



## PHYTOCHEMICAL PROFILING OF RHIZOME, PETIOLE AND LEAVES OF *CHRISTELLA DENTATA* (FORSSK.) BROWNSEY & JERMY USING GC-MS ANALYSIS

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### ABSTRACT

The study focuses on the Gas Chromatography-Mass spectrometry (GC-MS) analysis of ethanolic extract of various plant parts of *Christella dentata* (Forssk.) Brownsey & Jermy. GC-MS chromatogram of leaf extracts showed 32 peaks corresponding to different phytochemical constituents present in the extract. The most prevalent compound was Hexadecanoic acid that possesses anti-inflammatory, antimicrobial and antioxidant properties. GC-MS spectrum of petiole extract revealed 26 peaks. Seven compounds identified in the petiole extract were found to have medicinal properties. 16 compounds were detected in the GC-MS spectrum of rhizome extract. Maximum occurrence as per area percentage was recorded for Hydroxy methyl furfural, a compound with high degree of bioactivity. The results obtained reveal the medicinal importance of different parts of *Christella dentata*. However, isolation and *in vitro* and *in-vivo* bioactivity studies of various compounds are essential for establishing the pharmaceutical potential of this plant species.

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### INTRODUCTION

Phytochemical profiling of different plant species forms a major area of research today owing to its great significance in the pharmacognostic industry. Drug designing and development depend largely on the identification, isolation and characterization of various secondary metabolites present in plants. Pteridophytes, a primitive group of land plants have not received much attention earlier in this respect though a number of these plant species reportedly possess medicinal properties. In recent years, phytochemical studies in pteridophytes have attained great importance in India and the volume of literature regarding the phytochemical analysis of various fern species is increasing day by day. *Christella dentata* (Forssk.) Brownsey & Jermy member of fern family Thelypteridaceae is regarded as a facultative wetland plant with widespread distribution. The plant usually prefers wet habitat and flourish well along the banks of streams, riverbeds and in swampy sites, in drains and also grows under overhanging cliffs (Brownsey and Perrie, 2016). The plant is edible (Kumar *et al.*, 2003) and is used to treat skin diseases in folk medicine (Kumar and Dash, 2012). Not much literature is available with regard to phytochemical analysis of this fern species. The current study focuses on the phytochemical characterization of *Christella dentata* through GC-MS analysis.

### MATERIALS AND METHODS

#### Collection and preparation of dried plant material

The study material *Christella dentata* (Forssk.) Brownsey & Jermy was collected from Thrissur District of Kerala, India. The collected materials were separated into leaves, petiole, and rhizome. These were reduced in size by chopping them into smaller pieces using a blade and then dried using hot air at 45°C. The dried material was ground to fine powder by a domestic grinder and stored in containers till further use.

#### Ethanol Extraction

5 gram powder each of leaves, petiole and rhizome was weighed out and transferred to separate conical flasks each containing 50ml ethanol. These were left to stand for a period of two days on magnetic stirrer. Ethanol extracts were obtained by sieving to separate the extracts from the residue. It was followed by rinsing the extracts with 5ml ethanol each time followed by filtration to complete the separation of the extract from the residue. The extract was then concentrated to 1ml by evaporation of the solvent. Concentrated sample was subjected to GC-MS analysis.

#### GC-MS Analysis

GC-MS analysis of the ethanolic extract of *Christella dentata* was performed using a Perkin-Elmer GC Clarus 500 system comprising an AOC-20i auto-sampler and a Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with a Elite-5MS (5% diphenyl/95% dimethyl poly siloxane) fused capillary column (30 × 0.25 µm ID × 0.25 µm df). The mass-detector used in this analysis was Turbo-Mass

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Gold-Perkin-Elmer, and the software adopted to handle mass spectra and chromatograms was a Turbo-Mass ver-5.2.

## RESULTS AND DISCUSSION

GC -M S analysis revealed that different parts of *Christella dentata* contain various compounds that show antioxidant, antibacterial and anti-inflammatory activities. GC-MS chromatogram analysis of the ethanolic extract of *Christella dentata* leaves showed 32 peaks corresponding to different phytochemical constituents present in the extract (fig.1 and table 1a).

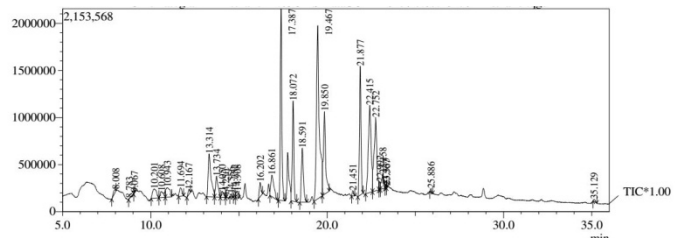


Fig 1 GC-MS spectrum of *Christella dentata* leaves

Table 1a Phytochemicals identified in the GC-MS analysis of ethanolic extracts of *Christella dentata* leaves

Peak#	R. Time	Area	Area%	Height	Height%	Name	Base m/z
1	8.008	366913	0.37	20401	0.18	5-Amino-L-propanoic N-Octadecyl-	144.05
2	8.783	70296	0.07	6571	0.06	2,3-DIHYDRO-BENZOFURAN	94.00
3	9.067	16193	0.02	6978	0.06	2-Cyclopenten-1-one, 5-hydroxy-2,3-dimethyl-	55.05
4	10.201	1517334	1.52	99750	0.86	2-Methoxy-4-vinylbenzol	150.10
5	10.608	103592	1.03	66626	0.57	DECAENOIC ACID	159.15
6	10.943	1710356	1.71	102953	0.88	(TRANS)-2-NONADECENE	55.00
7	11.694	886902	0.89	90829	0.78	1-(3,3-Dimethyl-but-1-ynyl)-1,2-dimethyl-3-methylene-cyclopropane	86.00
8	12.167	347007	0.35	50639	0.44	DODECANE, 1-CHLORO-	91.05
9	13.314	5138242	5.13	456829	3.92	DODECAENOIC ACID	73.00
10	13.734	2216781	2.21	224229	1.93	1-OCTADECANETHIOL	55.05
11	14.050	699598	0.70	66995	0.58	Asarone	208.10
12	14.250	25182	0.03	17072	0.15	(+)-ALPHA-TERPINEOL (P-MENTH-1-EN-8-OL)	59.00
13	14.556	140356	0.15	33386	0.29	Megastigmatrienone	109.10
14	14.753	33180	0.33	5702	0.50	6R-9R-3-Oxo-alpha-sinonol	108.10
15	14.908	601019	0.60	72000	0.62	8-PENTADECANONE	57.05
16	16.202	1054827	1.05	154590	1.33	Tetradecanoic acid	73.00
17	16.861	2797105	2.79	228811	1.97	(-)-LOLIOLIDE	111.10
18	17.387	11958140	11.94	2034405	17.48	Neophytadiene	68.10
19	18.072	6528974	6.52	1065897	9.16	Phytol	82.10
20	18.591	4663031	4.66	574352	4.93	Lidocaine	86.10
21	19.467	20695622	20.67	1841296	15.82	HEXADECANOIC ACID	73.05
22	19.850	7751582	7.74	880506	7.56	HEXADECANOIC ACID, ETHYL ESTER	88.05
23	21.451	325025	0.32	57746	0.50	Oxirane, hexadecyl-	71.10
24	21.877	8344866	8.33	1369534	11.77	PHYTOL ISOMER	71.05
25	22.415	10233850	10.23	927372	7.97	9-OCTADECENOIC ACID (Z)-	55.05
26	22.753	8222764	8.21	781228	6.71	(E)-9-Octadecenoic acid ethyl ester	55.05
27	22.992	301112	0.30	78253	0.67	9-Eicosone	67.05
28	23.158	1525601	1.52	159228	1.37	OCTADECANOIC ACID, ETHYL ESTER	88.05
29	23.333	141817	0.14	29078	0.25	cis-2-Ethyl-2-hexen-1-ol	213.15
30	23.367	20103	0.02	19262	0.17	1,2-Diazabicyclo[2.2.2]octan-3-one, 2-hydroxymethyl-	55.05
31	25.886	196083	0.20	41314	0.35	4,8,12,16-Tetramethylheptadecan-4-olide	99.05
32	35.129	172433	0.17	23466	0.20	Squalene	69.05
		100146016	100.00	11639408	100.00		

Table 1b Bioactivity of phytochemicals in the ethanolic extract of *Christella dentata* leaves

No	Compound	Biological activity
1	(-) Loliolide	Antioxidant activity
2	Asarone	Antibacterial, antidiabetic, antiadipogenic, insecticidal activity,
3	Hexadecanoic acid	Anti-inflammatory
4	Squalene	Antioxidant, antifungal anticancer, chemopreventive, anti-tumor, and sunscreen properties
5	Phytoliser	Antioxidant
6	Hexadecanoic acid, ethylester	Antioxidant, hypocholesteromic, nematocide, pesticide, androgenic
7	Lidocaine	Antiproliferative, antimicrobial, anesthetic

The various phytochemicals which contribute to the medicinal activities were shown in Table 1b. Of the 32 compounds identified, the most prevalent compound was Hexadecanoic acid (20.67%) that possesses anti-inflammatory, antimicrobial (Eva *et al.*, 2016) and antioxidant (Jagadeeswari *et al.*, 2012) properties. Anti-cancerous cytotoxic activity of Hexadecanoic acid against human colorectal carcinoma (HCT-116) was reported by Lokesh and Kannabiran (2017). GC-MS spectrum of petiole extract revealed 26 peaks (fig.2 and table 2a).

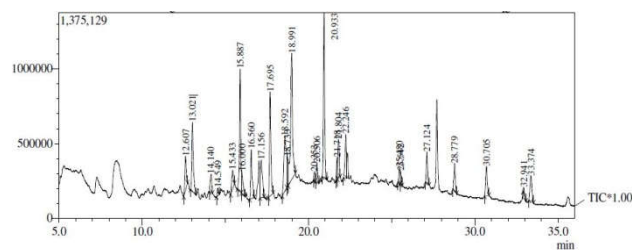


Fig 2 GC-MS spectrum of *Christella dentata* petiole

Table 2a Phytochemicals identified in the GC-MS analysis of ethanolic extracts of *Christella dentata* petiole

Peak#	R. Time	Area	Area%	Height	Height%	Name	Base m/z
1	12.607	1387993	2.96	228148	2.99	DODECAENOIC ACID	73.05
2	13.021	2967103	6.33	458443	6.01	E-14-Hexadecenal	55.05
3	14.140	714032	1.52	123579	1.62	8-PENTADECANONE	57.05
4	14.549	255097	0.54	53058	0.70	7-Hydroxy-6,9-dimethyl-5-methylene-decalylidene-azulene[4,5-b]furan-2,9-dione	123.10
5	15.433	905303	1.93	126545	1.66	Tetradecanoic acid	73.05
6	15.887	3986649	8.51	817673	10.72	E-15-Hepadecenal	55.05
7	16.000	922699	1.97	148674	1.95	DOTRIACONTANE	57.05
8	16.560	1958122	4.18	327730	4.30	Neophytadiene	68.05
9	17.156	1941732	4.14	249999	3.28	8-Octadecanone	57.05
10	17.695	4156251	8.87	697144	9.14	Lidocaine	86.10
11	18.592	3792386	8.09	364528	4.78	Pentadecanoic acid	73.05
12	18.733	942601	2.01	201567	2.64	Ethyl (+)-camphorcarboxylate	119.15
13	18.991	7042915	15.03	846733	11.10	1-OCTADECANETHIOL	55.05
14	20.353	245880	0.52	56281	0.74	10-Nonadecanone	55.05
15	20.506	673952	1.44	128074	1.68	Hexadecan-1-ol, trans-9-	83.10
16	20.933	5471442	11.67	1102907	14.46	Phytol	71.05
17	21.718	618308	1.32	144643	1.90	9,12-Octadecadienoic acid, methyl ester	67.05
18	21.804	1298864	2.77	259714	3.40	(E)-9-Octadecenoic acid ethyl ester	55.05
19	22.246	17199192	3.82	292553	3.84	1-Hexacosanol	55.05
20	25.480	465645	0.99	104706	1.37	n-Tetracosanol-1	97.15
21	25.542	430740	0.92	89098	1.17	DODECANE, 1-HODO	57.05
22	27.124	1048961	2.24	208918	2.74	PENTATRIACONTANE	57.05
23	28.779	1029880	2.20	182678	2.39	PENTACOSANE	57.10
24	30.705	1338140	2.86	198267	2.60	TETRAE-TETRACONTANE	57.05
25	33.941	170886	0.36	43392	0.57	Eicosane	57.05
26	33.374	1311414	2.80	172909	2.27	Squalene	69.05
		46868847	100.00	7627951	100.00		

Table 2b Bioactivity of phytochemicals in the ethanolic extract of *Christella dentata* petiole

No	Compound	Biological activity
1	Pentacosane	Antibacterial activity
2	8 pentadecanone	Hypocholesterolemic activity
3	Neophytadiene	Antioxidant, antibacterial, anti-inflammatory, antidiabetic, disinfectant
4	Lidocaine	Medication for numb tissue
5	Ethyl (+) camphorcarboxylate	Antitumor activity
6	Pentadecanoic acid	Flavouring agent
7	Phytol	Antioxidant, antinociceptive

Seven compounds identified in the petiole extract were found to have medicinal properties as given in table 2b. 16 compounds were detected in the GC-MS spectrum of rhizome extract (fig.3, table 3a). Maximum occurrence (59.84%) as per area percentage was registered for Hydroxy methyl furfural, a compound with high degree of bioactivity (table 3b). The potential of Hydroxy methyl furfural as a novel natural antioxidant with eventual applications in cancer chemoprevention was suggested by Ling *et al.* (2013). Rekha (2017) analyzed antioxidant properties of various plant parts of *Christella dentata* and reported maximum antioxidant property with IC50 value of 26µg/ml for the ethanolic extract of rhizome and it can be suggested that the presence of Hydroxy methyl furfural in rhizome in higher proportion contributes to the greater antioxidant property of rhizome compared to other plant parts.

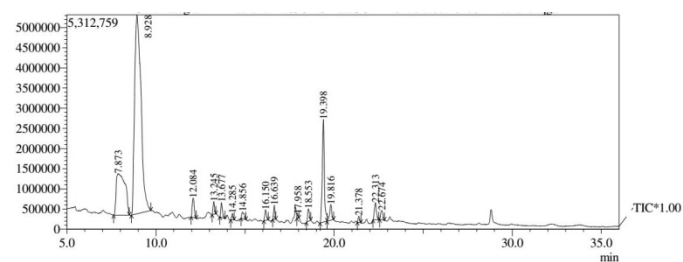


Fig 3 GC-MS spectrum of *Christella dentata* rhizome

Table 3a Phytochemicals identified in the GC-MS analysis of ethanolic extract of *Christella dentata* rhizome

Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	7.873	35831575	17.38	1034064	8.46	BENZOFURAN, 2,3-DIHYDRO-	91.05
2	8.928	123331326	59.84	4928880	40.33	5-Hydroxymethylfurfural	97.05
3	12.084	3865755	1.88	483339	3.96	1-DODECANOL	55.05
4	13.245	1888829	0.92	315751	2.58	DODECANOIC ACID	73.05
5	13.677	2328991	1.13	369314	3.02	E-14-Hexadecenal	55.05
6	14.285	931214	0.45	159049	1.30	CIS-ISOAPIOLE	222.10
7	14.856	1638206	0.79	168191	1.38	8-PENTADECANONE	57.05
8	16.150	1737614	0.84	264158	2.16	Tetradecanoic acid	73.05
9	16.639	1682442	0.82	350391	2.87	E-15-Heptadecenal	55.05
10	17.958	502179	0.24	96466	0.79	6-Tridecanone	58.05
11	18.553	2090469	1.01	310886	2.54	Lidocaine	86.10
12	19.398	20525575	9.96	2543335	20.81	HEXADECANOIC ACID	73.05
13	19.816	3387885	1.64	388505	3.18	Ethyl 14-methylhexadecanoate	88.05
14	21.378	871834	0.42	154002	1.26	n-Nonadecanol-1	55.05
15	22.674	3506750	1.70	421096	3.45	cis-Vaccenic acid	55.05
16	22.674	1995022	0.97	232531	1.90	9-OCTADECENOIC ACID (Z)-	55.05
		206115666	100.00	12219958	100.00		

Table 3b Bioactivity of phytochemicals in the ethanolic extract of *Christella dentata* rhizome.

No	Compound	Biological activity
1	Hydroxy methyl furfural	Antioxidant, antiproliferative, ameliorative effect, protect human vein epidermal cells against water and glucose, improve acute liver injury,
2	Benzofuran-2,3 dihydro	Antimicrobial, antifungal, anti-inflammatory, antidepressant, anticonvulsant, antitumor, antidiabetic, antioxidant, antitubercular
3	Cis Vaccenic acid	Used in cosmetic
4	Tetradecanoic acid	Anti-cancerous, nematocidal, hypocholesteremic,
5	1-Dodecanol	Antimicrobial
6	6-Tridecanone	insect resistant
7	Cis-isoapiole	Anticancerous, antibacterial, antioxidant
8	lidocaine	Antiproliferative, antimicrobial, anesthetic

From the current analysis it can be concluded that all parts of *Christella dentata* possess various compounds with medicinal properties, advocating the possible application of this plant in the drug discovery against various ailments. However, isolation of various bioactive compounds and *in vitro* and *in vivo* study of their bioactivities are pre-requisites for establishing the pharmaceutical potential of this plant species.

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