Research Article

Qualitative and Quantitative Phytochemical Analysis in Four Pteridophytes

Kalpana Devi Rajesh[1]*, Subramani Vasantha[1], Nakulan Valsala Rajesh[2], Annamalai Panneerselvam[1]
[1]Department of Botany and Microbiology, A.V.V.M Sri Pushpam College (Autonomous), Poondi, Thanjavur, Tamil Nadu, India.
[2]Research Assistant, Department of Animal Husbandry, Exotic Cattle Breeding Farm, Eachenkottai, Thanjavur, Tamil Nadu, India.
***Corresponding author's E-mail:** kalpanafern@gmail.com

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ABSTRACT

The objective of the present study was to find out the presence of phytochemicals in the aqueous, ethanol and petroleum ether extracts of four ferns *Actinopteris radiata, Drynaria quercifolia, Dryopteris cochleata* and *Pityrogramma calomelanos* by both qualitative and quantitative screening methods. In qualitative analysis, the phytochemical compounds such as Tannins, Saponins, Flavonoids, Quinones, Phenols, Terpenoids, Alkaloids, Glycosides, Cardio glycosides**,** Coumarins, Betacyanin, Anthocyanin and Steroids were screened. In quantitative analysis, the phytochemical compounds such as Total Tannin and Total Phenol were quantified. The ethanolic fern extract performed well to show positivity rather than aqueous and petroleum ether fern extracts for the 13 phytochemical qualitative tests. Ethanolic extract showed strong positivity for all 13 phytochemical tests. Aqueous fern extract showed positivity for the phytochemical tests in the four ferns. Petroleum ether fern extracts showed almost negativity in the four fern species studied. Ethanolic fern extract showed strong positivity for phenol and tannin in all the four fern species. In quantitative analysis the important secondary metabolite for Anthelmintic activity such as total phenol and total tannin content were tested. The ethanolic fern extract of total tannin and total phenol content were highest in *Pityrogramma calomelanos* and least in *Drynaria quercifolia.* More active compounds will be isolated from the selected fern and a comparative study should be analyzed for medicinal purpose in future

Keywords: Actinopteris radiata, Drynaria quercifolia, Dryopteris cochleata, Phytochemicals, Pityrogramma calomelanos, Pteridophytes.

INTRODUCTION

The use of traditional medicines holds a great promise as an easily available source as effective medicinal agents to cure a wide range of ailments among the people particularly in tropical developing countries like India. In this context, the people consume several plant or plant derived formulations to cure helmintic infections.[1] 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.[2,3] Studies have shown that many of these antioxidant compounds posses antitumor, anti inflammatory, anti atherosclerotic, anti mutagenic, anticarcinogenic, anti bacterial, anti viral and anti-parasitic activities.[4, 5]

In recent years, secondary metabolites (phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents.[6] Thus, it is anticipated that phytochemicals with adequate anti-parasitic efficacy will be used for the treatment of various nematode infections.[7] Considering the rich diversity of Indian medicinal plants including Pteridophytes, it is expected that, the screening of plant extract may be beneficial for humans and animal diseases.[8] The aim of this study was to evaluate the phytochemicals from aqueous, ethanolic and petroleum ether extracts of four fern species.

MATERIALS AND METHODS

Collection of plant materials

Fresh whole ferns (fronds, stems and rhizomes) were collected randomly 2 No's (*Actinopteris radiata* and *Pityrogramma calomelanos*) from the region of Southern Western Ghats (Kanyakumari) and 2 Nos (*Drynaria quercifolia* and *Dryopteris cochleata*) from Eastern Ghats (Kolli hills) and their identification was confirmed with the help of herbarium specimens in Scott Christian College, Nagercoil. Four (4) collected pteridophytes were washed in running tap water and cut into small pieces. Firstly, the plant materials were shade dried and then in Hot air oven at 55-60^{o}C. Dust was prepared by pulverizing the dried leaves, stems and roots with the help of mixer. A 25- mm mesh diameter sieve was used to obtain fine dust and preserved them into airtight plastic container, labeled, till their use in extract preparation.

Qualitative Phytochemical analysis

Phytochemical studies of the collected ferns were carried out in Poonga Biotech Research Laboratory, Arumbakkam, Chennai. Phytochemical screening of the extracts was carried out according to standard procedure.[9]

The Qualitative phytochemical analysis was carried out from the whole parts of plant material powdered and was extracted by the distillation method using soxhlet apparatus.[10] Different solvent systems were used for the separation of chemicals especially to study the

Anthelmintic property of the Pteridophytes (*Actinopteris radiata, Drynaria quercifolia, Dryopteris cochleata,* and *Pityrogramma calomelanos*) according to the polarity (Aqueous, Ethanol and Petroleum ether), to identify the major natural chemical groups such as Tannins, Saponins, Flavonoids, Quinones, Phenols, Terpenoids, Alkaloids, Glycosides, Cardio glycosides**,** Coumarins, Betacyanin, Anthocyanin and Steroids.

Test for alkaloids

Mayer's reagent

Mercuric chloride (0.355 g) was dissolved in 60 ml of water and 5 g of potassium iodide was dissolved in 20 ml water. Two solutions were mixed and volume was made up to 1000 ml with distilled water.

One ml of the Plant extract (0.5 g) was mixed with about 1 ml of 1% HCl, warmed and filtered. 2 ml of filtrate were treated separately with Mayer's reagent. Turbidity or precipitation, green colour was observed to indicate the presence of alkaloids.

Test for Anthocyanin and Betacyanin

To 2 ml of the leaf extract, 1 ml of 2N sodium hydroxide was added and heated for 5 min at 100°C. Formation of bluish green colour indicates the presence of anthocyanin and formation of yellow colour indicates the presence of betacyanin.

Test for cardio glycosides

One ml of the leaf extract, added with 2 ml of glacial acetic acid and few drops of 5 % ferric chloride were added. This was under layered with 1 ml of concentrated sulphuric acid. Formation of brown ring at interface indicates the presence of cardio glycosides.

Test for Coumarins

One ml of the plant extract (0.5 g) was taken in a small test tube and covered with filter paper moistened with 1 N NaOH. The test tube was placed for few minutes in boiling water. Then the filter paper was removed and examined in UV light for yellow fluorescence to indicate the presence of Coumarins.

Test for flavonoids

3 ml of the leaf extract was mixed with 4 ml of 1N NaOH in a test tube. Formation of dark yellow colour was observed which indicated the presence of flavonoids.

Test for Glycosides

Two ml of the leaf extract was added with three ml of chloroform and 1 ml of 10% ammonium solution was added. Formation of pink colour indicates the presence of glycosides

Test for Phenols

To 1 ml of the leaf extract, 2 ml of distilled water followed by 0.5 ml of sodium carbonate and Folin Ciocalteau's reagent (0.5 ml) added to the extract. Formation of blue / green colour indicates the presence of phenols

Test for Quinones

To 1ml of the leaf extract, add 1 ml of concentrated sulphuric acid. Formation of red colour indicates the presence of quinones.

Test for Saponins

Plant extract (0.5 g) was dissolved in 2 ml of boiling water in a test tube, allowed to cool and shaken well to mix thoroughly. The appearance of foam indicates the presence of Saponins.

Test for Steroids

To 1 ml of the leaf extract, 2 ml of chloroform and 1 ml of Sulphuric acid (H_2SO_4) were added. Formation of reddish brown ring at interface indicates the presence of steroids.

Test for tannins

About 0.5 g of plant extract was boiled in 20 ml of distilled water in a test tube and then filtered. 1ml of the leaf extract added with 5 % $FeCl_3$ (1 ml) was added to the filtrate. Appearance of brownish green coloration showed the presence of tannins.

Test for Terpenoids

To 1 ml of the leaf extract add 2 ml of chloroform, and then add 1.5 ml concentrated Sulphuric acid carefully. Formation of reddish brown colour at the interface indicates the presence of Terpenoids.

Quantitative phytochemical analysis

Quantitative phytochemical analysis was studied for Total Phenol and Total Tannin content, which were responsible for the major Anthelmintic activity of the ferns under study. For the quantification 4 ferns having strong positive for phenols and tannin content (*Actinopteris radiata, Drynaria quercifolia, Dryopteris cochleata* and *Pityrogramma calomelanos*) were determined and quantified by standard procedure.[11]

Determination of total tannins

Tannin content of the given sample was estimated by following the standard procedure.[12, 13] The sample extract (1 ml) was mixed with Folin-Ciocalteau's reagent (0.5 ml), followed by the addition of saturated Na_2CO_3 solution (1 ml) and distilled water (8 ml). The reaction mixture was allowed to stand for 30 min at room temperature. The supernatant was obtained by centrifugation and absorbance was recorded at 725 nm using UV-Visible Spectrophotometer. Increasing concentrations of standard tannic acid was prepared and the absorbance of various tannic acid concentrations was plotted for a standard graph. The tannin content was expressed as mg tannic acid equivalent per 100 gram of the sample.

Determination of total phenol

The Folin–Ciocalteau reagent method has been used for the estimation of total phenolic extracts quantities.[14] Different concentrations of sample extracts of the plant have been prepared and then 100 µL have been taken from each concentration and mixed with 0.5 mL of Folin–Ciocalteau reagent (1/10 dilution) and 1.5 mL of Na_2CO_3 2% (w/v). The blend was incubated in the dark at room temperature for 15 min. The absorbance of blue-colored solution of all samples was measured at 765 nm. The results were expressed in mg of gallic acid equivalent (GAE) per g of dry weight of plant powders.

Table 1: Qualitative analysis of phytochemicals in four fern species

Plants name	Solvents	Tannins	Saponins	Flavonoids	Quinones	Glycosides	Cardio glycosides	Terpenoids	Phenol	Coumarins	Steriods	Alkaloids	Anthocyanin	Betacyanin
A.radiata	A	+	-	+	+	-	+	-	+	+	-	+	-	+
	E	++	+	++	++	-	+	+	++	+	+	+	-	+
	P	-	+	-	-	-	-	+	-	-	+	-	-	-
D.quercifolia	A	+	+	+	+	-	+	-	+	-	-	-	-	+
	E	++	++	+	++	-	+	+	++	+	+	-	-	+
	P	-	-	-	-	-	+	-	-	-	+	-	-	-
D.cochleata	A	++	+	+	++	-	+	-	+	+	-	-	-	+
	E	++	-	+	++	-	++	-	++	-	+	+	-	+
	P	-	-	-	-	-	-	-	-	-	-	-	-	-
P.calomelanos	A	+	+	+	+	-	-	+	+	+	-	-	-	+
	E	++	+	+	+	+	+	++	++	+	++	+	-	+
	P	-	-	-	-	-	-	-	-	-	-	-	-	-

++: Strong Positive; +: Positive; '-' : Negative

Table 2: Quantitative analysis of phytochemicals (mg/g) in four fern species

Phytochemicals	Ar	Dq	Dc	Pc
Total tannin	12.189 ± 0.258	6.332 ± 0.187	9.405 ± 0.299	17.181 ± 0.441
Total phenol	10.962 ± 0.327	7.131 ± 0.184	8.912 ± 0.310	13.581 ± 0.481

Ar: Actinopteris radiata; Dq: Drynaria quercifolia; Dc: Dryopteris cochleata; Pc: Pityrogramma calomelanos

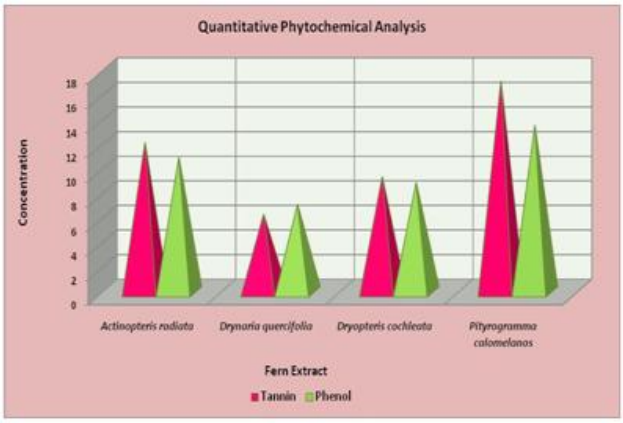

Figure 1: Quantitative Phytochemical Analysis

RESULTS AND DISCUSSION

Qualitative phytochemical analysis

In qualitative analysis of aqueous, ethanolic and petroleum ether extracts of *Actinopteris radiata, Drynaria quercifolia*, *Dryopteris cochleata, Pityrogramma calomelanos,* aqueous extract of *Dryopteris cochleata* alone showed strong positivity (++) for phytochemicals tannin and Quinones. Aqueous extract of other 3 ferns showed positivity (+) for major phytochemicals (Table 1). Ethanolic extract of all the 4 ferns showed strong positivity (++) for major phytochemicals. The important phytochemicals like tannin and phenol are well expressed in ethanolic extracts of the 4 ferns studied. However petroleum ether performed poorly and showed negativity (-) in almost all phytochemicals, especially *Dryopteris cochleata* and *Pityrogramma calomelanos* showed complete negativity for the 13 phytochemicals studied.

Quantitative phytochemical analysis

Total phenol and total tannins were subjected for quantitative analysis using the four fern extracts showed *Pityrogramma calomelanos* had maximum tannin content (17.181 ± 0.441 mg TAE/g) and highest phenol (13.781 ± 0.481 mg GAE/g) content. However *Drynaria quercifolia* (Table 2 and Fig 1) had least tannin (6.332 ± 0.187 mg TAE/g) and least phenol (7.131 ± 0.184 mg GAE/g) content.

Mithraja *et al.*[15] performed phytochemical screening of some important medicinal Pteridophytes of Western Ghats with the solvents acetone, benzene, chloroform, aqueous, ethanol and petroleum ether extracts of the whole plants of *D.heterophyllum, D.linearis, B.orientale, C.thalictroides, H.arifolia, L.ensifolia, N.multiflora,*

P.calomelanos, P.confusa and leaves and rhizomes of *D.quercifolia.* Analysis revealed tannin (47/66 extracts) presence in all tested plants and opined tannin containing drugs were found to posses anti-parasitic property which was in confirmation with our present study. Kumudhavalli and Jaykar[16] 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 aqueous, ethanolic and petroleum ether extract of ferns are screened for phytochemical analysis and ethanolic solvent extract expressed well to exhibit strong positivity for 13 phyto constituents studied. Muraleedharannair *et al.*[17] 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.lunmulatum, C.dentata* and *C.parasitica* showed varied degree of phyto-constituents with reference to solvent of the plant extracts. Sreejith *et al.* (2013)[18] performed phytochemical, anti-oxidant and anthelmintic activities of various leaf extracts of *Flacourtia sepiaria* Roxb using the solvent petroleum ether, ethyl acetate, methanol and chloroform extract. Jadhav *et al*.[19] conducted phytochemical studies in eleven species of ferns from Satara district of Maharashtra for the determination of chlorophylls, carotenoids and polyphenols. Santos *et al.*[20] reviewed on phytochemicals in Pteridophytes growing in Brazil. But our present study is on the phytochemical composition for Anthelmintic medicinal property of Pteridophytes. Rajurkar and Kunda (2012)[21] 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, quantitative analysis showed highest tannin (17.181 ± 0.441 mg TAE/g) and highest phenol (13.781 ± 0.481 mg GAE/g) content in *Pityrogramma calomelanos* and least tannin (6.332 ± 0.187 mg TAE/g) and least phenol (7.131 ± 0.184 mg GAE/g) content in *Drynaria quercifolia*.

CONCLUSION

In the present study among the four fern extract, ethanolic solvent performed well to show the phytochemicals rather than aqueous and petroleum ether extract. Quantitative analysis showed highest content of tannin and phenol in *Pityrogramma calomelanos* fern extract followed with least content of tannin and phenol in *Actinopteris radiata, Dryopteris cochleata* and *Drynaria quercifolia.* This study is an output for further research in application of this fern extract for Anthelmintic property especially *Pityrogramma calomelanos* holding higher phenolic and tannin content. Isolation and identification of the active compound from the selected fern using chromatographic and spectroscopic techniques are essential

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