Current Applied Science and Technology e-ISSN: 2586-9396

Curr. Appl. Sci. Technol. 2025, Vol. 25 (No. 6), e0264884

Research article

LC/MS Analysis and ACE Inhibition Activity of Aqueous and Solvent Extracted Fractions of *Selaginella bryopteris* from Different Geolocations

Utkarsha Srivastava and Ashwani Mathur*

Department of Biotechnology, Jaypee Institute of Information Technology, Noida, India

Received: 1 October 2024 , Revised: 13 February 2025 Accepted: 25 February 2025, Published: 22 April 2025

Abstract

Pteridophytes from different demographic locations are attracting scientific interest for exploration of environment mediated changes in their phytocompound compositions, with therapeutic and commercial importance. *Selaginella bryopteris* (L.) Baker is one such lithophytic pteridophyte that is endemic to Indian sub-continent. It is rich in a range of phytocompounds, including flavonoids and phenolics, some of which have been reported for their therapeutic properties including neuroprotection. The current work focused on a comparison of the composition and angiotensin converting enzyme (ACE) inhibition activities of three different geographical variants of *S. bryopteris* from Indian deccan terrain. The phytochemical and extraction yield analysis revealed the highest methanolic extract of 81.3 mg/g of dry plant. The methanolic fraction of germplasm was analysed by liquid chromatography-mass spectrometry and was rich in flavonoids and phenolics. The presence of important flavonoids, such as narignenin, may also be responsible for relatively high ACE inhibition activity of nearly 91%, which was found for all the extracts. In-silico toxicity analysis using ProTox 3.0 revealed the presence of less toxic phytocompounds (class 4 and 5). The pharmacokinetic studies using Swiss ADME suggested the pivotal role of phytocompounds in cardioprotective and neuroprotective properties. The study may be further extended for optimization of cultivation strategies for generation of phytocompound rich plant biomass.

Keywords: *Selaginella bryopteris*; ACE inhibition; geolocation; LC/MS; pharmacology

1. Introduction

Selaginella bryopteris (L.) Bak is a lithophytic pteridophyte growing in the colder climatic regions with sufficient water availability. The plant is found in varied regions, in India, extending from the Himalayas in the north to Arunachal Pradesh in the east and Andhra Pradesh in the south. It is known as a resurrection herb due to its drought resistant properties that enable the plant to protect itself from desiccation when water in insufficient supply and revive when optimum conditions are restored. This plant has long been reported for its therapeutic properties in ancient scriptures of South-east Asian countries such as

*Corresponding author: E-mail: Ashwani.mathur@mail.jiit.ac.in
https://doi.org/10.55003/cast.2025.264884

the Ramayana and the Charaka Samhita and have also been prescribed in Chinese traditional medicine, for its therapeutic properties (Pandey et al., 2017).

The resurrection properties of this plant have gained scientific attention leading to a need to explore its underlying mechanism and under-explored therapeutic potential. Currently, other species of this genus including *S. tamariscina*, *S. doederleinii*, *S. uncinate* and *S. moellendorffi* have been explored extensively for their hepato-protective, neuro-protective, antioxidant, antimicrobial and anticancer properties among others (Adnan et al., 2021). The limited evaluation of this plant and the exploration of other species from this genus have highlighted the presence of a rich flavonoid pool in the plant with the predominant presence of bioflavonoids. Some commonly reported phytocompounds manifesting therapeutic properties include amentoflavone and its derivatives, Sciadopitysin, naringenin, hinokiflavone, and campesterol and its derivatives (Adnan et al., 2021, Gautam et al., 2023).

The quest to explore the phytocompound catalogue and therapeutic properties of extracts from different germplasm is paving the way for regulating cultivation strategies and potential therapeutic benefits. The research gap can be addressed by exploring the consortia of phytocompounds in the extracts of *S. bryopteris* and the assessment of angiotensin converting enzyme (ACE) inhibition properties via a strategy towards growth, cultivation and regulation of phytocompound yield. The ACE inhibition assay can be considered as an initial screening strategy for *in vitro* analysis of cardioprotective compounds. ACE forms a part of the renin-angiotensin system which plays an important role in regulating hypertension, a major factor responsible for cardiovascular diseases (Cutrell et al., 2023). Hypertension and cardiovascular diseases have been accountable for nearly 32% of deaths globally as of 2019, making it important to find novel therapeutically important ACE inhibitors to control hypertension and associated cardiovascular diseases (WHO, 2021).

The present study is, therefore, important in expanding the therapeutic potential of *S. bryopteris* beyond its known efficacy as a neuro-modulator. Although the exact mechanism of action of the plant extract as an ACE inhibitor was out of scope for the present study but it can be evaluated in future studies. The plant materials were procured from three different geographical locations of India namely, Bengaluru in Karnataka (SBK), Kolhapur in Maharashtra (SBM) and Chennai in Tamil Nadu (SBT) and extracted in three different solvents, water (D), methanol (M) and acetone (A). An overview of the present study is depicted in Figure 1.

2. Materials and Methods

2.1 Chemicals

HPLC grade chemicals from Qualigens were used in the present study. Methanol (CAS No. 67-56-1), acetone (CAS No. 67-64-1) and sulfuric acid (CAS No. 7664-93-9) were used.

The plant materials were procured from three different geographical locations in India, namely Bengaluru in Karnataka, Kolhapur in Maharashtra, and Chennai in Tamil Nadu. Samples from Bengaluru and Chennai were recieved as potted plants while the plant from Kohlapur was received as a dried plant which was revived upon soaking the plant in water for a period of 24 h. The samples were identified at Dr. Y.S. Parmar University of

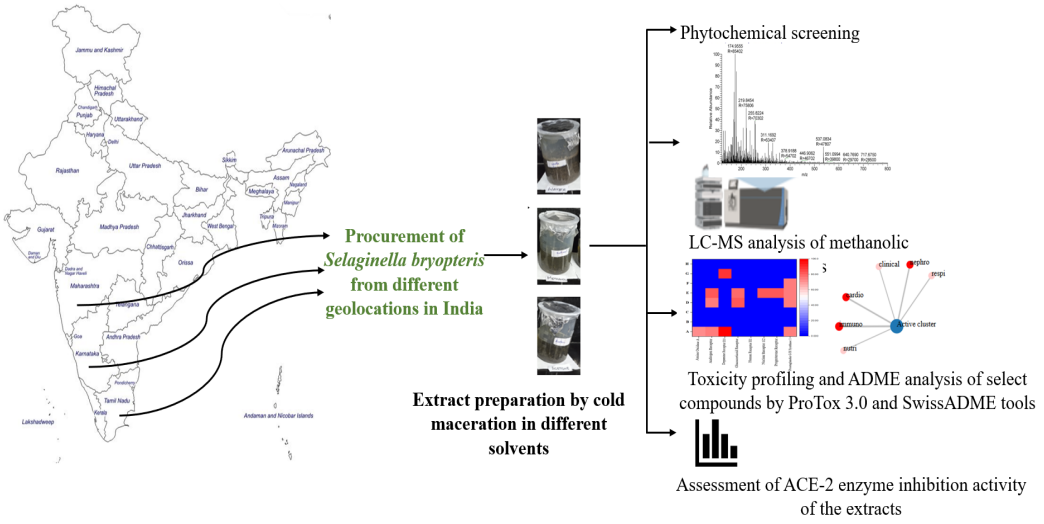

Figure 1. Overview of current study

Horticulture and Forestry, Himachal Pradesh, India. The whole plant material of *S.bryopteris* was used for extraction of phytochemicals by cold maceration technique in four different solvents, water, ethanol, methanol and acetone as per the method of Sarvade et al. (2020) with modifications. Briefly, coarsely ground plant material was soaked in extraction solvent in the ratio of 3:20 (w/v) for a period of two days, after which the extract was filtered, air dried for 48-72 h until a powdery mass was obtained, and stored at -20°C until further use.

2.2 Phytochemical analysis of plant extract

2.2.1 Saponins

The qualitative estimation of saponin was performed in all extracts using a froth test as reported previously by Tiwari et al. (2011) with modifications. Briefly, distilled water was added to each extract in the ratio of 1:1 by volume, followed by vigorous shaking. The formation of stable froth, persisting for 5-10 min confirmed the presence of saponins.

2.2.2 Tannins

The qualitative estimation of tannin was performed using Braymer's ferric chloride test as previously reported by Auwal et al. (2014) with modifications. Briefly, ferric chloride solution (10% w/v) was added to the extract in the ratio of 2:1 (by volume) for the appearance of greenish-black or bluish-green color solution.

2.2.3 Alkaloids

The qualitative estimation of alkaloids was confirmed by Drangendroff reagent test as reported earlier by Sharma et al. (2020). In brief, a small amount of extract (approximately 2 mL) was initially warmed with 2% sulfuric acid (v/v) and a few drops of Drangendroff

reagent was added to it. The formation of orange red precipitate confirmed the presence of alkaloids.

2.2.4 Steroids

The qualitative estimation of steroids was performed by modified Salkowski test method as reported previously by Auwal et al. (2014). Briefly, to a small amount of extract (approximately 0.2 g) nearly 5mL of chloroform was added and then filtered. A few drops of concentrated sulfuric acid was added to the filtrate. The appearance of reddish ring at the interface confirmed the presence of steroids.

2.2.5 Anthraquinones

The qualitative estimation of anthraquinones was done via Borntrager's reaction as reported by Auwal et al. (2014) but with modifications. A small amount of the extract (nearly 0.2 g) was dissolved in chloroform and boiled. The solution was filtered and 10% (v/v) ammonia solution was added to the filtrate at a volume ratio of 1:1. The formation of pink color in the upper layer confirmed the presence of anthraquinones.

2.2.6 Reducing sugars

The qualitative estimation of reducing sugars was done by Fehling's test as reported earlier by Auwal et al. (2014). Equal volumes of Fehling's solution I and II were added together to make Fehling's working solution. Fehling's solution was added to the different extracts in the ratio of 1:2 (v/v) and boiled in water bath. The development of a brick-red precipitate confirmed the presence of reducing sugars in the extracts.

2.2.7 Total flavonoid content

The quantification of total flavonoid content (TFC) was done by the aluminium chloride method as reported by Jagadish et al. (2009) but with alterations. Quercetin (1mg/mL) prepared in 95% (v/v) methanol was used as standard in different concentrations. Equal concentrations of the extract and 2% (v/v) $AlCl_3$ were mixed together. The incubation of the reaction mixture was done for 10 min at room temperature. Absorbance of the mixture was taken at a wavelength of 367 nm. Blanks were prepared in individual solvents and absorbance for the same were also taken. The total flavonoid content was expressed in terms of relative amount of flavonoid as quercetin equivalent per gram of extract (mg QE/g).

2.2.8 Total phenol content

The total phenolic content (TPC) in the extract was estimated quantitatively using Folin-Ciocalteu (FC) reagent test as mentioned earlier by Ainsworth et al. (2007). Gallic acid (1 mg/mL) in 95%(v/v) methanol was used as standard. Different concentrations were prepared from it. To 100 µL of extract, 200 µL 10% (v/v) FC reagent was added followed by addition of 800 µL 0.7M sodium carbonate solution. The mixture was incubated for 2 h at room temperature. The absorbance of the mixture was taken at 765 nm. Blanks were prepared in individual solvents and absorbance for the same were also taken. The total phenol content was expressed in terms of relative amount of phenolics as gallic acid equivalent per gram of extract (mg GAE/g).

2.3 Liquid chromatography/mass spectrometry

Liquid chromatography/mass spectrometric (LC/MS) analysis was done for the methanolic extracts of all plants. Qualitative evaluation of compounds in the extracts was done by Dionex Ultimate 3000 HPLC system from Thermo Fischer Scientific. Hypersil gold C-18 column was used (2.1 mm x 100 mm, 3.0 µm) with a column temperature maintained at 25°C. The flow rate in the LC column was maintained at 0.35mL/min for a duration of nearly 1 h. Formic acid in water (0.1%) was used as buffer A while formic acid in acetonitrile (0.1%) was used as buffer B. The chromatogram was analyzed for major peaks and area under the curve. The focus of the study was on the identification of compounds reported in *S. bryopteris* and its sister species. Additionally, the present work focused on the analysis of unexplored therapeutic properties of the major flavonoids and biflavonoids which could tentaively be identified by the LC/MS analysis. The major flavonoid group of compounds were shorlisted based on their area under the LC/MS chromatogram and cross-referenced with their reported chemical formula and organism in which they were present.

2.4 Toxicity profiling and ADMET analysis

The selected compounds identified by the LC/MS analysis were evaluated further for their pharmacokinetic activity using Swiss-ADME tool developed and maintained by the Swiss Institute of Bioinformatics (SIB) (Daina et al., 2017). The toxicity profiling of these compounds was done using ProTox 3.0 tool. The lethal dose (LD_{50}) and possible targets were identified using these tools.

2.5 Angiotensin converting enzyme inhibition assay

The ACE inhibition activity assay involves evaluating the ability of a compound to inhibit angiotensin-converting enzyme (ACE). It was performed as per the method by Cushman and Cheung (1971) with modifications (El-Sohaimy et al., 2022). A substrate solution of N-hippuryl-histidyl-leucine (HHL) at 3mM concentration was prepared in a borate buffer (~0.2 M, pH 8.3 with sodium chloride). ACE enzyme from rabbit lung was dissolved in borate buffer (~0.2 M, pH 8.3 without sodium chloride). Captopril was used as the standard and the color reagent used was cyanuric chloride in 1,4-dioxane. A mixture of substrate with buffer and enzyme stock (4:22:1 v/v) was incubated with test samples (0.5 mg/mL) and standard (volume same as the amount of enzyme) for the optimized time of 5-10 min at 37°C followed by the addition of color reagent in the ratio of 1:7 (v/v) to the mixture. Absorbance was recorded at 405 nm. The percentage ACE inhibition activity was calculated using equation (1):

$$\text{ACE inhibition percentage} = \frac{A - B}{A - C} \times 100 \quad (1)$$

Where, A is the absorbance of Hippuryic acid without any inhibitors,
B is the absorbance of Hippuryic acid with ACE enzyme inhibitors,
C is the absorbance of Hippyuric acid without ACE enzyme.

2.6 Statistical analysis

All the results were verified statistically using Data Analysis tool of Microsoft Corporation (2023), Microsoft Excel (version 2310). All the studies were performed in triplicate. The total phenol content and total flavonoid content were reported as mean±S.D. The linear regression coefficient (R^2) for all the studies was evaluated using Microsoft Excel. Single factor ANOVA with a p-value of less than 0.05 was used to test the significance of data. Student's t-test was performed for all the samples.

3. Results and Discussion

3.1 Extraction yield and phytochemical screening

Previous studies extensively reported the abiotic and biotic factor mediated changes in plant morphology and phytocompound composition (Liu et al., 2016). In the current study, the analysis of extracts of three different germplasms (demographic variants) of *S. bryopteris* showed variation in the extraction yield and phytocompound composition (Table 1). SBM-M had the highest extraction yield (81.3 mg/g dry plant) and % ACE inhibition (90.63±4.18), whereas SBT-A gave highest TPC of 53.89±1.92 (mgGAE/g) and TFC of 219.23±1.44 (mgQE/g). Among all extracts of SBK, SBK-D had the highest extraction yield (40.9 mg/g dry plant). The possible reason may be attributed to the different composition of the phytochemical consortia, which was influenced largely by the environmental factors. A high temperature of 30°C to 40°C is considered to inhibit flavonoid biosynthesis by suppressing the genes responsible for the expression of flavonoids but Bengaluru, Kolhapur and Chennai experience lower temperatures ranging from 20°C to 30°C with tropical climatic conditions which may be a possible reason for the variation seen in the flavonoid and phenolic content of all the extracts (Shi et al., 2022). Additionally, the variation in the precipitation at these geolocations, is also a determining factor of the biosynthesis of phytocompounds. The methanolic extracts of all the plants were found to be rich in tannins, alkaloids, steroids and reducing sugars. Some of these phytocompounds and their ability to solubilize in different solvents is pivotal when developing a phytochemical consortium. Previously, Iloki-Assanga et al. (2015) and Tourabi et al. (2023) showed similar variations in the nature of phytochemicals among different solvents. The results further strengthen the rationale of variation in the nature of compounds extracted in different solvents (Thouri et al., 2017). In the light of limited study available with respect to *S. bryopteris*, to the best of our knowledge, the study further strengthens the rationale of exploring the nature of phytocompounds in the methanolic extract of the germplasms using LC/MS.

3.2 Total phenol content, total flavonoid content and ACE inhibition activity

Flavonoids and phenols are two major groups of phytocompounds known for their catalogues of bioactive properties (Mutha et al., 2021). The results in the study showed that flavonoids and phenols were comparatively higher in acetone extracts for Maharashtra and Tamil Nadu and in methanol extract of Karnataka (Table 1). All the plants extracts

Table 1. Extraction yield, phytochemical evaluation and ACE inhibition (%) of *S. bryopteris* extract from three different geolocations. * Here '++' represents strongly present, '+' means weak color or precipitate development observed while '– 'represents absent. ND signifies 'Not Determined'.

	Extraction yield (mg/g of dry plant)	Qualitative Phytochemical Screening						Quantitative Phytochemical Screening		% ACE inhibition
		Saponins	Tannins	Alkaloids	Anthraquinones	Steroids	Reducing sugar	TPC (mg GAE/g)	TFC (mg QE/g)	
SBK-D	40.9	+	-	+	-	-	+	12.64±0.57	20.66±0.34	84.50 ± 2.97
SBK-M	23.4	-	++	++	-	+	+	48.22±1.43	210.33±1.83	32.75 ± 0.17
SBK-A	12.4	-	++	++	-	-	+	34.09±2.95	164.30±0.56	37.89 ± 1.54
SBT-D	26.7	-	-	+	-	-	+	5.41±1.03	6.03±0.33	69.03 ± 0.30
SBT-M	61	-	++	+	-	+	-	29.73±0.69	168.68±0.59	ND
SBT-A	10.6	-	-	+	-	-	-	53.89 ±1.92	219.23±1.44	66.68±0.04
SBM-D	44.4	-	-	+	-	-	+	6.17±2.19	8.53±1.03	51.84 ± 1.97
SBM-M	81.3	-	++	+	-	+	+	38.16±0.44	195.85±1.00	90.63 ± 4.18
SBM-A	32.9	-	++	+	-	-	-	36.15±1.80	194.63±0.86	ND

* The codes used in Table are as follows: SBK, SBT and SBM signify *Selaginella bryopteris* obtained from Karnataka, Tamil Nadu and Maharashtra States of India, respectively. The 'D', 'M' and 'A' of each geolocation signify the water, methanolic and acetone extracts of the respective germplasm.

showed better ACE inhibition activity when compared to the ACE inhibition potential of standard drug, Captopril, which showed only 17% of inhibition potential, suggesting the synergistic role of phytocompounds in ACE inhibition. The comparative assessment of phytochemical profile and ACE inhibition in different aqueous and non-aqueous extracts showed comparatively higher yield in methanol. However, the variation in the ACE inhibition activity may be attributed to the diversity in the pool of phenolics, flavonoids and other phytocompounds. The presence of compounds such as naringenin in the plant extract may be a major contributor to the ACE inhibition activity of *S. bryopteris*. The ACE inhibitory activity of naringenin was studied by Oyagbemi et al. (2020) on male Wistar rats. The results showed a decrease in the reactive oxygen species (ROS) and a decrease in the antioxidant activity of the organism. Naringenin was reported to ameliorate this condition by decreasing the ACE inhibition activity. Additionally, the presence of a rich flavonoid pool may contribute to the ACE inhibition activity of the plant extract, although its exact mechanism of action is not yet explored (Balasuriya & Rupasinghe, 2011). The results highlight the prominent impact of nature of solvents and their pivotal role in the type of phytocompounds getting solubilized suggesting that a higher yield of flavonoids is not the only representative of the therapeutically active compounds in the extract. The impact of environmental factors on the biosynthesis of secondary metabolites cannot be ignored. These secondary metabolites also play a major role in determining the therapeutic activity of the plant extract including the ACE inhibition activity (Pant et al., 2021).

3.3 LC/MS analysis

The LC/MS analysis has been instrumental in elucidating the nature of phytocompounds and their chemical structure and enhancing further purification strategies (Kumar, 2017). The results showed the presence of 15-20 major peaks in the different methanolic samples (Figures 2-4). The current study highlighted the screening of phytocompounds using ChemSpider structure database and Metabolika pathway library (Ehlers et al., 2023). The raw data analysis was done using ThermoFisher Scientific Compound Discoverer 3.3. The signature compounds of *S. bryopteris*, such as sciadopitysin, amentoflavone and their derivatives along with other major flavonoids were identified. Table 2 shows the major compounds identified with their maximum area and RT (in min). It also highlights the absence of these major compounds in the methanolic extract from Tamil Nadu showing the difference in the phytocompound consortia of this plant sample. The results also reported a dominant presence of flavonoid group, which was previously reported by Guerrero et al. (2012), for their role in regulating ACE-2 enzyme activity. The extract rich in these compounds can be a potential source for anti-cancer, hepatoprotective and anti-inflammatory properties. Apart from their therapeutic potential, these compounds also find roles in other industries including nutraceutical and cosmetics (Javed et al., 2024). Compounds such as amentoflavone find major applications in cosmetic industry due to their high antioxidant potential. Similarly, alpha-tocopherol also finds major application in cosmetic industry as a source of vitamin E or substitute. The major compounds analyzed using LC/MS were reported for their presence in previous studies on *Selaginella* species (Table 2). Furthermore, the selected compounds were screened for their toxicity and in-silico therapeutic properties using SwissADME and ProTox 3.0.

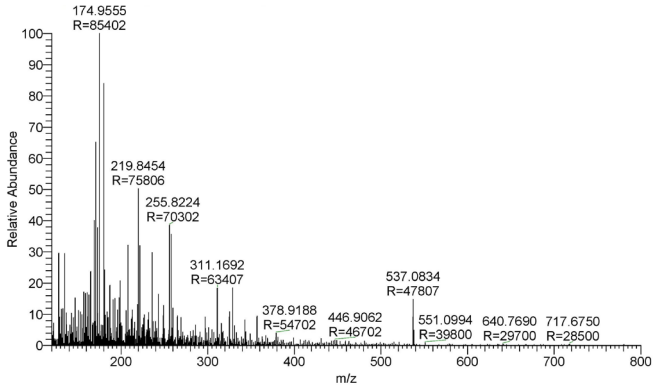

Figure 2. LC/MS chromatogram for sample from Karnataka (SBK) in the negative ionization phase

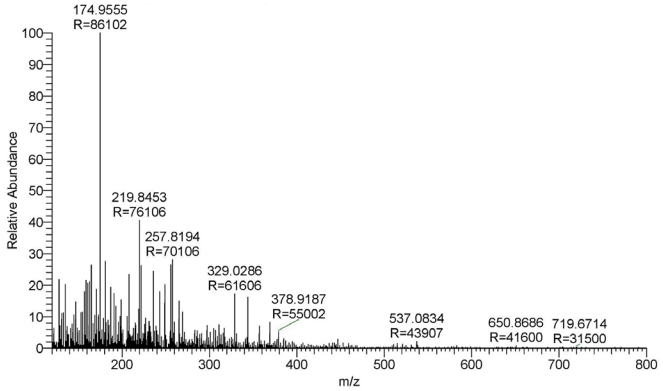

Figure 3. LC/MS chromatogram for sample from Maharashtra (SBM) in negative ioniztion phase

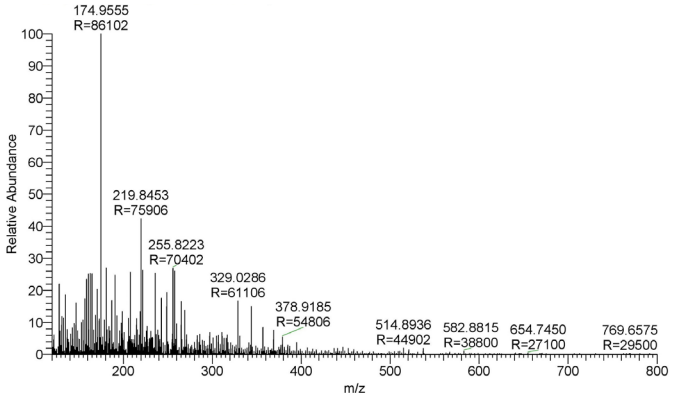

Figure 4. LC/MS chromatogram of sample from Tamil Nadu (SBT) in negative ionization phase

Table 2. Common compounds reported in the methanolic extracts of plants from different geolocations by LC/MS with their predicted LD_{50} and toxicity class (from ProTox 3.0 tool) and reference of *Selaginella* species in which they have been reported. **'**NA**'** indicates-Not applicable implying the compound was not detected in that sample.

Code	Compound	Molecular Formula	Synonymous Name (as identified in Literature)	Area under the Peak			RT (in min)			Predicted LD50 (mg/kg)	Predicted Toxicity Class	Reference
				SBK	SBM	SBT	SBK	SBM	SBT			
A	Amento-flavone	$C_{30}H_{18}O_{10}$	Amentoflavone	917092221.7	4183943421	NA	10.06	8.125	NA	3919	5	Weng and Noel (2013)
B	Volkensi-flavanone	$C_{30}H_{20}O_{10}$	Volkensiflavone(Naringenin yl (4 ,O,3) kaempferol)	68376048.67	109042545.1	NA	8.86	11.195	NA	2000	4	Swamy et al. (2006)
C	2,3-dihydro-4',4'''-di-O-methyl amentoflavone	$C_{32}H_{24}O_{10}$	2,3-Dihydroisoginkgetin	NA	NA	27134183.76	NA	NA	11.706	5000	5	Weng and Noel (2013)
D	2-(3-Hydroxy-3,7,11,15-tetramethylhexadecyl)-3,5,6-trimethyl-1,4-benzoquinone	$C_{29}H_{50}O_3$	alpha-Toco quinone	4476416.032	NA	NA	22.404	NA	NA	1680	4	Zeng et al. (2018)
E	(22R,23R,24S)-22,23-Dihydroxyergost-4-en-3-one	$C_{28}H_{46}O_3$	22,23-Dihydroxy-campest-4-en-3-one	276588492.5	139992947.2	11376832.45	22.175	22.401	22.412	1860	4	Cheon et al. (2013)
F	7-{[4,5-dihydroxy-6-(hydroxymethyl)-3-{[(2S,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxy}oxan-2-yl]oxy}-5-hydroxy-2-(4-hydroxyphenyl)-3,4-dihydro-2H-1-benzopyran-4-one	$C_{27}H_{32}O_{14}$	Naringenin	NA	2379289.998	40579376.96	NA	1.276	1.281	2300	5	Swamy et al. (2006)
G	Sciadopitysin	$C_{33}H_{24}O_{10}$	Sciadopitysin	1586431332	1455096742	155321207.7	14.18	14.353	14.374	4000	5	Demehin et al. (2022)

3.4 Toxicity and pharmacokinetic studies

Phytocompounds have been explored in recent studies for their toxicity in dose-dependent manner (Guldiken et al., 2018). ProTox 3.0 tool was used for the elucidation of phytocompound toxicity and categorization to different toxicity levels (Banerjee et al., 2024). The in-silico pharmacokinetic properties of selective phytocompounds in the methanolic extract were explored for their role in toxicity. The current study compared the toxicity of selected phytocompounds as listed in Table 2. The results showed that majority of compounds such as amentoflavone, volkensiflavone, naringenin and sciadopitysin were analyzed to be of toxicity classes 4 and 5 (Table 2) when analyzed by ProTox 3.0 online tool. Further analysis showed that the average pharmacophore fit of most of these compounds was 0% for some selected targets (Figure 5) suggesting that the phytocompounds selected may not be toxic to these common toxicity targets, the binding to which may result in adverse drug reactions. Pharmacokinetic analysis was performed using SwissADME and showed high bioavailability score (0.55) and no inhibition to cytochromeP450 (Table 3). A previous study by DeGoey et al. (2018) reported that bioavailability score may dominate Lipinski rule of five for selection of potential drug molecules. The results suggest that the selected compounds may be evaluated further for their use as bioactive molecules of therapeutic importance in a dose-dependent manner. Further studies would be important to validate these predictions. A high average similarity to known ligands is also indicative of similar biological activity, as reported previously by Utomo et al. (2020) in a study on the efficiency of naringenin ($C_{27}H_{32}O_{14}$) as a natural inhibitor of ACE-2 enzyme by molecular docking, while cardioprotective properties of amentoflavone were explored in a study by Alherz et al. (2022).

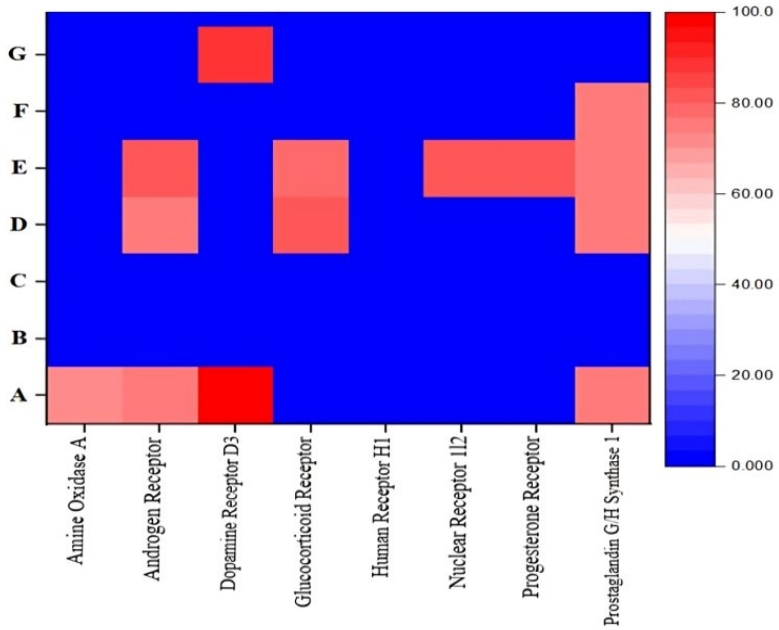

Figure 5. A heat map of pharmacophore fits of selected compounds with common toxicity targets. Code names used are as per the codes mentioned in Table 2.

Table 3. Predicted toxicity interaction with selected CYPs molecular targets (Molecule code is mentioned in Table 2)

Molecule	Silicos-IT class	GI absorption	BBB permeant	Pgp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor
A	Poorly soluble	Low	No	No	No	No	No	No	No
B	Poorly soluble	Low	No	No	No	No	Yes	No	No
C	Poorly soluble	Low	No	No	No	No	Yes	No	No
D	Poorly soluble	Low	No	Yes	Yes	No	No	No	No
E	Moderately soluble	High	No	No	No	No	No	No	No
F	Soluble	Low	No	Yes	No	No	No	No	No
G	Insoluble	Low	No	No	No	No	No	No	No

4. Conclusions

The paradigm shift from the well explored angiosperms to pteridophytes is evident by the growing number of research studies focusing on different genera. The current study explored the environment mediated changes in the phytochemical composition and ACE inhibition of not so well explored pteridophyte, *S. bryopteris.* The flavonoid content was found to be approximately 3 to 4 times higher than the phenolic content for all the extracts. The ACE inhibition was found to be highest for methanolic extracts of *S. bryopteris* from Maharashtra (90.63%). Dynamic biotic and abiotic environment is pivotal in causing significant variations in plant growth and phytocompound yield for three different geological variants of the pteridophyte, which were compared for the first time. The results revealed that significant variations in the phytocompounds pool were associated with toxicity and therapeutic potential, phytocompound yield and the ACE inhibition activity of the methanolic extract. The quest to explore the phytocompound mediated ACE inhibition paves the way for further exploration of the pteridophyte as a potential source of natural drugs. Furthermore, the study will be useful for the estimation of signature phytocompounds, their purification and dose dependent bioactivity assessment.

5. Acknowledgements

The authors acknowledge Central Instrumentation Facility, University of Delhi, for LC/MS analysis and Jaypee Institute of Information Technology, Noida, India for the provision of infrastructure for carrying out the work.

6. Conflicts of Interest

The authors declare that they have no conflict of interest.

ORCID
Utkarsha Srivastava https://orcid.org/0000-0003-1009-3338
Ashwani Mathur https://orcid.org/0000-0002-3858-8271

References

Adnan, M., Siddiqui, A. J., Arshad, J., Hamadou, W. S., Awadelkareem, A. M., Sachidanandan, M., & Patel, M. (2021). Evidence-based medicinal potential and possible role of *Selaginella* in the prevention of modern chronic diseases: Ethnopharmacological and ethnobotanical perspective. *Records of Natural Products*, 15(5), 330-355. http://doi.org/10.25135/rnp.222.20.11.1890

Ainsworth, E. A., & Gillespie, K. M. (2007). Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin– Ciocalteu reagent. *Nature Protocols*, 2(4), 875-877. https://doi.org/10.1038/nprot.2007.102

Alherz, F. A., El-Masry, T. A., Negm, W. A., & El-Kadem, A. H. (2022). Potential cardioprotective effects of Amentoflavone in doxorubicin-induced cardiotoxicity in mice. *Biomedicine and Pharmacotherapy*, 154, Article 113643. https://doi.org/10.1016/j.biopha.2022.113643

Auwal, M. S., Saka, S., Mairiga, I. A., Sanda, K. A., Shuaibu, A., & Ibrahim, A. (2014). Preliminary phytochemical and elemental analysis of aqueous and fractionated pod extracts of *Acacia nilotica* (Thorn mimosa). *Veterinary Research Forum,* 5(2), 95-100.

Balasuriya, B. W. N., & Rupasinghe, H. P. V. (2011). Plant flavonoids as angiotensin converting enzyme inhibitors in regulation of hypertension. *Functional foods in health and disease*, 1(5), 172-188. https://doi.org/10.31989/ffhd.v1i5.132

Banerjee, P., Kemmler, E., Dunkel, M., & Preissner, R. (2024). ProTox 3.0: a webserver for the prediction of toxicity of chemicals. *Nucleic Acids Research*, 52(W1), W513-W520. https://doi.org/10.1093/nar/gkae303

Cheon, J., Fujioka, S., Dilkes, B. P., & Choe, S. (2013). Brassinosteroids regulate plant growth through distinct signaling pathways in Selaginella and Arabidopsis. *PLoS One*, 8(12), Article e81938. https://doi.org/10.1371/journal.pone.0081938

Cushman, D. W., & Cheung, H. S. (1971). Spectrophotometric assay and properties of the angiotensin-converting enzyme of rabbit lung. *Biochemical Pharmacology*, 20(7), 1637-1648.

Cutrell, S., Alhomoud, I. S., Mehta, A., Talasaz, A. H., Van Tassell, B., & Dixon, D. L. (2023). ACE-Inhibitors in Hypertension: A historical perspective and current insights. *Current Hypertension Reports*, 25(9), 243-250.

Daina, A., Michielin, O., & Zoete, V. (2017). SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific Reports*, 7(1), Article 42717. https://doi.org/10.1038/srep42717

DeGoey, D. A., Chen, H. J., Cox, P. B., & Wendt, M. D. (2018). Beyond the rule of 5: lessons learned from AbbVie's drugs and compound collection: miniperspective. *Journal of medicinal chemistry*, 61(7), 2636-2651. https://doi.org/10.1021/acs.jmedchem.7b00717

Demehin, A. A., Thamnarak, W., Lamtha, T., Chatwichien, J., Eurtivong, C., Choowongkomon, K., Chainok, K., Ruchirawat, S., & Thasana, N. (2022). Siamenflavones A-C, three undescribed biflavonoids from *Selaginella siamensis*

Hieron. and biflavonoids from spike mosses as EGFR inhibitor. *Phytochemistry*, 203, Article 113374. https://doi.org/10.1016/j.phytochem.2022.113374

Ehlers, M., Uttl, L., Riedl, J., Raeke, J., Westkamp, I., Hajslova, J., Brockmeyer, J., & Fauhl-Hassek, C. (2023). Instrument comparability of non-targeted UHPLC-HRMS for wine authentication. *Food Control*, 144, Article 109360. https://doi.org/10.1016/j.foodcont.2022.109360

El-Sohaimy, S. A., Shehata, M. G., Mathur, A., Darwish, A. G., El-Aziz, N. M. A., Gauba, P., & Upadhyay, P. (2022). Nutritional evaluation of sea buckthorn "*Hippophae rhamnoides*" berries and the pharmaceutical potential of the fermented juice. *Fermentation*, 8(8), Article 391. https://doi.org/10.3390/fermentation8080391

Gautam, A., Pal, L. C., Rao, C. V., & Kumar, V. (2023). The role of Indian magical herb *Selaginella bryopteris* L. (Selaginaceae) in pharmacotherapeutic perspective: An overview. *Pharmacognosy Journal*, 15(1), 14-20. https://doi.org/10.5530/pj.2023.15.3

Guerrero, L., Castillo, J., Quinones, M., Garcia-Vallve, S., Arola, L., Pujadas, G., & Muguerza, B. (2012). Inhibition of angiotensin-converting enzyme activity by flavonoids: structure-activity relationship studies. *PloS One*, 7(11), Article e49493. https://doi.org/10.1371/journal.pone.0049493

Guldiken, B., Ozkan, G., Catalkaya, G., Ceylan, F. D., Yalcinkaya, I. E., & Capanoglu, E. (2018). Phytochemicals of herbs and spices: Health versus toxicological effects. *Food and Chemical Toxicology*, 119, 37-49. https://doi.org/10.1016/j.fct.2018.05.050

Iloki-Assanga, S. B., Lewis-Luján, L. M., Lara-Espinoza, C. L., Gil-Salido, A. A., Fernandez-Angulo, D., Rubio-Pino, J. L., & Haines, D. D. (2015). Solvent effects on phytochemical constituent profiles and antioxidant activities, using four different extraction formulations for analysis of *Bucida buceras* L. and *Phoradendron californicum*. *BMC Research Notes*, 8, Article 396. https://doi.org/10.1186/s13104-015-1388-1

Jagadish, L. K., Krishnan, V. V., Shenbhagaraman, R., & Kaviyarasan, V. (2009). Comparitive study on the antioxidant, anticancer and antimicrobial property of *Agaricus bisporus* (J.E. Lange) Imbach before and after boiling. *African Journal of Biotechnology*, 8(4), 654-661.

Javed, A., Azmat, R., Batool, M., Aqib, A. I., Hussain, S. A., & Ishtiaq, A. (2024). Hepatoprotective potential of sciadopitysin against paraquat induced liver damage in rats. *Journal of King Saud University-Science*, 36(8), Article 103328. https://doi.org/10.1016/j.jksus.2024.103328

Kumar, B. R. (2017). Application of HPLC and ESI-MS techniques in the analysis of phenolic acids and flavonoids from green leafy vegetables (GLVs). *Journal of Pharmaceutical Analysis*, 7(6), 349-364. https://doi.org/10.1016/j.jpha.2017.06.005

Liu, W., Yin, D., Li, N., Hou, X., Wang, D., Li, D., & Liu, J. (2016). Influence of environmental factors on the active substance production and antioxidant activity in *Potentilla fruticosa* L. and its quality assessment. *Scientific Reports*, 6(1), Article 28591. https://doi.org/10.1038/srep28591

Mutha, R. E., Tatiya, A. U., & Surana, S. J. (2021). Flavonoids as natural phenolic compounds and their role in therapeutics: An overview. *Future Journal of Pharmaceutical Sciences*, 7, Article 25. https://doi.org/10.1186/s43094-020-00161-8

Oyagbemi, A. A., Omobowale, T. O., Adejumobi, O., A., Owolabi, A., M., Ogunpolu, B. S., Falayi, O. O., Hassan, F. O., Ogunmiluyi, I. O., Asenuga, E. R., Ola-Davies, O. E., Soetan, K. O., Saba, A. B., Adedapo, A. A., Nkadimeng, S. M., McGaw, L. J., Oguntibeju, O. O., & Yakubu, M. A. (2020). Antihypertensive power of Naringenin is mediated via attenuation of mineralocorticoid receptor (MCR)/ angiotensin converting enzyme (ACE)/ kidney injury molecule (Kim-1) signaling pathway. *European Journal of Pharmacology*, 880, Article 173142. https://doi.org/10.1016/j.ejphar.2020.173142

Pandey, S., Shukla, A., Pandey, S., & Pandey, A. (2017). An overview of resurrecting herb 'Sanjeevani' (*Selaginella bryopteris*) and its pharmacological and ethnomedicinal uses. *The Pharma Innovation Journal*, 6(2), 11-14.

Pant, P., Pandey, S., & Dall'Acqua, S. (2021). The influence of environmental conditions on secondary metabolites in medicinal plants: A literature review. *Chemistry and Biodiversity*, 18(11), Article e2100345. https://doi.org/10.1002/cbdv.202100345

Sarvade, D. D., Rakesh, G., Shukla, V. J., & Acharya, R. (2020). Quantification of total alkaloid, tannin, flavonoid, phenolic, and chlorogenic acid contents of *Leea macrophylla* Roxb. Ex Hornem. *International Journal of Green Pharmacy*, 14(2), 138-145.

Sharma, T., Pandey, B., Shrestha, B. K., Koju, G. M., Thusa, R., & Karki, N. (2020). Phytochemical screening of medicinal plants and study of the effect of phytoconstituents in seed germination. *Tribhuvan University Journal*, 35(2), 1-11. https://doi.org/10.3126/tuj.v35i2.36183

Shi, Y., Yang, L., Yu, M., Li, Z., Ke, Z., Qian, X., Ruan, X., He, L., Wei, F., Zhao, Y., & Wang, Q. (2022). Seasonal variation influences flavonoid biosynthesis path and content, and antioxidant activity of metabolites in *Tetrastigma hemsleyanum* Diels & Gilg. *PLoS One*, 17(4), Article e0265954. https://doi.org/10.1371/journal.pone.0265954

Swamy, R. C., Kunert, O., Schühly, W., Bucar, F., Ferreira, D., Rani, V. S., Kumar, B. R., & Rao, A. V. N. A. (2006). Structurally unique biflavonoids from *Selaginella chrysocaulos* and *Selaginella bryopteris*. *Chemistry and Biodiversity*, 3(4), 405-413. https://doi.org/10.1002/cbdv.200690044

Tiwari, P., Kumar, B., Kaur, M., Kaur, G., & Kaur, H. (2011) Phytochemical screening and extraction: a review. *Internationale Pharmaceutica Sciencia* 1(1), 98-106.

Thouri, A., Chahdoura, H., El Arem, A., Omri Hichri, A., Ben Hassin, R., & Achour, L. (2017). Effect of solvents extraction on phytochemical components and biological activities of Tunisian date seeds (var. Korkobbi and Arechti). *BMC Complementary and Alternative Medicine*, 17, Article 248. https://doi.org/10.1186/s12906-017-1751-y

Tourabi, M., Metouekel, A., Ghouizi, A. E. L., Jeddi, M., Nouioura, G., Laaroussi, H., Hosen, M. E., Benbrahim, K. F., Salamatullah, A. M., Nafidi, H.-A., Wondmie, G. F., Lyoussi, B. & Derwich, E. (2023). Efficacy of various extracting solvents on phytochemical composition, and biological properties of *Mentha longifolia* L. leaf extracts. *Scientific Reports*, 13(1), Article 18028. https://doi.org/10.1038/s41598-023-45030-5

Utomo, R. Y., Ikawati, M., & Meiyanto, E. (2020). Revealing the potency of citrus and galangal constituents to halt SARS-CoV-2 infection. *Preprints*, 2020, Article 2020030214. https://doi.org/10.20944/preprints202003.0214.v1

Weng, J.-K., & Noel, J. P. (2013). Chemodiversity in *Selaginella*: a reference system for parallel and convergent metabolic evolution in terrestrial plants. *Frontiers in Plant Science*, 4, Article 119. https://doi.org/10.3389/fpls.2013.00119

WHO. (2021, June 11). *Cardiovascular diseases (CVDs).* https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds)

Zeng, X., Zhang, P., He, W., Qin, C., Chen, S., Tao, L., Wang, Y., Tan, Y., Gao, D., Wang, B., Chen, Z., Chen, W., Jiang, Y. Y., & Chen, Y. Z. (2018). NPASS: natural product activity and species source database for natural product research, discovery and tool development. *Nucleic Acids Research*, 46(D1), D1217-D1222. https://doi.org/10.1093/nar/gkx1026