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Research Article

QUALITATIVE AND QUANTITATIVE ANALYSIS OF PHYTOCHEMICALS IN FIVE *PTERIS* SPECIES

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ABSTRACT

Objectives: The objective of the present study was to find out the presence of phytochemicals in the methanol extracts of five ferns in Pteridaceae family such as *Pteris argyreae* T. Moore*, Pteris confusa* T.G. Walker*, Pteris vittata* L., *Pteris biaurita* L., and *Pteris multiaurita* Ag., by both qualitative and quantitative screening methods.

Methods: In qualitative analysis, the phytochemical compounds such as steroids, reducing sugars, triterpenoids, sugars, alkaloids, phenolic compounds, flavonoids, saponins, tannins, anthroquinones and amino acids were screened in five ferns extracts by using standard methods.

Results: The methanol extract of the fern *Pteris biaurita* showed positive results for 10 phytochemical tests. *Pteris vittata* extract exhibited positive results for 9 tests. In methanol extracts of the *Pteris argyreae, Pteris confusa* showed positive results for 7 tests and in the extract of *Pteris multiaurita* 5 phytochemical tests were positive. In quantitative analysis the important secondary metabolites such as alkaloids, phenolic compounds, flavonoids, saponins and tannins were tested in all the ferns extracts. The methanol extract of *Pteris biaurita* showed highest amount of phytochemicals when compared with other solvent extracts.

Conclusions: More active compounds will be isolated from the selected ferns and they may be used for medicinal purposes in future.

Keywords: Phytochemicals, Pteridaceae, Ferns, Qualitative and quantitative screening

INTRODUCTION

Plants are endowed with various phytochemical molecules such as vitamins, terpenoids, phenolic acids, lignins, stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains, and other metabolites, which are rich in antioxidant activity [1,2]. Studies have shown that many of these antioxidant compounds possess anti-inflammatory, antiatherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial, and antiviral activities [3,4]. The ingestion of natural antioxidants has been associated with reduced risks of cancer, cardiovascular disease, diabetes, and other diseases associated with ageing [5,6]and in recent years, there has been a worldwide trend towards the use of the natural phytochemicals present in berry crops, teas, herbs, oilseeds, beans, fruits and vegetables [7-9].

In recent years, secondary plant metabolites (phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents [10]. Thus, it is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of bacterial infections [11]. Pteridophytes are not infected by microbial pathogens, which may be one of the important factors for the evolutionary success of pteridophytes and the fact that they survived for more than 350 million years. Considering the rich diversity of Indian medicinal plants including Pteridophytes, it is expected that, the screening of plant extract for antibacterial activity may be beneficial for humans and plants diseases [12]. The aim of this study was to evaluate the phytochemicals from methanol extracts of five Pteris species.

MATERIALS AND METHODS

Collection of plant materials

Fresh leaves (fronds) of the selected ferns were collected randomly from the region of southern Western Ghats and their identification was confirmed with the help of herbarium specimens in Xavier's College Herbarium, St. Xavier's college, Palayamkottai. Fresh leaves were washed; shade dried and then powdered using the blender and stored in air tight bottles.

Methanol extraction

10 g of each plant powder was added to 100 ml of methanol in a conical flask and plugged with cotton wool . After 24 hours the supernatant was collected and the solvent was evaporated to make the crude extract and stored at 4°C.

Qualitative phytochemical analysis

Qualitative phytochemical analysis of methanol extracts of *P. argyreae, P. confusa, P. vittata, P. biaurita* and *P. multiaurita* was conducted following the standard procedures [13].

Quantitative phytochemical analysis

The phytochemicals which are present in the methanol extracts of *P. argyreae, P. confusa P. vittata, P. biaurita* and *P. multiaurita* were determined and quantified by standard procedures.

Determination of total phenolic compounds

100 mg of the extract of the sample was weighed accurately and dissolved in 100 ml of triple distilled water (TDW). 1 ml of this solution was transferred to a test tube, then 0.5 ml 2N of the Folin-Ciocalteu reagent and 1.5 ml 20% of Na_2CO_3 solution was added and ultimately the volume was made up to 8 ml with TDW followed by vigorous shaking and finally allowed to stand for 2 hours after which the absorbance was taken at 765 nm. These data were used to estimate the total phenolic content using a standard calibration curve obtained from various diluted concentrations of gallic acid [14].

Determination of total flavonoids

The method is based on the formation of the flavonoids - aluminium complex which has an absorptivity maximum at 415nm. 100µl of the plant extracts in methanol (10 mg/ml) was mixed with 100 µl of 20 % aluminum trichloride in methanol and a drop of acetic acid, and then diluted with methanol to 5ml. The absorption at 415 nm was read after 40 minutes. Blank samples were prepared from 100 ml of plant extracts and a drop of acetic acid, and then diluted to 5ml with methanol. The absorption of standard rutin solution (0.5 mg/ml) in methanol was measured under the same conditions. All determinations were carried out in triplicates [15].

Determination of total alkaloids

5 g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on

a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed [16].

Determination of total tannins

500 mg of the sample was weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 h in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtered was pipetted out into a test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.I N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min [17].

Determination of total saponins

The samples were ground and 20 g of each were put into a conical flask and 100 cm^3 of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separatory funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded.

The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight; the saponin content was calculated [18].

RESULTS AND DISCUSSION

Qualitative phytochemical analysis

In qualitative analysis of methanol extracts of *P. argyreae, P. confusa, P. vittata, P. biaurita* and *P. multiaurita* exhibited positive results for ten phytochemical tests. 10 phytochemical tests were positive in methanol extract of the fern *P. biaurita*. In *P. vittata* extract 9 tests were positive. In methanol extracts of the ferns *P. argyreae* and *P. confusa* 7 tests were positive. 5 tests were positive in *P. multiaurita* extract. Maximum tests were positive in methanol extracts of *P. biaurita* followed by *P. vittata*, *P. argyreae* and *P. confusa.* Minimum tests were positive in *P. multiaurita* extract (Table 1).

Table 1: Phytochemical analysis of five *Pteris* species

Compounds	*Pc*	*Pv*	*Pa*	*Pb*	*Pm*
Steroids	+	+	+	+	+
Triterpenoids	-	+	+	+	-
Reducing sugars	+	+	-	-	-
Sugars	-	-	-	+	-
Alkaloids	+	+	+	+	+
Phenolic compounds	+	+	+	+	+
Flavonoids	+	+	+	+	+
Catechins	-	-	-	-	-
Saponins	+	+	+	+	+
Tannins	+	+	+	+	-
Anthroquinones	-	-	-	+	-
Amino acids	-	+	-	+	-

Pc- *Pteris confusa* ***Pv-*** *Pteris vittata* ***Pa-*** *Pteris argyreae* ***Pb-*** *Pteris biaurita* ***Pm-*** *Pteris multiaurita*

Phytochemical compounds such as steroids, reducing sugars, triterpenoids, sugars, alkaloids, phenolic compounds, flavonoids, saponins, tannins, anthroquinones and amino acids were screened in five ferns extracts. Among these compounds alkaloids, phenolic compounds, flavonoids, saponins and tannins are important secondary metabolites and are responsible principles for medicinal values of the respective plant. These five compounds were present in all the extracts except *P. multiaurita*. Tannin was absent in *P. multiaurita* extract. All the extracts were subjected to further analytical tests for the quantification of phytochemical compounds.

Quantitative phytochemical analysis

The amount of phytochemicals which are found in the five ferns extract was quantitatively determined by standard procedures.

All the extracts of *Pteris* species showed different amount of phytochemicals. Among the five components flavonoids content was highest in all the selected ferns followed by alkaloids and phenolic compounds (Table 2). The amount of tannins and saponins was very low in the ferns extract.

Table 2: Quantitative analysis of phytochemicals (mg/g) in five *Pteris* species

Phytochemicals	*Pc*	*Pv*	*Pa*	*Pb*	*Pm*
Alkaloids	10.05 ± 0.10	12.10 ± 0.15	11.55 ± 0.30	16.40 ± 0.35	09.50 ± 0.15
Flavonoids	12.15 ± 0.50	14.20 ± 0.15	13.25 ± 0.40	17.55 ± 0.10	11.25 ± 0.10
Phenolics	09.33 ± 0.15	10.45 ± 0.10	08.05 ± 0.15	13.25 ± 0.50	07.10 ± 0.20
Saponins	06.22 ± 0.14	08.40 ± 0.45	07.10 ± 0.25	11.10 ± 0.65	05.40 ± 0.25
Tannins	03.06 ± 0.10	05.30± 0.15	04.60 ± 0.65	06.25 ± 0.40	-

Pc- *Pteris confusa* ***Pv-*** *Pteris vittata* ***Pa-*** *Pteris argyreae* ***Pb-*** *Pteris biaurita* ***Pm-*** *Pteris multiaurita*

P. confusa extract contained 10 mg of alkaloids, 12 mg of flavonoids, 9 mg of phenolic compounds, 6 mg of saponins and 3 mg of tannins. In *P. vittata* extract 12 mg of alkaloids, 14 mg of flavonoids, 10 mg of phenolic compounds, 8 mg of saponins and 5 mg of tannins were found. In *P. argyreae* extract 11 mg of alkaloids, 13 mg of flavonoids, 8 mg of phenolic compounds, 7 mg of saponins and 4 mg of tannins were observed. *P. biaurita* extract contained 16 mg of alkaloids, 17 mg of flavonoids, 13 mg of phenolic compounds, 11 mg of saponins and 6 mg of tannins. In *P. multiaurita* extract 9 mg of alkaloids, 11 mg of flavonoids, 7 mg of phenolic compounds and 5 mg of saponins were found.

Kumudhavalli and Jaykar (2012)[19] evaluated the petroleum ether, chloroform, acetone, ethanol and aqueous extracts of the fern *Hemionitis arifolia* for preliminary phytochemical screening. The ethanolic and aqueous extracts showed the presence of flavonoids, carbohydrates, phenolic compounds and sterols were the major phyto constituents. In the present study methanol extracts of ferns are screened for phytochemical analysis. Muraleedharannair *et al*. (2012)[20] examined the phyto-constituents of *Adiantum caudataum, Adiantum latifolium, Adiantum lunulatum, Christella dentate* and *Christella parasitica*, to provide chemical marker and inter-specific variation between the medicinally important genuses.

A total of five plants and 30 extracts were examined for the phytochemical screening. The crude extracts of *A. caudataum, A. latifolium, A. lunulatum, C. dentata* and *C. parasitica* showed varied degree of phyto-constituents with reference to solvents of the plant extracts. Pragada *et al*. (2011)[21] carried out Preliminary phytochemical analysis and quantification of total phenols, *in-vitro* antioxidant and antibacterial activities of the hydro alcoholic (70% ethanol) extract of *Acalypha indica.* Ramamoorthy *et al*. (2011)[22] screened the methanol extract of roots of *Gentiana kurroo* Royle (Gentianaceae) an important and endemic medicinal plant of Kashmir Himalaya for the presence of various bioactive plant metabolites and analgesic activity. The phytochemical analysis revealed the Presence of tannins, alkaloids, saponins, cardiac glycosides, terpenes, flavonoids, phenolics, and carbohydrates. Rajurkar and Kunda (2012) [23] screened *Adiantum capillus - veneris* for phytochemicals and metal content. The Soxhlet extraction of *Adiantum capillus veneris* showed the presence of phenolics and terpenoids (2.73 %), fats and waxes (0.20 %), alkaloids (0.53 %), quaternary and Noxides (26.33 %) and fiber (67.23 %). But in the present study, highest amount of alkaloids (16mg), flavonoids (17mg) and phenolics (13mg) are quantified in the selected *Pteris* species.

CONCLUSIONS

In the present study all the ferns extracts showed the presence of alkaloids, flavonoids and saponins. This study also leads to the further research in the way of isolation and identification of the active compound from the selected fern using chromatographic and spectroscopic techniques.

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