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ORIGINAL ARTICLE

Qualitative Phytochemical Screening and Identification of Phytoconstituents from *Ceratopteris thalictroides* (L.) Brogn by FTIR and GC-MS.

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ABSTRACT
Plant-based drugs are the premise of some of the contemporary-day prescribed drugs we use nowadays for our diverse ailments. This study aimed to find out the bioactive chemical constituents of Ceratopteris thalictroides. A qualitative phytochemical evaluation become carried out for the detection of alkaloids, steroids, coumarin, tannins, saponins, flavonoids, quinone, anthroquinone, phenol, protein, xanthoprotein, carbohydrate, glycosides, catechin, sugar and terpenoids. FTIR became implemented and IR spectrum in mid-infrared vicinity 400-4000cm^{-1} became used for discriminating and to identify diverse user groups present withinside the medicinal plant. GC-MS analysis of the whole plant methanol extract of C. thalictroides was performed and a total of 28 compounds were identified. From the results, it is evident that C. thalictroides contains various phyto-components and is recommended as a plant of phytopharmaceutical importance.
Keywords*: Ceratopteris thalictroides, phytochemical screening, FTIR analysis and GC-MS analysis.*

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INTRODUCTION

Phytochemicals are organic, non-nutritive, obviously happening chemical substances observed in plant ingredients and have shielding or ailment preventive properties. They are nonessential nutrients, which means that they may be now no longer required with the aid of using the human body for maintaining life. It is well-known that plant produces these chemicals to protect them but recent research demonstrates that they can also be protecting human health from hazards [1]. The plant-based natural chemicals can be extracted from different parts of the plant such as leaves, fruits, flowers, seeds, roots, stems, barks, rhizomes etc. which reveal that every part of the plant contains this biologically active components [2]. In recent years, treatments through medicinal plants got tremendous attention worldwide [3]. Like angiosperms plants, phytochemical studies of Pteridophytes plants do not work out extensively [4]. Pteridophytes existed from the Palaeozoic era and they have faced many stochastic disturbances that led them to adapt to many serious changes in the environment [5]. Hence, Pteridophytes are predicted to contain several powerful secondary metabolites than other plants. Many beneficial phytochemicals or secondary metabolites, for instance, alkaloids, flavonoids, phenols, steroids, triterpenoids, various amino acids and fatty acids had been documented in the Pteridophytes [6,7]. Besides, they also contain unique phytochemicals, yet not found in higher plants [8]. In the recent past, many traditional medicinal Pteridophytes were analysed and reported to have various bioactivities, such as antioxidant, anticancer, antidiabetic, antiviral, anti-inflammatory, wound healing, antimicrobial and anti-Alzheimer activities [9]. Nowadays there is a global renaissance of medicinal plants research and great emphasis is being laid on exploring bioactive compounds and biological activities of plants owing to the natural origin, cost-

effectiveness and lesser side effects [10]. Therefore, in the present work, a qualitative phytochemical evaluation was done through preliminary phytochemical screening, FTIR and GC-MS in *Ceratopteris thalictroides*, a Pteridophyte plant species gathered from Puthalam, Kanyakumari District, Tamil Nadu, India to discover their medicinal value. *C. thalictroides* occurs in semi-shaded localities mostly rooted in mud, occasionally free-floating and common in paddy fields, ponds [11,12]. The fronds of *C. thalictroides* are used as a poultice in skin diseases [13]. In Andhra Pradesh, Chenchus of Nallamalais used leaf powder of *C. thalictroides* together with turmeric to treat unhealed wounds [14]. The fronds of *C. thalictroides* are used as a vegetable [15,16]. The whole plant parts are ground into a paste and mixed with turmeric. The mixture is applied over the affected places to cure skin diseases and wounds [17,18]. In Madagascar, *C. thalictroides* leaves are eaten as a salad or cooked as a vegetable; while in Swaziland, leaves are eaten as leafy vegetables [19]. The uncurled fronds are eaten as a salad or as an alternative for *Asparagus*. The tribal people use the plant as a poultice for skin problems [20].

MATERIAL AND METHODS

Materials

The plant material, *C. thalictroides*, a Pteridophyte belongs to the family Pteridaceae, was collected from Puthalam, Kanyakumari District, Tamil Nadu, India. Field photographs were taken by using a digital camera (Canon SLR 1200D). Care was taken to select healthy and mature plants. The collected specimen was properly processed in the laboratory for the preparation of herbarium [21]. The herbarium has been deposited at the PG & Research Department of Botany, V.O. Chidambaram College, Tuticorin, Tamil Nadu (VS-VV-01). The accrued specimen became diagnosed with the assist of floras like *The Ferns of India* (*Enumeration*, *Synonyms* & *Distribution*) [22], *Pteridophyte in Andhra Pradesh, India* [23] and *Pteridophytes of Karnataka State*, India [24]. After identity and verification of accurate identification, similar affirmation of identification become carried out at Botanical Survey of India, Southern Circle, Coimbatore, Tamil Nadu, India. The whole plant material of *C. thalictroides* was cut into small fragments and shade-dried. The shade-dried plant material was powdered by using a blender and sieved (sieve number 60) to get uniform particles. The final uniform powder of the plant was kept in air-tight containers and was used for further experimental studies.

Preparation of the Extract for Preliminary Phytochemical Screening

The extraction and protection of the extract had been executed in step with the technique of Kavitha [25] with a few modifications. About 25g of dried coarse powdered samples had been weighed and subjected to 250ml of successive solvent extraction. The extraction was done with the following solvents in increasing order of polarity viz., petroleum ether, benzene, chloroform, ethyl acetate, ethanol and methanol in a Soxhlet extractor for 12h. All the extracts were filtered through Whatman No. 41 filter paper separately. The acquired filtrate became evaporated in a vacuum rotary evaporator under reduced pressure at 40ºC till the filtrate became decreased to 1/3 of the beginning filtrate volume. The filtrate became amassed in the petri dish and to evaporate the remaining solvent, the extracts had been kept in an oven at a temperature of 40-50°C for 8h. The dried extract from the petri dish was scraped and transferred to the Eppendorf tube and was used for analysis. A part of dried extract powder was re-dissolved in 50% (v/v) different solvents separately each containing 2mg/ml extract and stored in an airtight container.

Preliminary Phytochemical Screening in Different Extracts

The methods described by Aiyelaagbe and Osamudiamen [26] were used to find out the presence of saponin and anthraquinone in the extracts. The method of Koperuncholan and Ahmed John [27] was adopted to find out the presence of catechin. The methods described by Saklani *et al* [28] were used to test the presence of alkaloids, steroids, flavonoids and proteins. The presence of coumarin, tannins, phenols and xanthoprotein were tested by the method of Suman Kumar *et al* [29] and the presence of reducing sugar and terpenoids were tested by the method of Sharma *et al* [30]. Carbohydrate presence was tested by the method of Leela Shivaranjani *et al* [31] while for quinones and glycosides the method of Jayapriya and Shoba [32] was adopted.

Preparation of the Extract for FTIR and GC-MS Analysis

The coarse powder (20g) of the plant material was weighed and put into the brown glass bottle. Then the solvent methanol (200ml) was added to it. Then the bottle became sealed with aluminium foil and kept in a laboratory shaker at room temperature, and the bottles have been shaken for 12h. The extract was then filtered through Whatman filter paper No. 41 along with 2g sodium sulphate to eliminate the sediments and strains of water withinside the filtrate. Before filtering, the filter paper along with sodium sulphate was wetted with methanol. The received filtrate was evaporated in a vacuum rotary evaporator under reduced pressure at 40ºC till the filtrate was decreased to one-third of the beginning filtrate volume. The

extract contained both polar and non-polar Phyto components of the plant material used and was used for GC-MS analysis [33]. A part of the filtrate was collected in a petri dish and then it was kept in an oven at a temperature of 40-50°C for 8h. The dried extract from the petri dish was scraped and transferred to the Eppendorf tube and was used for FTIR analysis.

Fourier-transform Infrared (FTIR) Spectroscopic Analysis

FTIR spectroscopic evaluation of the methanolic extract powder was performed through the potassium bromide (KBr) pellet method. 1mg of the methanolic extract powder was blended with 100mg of dry potassium bromide (1:100 ratio) after which the combination was compressed one after the other right into a pellet through the usage of a hydraulic press (5000-10000 PSI). The compressed pellet was put into the sample holder and the FTIR (Systronics166) spectra of the sample were recorded in the range of 400-4000cm^{-1}. To alleviate the moisture content in the sample, a blank disc was put in the reference beam [34].

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The sample was investigated through Gas Chromatography-Mass Spectrometry/Mass Spectrometry Electron Ionization (GC-MS/EI) mode. The GC-MS/MS is a Scion 436- GC Bruker version coupled with a Triplequadruple mass spectrophotometer with fused silica capillary column BR-5MS (5% Diphenyl / 95% Dimethylpolysiloxane) and Length: 30m; Internal diameter: 0.25mm; Thickness: 0.25µm. Helium gas (99.999%) was used as the carrier gas at a consistent flow rate of 1ml/min (split ratio of 10:1). The methanol extracts of the sample (2µl) were injected into the column. The injector and ion source temperatures were maintained at 250°C and 280°C, respectively. The oven temperature was programmed from 110°C (isothermal for 2min), with an increase of 10°C/min, to 200°C, then 5°C/min to 280°C, finishing with a 9min isothermal at 280°C and overall GC running time became 41min. This final increase was to clean the column from any residues. The mass spectrometer was operated in the positive electron ionization (EI) mode with an ionization energy of 70eV. The solvent delay was 0-3min. A scan interval of 0.5 seconds and fragments from m/z 50 to 500 Da was programmed. The inlet temperature was set at 280°C and the source temperature was set at 250°C. The relative per cent quantity of every component was calculated by comparing its average peak area to the whole area. Software adapted to handle mass spectra and chromatograms was MS Work station 8. The National Institute Standard and Technology (NIST) Version 2.0 Library Database was used for identifying the chemical components [33].

RESULTS AND DISCUSSION

Preliminary Phytochemical Screening

Preliminary phytochemical screening of plants is vital in the detection of bioactive principles that is a brand-new source of therapeutically and industrially precious compounds that can result in the invention of new drugs. In the prevailing study, the presence of 16 phytochemicals was screened in the petroleum ether, benzene, chloroform, ethyl acetate, ethanol and methanol extracts of the whole plants of *C. thalictroides* and the results are shown in Table 1.

Besides, the present study was also performed to become aware of the excellent extraction solvent, which may be used to extract the most quantity of the phytochemicals from the *C. thalictroides* dried whole plants. Among all the solvents, the highest number of compounds were detected in methanol extract (13 compounds).

FTIR Analysis

Fig. 1 shows FTIR spectra of whole plant methanolic extract of *C. thalictroides*. The spectra were interpreted using vibrational group frequencies characteristic of the most common functional groups and structural components [35,36] and the interpreted data were shown in Table 2. Phosphine is used to kill insect pests in grain, without affecting grain viability [37]. In plants, the alkanes are found in the plant cuticle and epicuticular wax of many species. Alkanes protect the plant against water loss, prevent the leaching of important minerals by the rain, and protect against bacteria, fungi, and harmful insects [38]. The aromatic amines are used in dyes, as antioxidants, and as precursors of pharmaceutical products [39].

GC-MS Analysis

The compounds present in the methanolic extract of whole plant powder of *C. thalictroides* were identified by GC-MS analysis. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) were presented in Table 3. Interpretation of mass spectrum of GC-MS was done using the database of NIST. The mass spectrum of the unknown component was compared with the spectrum of the known component stored in the NIST library. Major components were identified with authentic standards and recorded from computerized libraries. Twenty-eight compounds were identified in the methanolic extract by GC-MS.]

Table 1. Preliminary Phytochemical Screening of *Ceratopteris thalictroides* Whole Plant Powder

Compounds	Name of the Extract					
	Petroleum Ether	Benzene	Chloroform	Ethyl Acetate	Ethanol	Methanol
Alkaloids	-	-	+	+	+	+
Steroids	+	+	+	+	+	+
Coumarin	-	+	+	+	-	+
Tannins	-	-	+	-	-	+
Saponins	+	+	-	+	+	+
Flavonoids	+	+	-	-	-	+
Quinone	+	+	+	+	-	+
Anthroquinone	+	-	+	-	+	-
Phenol	+	+	+	+	+	+
Protein	-	+	+	-	-	+
Xanthoprotein	-	+	+	-	-	+
Carbohydrate	+	+	-	+	+	-
Glycosides	+	-	-	+	+	+
Catechin	+	+	+	+	-	+
Sugar	+	+	-	-	-	-
Terpenoids	-	-	+	-	-	+

Table 2. FTIR Spectrum Analysis of Methanolic Extracts of *Ceratopteris thalictroides*

Wavenumber (cm^{-1}) (Test sample)	Wavenumber (cm-1) (Reference article)	Functional Group/ Assignment	Phyto-compounds Identified
3788.75	> 3500	Non-bonded, O-H stretch	Hydroxyl group
3411.86	3415–3380	N-H stretch	Aromatic primary amine
2923.43	2935 – 2915	Methylene C-H asym. Stretch	Alkane
2853.19	2865 – 2845	Methylene C-H sym. Stretch	Alkane
2361.69	2280 – 2440	P-H phosphine	Phosphorous functions
2339.90	2280 – 2440	P-H phosphine	Phosphorous functions
1742.58	1760 – 1740	Alkyl carbonate	Carbonyl compound
1635.21	1680 – 1620	C=C stretch	Alkene
1461.31	1470 – 1430	Methyl C-H asym	Alkene
1381.04	1385 – 1380	gem-Dimethyl or "iso"- (doublet)	Alkane
1268.58	1270 – 1230	aryl -O stretch	Aromatic ethers
1156.72	1210 – 1150	C-N stretch	Tertiary amine
1104.06	1140 – 1070	C-O stretch	Cyclic ethers, large rings
1034.58	1055 – 1020	Silicone (Si-O-Si)	Silicon-oxy compounds
816.06	840 – 815	Nitrate ion	Common inorganic ions
779.21	800 – 700	C-Cl stretch	Aliphatic chloro compound
718.12	800 – 700	C-Cl stretch	Aliphatic chloro compound
669.86	680 – 610	C-H bend	Alkyne
468.90	500 – 430	S-S stretch	Aryl disulfides

Table 3. Bioactivity of phytocompounds identified in the methanol extracts of *Ceratopteris thalictroides*

RT	Compound Name	Molecular formula	MW	Peak area %	Compound nature	**Activity
3.93	Chlorozotocin	$C_9H_{16}ClN_3O_7$	313	3.15	Nitrosourea	Antimicrobial, Anti-inflammatory, Anticancer, Antioxidant
4.32	Trans-2-undecenoic acid	$C_{11}H_{20}O_2$	184	1.50	Unsaturated fatty acid	No activity reported
5.05	Decane, 2,4,6 trimethyl-	$C_{13}H_{28}$	184	7.70	Alkane	No activity reported
5.63	7-Diethoxymethylbicyclo [1.2.0] heptan-3-one	$C_{12}H_{20}O_3$	212	4.97	Ketone	No activity reported
8.08	Decanoic acid, ethyl ester	$C_{12}H_{24}O_2$	200	3.28	Saturated fatty acid ester	No activity reported

8.17	Tetradecane	$C_{14}H_{30}$	198	7.28	Alkane	No activity reported
10.82	Pterin 6 Carboxylic acid	$C_7H_5N_5O_3$	207	4.72	Aromatic nitrogen	Colour pigments
13.17	tert-Hexadecanethiol	$C_{16}H_{34}S$	258	2.88	Sulfur compound	Antimicrobial, Insecticide
13.41	7-Hexadecyn-1-ol	$C_{16}H_{30}O$	238	0.46	Unsaturated alcoholic compound	No activity reported
13.61	Z-(13,14 Epoxy tetradec-11en-1-ol acetate	$C_{16}H_{28}O_3$	268	5.65	Acetate compound	No activity reported
13.90	1,1-Dichloro-2-methyl-1 (4,4 diformyl-1,1-	$C_{10}H_{10}Cl_2O_2$	232	1.53	Chlorine compound	Antimicrobial
14.13	9,12 Octadecadienoylchloride, (Z,Z)	$C_{18}H_{31}ClO$	298	0.94	Linoleic acid chloride compound	Anti-inflammatory, Hypocholesterolemic, Cancer preventive, Hepatoprotective, Nematicide, Insectifuge, Antihistaminic, Antieczemic, Antiacne, 5-Alpha reductase inhibitor, Antiandrogenic, Antiarthritic
14.49	Albuterol	$C_{13}H_{21}NO_3$	239	1.40	Bronchodilator	Used to cure asthma
15.13	Dasycarpidan-1-methanol, acetate (ester)	$C_{20}H_{26}N_2O_2$	326	5.69	Indole alkaloid	Antibacterial, Antifungal, Anticancer, Antihypertensive, Antioxidant, Anti-inflammatory
15.42	B-Vatirenene	$C_{15}H_{22}$	202	5.57	Sesquiterpenoid compound	Antimicrobial, Anti-inflammatory, Antioxidant, Anticancer, Analgesic
15.54	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	2.11	Palmitic acid ethyl ester	Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Anti-androgenic, Flavor, Hemolytic 5-Alpha reductase inhibitor
15.64	Geranyl isovalerate	$C_{15}H_{26}O_2$	238	1.40	Sesquiterpene alcohol compound	Antimicrobial, Anti-inflammatory, Analgesic, Antioxidant, Anticancer, Analgesic
15.98	5,8,11-Heptadecatriynoic acid, methyl ester	$C_{18}H_{24}O_2$	272	3.00	Unsaturated fatty acid compound	No activity reported
17.17	Linolenin, 1-mono	$C_{21}H_{36}O_4$	352	2.68	Linolenic acid compound	Anti-inflammatory, Hypocholesterolemic Cancer preventive, Hepatoprotective, Nematicide, Insectifuge, Antihistaminic, Antieczemic, Antiacne, 5-Alpha reductase inhibitor Antiandrogenic, Antiarthritic
17.95	Cis-5,8,11,14,17-Eicosapentaenoic acid	$C_{20}H_{30}O_2$	302	0.44	Unsaturated fatty acid compound	Cardioprotective
18.43	Retinai, 9 cis-	$C_{20}H_{28}O$	284	3.42	Vitamin A compound	Skin care, Anti-inflammatory, Eye cure
19.11	Cholestan-3-ol, 2-methylene, (3β,5α)-	$C_{28}H_{48}O$	400	0.68	Steroid	Antimicrobial, Anti-inflammatory, Anticancer, Antiasthma, Diuretic, Hepatoprotective
19.81	Octadecanal, 2-bromo-	$C_{18}H_{35}BrO$	346	1.54	Aldehyde compound	Antimicrobial, Anti-inflammatory
22.39	Palmitoleic acid	$C_{16}H_{30}O_2$	254	2.93	Unsaturated fatty acid	No activity reported
22.93	DL-Phenylalanine, N-Chlorodiflurocetyl-ethyl ester	$C_{13}H_{14}ClF_2NO_3$	305	20.8	Amino acid compound	Used in skin diseases, Parkinson disease, rheumatoid arthritis and in weight loss
23.22	Isopropyl linoleate	$C_{21}H_{38}O_2$	322	0.97		Anti-inflammatory,

					Linoleic acid ester	Hypocholesterolemic Cancer preventive, Hepatoprotective, Nematicide, Insectifuge, Antihistaminic, Antieczemic, Antiacne, 5-Alpha reductase inhibitor, Antiandrogenic, Antiarthritic
26.71	2,3-Dihydroxypropyl elaidate	$C_{21}H_{40}O_3$	356	1.87	Oleic acid ester	Anti-inflammatory, Antiandrogenic, Cancer preventive, Dermatitigenic Hypocholesterolemic, 5-Alpha reductase inhibitor Anemiagenic, Insectifuge, Flavor
35.77	Pregnan-18-oic acid, 20-hydroxy-, (5α)-	$C_{21}H_{34}O_3$	334	1.39	Steroid	Antimicrobial, Anti-inflammatory, Anticancer, Antiasthma, Diuretic, Hepatoprotective

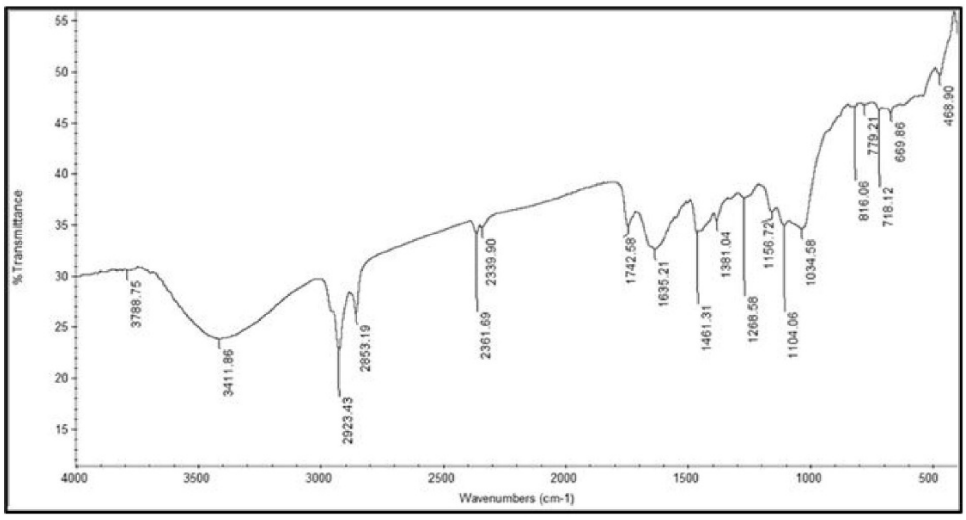

Fig: 1-FTIR Spectrum of Methanolic Extract of Ceratopteris thalictroides

CONCLUSION

The phytochemical screening of diverse solvent extracts and FTIR and GC-MS evaluation of methanol extracts of *Ceratopteris thalictroides* confirmed the existence of diverse bioactive compounds. The biological activities of a few diagnosed Phyto components used for antimicrobial, antifungal, antioxidant, anti-inflammatory, anticancer, diuretic antiasthma, antiarthritic. The research findings have shown that the whole plant extract of *C. thalictroides* is extensively rich in secondary metabolites. The whole plant has a high potential for a vast number of bioactive compounds which justified its use for the treatment of various ailments by traditional practitioners. These findings have formed a scientific basis for the ethnomedical usage of the plant. However, isolation of the individual phytochemical constituents and subjecting it to biological activity and toxicity profile may be significant further for the finding of a novel drug or a lead compound.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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