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Phytochemical Profiling and LC/MS Analysis of *Selaginella bryopteris* (L.) Baker

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Abstract

Selaginella bryopteris (L.) Baker, an endemic pteridophyte is found restricted to the Indian subcontinent mostly because of its environmental requirements of cool temperatures and optimum water availability. It is a drought-resistant species and shows curling in limited water supply reviving when water conditions are restored. This property makes this species a suitable plant for exploration of medicinal properties, although its restricted occurrence has limited this evaluation. The current study explores the role of hydrophobic (acetone) and hydrophilic solvents on the phytochemical profiles of the extracts from the dried Selaginella bryopteris, from northern terrain of India. The study is pivotal considering the demographic and environment mediated changes in the compositions of the plant. The results have shown comparatively higher yield of phenolic and flavonoid, to be 36.67 mg/mL and 96.17 mg/mL, respectively in the methanolic extract of the plant. In the absence of limited studies on high yield extraction of the phytocompounds in methanolic extract, further characterization of the extract was performed using LC/MS analysis. The LC/MS analysis revealed a rich consortium of diverse catalogues of compounds including flavonoids and biflavonoids. Some of the key compounds observed, and are known for therapeutic potential, such as anticancer, antioxidant and antifungal activities are Gingerol, Sciadopitysin, Ginkgetin, and Amentoflavone, to name a few. The presence of such rare phytocompounds obtrudes the methanolic extracts a pool of therapeutic compounds consortia that may be evaluated for the bioactivities including therapeutic potential against different model systems. The study will provide an avenue for quantification of the different compounds and their assessment of dose dependent therapeutic potential.

Keywords: *Selaginella bryopteris*, amentoflavone, sciadopitysin, LC/MS, phytoconstituents

INTRODUCTION

The pteridophyte *Selaginella bryopteris* (L.) Baker is a lithophyte. It is found growing in areas with colder climate conditions and sufficient water supply. This environmental requirement has restricted its growth making it endemic to the Indian subcontinent. In India, it is found in various regions including the Himalayas and the hilly terrains of Arunachal Pradesh and Andhra Pradesh. It has also been mentioned in various ancient texts for its medicinal properties, the most prominent one being the Ramayana [1].

Selaginella bryopteris shows drought-resistant properties by curling in water deficit conditions and reviving again when its water requirements are met. This resurrection property has gained scientific interest mostly because of its unexplored mechanism and under-explored medicinal properties. *S.tamariscina*, *S. doederleinii*, *S. uncinate*, *S. moellendorffi*, and other species of this genus are currently being explored for their therapeutic properties as hepatoprotective

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potential, neuroprotective ability and antimicrobial and antioxidant ability to name a few. The limited exploration of this species reveals the presence of a rich flavonoid phytocompound pool [2]. The current study, therefore, aims to explore the diversity in phytocompound pool of *Selaginella bryopteris*.

MATERIALS AND METHODS

Procurement of Plant Sample and Extract Preparation

The whole plant of *Selaginella bryopteris* (L.) Baker was procured from Delhi, India. The plant was identified at Dr. Y.S. Parmar University of Horticulture and Forestry, Solan, India. The plant was carefully washed air dried and coarsely ground, equal amounts of which were dipped in distilled water, methanol and acetone in the ratio of 3:20 (w/v). The mixture was kept on rotation at a constant temperature of 37°C for a period of 2 days while changing the solvent after every 24 hours. The solvent was filtered and air dried and the extractive yield was calculated using the formula mentioned in Equation (1) [3]. The powdered extract, so obtained, was dissolved in methanol or water and stored at –20°C until further use.

$$Extractive\ yield\ (\%) = \frac{\text{Weight of the extract (in g)}}{Weight\ of\ the\ dry\ plant\ material\ (in\ g)} \times 100 \quad (1)$$

Qualitative Phytochemical Screening

1. *Saponins:* The method reported by [4] was used for the froth test estimation of saponins in the extracts. Equal volumes of distilled water and extract were mixed and checked for the presence of froth. Occurrence of persistent and stable froth confirmed the presence of saponins.
2. *Tannins:* Braymer's ferric chloride test was used for the determination of presence of tannins [5]. The appearance of greenish black or bluish green color in the mixture of extract and 10% (w/v) ferric chloride solution confirmed the presence of tannins.
3. *Alkaloids:* Drangendroff reagent test was used to test the presence of alkaloids as reported by Sharma et al. (2020) [14]. A few drops of Drangendroff reagent were added to a previously warmed mixture of extract and sulfuric acid (2% v/v). The development of orange red precipitate confirmed the presence of alkaloids.
4. *Reducing Sugars:* The presence of reducing sugar was confirmed by [6]. Fehling's working solution was prepared by mixing equal volumes of Fehling's solution I and II. It was added to the extract and the mixture was boiled and checked for the development of brick-red precipitate which confirmed the presence of reducing sugar.
5. *Anthraquinones:* The presence of anthraquinones was confirmed by Borntrager's reaction [7]. Briefly, a small amount of extract was dissolved in chloroform, boiled and the mixture filtered. The filtrate was mixed with equal volume of 10% (v/v) ammonia solution. The development of pink color in the upper layer established the presence of anthraquinones.
6. *Steroids:* Salkowski test method was used to check the presence of steroids [8]. Chloroform was added to a small amount of plant extract and filtered. A few drops of concentrated sulfuric acid were added to the filtrate and checked for the appearance of reddish ring at the interface.

Quantitative Phytochemical Screening

1. *Total Phenol Content (TPC):* Folin-Ciocalteu (FC) reagent test was used for the quantitative estimative of phenol content in the extract by the procedure mentioned by [9]. Different concentration of gallic acid in methanol was used as standard. FC reagent (10% v/v) was added to the extract in the ratio of (2:1) preceded by the addition of 0.7M sodium carbonate solution and incubated for 2 hours at room temperature. The absorbance of the mixture was taken at 765nm. The TPC content was expressed as Gallic Acid Equivalent (mg GAE/g).
2. *Total Flavonoid Content (TFC):* Aluminum chloride method for the detection of total flavonoid content (TFC) was used as demonstrated by [10]. Quercetin was used as standard. Aluminium chloride (2% v/v) was mixed with equal volume of extract and incubated in dark for 10 minutes. The absorbance of the mixture was taken at 367nm. The TFC was expressed as Quercetin Equivalent (mg QE/g).

LC/MS analysis

The methanolic extract was subjected to liquid chromatography/mass spectrometry (LC/MS) analysis for analysis of possible phytocompound consortia in the extract. Dionex Ultimate 3000 HPLC system from Thermo Fischer Scientific was used for this purpose. Column used was hypersil gold C-18 with dimensions of 2.1 mm x 100 mm, 3.0 µm and its temperature was maintained at 25°C. Formic acid and Forminc acid in acetonitrile were used as Buffer A and Buffer B, respectively. The flow rate was maintained at 0.35 ml/minute.

RESULTS AND DISCUSSION

Extractive Yield and Phytochemical Screening

The extractive yield was the highest in methanolic solvent highlighting the impact of solvent system in extraction of phytocompounds as shown in Table 1. The results circumvent the outcomes previously reported to support the role of solvent used for efficient extraction of phytocompounds [10]. The qualitative phytochemical profiling of the extracts also shows prominent variations although anthraquinones and steroids were found to be absent in all the extracts while alkaloids were present in all. Additionally, the methanolic and acetone extracts showed similar phytochemical profile, although a huge variation was observed in the extractive yield making methanol a suitable solvent system for extraction.

Table 1. Extractive yield and the qualitative estimation of phytocompound in all the extracts.

Sample name	Water Extract	Methanol Extract	Acetone Extract
Extractive yield (%)	2.65 %	11.41%	1.3%
Saponins	–	–	–
Tannins	–	+	+
Alkaloids	+	+	+
Steroids	–	–	–
Reducing sugar	+	–	–
Anthraquinones	–	–	–

Note: '+' means present and '–' means absent.

Quantitative Phytochemical Screening

The quantitative assessment of phenol and flavonoid content in all the extracts is mentioned in Figure 1 below. The results depict that the methanol extracts also showed a higher phenol and flavonoid content as compared to acetone and water extracts. This further supported methanol to be a better solvent for the extraction of phytocompounds from *Selaginella bryopteris* (L.) Baker.

The presence of higher flavonoid content is also indicative of the therapeutic potential of the plant including but not limited to antioxidant, anticancer, antiaging, antimicrobial, and neuroprotective property [11]. The present results also align with the therapeutic properties reported previously for *Selaginella bryopteris* which may be attributed to the higher flavonoid content in the species [12]. The LC/MS analysis of the methanolic extract was further undertaken to explore the phytochemical consortia in this extract.

LC/MS Analysis of Plant Extract

The LC/MS analysis of the methanolic extract, undertaken at University of Delhi, South Campus, revealed a pool of phytocompounds. The LC/MS chromatogram is depicted in Figure 2.

The LC/MS analysis revealed a list of approximately 1000 compounds. Most of these compounds had previously been reported in genus Selaginella. Table 2 shows a list of major phytocompounds identified from LC/MS analysis with the species and their potential medicinal role as reported in literature.

The LC/MS analysis also revealed some other phytocompounds as morelloflavone and pinellic acid which possess medicinal properties [13]. The phytocompound evaluation was in accordance with the flavonoid content evaluation observed in the extracts.

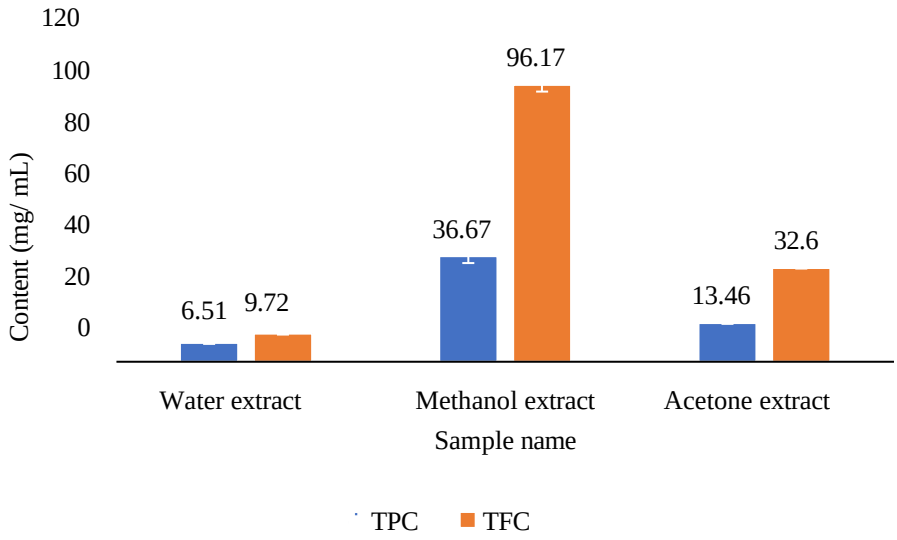

Figure 1. Quantitative estimation of TPC and TFC in plant extracts.

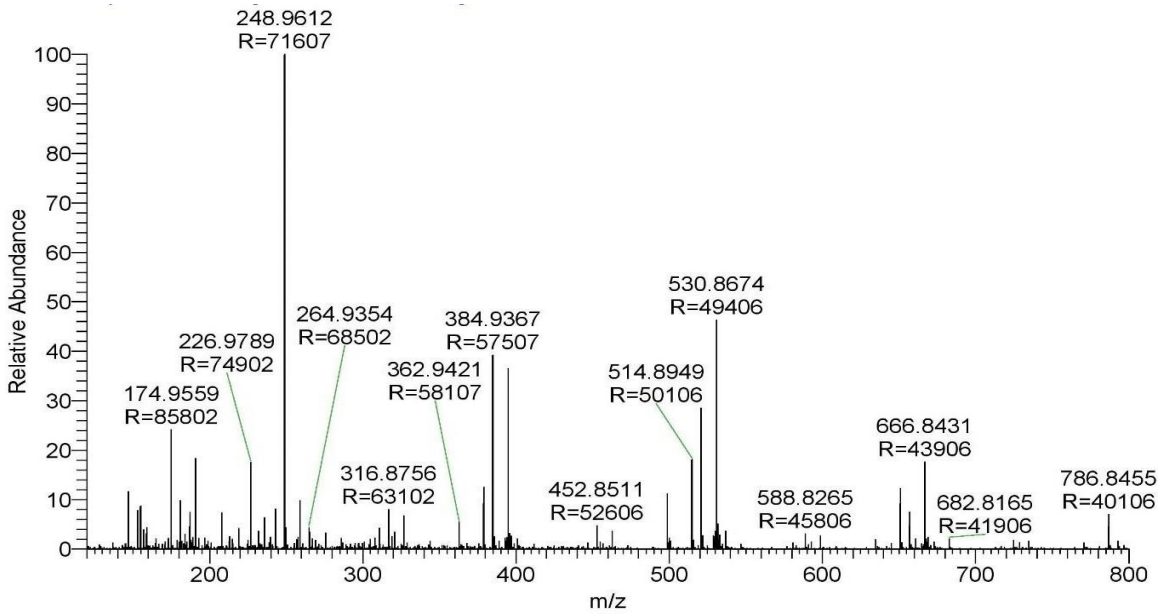

Figure 2. Chromatogram for LC-MS analysis of methanolic extract of *S. bryopteris.*

Table 2. List of phytocompounds identified from LC/MS analysis.

S.N.	Phytocompound	Species Reported in	Medicinal Property	Reference
1.	Gingerol	*Zingiber officinale*	Anticancer	[14]
2.	Sciadopitysin	*Selaginella bryopteris*	Antifungal	[1]
3.	Oleic acid	*Selaginella willdenowii (Desv.) Baker*	Antitumor, antidiabetics and anticancer	[15]
4.	Ginkgetin	*Selaginella Moellendorffii*	Cytotoxicity	[16]
5.	Amentoflavone	*Selaginella tamariscina*	Anti-inflammatory, anti-oxidation, anti-cancer	[17]
6.	Bilobetin	*Selaginella Moellendorffii*	Antihyperlipidemic and anti-proliferative activities	[5, 18]

CONCLUSIONS

The medicinally important pteridophyte, *Selaginella bryopteris*, is found endemic to the Indian terrain and therefore remains underexplored for its therapeutic importance. The present study focuses on the phytocompound consortia of a particular germplasm of *Selaginella bryopteris*. The study also highlights the role of solvent for appropriate extraction of phytocompounds from the species. The extraction yield was found to be highest in the methanolic extract of the plant which was supplemented with a greater flavonoid and phenolic content in this extract. Upon further exploring the methanolic extract by LC/MS, a rich pool of phytochemicals was revealed which included mostly flavonoids and biflavonoids, as amentoflavone, morelloflavone and sciadopitysin which have reported for their therapeutic property. Further evaluation of the amount of these important flavonoids and their role in governing the medicinal potency of this species needs to be explored.

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Conflict of Interest

The authors declare no conflict of interest.

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