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Phytochemical characterization and GC-MS analysis of methanolic leaf extracts of *Coscinium fenestratum* (Gaertn.) Colebr

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ABSTRACT

In the present study, phytochemical analysis of methanolic leaf extracts of Coscinium fenestratum (Gaertn.) Colebr was carried out. GC-MS and Phytochemical analysis of the leaves of this taxon is reported for the first time. The leaves showed the presence of alkaloids, tannins, phenol, flavonoids, steroids, terpenoids and coumarins. The GC-MS analysis of the Coscinium fenestratum extract shows the presence of 54 bioactive compounds. The major constituents were cis-9-Hexadecenal (34.98, n-Hexadecanoic acid (20.14%), Hexadecanoic acid, trimethylsilyl ester (5.78%), 9, 12, 15-Octadecatrienoic acid, (Z, Z, Z) - (5.55%), 3-9-Octadecenoic acid (Z)-, methyl ester (4.67%) and Hexadecanoic acid, methyl ester (3.27%) which revealed a broad spectrum of medicinal properties and antioxidant activities.

Keywords: *Coscinium fenestratum*, Phytochemical screening, GC-MS analysis.

INTRODUCTION

Coscinium fenestratum (Gaertn.) Colebr. (Menispermaceae) commonly known as Maramanjal in Tamil is a plant of South Asian origin. The plant has been mainly used for treating diabetes mellitus in the traditional Ayurvedic and Siddha systems of medicine [1]. The stem contains berberine, ceryl alcohol, hentriacontane, sitosterol, palmitic acid, oleic acid and saponin, together with some resinous material. Isolation of tertiary alkaloids, berlambine, dihydroberlambine and noroxyhydrastinine from the roots has been reported [2]. The stem is used for dyspepsia, and as a febrifuge. Its hypotensive [3] and hepatoprotective [4] actions have also been reported. There were no previous reports on the GC-MS analysis of methanolic leaf extract of *C. fenestratum.* In the present investigation we attempted to conduct GC-MS analysis and Phytochemical analysis of methanolic extract of *C. fenestratum*

MATERIALS AND METHODS

Plant material

The leaves of *Coscinium fenestratum* were collected from the natural population growing in the Courtallam forest Area, Tirunelveli district, Tamil Nadu, India, during December 2015. The plant sample was carried to the Botany Research Laboratory and voucher specimen of the plant was deposited in the Botany research laboratory of V.H.N.S.N.College (Autonomous) for further references.

Preparation of leaves extracts
The plant leaves were cleaned, washed, shade dried and powdered for the phytochemical studies and extraction method using an organic polar solvent methanol. 30g of the dried leaves sample was taken in a conical flask and 200ml of methanol was added. The conical flask was kept on mechanical shaker for 24 hours, after which the extract was filtered through Whatman No: 1 filter paper in to a glass beaker and then allowed to dry by incubation in an oven at 64.7ºC. The dried extract was recovered and stored in refrigerator for further analysis.

Phytochemical Screening
Qualitative phytochemical analysis
The dried leaf extracts were subjected to GC-MS analysis and qualitative phytochemical screening for the identification of various classes of active biochemical constituents.

Test for Alkaloids (Mayers' Test)
To 1 ml of leaf extract, 6 drops of Mayer's reagent was added. The formation of yellowish creamish precipitate indicated the presence of alkaloids [5, 6].

Test for Saponins (Foam Test)
1ml of leaf extract was mixed with 5ml of distilled water. The contents were heated in a boiling water bath. Frothing indicated the presence of saponins [5, 6].

Test for Tannins (Braymers Test)
1ml of the leaf extract was mixed with 2 ml of water. To this, 2 drops of 5% ferric chloride solution was added. Appearance of dirty green precipitate indicated the presence of tannins [5, 6].

Test for Steroids (Salkowski Test)
To 2ml of the leaf extract, 2 ml of chloroform was added followed by concentrated sulphuric acid. Formation of reddish brown ring at the junction showed the presence of steroids. [7].

Test for Terpenoids
2ml of the leaf extracts was to with 2 ml of acetic acid. Then concentrated sulphuric acid was added. The formation of deep red color showed the presence of steroids [7].

Test for Coumarins
2 ml of the leaf extract was taken and 3 ml of 10% sodium hydroxide was added. The development of yellow coloration indicated the presence of coumarins [7].

Test for Catechins
2 ml of alcoholic leaf extract solution was treated with few drops of Echrilich reagent and few drops of concentrated HCL. The appearance of pink color indicated the presence of catechins [7].

Test for flavonoids
1ml of the leaf extracts was added with 1ml of sulphuric acid. Orange color formation confirmed the presence of flavonoids [8, 9].

Test for Quinones
1ml of the leaf extract was treated with 5ml of HCL. Formation of yellow color precipitate indicated the presence of quinone [8, 9].

Test for Phenols
1 ml of the leaf extracts was treated with 3% ferric chloride. The appearance of deep blue color, showed the presence of phenol [8, 9].

GC-MS Analysis
GC-MS analysis of these extracts was performed using a Perkin-Elmer GC Clarus 500 system and Gas chromatograph interfaced to a Mass spectrometer GC-MS equipped with a Elite-I, fused silica capillary column (30mmX0.25mm 1D X 1 iMdf, composed of 100% Dimethyl poly siloxane). For GC-MS detection, an electron

ionization system with ionizing energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas a at constant flow rate of 1ml/min and at an injection volume was 2il (split ratio of 10:1); The injector temperature was 250° C and Ion-source temperature was 280° C. The oven temperature was programmed from 110° C (isothermal for 2 min), with an increase of 10° C/min to 200° C, then 5° C/min to 280° C, ending with a 9 min isothemal at 280° C. Mass spectra were taken at 70 eV with a scan interval of 0.5 seconds and fragments length varied from 45 to 450 Da. Total GC running time was 36 minutes. The relative % amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was Turbomass. Interpretation on mass spectrum GC-MS was conducted using the database of National Institute of Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of unknown component was compared with the spectrum of the known components stored in the NIST library. The Name, Molecular weight and structure on the components of the test materials were ascertained.

Identification of compounds

The characteristics of the compounds identified in the extracts were elucidated by the comparison of their retention time and mass spectra fragmentation patterns with that of compounds stored in the computer library and also with published literatures. NIST and Wiley Library were used for matching the identified components from the plant material.

RESULTS AND DISCUSSION

Table 1: preliminary phytochemical screening of *Coscinium fenestratum*

S.No	Constituents	Methanol extracts
1.	Alkaloid	+
2.	Catechin	-
3.	Coumarin	+
4.	Flavonoid	+
5.	Phenol	+
6.	Quinone	-
7.	Saponin	-
8.	Steroid	+
9.	Tannin	+
10.	Terpenoid	+

+ = indicates presence of phytochemicals, - = indicates absence of phytochemicals.

The present study carried out on the methanol leaf extracts of *C. fenestratum* revealed the presence of medicinally active constituents. The phytochemical constituents of the leaves investigated are summarized in Table 1. Alkaloids, tannins, phenol, flavonoids, steroids, terpenoids and coumarins were present in the leaf extract. Catechins, quinones and saponins were found to be absent. Alkaloids have a bitter taste and many are toxic to other organisms [10]. Flavonoids are a group of polyphenolic compounds which influence the radical scavenging activity, inhibit hydrolytic and oxidative enzymes and also act as anti-inflammatory agent [11]. The flavonoids show antioxidant activity and their effects on human nutrition and health is considerable. The mechanism of action of flavonoids are through scavenging or chelating process [12, 13], Phenolic compounds prevent oxidative damage in living systems [14, 15]. The presence of tannins suggests the ability of this plant to play a major role as an antidiarrhoeal and antihemorrhagic agent [16]. In the present study, the leaf of *C. fenestratum* was preliminarily screened for the phytochemicals. The methanolic extract was found to be rich in all the phytoconstituents. An earlier report on the phytochemical analysis of *C. fenestratum* stem extract suggests that it possesses very high levels of alkaloids and flavonoids, phenols, terpenes and tannins and has medicinal uses [17].

Table 2: Shows GC-MS analysis of methanolic extract of leaf of *C. fenestratum*

Peak	R.time	Area%	Name
1	9.512	0.44	Cyclododecane
2	10.775	0.61	1,3-propanediol, 2-ethyl-2-(hydroxymethyl)-
3	11.808	0.16	2-bromotetradecane
4	12.084	1.23	Cetene
5	12.165	0.67	Tridecane
6	12.766	0.08	3,9-Dodecadiyne
7	12.915	0.09	N-tridecylcyclohexane
8	13.025	0.08	2-furanmethanol, 5-ethenyltetrahydro-.alpha.,.alpha.,5-tri
9	13.080	0.41	8-pentadecanone
10	14.142	0.13	Octadecanoic acid
11	14.316	0.10	Tridecane, 3-methylene-
12	14.375	0.66	1-nonadecene
13	14.441	0.34	Heptadecane
14	14.817	0.02	1-(3,3-dimethyl-1-butynyl)-1
15	14.886	0.85	2-hexadecen-1-ol, 3,7,11,15-tetramethyl-, [r-
16	15.148	0.12	9-octadecen-1-ol, (z)-
17	15.283	0.27	8-pentadecanone
18	15.343	0.37	3,7,11,15-tetramethyl-2-hexadecen-1-ol
19	15.786	3.27	Hexadecanoic acid, methyl ester
20	16.067	0.16	9-octadecenoic acid (z)-
21	16.242	20.14	N-hexadecanoic acid
22	16.440	0.17	1-octadecanol
23	16.496	0.13	Tetratriacontane
24	16.783	0.06	11-eicosaenoic acid 1tms
25	16.963	5.78	Hexadecanoic acid, trimethylsilyl ester
26	17.113	0.20	3-heptadecen-5-yne, (z)-
27	17.182	0.60	17-octadecen-14-yn-1-ol
28	17.261	1.49	Octadecanoic acid, 2-propenyl ester
29	17.472	1.14	9,12-octadecadienoic acid (z,z)-, methyl ester
30	17.518	4.67	9-octadecenoic acid (z)-, methyl ester
31	17.635	3.79	9,12,15-octadecatrienoic acid, methyl ester, (z,z,z)-
32	17.734	1.34	Octadecanoic acid, methyl ester
33	17.798	0.35	Methyl all-cis-9,12,15-octadecatrienoate
34	18.004	34.98	Cis-9-hexadecenal
35	18.110	5.55	9,12,15-octadecatrienoic acid, (z,z,z)-
36	18.171	0.63	Octadecanoic acid
37	18.265	0.27	9,12-tetradecadien-1-ol, acetate, (z,e)-
38	18.550	0.02	Pteridine-2,4-Diamine
39	18.619	0.74	Cis-5,8,11-eicosatrienoic acid, trimethylsilyl ester
40	18.747	0.62	1,e-8,z-10-hexadecatriene
41	18.900	0.39	4,7-methano-5h-inden-5-one, octahydro-
42	18.948	2.08	1,3-cyclohexadecanedione, 6-nitro-
43	19.094	1.37	9,12,15-octadecatrien-1-ol, (z,z,z)-
44	19.192	0.20	Octadecanoic acid, 2-propenyl ester
45	19.315	0.16	Methyl (z)-5,11,14,17-eicosatetraenoate
46	19.462	0.39	Docosanoic acid
47	19.759	0.13	Heptacosanoic acid
48	21.156	0.16	7-hexadecenal, (z)-
49	21.893	0.53	2-methyl-z,z-3,13-octadecadienol
50	22.106	0.29	Cis,cis,cis-7,10,13-hexadecatrienal
51	24.908	0.73	Cis-9-hexadecenal
52	25.067	0.19	Ethyl (9z,12z)-9,12-octadecadienoate #
53	25.168	0.07	Cis,cis,cis-7,10,13-hexadecatrienal
54	31.807	0.60	Cholest-5-en-3-ol (3.beta.)-, carbonochloridate
		100.00	

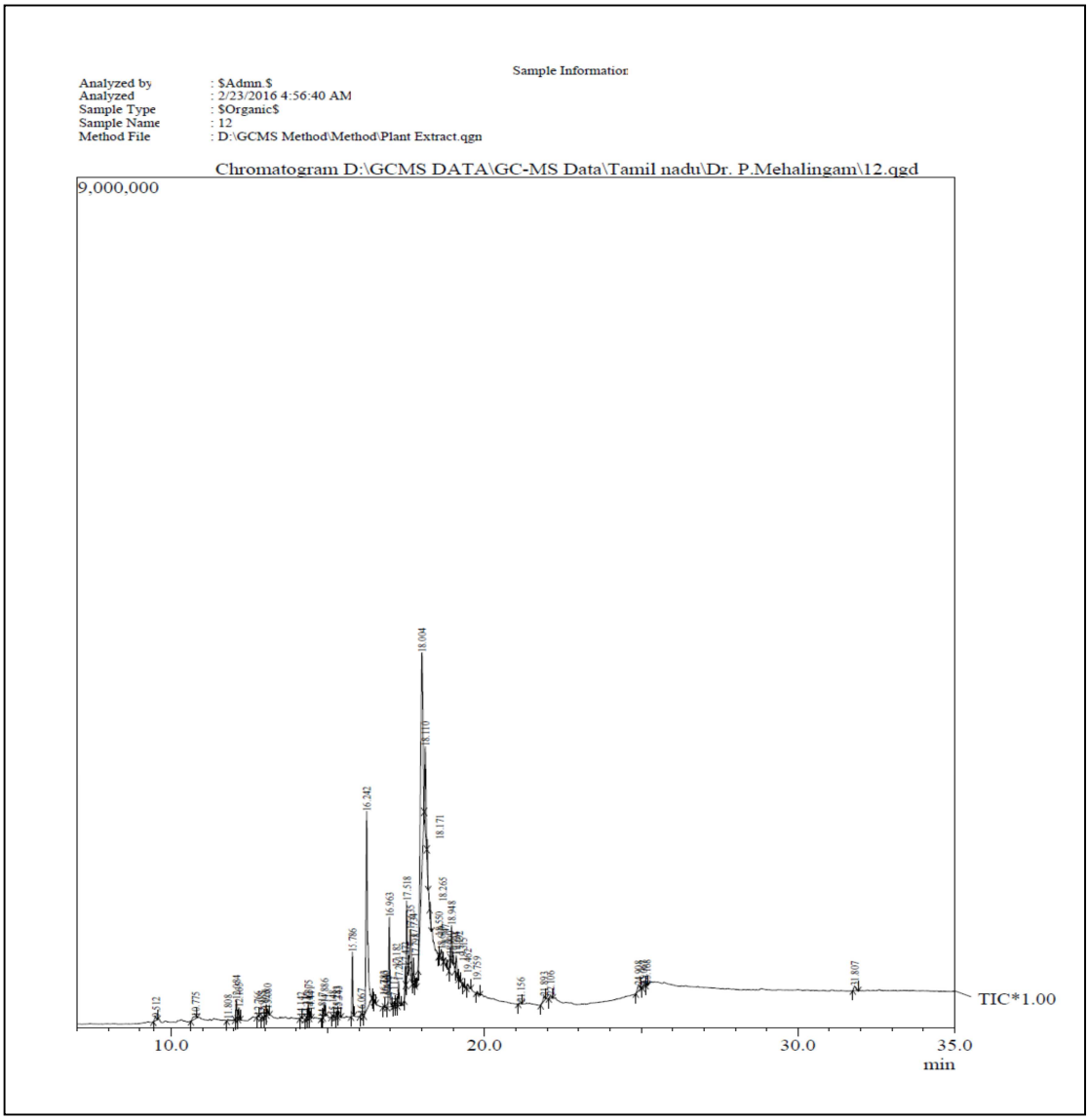

Fig. 1: Shows GC-MS Chromatogram of methanolic leaf extract of *C. fenestratum*

The phytoconstituent rich methanolic extract of *C. fenestratum* leaf was subjected to Gas Chromatography-Mass spectrometry analysis. The result revealed the presence of 54 compounds (Fig 1& Table 2). The major constituents were found to be cis-9-Hexadecenal (34.98, n-Hexadecanoic acid (20.14%), Hexadecanoic acid, trimethylsilyl ester (5.78%) 9, 12, 15-Octadecatrienoic acid, (Z, Z, Z) - (5.55%), 3-9-Octadecenoic acid (Z)-, methyl ester (4.67%), Hexadecanoic acid, methyl ester (3.27%) and other chemical constituents with less than 1% peak area. In a previous report by Jagadeep chandra and Lakshmidevi., 2015 [18] the phytoconstituent rich methanolic extract of *Cyclea peltata* (Menispermaceae) subjected to GC-MS analysis revealed the presence of 6 compounds. A similar report for chemical composition analysis of essential oil of *Cissampelos owariensis* presence 11 compounds by Olajide Olutayo et al., 2013 [19]. The presence of various bioactive compounds justifies the use of the flower of *C. fenestratum* for various ailments by traditional practitioners.

CONCLUSION

The investigation concluded that the strong extraction capacity of methanol could have produced high number of active constituents which are responsible for the many biological activities of the plant. Here it may be might be

utilized for the development of traditional medicines. Further investigation is in needed to discover novel active compounds from the medicinal plants which may create a new way to treat many incurable diseases.

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