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Phytochemical screening for active compounds in *Ceratopteris thalictroides* (L.) Brogn

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Abstract
The plant (whole) *Ceratopteris thalictroides* (L.) Brogn collected from Puthalam, Kanyakumari District, Tamil Nadu, India, were analyzed for the presence of different phytochemicals. The aim of our study is to screen the biologically active compounds in plant material, *C. thalictroides*. Phytochemical methods of screening proven the presence of alkaloids, steroids, coumarin, tannins, saponins, flavonoids, quinone, anthroquinone, phenol, protein, xanthoprotein, carbohydrate, glycosides, catachin, sugar and terpenoids in the extracts of the whole plants. The phytochemical composition of the whole plants indicate their medicinal properties.

Keywords: Phytochemical, pteridophyte, *Ceratopteris thalictroides*

Introduction
Like angiosperms plants, phytochemical studies of fern plants do not worked out extensively. From phytochemical analyses, it has been observed that fern plants contain higher levels of carbohydrate, amino acids, protein, lipid and secondary metabolites and these are economically useful product of any region [1]. Phytochemical characterization of plant material is important as it relates to the therapeutic actions. It is perhaps obvious that different species of plants would have different chemical constituents.
Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. Phytochemicals are organic, non-nutritive, naturally occurring chemicals found in plant foods. They are nonessential nutrients, meaning that they are not required by the human body for sustaining life. It is well-known that plant produces these chemicals to protect them but recent research demonstrates that they can also protect humans against diseases.
Further the importance of secondary metabolites like phenolic compounds and alkaloids as medicinal value has been highlighted. Several studies [2, 3, 4, 5] were made to evaluate the importance of ferns from chemical and pharmacological aspects. Humans use secondary metabolites as medicines, flavourings, and recreational drugs.
Ceratopteris thalictroides occurs in semi shaded localities mostly rooted in mud, occasionally free floating and common in paddy fields, ponds [6, 7, 8]. The fronds of *C. thalictroides* are used as a vegetable [9, 10]. The fronds of *C. thalictroides* are used as poultice in skin diseases [11]. The uncurled fronds are eaten as a salad or as a substitute for asparagus. The tribal people use the plant as a poultice for skin problems [12]. The whole plant parts are ground into paste and mixed with turmeric. The mixture is applied over the affected places to treat cure skin diseases and wounds [13, 14]. In Madagascar *C. thalictroides* leaves are eaten as salad or cooked as vegetable; whereas in Swaziland, leaves are eaten as leafy vegetable [15].

Materials and Methods
Plant Material
The plant material, *Ceratopteris thalictroides* (L.) Brogn., was collected from Puthalam (8.106488; 77.46), Kanyakumari District, Tamil Nadu, India and was authenticated at Botanical Survey of India, Southern circle, Coimbatore, Tamil Nadu; a voucher specimen (VS-VV-01) has been deposited in the Department of Botany, V.O. Chidambaram College, Tuticorin.

Preparation of Plant Extract
The collected plant material was washed under running tap water followed by sterilized distilled water to remove the soil and dust particles. The damaged parts of the plant was removed, cut into small pieces and then shade dried. The shade dried plant pieces were ground to coarse powder with the help of an electric grinder. After that the powdered plant material was stored in air-tight container for further investigation.

The coarse powder was subjected to extraction in 250ml each of petroleum ether, benzene, chloroform, ethyl acetate, ethanol and methanol solvents separately. The coarse powder (10g) of the plant material was weighted and put into the brown glass bottles. Then the solvents were added to it. Then the bottles were sealed with aluminium foil and kept in laboratory shaker at room temperature, and the bottles were shaken for one week. Finally the extract was filtered through many layers of muslin cloth for coarse filtration. The coarse filtrate was than filtered through Whatman number 1 filter paper. The obtained filtrate was evaporated in a vacuum rotary evaporator under reduced pressure at 40ºC until the filtrate was reduced to one-third of the starting filtrate volume and the concentrated extracts were further evaporated to get dry extracts. A part of dry extracts were re-dissolved in dimethyl sufloxide (DMSO) and were stored in stopper glass bottles and another part was kept as such in air-tight bottles at 0ºC for further analysis.

Phytochemical Screening

The different solvent extracts of *Ceratopteris thalictroides* were used for screening the presence of alkaloids, steroids, coumarin, tannins, saponins, flavonoids, quinone, anthroquinone, phenol, protein, xanthoprotein, carbohydrate, glycosides, catachin, sugar and terpenoids according to standard procedures of Harborne [16], Brindha *et al*. [17], Trease and Evans [18] and Sofowara [19].

Screening for Alkaloids (Dragendroff's test)

2ml of the extract was mixed with 8ml of 1% HCl, warmed and filtered. Then the filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

Screening for Steroids (Liebermann Burchard test)

Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

Screening for Coumarin

2ml of the extracts was taken in test tubes. The mouth of the tube was covered with filter paper treated with 3ml of 1N NaOH solution. Test tube was placed for few minutes in boiling water and then the filter paper was removed and examined under the UV light for yellow fluorescence indicated the presence of coumarins.

Screening for Tannins

50mg of various solvent extract powder was dissolved in 10ml distilled water and filtered. 1% aqueous iron chloride ($FeCl_3$) solution was added to the filtrate. The appearance of intense green, purple, blue or black colour indicated the presence of tannins in the test samples.

Screening for Saponin

50mg of the various solvent extract powder was boiled in distilled water in a test tube in boiling water bath and filtered. 10ml of the filtrate was mixed with 5ml of distilled water and was shaken vigorously to the formation of stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously for the formation of emulsion thus a characteristic of saponins.

Screening for Flavonoids (Shinoda Test)

To the extract solution (5ml), added few fragments of magnesium ribbon and concentrated HCl drop wise. Appearance of red or orange red colour indicates the presence of flavonoids.

Screening for Quinone

1ml of the extract was mixed with 1ml of concentrated H_2SO_4. Appearance of red colour shows the presence of Quinone.

Screening for Anthroquinone (Borntrager's test)

50mg of extract powder was taken into a dry test tube and 5ml of chloroform was added and shaken for 5min. The extract was filtered through Whatman No 1 filter paper and the filtrate was shaken with equal volume of 10% ammonia solution. A pink violet or red colour in the ammoniacal layer (lower layer) indicates the presence of anthroquinone.

Screening for Phenols

The extract powder (50mg) was dissolved in 5 ml of distilled water. To this few drops of 10% ferric chloride solution was added. Appearance of blue or green colour indicates the presence of phenol compounds.

Screening for Protein

The extract powder (50mg) was dissolved in 10ml of distilled water and filtered through Whatman No. 1 filter paper. To the filtrate, 1ml of 40% NaOH was added. Then, 1 or 2 drops of 2% copper sulfate solution was added. Appearance of violet colour indicates the presence of proteins.

Screening for Xanthoprotein

One ml each of the various extracts were treated separately with few drops of concentrated HNO_3 and NH_3 solution. Formation of reddish orange precipitate indicates the presence of xanthoproteins.

Screening for Carbohydrates (Molisch Test)

To 2ml of extracts, 3 drops of α-naphthol (20% in ethanol) was added. Then 1ml of concentrated sulphuric acid was added along the side of the test tube. Reddish-violet ring at the junction of the two layers indicated the presence of carbohydrates.

Screening for Glycosides (Borntrager's test)

Extract powder (50mg) was mixed with concentrated H_2SO_4 (5ml.), then it was heated for 3 minutes, thereafter it was filtered, after that filtrate was mixed with 0.5ml of 10% NaOH and allowed to stand for 3 minutes. Appearance of reddish brown precipitate indicates the presence of glycosides.

Screening for Catachin

To the extracts, a few drops of Ehrlich's reagent and concentrated hydrochloric acid were added. Appearance of pink colour indicates the presence of catechin.

Screening for Reducing Sugar

For the presence of reducing sugars in the extract Fehling test was performed. An amount of 50mg of the extract powder was taken and added it to the equal volume of boiling Fehling solutions (A and B) in a test tube. A brick- red precipitates indicates the presence of reducing sugar

Screening for Terpenoids (Salkowski test)

5ml of various solvent extract was mixed in 2 ml of chloroform followed by the careful addition of 3ml concentrated sulfuric acid (H_2SO_4). A layer of the reddish brown colouration was formed at the interface thus indicating a positive result for the presence of terpenoids.

Result and Discussion

Preliminary phytochemical screening of plants is important in the detection of bioactive principles which is a new source of therapeutically and industrially valuable compounds that may lead to the discovery of new drugs. In the present study, the presence of sixteen phytochemicals were screened in the petroleum ether, benzene, chloroform, ethyl acetate, ethanol and methanol extracts of the whole plants of *Ceratopteris thalictroides* and the results are shown in Table 1.

Table 1: Phytochemical compounds detected in the plant extracts.

S. No.	Compounds	Petroleum Ether	Benzene	Chloroform	Ethyl Acetate	Ethanol	Methanol
1	Alkaloids	-	-	+	+	+	+
2	Steroids	+	+	+	+	+	+
3	Coumarin	-	+	+	+	-	+
4	Tannins	-	-	+	-	-	+
5	Saponins	+	+	-	+	+	+
6	Flavonoids	+	+	-	-	-	+
7	Quinone	+	+	+	+	-	+
8	Anthroquinone	+	-	+	-	+	-
9	Phenol	+	+	+	+	+	+
10	Protein	-	+	+	-	-	+
11	Xanthoprotein	-	+	+	-	-	+
12	Carbohydrate	+	+	-	+	+	-
13	Glycosides	+	-	-	+	+	+
14	Catachin	+	+	+	+	-	+
15	Sugar	+	+	-	-	-	-
16	Terpenoids	-	-	+	-	-	+

Presence or absence of certain important bioactive compounds in an extract is determined by colour reactions of the compounds with specific chemicals which act as dyes. This procedure is a simple preliminary pre-requisite before going for detailed phytochemical investigation. In India traditional communities like tribal and rural populations are frequently using the crude extracts of local plants for medicinal and other purposes. Crude extracts and medicines manufactured on the principles of natural compounds even by pharmaceutical companies, may lead to large scale exposure of humans to natural products. The first step towards this goal is the biological and phytochemical screening of plant extracts from traditional preparations used in popular medicine. Hence, in the present study, the crude extracts obtained by petroleum ether, benzene, chloroform, ethyl acetate, ethanol and methanol solvents were screened for the presence of phytochemicals.

The petroleum ether extract showed the presence of steroids, saponins, flavonoids, quinine, anthroquinone, phenol, carbohydrate, glycosides, catechin and reducing sugar. The benzene extract showed the presence of steroids, coumarin, saponins, flavonoids, quinine, phenols, protein, xanthoprotein, carbohydrate, catechin and reducing sugar. The chloroform extract showed the presence of alkaloids, steroids, coumarin, tannins, quinine, anthraquinone, phenol, protein, xanthoprotein, catechin and terpenoids. The ethyl acetate extract showed the presence of alkaloids, steroids, coumarin, saponins, quinon, phenol, carbohydrate, glycoside and catechin. The ethanol extract showed the presence of alkaloids, steroids, anthroquinone, phenols, carbohydrate and glycosides. The methanol extract showed the presence of alkaloids, steroids, coumarin, tannins, saponins, flavonoids, quinone, phenol, protein, xanthoprotein, glycosides, catechin and reducing sugar. Among the phytochemicals, steroids and phenol were detected in all the presently investigated solvent extracts.

This research findings highlights that due to the abundance of various phytochemicals in the *Ceratopteris thalictroides* methanolic plant extract, it holds the great potential to treat various human diseases and has profound medical applicability. The presence of these important phytochemicals in *C. thalictroides* signals their therapeutic potential. They may function in stimulating digestion, act as anti-inflammatory, reducing swelling and pain, antioxidant and venotonics, antibacterial and antifungal, diuretic property that enhance the elimination of waste products and toxins and enhancing mood to give a sense of well-being [20].

Besides the present study was also conducted with an objective to identify the best extraction solvent, which can be used to extract the maximum amount of the phytochemicals from the *Ceratopteris thalictroides* dried plant leaves. Among all the solvents, the highest number of compounds were detected in methanol extract (13 compounds).

Conclusion

A comparative study has been conducted with an aim to achieve the best extraction solvent for the extraction of phytochemicals from *Ceratopteris thalictroides* plant. The results from this study demonstrate that using methanol as extraction solvent results in the maximum extraction of phytochemicals.

Besides, *Ceratopteris thalictroides* have known to possess many phytochemicals which play an important role in fighting against pathogens. The phytochemical screening demonstrated the presence of the secondary metabolites i.e., alkaloids, steroids, coumarin, tannins, saponins, flavonoids, quinone, anthroquinone, phenol, glycosides, catachin and terpenoids in extracts of *Ceratopteris thalictroides*. The phytochemical analysis of the plants is also important in pharmaceuticals companies for the novel drugs for the treatment of various diseases. Further studies are needed to strengthen the disease fighting ability of the *Ceratopteris thalictroides*.

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