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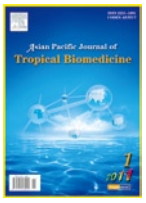

Document heading

Phytochemical studies on Azolla pinnata R. Br., Marsilea minuta L. and Salvinia molesta Mitch

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ABSTRACT

Objective: To evaluate the phytochemical properties of *Azolla pinnata* R. Br., *Marsilea minuta* L. and *Salvinia molesta* Mitch. **Methods:** The dried and powered leaves materials (50 g) were extracted successively with 250 mL of petroleum ether, ethyl acetate, methanol, chloroform, acetone, benzene and water by using Soxhlet extractor for 8 h at a temperature not exceeding the boiling point of the solvent. Phytochemical screening of the extracts was carried out according to the standard methods. **Results:** Out of eighteen tested extracts, eighteen extracts showed the presence of phenolics. Next to that, fourteen extracts were illustrated their existence of tannin. Ten extracts showed the occurrence of carbohydrates in the crude extracts of the selected plants. Steroid and saponin are present in eight extracts, next to that xanthoprotein is present in six extracts, followed by flavonoid and protein which are present in five extracts. Carboxylic acid showed its presence only in two extracts. **Conclusions:** From the results, it can be concluded that the three plants extracts show the presence of many bioactive compounds after extensive investigation. We recommend further research on these plants to quantify the concentration of these compounds. Further work will accentuate the isolation and characterization of active principles responsible for bio-efficacy and bioactivity.

1. Introduction

Medicinal plants being an effective source of both traditional and modern medicines are genuinely useful for primary health care. World Health Organization has advocated traditional medicine as safe remedies for ailments of microbial and non-microbial origin. For past few decades compounds from natural sources have been gaining importance because of the vast chemical diversity that they offer. This had led to phenomenal increase in the demand for herbal medicines in the last two decades and or need has been felt for ensuring the quality, safety and/ efficacy of herbal drugs. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important compounds of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic components. Plants have an 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*. Among them 12 000 have been isolated which are estimated to be less than 10% of the total. In India, many plants are widely used by all section of people either directly as folk remedies or indirectly in pharmaceuticals preparations of modern medicine. India is one of the richest countries in the world providing medicinal plants. The limitations associated with synthetic pharmaceutical products have opened avenues for 'Green Medicine' that is considered to be safe, more accessible and affordable too. To determine the potential and promote the use of herbal medicine, it is essentially required to intensify the research studies on traditional and folklore medicines[1]. Hence today there is a need to do research to produce new antimicrobial agents from plants as an alternative to available antibiotics because they are effective against resistant pathogen of plants and animals to avoid the threat of post antibiotic era. The knowledge of the chemical constituents of plants would further be valuable in discovering the actual value

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of folkloric remedies. Recently there has been growing interest in exploiting the biological activities of flora and fauna owing to their natural origin, cost effectiveness and lesser side effects[2,3]. Many pharmaceutical innovations are developed from a starting point of knowledge derived from the biological activities of natural organisms. At the global level plants have been examined for their phytochemical properties, antimicrobial and pharmacological activity and they proved their fruitfulness[4-10]. Very few studies were carried out on the phytochemical properties of pteridophytes from Kattakada, Trivandrum district, Kerala, Western Ghats, India. With this background an attempt was made to evaluate the phytochemical properties of *Azolla pinnata* (*A. pinnata*) R. Br., *Marsilea minuta* (*M. minuta*) L. and *Salvinia molesta* (*S. molesta*) Mitch.

2. Materials and methods

2.1. Plant material collection

Healthy and disease free entire plants of *A. pinnata* R. Br., *M. minuta* L. and *S. molesta* Mitch. were collected from Kattakada, Trivandrum district, Kerala, Western Ghats, India. Voucher specimen was deposited in Centre for Biodiversity and Biotechnology, Department of Botany, Nesamony Memorial Christian College, Marthandam-629 165, Tamil Nadu, India.

2.2. Extraction of the plant and preliminary phytochemical screening

The fresh materials were washed in tap water for 5 min and dried using blotting papers. The washed plant materials were air and shade dried for two weeks and pulverized to powder using mortar. The dried and powered leaves materials (50 g) were extracted successively with 250 mL of petroleum ether, ethanol, chloroform, acetone, 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 Whattman No. 1 filter paper and then concentrated in vacuum at 40 ℃ using rotary evaporator. The residues obtained were stored in a freezer at −70 ℃ for further tests[11]. Phytochemical screening of the extracts was carried out according to the standard methods[12-17].

3. Results

The crude extracts of *A. pinnata* showed diverse phyto-profiles with reference to solvents of the plant extracts. The aqueous extracts of *A. pinnata* demonstrated maximum occurrence of phyto-constituents (4/12), followed by acetone, benzene, chloroform and ethanol (3/12) (Table 1). The phenol is present in all the tested extracts of *A. pinnata*. The tannin showed its presence in the aqueous, benzene and ethanol extracts and saponin in benzene and chloroform extracts of *A. pinnata*. The carboxylic acid is present in acetone and ethanol extracts of *A. pinnata*. The flavonoid is present only in aqueous extracts of *A. pinnata*. The carbohydrate exists in aqueous, acetone and chloroform extracts of *A. pinnata* (Table 1).

The crude extracts of *M. minuta* illustrated various metabolites presence with reference to solvents of the plant extracts. The chloroform and petroleum ether extracts of *M. minuta* demonstrated maximum occurrence of phyto-constituents (7/12), next to that were acetone, benzene and aqueous (6/12) (Table 1). The phenol is present in all the tested extracts of *M. minuta*. The flavonoid is present in petroleum ether, acetone and benzene. The tannin showed its presence in all the extracts of *M. minuta* except ethanol. The coumarin and carbohydrate are present in chloroform, acetone, benzene and aqueous extracts of *M. minuta*. The steroid is present in chloroform, petroleum ether, aqueous and ethanol and saponin in petroleum ether, benzene and chloroform extracts of *M. minuta*. Xanthoprotein is present in petroleum ether, acetone and aqueous extracts of *M. minuta*. The chloroform and petroleum ether extracts of *M. minuta* demonstrated the presence of protein (Table 1).

The crude extracts of *S. molesta* demonstrated different metabolites presence with reference to solvents of the plant extracts. The chloroform extracts of *S. molesta* showed

Table 1
Preliminary phytochemical evaluation of *A. pinnata* R. Br., *M. minuta* L., and *S. molesta* Mitch.

Compounds	Extracts																	
	Acetone			Benzene			Chloroform			Water			Ethanol			Petroleum Ether		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Alkaloids	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Phenols	++	++	++	++	++	++	++	++	+	++	++	+	+++	++	+	+	++	+
Flavonoids	−	+	−	−	+	−	−	−	+	+	−	−	−	−	−	−	+	−
Saponins	−	−	−	+	+	+++	+	+++	+++	−	−	−	−	−	−	−	+	+
Proteins	−	−	−	−	−	+	−	+	+	−	−	−	−	−	−	−	+	+
Quinones	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Steroids	−	−	+++	−	−	+	−	++	+	−	+	−	−	++	++	−	+	−
Tannins	−	+	+	+	+	+	−	+	++	++	+	+	+	−	+	−	+	+
Xanthoprotiens	−	+++	+++	−	−	−	−	−	−	−	+	++	−	−	++	−	+	−
Carboxylic acids	+	−	−	−	−	−	−	−	−	−	−	−	++	−	−	−	−	−
Coumarins	−	+++	+	−	+	−	−	+	−	−	+	++	−	−	+++	−	−	++
Carbohydrates	+	+	−	−	+	−	++	+++	+++	+	+	++	−	−	+	−	−	−
Total	3	6	5	3	6	5	3	7	7	4	6	5	3	2	6	1	7	5

A: *A. pinnata* R. Br.; B: *M. minuta* L.; C: *S. molesta* Mitch.

maximum occurrence of phyto-constituents (7/12), next to that was ethanol (6/12), followed by acetone, benzene, aqueous and petroleum ether (5/12) (Table 1). The phenol and tannin are present in all the tested extracts of *S. molesta*. The steroid showed its presence in chloroform, ethanol, acetone and benzene extract of *S. molesta*. The coumarin is present in ethanol, acetone, aqueous and petroleum ether. The saponin showed its presence in chloroform, benzene and petroleum ether extracts of *S. molesta*. The flavonoid is present in chloroform extracts of *S. molesta*. The xanthoprotein showed its occurrence in ethanol, acetone and aqueous extracts of *S. molesta*. Protein is present in chloroform, benzene and petroleum ether extracts of *S. molesta*. The carbohydrate showed its existence in chloroform, aqueous and ethanol extracts of *S. molesta* (Table 1).

4. Discussion

The present study has screened the phytochemical properties of the three pteridophytes with eighteen extracts. The presence or absence of the phyto-constituents depends upon the solvent medium used for extraction and the physiological property of individual taxa. Out of eighteen tested extracts, eighteen extracts showed the presence of phenolics. Next to that, fourteen extracts illustrated their existence of tannin. Ten extracts showed the occurrence of carbohydrates in the crude extracts of the selected plants. Steroid and saponin are present in eight extracts, next to that is xanthoprotein which is present in six extracts, followed by flavonoid and protein which are present in five extracts. Carboxylic acid showed its presence only in two extracts. There are some studies on phytochemistry and pharmacology studies on pteridophytes from Western Ghats of Tirunelveli Hills, but there is no report on Western Ghats of Kerala[18-22]. The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, sterols, *etc*. Thus the preliminary screening tests may be useful and lead to the detection of bioactive principles and drug discovery. The present study revealed and supplemented the phytochemical properties of pteridophytes of Western Ghats of Kerala. Recently, a number of plants have been reported for antimicrobial properties across the world[23-28]. In the present investigation, three plants from India have been screened for phytochemical properties. The various phytochemical compounds detected are known to have beneficial importance in medicinal sciences. Plant based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, *etc*[29]. The medicinal actions of plants unique to particular plant species or groups are consistent with the concept that the combination of secondary products in a particular plant is taxonomically distinct[30]. 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 medicine[31,32]. For example, flavonoids are known to inhibit bacterial growth[33]. In the present study we observed the presence of the flavonoid in all the tested plants. It suggests that the selected three plants may be used to treat bacterial infection and diseases. Tannins are known to possess general antimicrobial and antioxidant activities[34]. Recent reports show that tannins may have potential value as cytotoxic and antineoplastic agents[35]. Tannin is almost present in 14 out of 18 extracts. It revealed the antimicrobial properties of the selected three plants, and the three plants may be used as antioxidant and anti-cancer agents. Other compounds like saponins also have antifungal properties[36,37]. Saponins are a mild detergent used in intracellular histochemistry staining to allow antibody access to intracellular proteins. In medicine, it is used in hypercholesterolemia, hyperglycemia, antioxidant, anticancer, antiinflammatory and weight loss, *etc*. It is also known to have anti-fungal properties[38]. Saponins have been implicated as a bioactive antibacterial agent of plants[39,40]. Saponin is also present in eight extract of plant. Steroids are known to be important for their cardiotonic activities and possess insecticidal and anti-microbial properties. In the present study saponin is present in the three plants. The present study paved a pathway to use the three plants for the anti-microbial and pharmacological activities.

Plant derived natural products such as flavonoids, terpenoids and steroids, *etc* have received considerable attention in recent years due to their diverse pharmacological properties including antioxidant and antitumor activity. Phenolics have anti-oxidative, anti-diabetic, anti-carcinogenic, anti-microbial, anti-allergic, anti-mutagenic and anti-inflammtory activities[41,42]. In the present study all the three plants and all the tested extracts showed the presence of phenolics in high concentrations. It is suggested that the selected three plants can be used as antioxidative, antidiabetic, anti-carcinogenic, ani-microbial, anti-allergic, anti-mutagenic and anti-inflammatory agents in the near future. In the present study steroid is present in *M. minuta* and *S. molesta* extracts. It is recommended that *M. minuta* and *S. molesta* extracts can be used as anti-insecticidal and anti-microbial agent. In view of all these phytochemical screening we find that the uses are associated with these compounds which are found in *A. pinnata*, *M. minuta* and *S. molesta* extracts. Therefore, we recommend further research on these plants to quantify the concentration of these compounds. Further work will accentuate the isolation and characterization of active principles responsible for bio-efficacy and bioactivity.

Conflict of interest statement

We declare that we have no conflict of interest.

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