi. Plant phenolics seems to be one of the important factors that evoke host plant alternation and the flavonoids are reported to exhibit various biological activities, including antioxidative and free radical scavenging activities [3,4]. 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 [5-8]. The excessive use of pesticides and chemicals in agriculture and consequent adverse impact on the health has prompted the scientists to look for newer molecules of plant origin. The Green Chemistry concept is gaining momentum in a big way and several research groups are active in this field of science.

There have been several reports on the phytochemical constituents of Azolla and some studies have been conducted on the phytochemical composition of pterodophytes from the Western Ghats of Kerala in the past [9-12]. Mithraja et al [13] characterized the phytochemical composition of Azolla pinnata. The identification and development of phenolic compounds or extracts from different plants has become a major area of health- and medical-related research [14]. The efficacy of plants as antimicrobial agents has been described [15]. Plant extracts can be used as natural fungicides to control pathogenic fungi, thus reducing the dependence on the synthetic fungicides [16]. The present study takes advantage of an observation that the aquatic pteridophyte *Azolla microphylla* changes its color of the fronds with respect to seasons. In summer the fronds are green in color and subsequently the color changes to red with the onset of winter (Fig 1). 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. The diversity of the Indian subcontinent with respect to environmental regimes such as light intensity and temperature will also

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be helpful in identifying the most optimal conditions for the production of the metabolites. Therefore in the present study we attempted to screen the phytochemical composition of the organism A. microphylla with respect to the seasons.

Fig. 1. *Azolla microphylla* plants showing differences in frond color with respect to seasons

2. Materials and methods

2.1 Test organism and growth conditions

A. microphylla maintained at the Centre for Collection, Conservation and Utilization of Blue Green Algae, Indian Agricultural Research Institute, New Delhi, India have been used in the present experiments. The plants were grown in cemented tanks of size (450 x150 cm) out door. Only healthy and disease free plants were used in the experiments. For comparison we used plants that were grown out-door during May and December, 2011.

2.2 Preparation of the extract 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 methanol (300 ml) for 16 h at a temperature that not exceeded the boiling point of the solvent. The filtrates were then concentrated in vacuum at 450C using rotary evaporator (Hahnshin Scientific Co.). The residues were then stored at -200C till further use. Preliminary phytochemical screening was conducted using the standard methods [17].

3. Results

Fig 1shows the fronds of A. microphylla multiplied outdoor during summer and winter months. The fronds of the organism remained green in color during the summer season and with the onset of winter the color was changed to red. Hence a phytochemical screening was conducted during both these seasons. The methanolic extracts prepared from the organism from two distinct seasons showed the same phytochemical profile despite the change in pigmentation pattern (Table 1). We have been able to observe the presence of five important constituents in the extracts. The extracts of the plants of both the seasons did not show the presence of any alkaloids, triterpenoids and amino acids. However, the extracts from both the seasons showed the presence of phenolics, tannins, steroids, anthraquinone glycosides and sugars. It was thus observed that despite the differences in the frond color the phytochemical composition based on qualitative tests remained the same.

Table. 1

Preliminary Phytochemical screening of the methanolic extracts of *Azolla microphylla*

Compound	Season I	Season II
Alkaloid	----	---
Tannins	+++	+++
Steroids	+++	+++
Triterpenoid	----	---
Anhraquinone glycosides	+++	+++
Phenols	+++	+++
Sugar	+++	+++
Amino acids	----	----

4. Discussion

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 [18, 19]. Methanolic extraction for the isolation of phenolic compounds from ginger plants has been attempted successfully [20]. Due to paucity of information regarding the phytochemical constituents of the aquatic pteridophyte *Azolla microphylla* in the present study screening of the phytochemical composition was attempted. Preliminary phytochemical screening revealed the presence of phenolics, tannins, anthraquinone glycosides and sugars confirming the findings of Mithraja et al [13]. Phenolic compounds of high value in the plant parts of T. montanum was observed [21]. These compounds are reported to have known bioactivity against a number of pathogens. It was also interesting to observe that despite change in the pigmentation of the organism in response to seasons the phytochemical profile remained the same. The phytochemical studies performed on Azolla show a great variety of metabolites which include phenolic compounds, phenylpropanoids, flavonoids, tannins and others [22-24]. Phenolic compounds are known

to have antioxidant properties for plants including Azolla ferns, established under stress conditions [25, 26]. There have been many reports on the antibacterial activity due to plant phenolics [27, 28]. 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 [29]. Presence of condensed tannins was reported in Azolla spp. [30, 31]. 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 [32]. The flavonoids are able to chelate some metals and consequently inhibit Fenton and Haber-Weiss reactions, which are important sources of active oxygen radicals [33, 34]. 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 [35]. 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 [36]. Potential antimicrobial activity of tannins and flavonoids has been established in the extracts of Pistaciaand Schinus spp [37].

The present study suggests the importance of *Azolla microphylla* as an important reservoir of several compounds with considerable antimicrobial as well as pharmacological properties. There have been some studies on the phytochemical composition of pterodophytes from the Western Ghats of Kerala in the past [7-10]. 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. The unique behavior of the test organism due to low temperatures during the winter months could be further studied. Further research studies are going on in our laboratory to quantify, isolate and characterize the bioactivity of the compounds. This will provide novel inputs regarding the chemical nature of the bioactive compounds from this important group of plants which otherwise has been exploited only for their biofertilizer potential.

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Conflict of interest statement

We declare that we have no conflict of interest.

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