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Full Length Research Article

Study on Phytochemical Screening of Some Selected Pteridophytes in Murshidabad District, West Bengal

Raja Kansabanik[1], Mithun Biswas[2], Sandip More[2] and Asim Mandal*[2]

[1] Department of Botany, Murshidabad University, Berhampore, Murshidabad - 742 101, West Bengal, India
[2] Department of Botany, Krishnath College, 1 No., Sahid Surya Sen Road, Gorabazar, Berhampore, Murshidabad - 742 101, West Bengal, India

Abstract

The main objective of the present study is to ascertain the presence of different phytochemicals in water, methanol, ethanol and acetone extracts of some selected species of pteridophytes by qualitative screening methods. The plant extracts are evaluated for the detection of certain phytochemicals such as alkaloids, glycosides, flavonoids, terpenoids, saponins, tannins, carbohydrates and proteins following standard methods. The result revealed the presence of several bioactive constituents which could be exploited for their potential applications for therapeutic purposes.

***Key words*:** Pteridophytes, Qualitative screening, Extraction, Phytochemicals, Murshidabad

Almost from the very beginning of human life, man has been connecting with plants. People from all continents have used medicinally important plants as a cure for several diseases since ancient times [1-2]. According to WHO, 80% of the population from the developing countries depends on traditional medicine system for their chief health care requirements [3]. People have known for over 2000 years that the pteridophytes are medicinal plants. However, in modern science of medicine, there are very few applications of pteridophytes compared to the angiosperms. Research on pteridophytes has developed over time in recent decades, and more information have been published on medicinal importance of pteridophytes. Pteridophytes are expected to comprise numerous effective secondary metabolites than other plants due to their existence from Paleozoic era [4]. Many useful phytochemicals or secondary metabolites for example, alkaloids, flavonoids, phenols, steroids, terpenoids, amino acids and fatty acids have been reported in pteridophytes.

A few prior investigations have also reported the presence of alkaloids in pteridophytes like *Pteridium aquilinum* [5], *Adiantum venustum* [6] and *Equisetum arvense* [7]. Literature survey has also admitted the presence of saponins in various ferns species, like *Adiantum venustum* [8] and *Equisetum arvense* [7]. *Pteridium aquilinum* [5], *Asplenium septentrionale* [9] are some of the fern species in which flavonoids have been detected previously. Shakir Ullah *et al*. [10], Rabiea Tanzin *et al*. [11], Shakoor *et al*. [12], Pan *et al*. [13], Rajesh *et al*. [14] and few other researchers had researched on qualitative phytochemical analysis in different pteridophytes. According to the preliminary check-list of pteridophytes [15-17], within present day political boundaries of India about 191 pteridophyte genera including more than 1250 species exist in India. The synergistic interaction among crude extracts or the active compounds improves the herbal or drug formulations. The objective of this study is to provide basic information for scientific research on medicinally important pteridophytes and to categorize them. In this study, a qualitative phytochemical screening was carried out in selected 10 species out of 21 species of pteridophytes in Murshidabad district, West Bengal, India.

MATERIALS AND METHODS

Collection of plant materials

The plant materials for the present study were collected from different areas of Murshidabad district, West Bengal, India. The identity of collected specimens was authenticated by Dr. Asim Mandal, Assistant professor of Botany, Krishnath College, Berhampore, Murshidabad.

Processing of plant materials

The plant materials used for phytochemical screening were thoroughly washed off under running tap water to get rid of all the debris and soil and then shade dried at room temperature. The air-dried plant material was finely packed in air tight polythene bags for further use.

Preparation of extracts

The plant extracts were prepared by standard methods. 5 grams of each plant sample was grinded by Mortar pestle in 50

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Correspondence to: Asim Mandal, Department of Botany, Krishnath College, 1 No., Sahid Surya Sen Road, Gorabazar, Berhampore, Murshidabad - 742 101, West Bengal, India, Tel: +91 9002524805; E-mail: asimbotvu@yahoo.com

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ml of water, ethanol, methanol and acetone. Each mixture was stirred carefully using a sterile glass rod. The extract was filtered through Whatman No. 1 filter paper. The filtrates so obtained were then stored in cool and dry place for further analysis using the standard procedures [18].

Screening procedure

The tests performed for the phytochemical screening are listed below.

a) *Tests for alkaloids*

Dragendroff's test

2 ml of Dragendroff's reagent is added to 1 ml of extract. Orange red precipitate is formed indicating the presence of alkaloids.

Wagner's test: 1 ml of Wagner's reagent is added to 1 ml of extract. The formation of reddish-brown precipitate denotes the presence of alkaloids.

Mayer's test: 2 ml of Mayer's reagent is added to 1 ml of extract. Formation of dull white precipitate indicated the presence of alkaloids.

b) *Tests for glycosides*

Keller- Killiani test: To 1 ml of extract, 1 ml of glacial acetic acid containing traces of ferric chloride and 1ml of concentrated sulphuric acid was added carefully. Appearance of brown ring at the interface indicates the presence of glycosides. A violet ring may also appear below that brown ring.

Borntrager's test: To 1 ml of extract, 1 ml of benzene and 0.5 ml dilute ammonia solution were added. A reddish pink colour detects the presence of glycosides.

Baljet test: To 1 ml of extract, 1 ml of sodium picrate is added. Appearance of yellow to orange colour confirms the presence of glycosides.

c) *Test for tannins*

Ferric chloride test: To 1 ml of extract, 1 ml of 5% ferric chloride (prepared in ethanol) solution was added. Blue black or dark green colour appeared.

Lead acetate test: On addition of 0.5 ml of 1% lead acetate solution to the extract white precipitate appeared.

Dilute HNO_3 test: On addition of few drops of dilute HNO_3 solution to the extract reddish colour appeared.

d) *Tests for flavonoids*

Shinoda test: To 1 ml of extract, few drops of concentrated HCl were added. To this solution 0.5 gram of magnesium turnings were added. Observance of pink coloration showed the presence of flavonoids.

Lead acetate test: To the 1 ml of extract, lead acetate solution was added. Formation of yellow precipitate indicated the presence of flavonoids.

Ferric chloride test: To 1 ml of extract, 1ml of ferric chloride (5% in water) was added. Formation of brown colour confirmed the presence of flavonoids.

e) *Tests for saponins*

Foam test: 1 ml of extract was shaken vigorously with 20 ml of distilled water for 5- 10 minutes in graduated cylinders. Formation of one centimeter layer of foam showed the presence of saponins.

Honey comb test: To the 5 ml of extract, few drops of sodium bicarbonate were added and shaken well. Honey comb like frothing indicated the presence of saponins.

f) *Tests for terpenoids*

Trichloroacetic acid test

To 1 ml of extract, 2 ml of trichloroacetic acid was added. Formation of coloured precipitate indicated the presence of terpenoids.

Salkowski test: 1 ml of extract was mixed with 2 ml of chloroform and concentrated sulphuric acid was added carefully along the sides of tube to form a layer. A reddish brown coloration of the interface was formed to confirm positive results for the presence of terpenoids.

g) *Test for carbohydrates*

Molisch's test

1 ml of extract was treated with few drops of Molisch's reagent (α-naphthol, 20% in ethyl alcohol). Then about 1 ml of concentrated sulphuric acid was added belatedly along the sides of the tube. Formation of violet colour shows the presence of carbohydrates.

Fehling's test: 1 ml of Fehling's A (Copper sulphate in distilled water) and 1 ml of Fehling's B (Potassium tartrate and sodium hydroxide in distilled water) reagents were mixed and boiled for a minute. Then equal volume of test solution was added to the above mixture. The solution was heated in a boiling water bath. Brick red precipitate was observed, showing the presence of carbohydrates.

h) *Tests for proteins and free amino acids*

Xanthoproteic test

1 ml of extract was treated with 1 ml of concentrated nitric acid solution. Formation of yellow colour shows the presence of proteins.

Ninhydrin test

2 ml of extract was treated with 1 ml of Ninhydrin solution. The mixture was boiled on a water bath. Appearance of blue to purple colour denotes the presence of amino acids.

RESULTS AND DISCUSSION

In the present study, 10 pteridophytes species were screened for the phytochemical constituents. Screening was performed with aqueous, ethanol, methanol and acetone extracts of the plants that make up a total of 40 extracts. In this preliminary study, out of 40 tested extracts, proteins were detected in 34 extracts and carbohydrates in 32 extracts. 30 extracts showed the presence of tannins. Next to that, 27 extracts tested positive for saponins. Flavonoids and terpenoids were detected in 25 and 23 extracts respectively. Alkaloids were observed in 22 extracts and 17 extracts showed occurrence of glycosides in the crude extracts of the investigated plants [19-20]. The results of qualitative phytochemical screening are summarizing in (Table 1).

Table 1 The qualitative phytochemicals analysis of extracts of pteridophytes species in different solvent system

Plant name	Solvents	Alkaloids	Glycosides	Tannins	Flavonoids	Saponins	Terpenoids	Carbohydrate	Protein
Adiantum caudatum Klotzsch	Water	+	-	+	-	+	-	+	-
	Ethanol	+	-	+	-	+	+	+	-
	Methanol	+	-	+	+	+	+	+	-
	Acetone	+	-	+	-	+	+	+	+
Ampelopteris prolifera (Retz.) Copel.	Water	-	+	-	+	+	+	+	+
	Ethanol	-	+	+	+	-	+	+	+
	Methanol	-	+	+	+	+	+	+	+
	Acetone	-	+	-	-	-	+	+	+
Azolla microphylla Kaulf	Water	-	+	+	-	+	+	+	+
	Ethanol	+	+	+	-	+	-	-	+
	Methanol	-	-	+	+	+	-	-	+
	Acetone	-	-	+	-	-	-	-	+
Christella dentata (Forssk.) Brownsey & Jermy	Water	+	+	+	+	+	+	+	+
	Ethanol	+	+	-	-	+	-	-	-
	Methanol	+	+	+	+	+	-	-	-
	Acetone	+	-	+	-	-	-	-	-
Diplazium esculentum (Retz.) Sw.	Water	-	-	+	+	-	-	+	+
	Ethanol	+	-	+	+	-	-	+	+
	Methanol	-	-	+	+	-	-	+	+
	Acetone	-	-	+	+	+	-	+	+
Dryopteris filix-mas (L.) Schott	Water	-	+	-	+	+	+	+	+
	Ethanol	-	+	-	-	-	+	+	+
	Methanol	-	+	-	-	-	+	+	+
	Acetone	-	+	-	-	-	+	+	+
Marsilea quadrifolia L.	Water	+	+	+	+	+	-	+	+
	Ethanol	-	+	+	+	+	-	+	+
	Methanol	+	+	+	+	+	-	+	+
	Acetone	-	-	+	-	+	-	+	+
Nephrolepis cordifolia (L.) K. Presl	Water	+	+	+	+	+	+	+	+
	Ethanol	+	-	+	+	+	+	+	+
	Methanol	+	-	+	+	+	+	+	+
	Acetone	+	-	+	+	+	+	+	+
Pteris vittata L.	Water	+	-	-	-	+	-	+	+
	Ethanol	+	-	+	+	-	-	+	+
	Methanol	+	-	+	+	-	+	+	+
	Acetone	-	-	+	-	-	+	+	+
Selaginella bryopteris L.	Water	+	-	-	+	+	+	-	+
	Ethanol	+	-	-	+	+	+	-	+
	Methanol	+	-	+	+	+	+	+	+
	Acetone	+	-	+	+	+	+	+	+

(+) indicates presence and (-) indicates absence

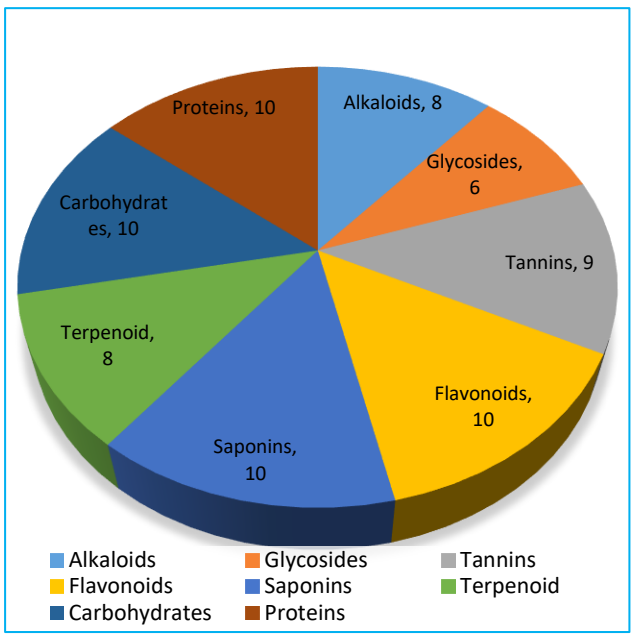

Fig 1 Distribution of phytochemicals within species

Out of 10 pteridophytes species, alkaloids are present in 8 (80%); glycosides in 6 (60%); tannins in 9 (90%); flavonoids in 10 (100%); saponins in 10 (100%); terpenoids in 8 (80%); carbohydrates in 10 (100%) and proteins were detected in 10 (100%) species. The maximum numbers of phytochemicals are reported in *Azolla microphyla*, *Christella dentata* and *Nephrolepis cordifolia*, and least number in *Diplazium esculentum* and *Dryopteris filix-mas* [21].

The phytochemical constituent utilizing four different solvents and their distribution within species is shown by the pie chart (Fig 1).

Conflict of interest

The authors asserted no conflict of interest. This research received no external funding.

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CONCLUSION

The study uncovered the presence of numerous medicinally active constituents in 10 species researched, proposing that few pteridophyte species can synthesize valuable metabolites. Preliminary phytochemical screening showed the presence of flavonoids, saponins, proteins and carbohydrates in all the plants investigated, whereas 9 species showed the presence of tannins, 8 species showed the presence of alkaloids and terpenoids, only 6 have depicted positive tests for Glycosides. Various evidences accumulated in earlier studies additionally confirmed the presence of bioactive phytoconstituents in pteridophytes. This information will draw in the consideration of ethno-botanist, phytochemists and pharmacologists for further critical examination of a few medicinal plants present in Murshidabad district, West Bengal, India. Additionally, the outcomes obtained in the present study are empowering and will give impulse for further research on phytochemical screening and extraction of pteridophytes in different parts of India.

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