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Original Article

GC-MS ANALYSIS OF METHANOLIC STEM EXTRACT OF *GYNOCHTHODES RIDSDALEI*, RAZAFIM AND B. BREMER, AN ENDEMIC, ENDANGERED MEDICINAL PLANT OF SOUTHERN WESTERN GHATS

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ABSTRACT

Objective: The present research study was undertaken to determine the presence of bioactive components present in the methanolic stem extract of *Gynochthodes ridsdalei* using Gas Chromatography–Mass Spectrometry (GC-MS) analysis.

Methods: the Fresh stem of *Gynochthodes ridsdalei* collected from the forest areas of Ponmudi region of Thiruvananthapuram district of Kerala state, India was used. The mass spectrum GC-MS of the crude methanolic extract was estimated using the database of National Institute of Standard and Technology (NIST).

Results: The active principles with their retention time, peak area, molecular formula, molecular weight, structure and category of the compound were predicted. The analysis revealed the presence of 52 bioactive components. Most of the identified compounds are basically biological important. The components were identified by comparing their retention time and peak area with that of literature and by interpretation of mass spectra. The phyto components screened were of biological importance. Some of them were sterols, anthraquinones, vitamins etc.

Conclusion: The result reveals the existence of various bioactive compounds and validates the earlier reports of therapeutic importance of the plant. *Gynochthodes ridsdalei* is recommended as a plant of phytochemical and pharmaceutical importance.

Keywords: *Gynochthodes ridsdalei*, *Morinda reticulata*, endangered, southern Western Ghats, gas chromatography

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INTRODUCTION

The use of medicinal plants has gained considerable importance in our day to day life since ancient times. Traditional medicine is the sum total of knowledge, skills and practices based on the theories, beliefs, experiences indigenous to different cultures that are used to maintain health as well as to diagnose, improve or treat physical and mental illness. The therapeutic use of some plants against critical human illnesses predates recorded history and represents the most significant direct antecedent to modern medicine [1]. Medicinal plants are rich resources of ingredients which can be used in drug development and synthesis. Many higher plants are a major source of secondary metabolites which are used for many medicinal purposes. *Gynochthodes ridsdalei* (Syn*: Morinda reticulata*) is a large woody climbing shrub with coriaceous reticulate leaves belonging to the family Rubiaceae. The plant is endemic to southern Western Ghats [2]. It forms an important component in a variety of herbal formulation in traditional medicine [3]. Plants belonging to family Rubiaceae are known to contain a substantial amount of anthraquinones especially in the roots [4] and are characterised by brightly coloured anthraquinones that have been used in the past for various dyeing purpose. The screening of plant extracts is an innovative method to find therapeutically important compounds which will help to develop novel drugs [5]. Gas Chromatography–Mass Spectrometry (GC-MS) analysis is used for the direct analysis of bioactive components in traditional medicine and for separation and analysis of multi-component mixtures such as essential oils, hydrocarbons etc [6].

MATERIALS AND METHODS

Plant material

Fresh stem of *Gynochthodes ridsdalei* collected from the forest areas of Ponmudi region of Thiruvananthapuram district of Kerala state, India was used. The taxonomical identification of the plant was done using authentic literature [7, 8]. A voucher specimen was deposited at the Herbarium of Department of Botany, University of Kerala, Kariavattom (KUBH No. 8095).

Preparation of plant extract

The collected stem was chopped and shade dried under room temperature for 7 d and then milled into coarse powder by the mechanical grinder. About 10 gm of the powdered stem sample was subjected to Soxhlet extraction using 200 ml methanol. The extract was concentrated using rotary evaporator (Superfit rotavap) under reduced pressure and stored in the refrigerator until further use. Two microliters of the extract were employed in GC–MS analysis for analysis of different compounds.

GC–MS analysis

The analysis of the extract was performed using GC–MS (Model: GC MS-QP 2010, Shimadzu, Tokyo, Japan) equipped with a VF 5 ms fused silica capillary column of 30 m length, 0.25 mm diameter and 0.25 µm film thickness. For GC–MS detection, electron ionization energy of 70eV was used. The carrier gas was helium (99.9%) and used at constant flow rate of 1.2 ml/min. Injector and mass transfer line temperature were set at 200 ° C and 255 ° C respectively. The oven temperature was set from 70 to 300 ° C at 10 °C/min for 9 min. One microliter of the sample was injected in a split mode with a scan range of 40-1000 m/z. The total running time of GC–MS was 35 min. The relative percentage amount of each component was calculated by comparing its average peak area normalization [9].

Identification of the components

Elucidation of mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) and Wiley Spectra Libraries. The spectrum of the unknown component was compared with the spectrum of known components, which was stored in the NIST library source [10]. The name, molecular weight and molecular mass of the identified compounds were further confirmed by comparison of their retention indices with literature data. For quantitative analysis, compounds concentrations (as % content) were calculated by integrating their corresponding chromatographic peak area.

RESULTS AND DISCUSSION

The bioactive components present in the methanolic stem extract of *G ridsdalei* were identified by GC–MS analysis. The gas chromatogram shows the relative concentrations of various compounds getting eluted as a function of retention time (fig. 1). Identification of the compounds was accomplished by comparing their mass spectra and retention indices with those given in the literature and those authentic samples. The active principles with their retention time (RT), molecular formula, molecular weight (MW), concentration (%), nature of the compound and their biological activities are presented in (table 1) and are listed by their order of retention times. The heights of the peak indicate the relative concentrations of the compounds present in *G ridsdalei*.

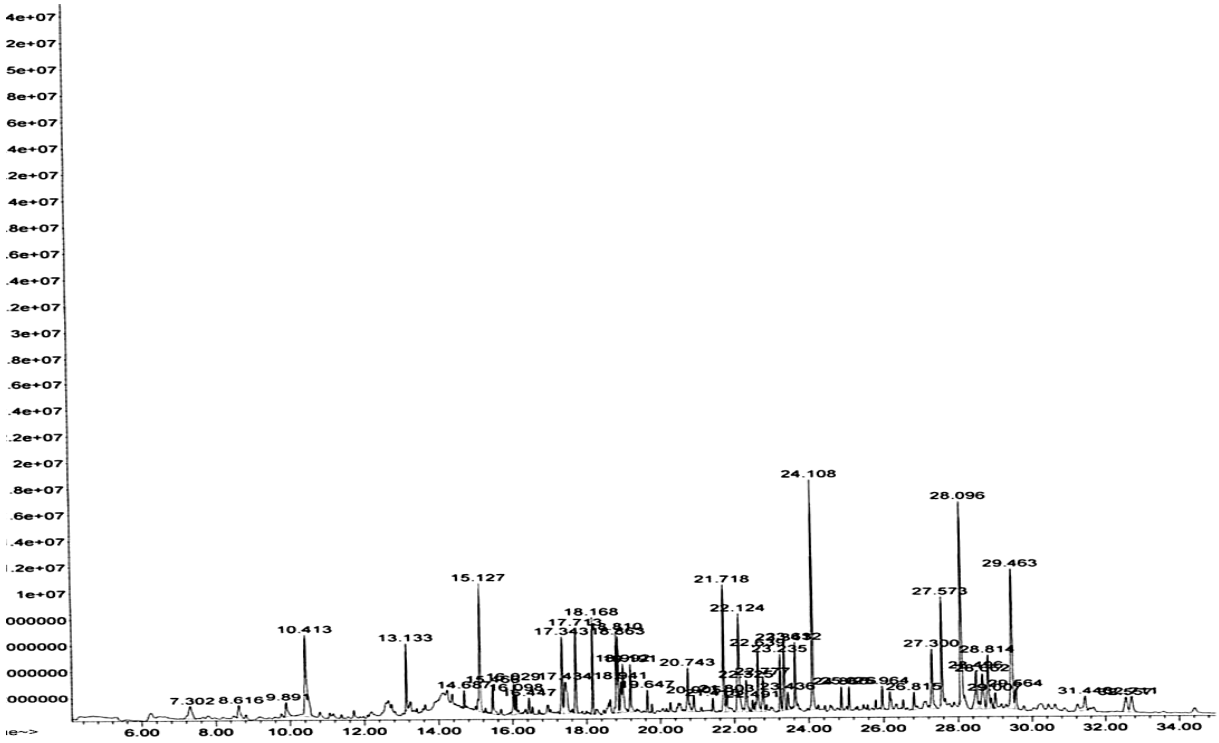

Fig. 1: GC-MS Chromatogram of methanolic stem extract of *Gynochthodes ridsdalei*

Table 1: Phytocomponents identified in the methanolic stem extract of *G. ridsdalei* by GC-MS

S. No.	Retention time	Peak area%	Name of the compound	Molecular formula	Molecular weight	Nature of compound	Uses
1	7.303	1.16	1,3-Benzenediol, 5-chloro-	$C_6H_8O_4$	144.1253	Phenol (Resorcinol)	Diazodyes, Dermatology
2	8.618	0.83	5-Hydroxymethylfurfura Benzene methanol, 3-fluoro-	$C_6H_6O_3$	126.1100	Organic compound	Baking industry
3	9.889	0.80	2-Methoxy-4-vinylphenol 3-Methoxyacetophenone	$C_7H_7NO_4$	169.136	Phenol	Flavoring agent
4	10.416	5.09	Phenol, 2,6-dimethoxy- 3-Amino-2,6-dimethoxypyridine	$C_8H_{10}O_3$	154.1632	Syringol Phenol	Smoky aroma in foods
5	13.135	1.60	1,Butanol, 3-methyl, formate	$C_6H_{12}O_2$	116	Alcoholic compound	Antimicrobial
6	14.688	0.39	Phenol, 2,6-dimethoxy-4-(2-propeny D-3-Hydroxy-4-methoxycinnamic acid	$C_{11}H_{14}O_3$	194.230	Phenol Eugenol	Perfumary dentistry
7	15.126	4.34	4-((IE)-3-Hydroxy-l-propenyl)-2-me thoxyphenol	$C_{10}H_{22}O_3$	$C_{10}H_{12}O_3$	Coniferyl alcohol	Fungal growth inhibitor
8	15.468	1.00	3,5-Dimethoxy-4-hydroxyphenylacetic acid	C8H8O3	152.147	Acid	Synthesis of atenolol
9	16.032	1.02	2,5-Diethoxyaniline	$C_6H_{11}N$	121.18	Aniline	Precursor to crystal violet dye
10	16.099	0.56	6-Octen-l-ol	$C_{10}H_{20}O$	156.27	Citronellol (monoterpenoid)	Insect repellents, perfumary
11	16.448	0.42	3-Methyl-l-penten-4-yn-3-ol	$C_6H_{10}O$	80.128	Tertiary hexanol	Hypnotic/sedative
12	17.340	2.58	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.4241	Palmitic acid	Antioxidant hypocholesterolemic, nematicide
13	17.436	2.29	Scopoletin	$C_{10}H_8O_4$	192.16	Coumarin	Used in food making
14	17.711	2.69	Squalene	$C_{30}H_{50}$	410	Triterpene	Antimicrobial,

							antioxidant, antitumour
15	18.172	2.24	1-Butanol,3-Methyl, Formate	$C_6H_{12}O_2$	116	Alcoholic	Antimicrobial
16	18.810	2.35	9,10-Anthracenedione,	$C_{14}H_8O_2$	208.22	Anthraquinone	Dyes
17	18.862	1.98	Phytol	● C $_{20}H_{40}O$	296	Diterpene	Anticancer, antimicrobial, anti-inflammatory
18	18.944	1.01	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.4455	Linoleic acid (carboxylic acid)	Antioxidant effect, paints, varnishes
19	18.989	1.72	Oleic Acid cis-13-Octadecenoic acid cis-Vaccenic acid	$C_{18}H_{34}O_2$	282.4614	Fatty acid	Reducing blood pressure
20	19.189	1.48	Octadecanoic acid	$C_{18}H_{36}O_2$	284.4772	Stearic acid	Dietary supplements
12	19.650	0.57	1-Hydroxy-2-methylanthraquinone l-Hydroxy-4-methylanthraquinone	$C_{15}H_{10}O_3$	238.242	Anthraquinone	Dyes, Medicinal Importance
22	20.742	1.76	9,10-Anthracenedione, 2-hydroxy-l-methoxy	$C_{19}H_{18}O_7$	358.342	Anthraquinone	Dyes, Medicinal importance
23	20.905	0.64	9-Octadecenamide, (Z)—	$C_{18}H_{35}NO$	281.4766	Amide	For depression, sleep disorders
24	21.418	0.40	1,2,4-Benzenetricarboxylic acid, 5-methyl-, trimethyl ester-	$C_{12}H_{12}O_6$	252.2201	Trimellitic acid	Unknown
25	21.715	4.03	2-(Hydroxymethyl) anthraquinones 1,4,7-Trimethyl-2-azafluorene 4-Propylxanthen-9-one	$C_{15}H_{10}O_3$	238.238	Anthraquinone	Dyes, Medicinal importance
26	21.804	0.65	9,10-Anthracenedione, 1,5-dimethoxybenzopyrenol	$C_{14}H_{10}N_2O_2$	238.241	Anthraquinone	Dyes, Medicinal importance
27	22.124	4.42	1-Hydroxy-4-methylanthraquinone 3-Phenoxy-2H-chromen-2-one 2,6-Diaminoanthraquinone	$C_{15}H_{10}O_3$	238.242	Anthraquinone	Dyes, Medicinal importance
28	22.324	1.32	1-Hydroxy-4-methylanthraquinone 9,10-Anthracenediol, 2-ethyl-	$C_{15}H_{10}O_3$	238.242	Anthraquinone	Dyes, Medicinal importance
29	22.488	0.35	Docosanoic acid	$C_{22}H_{44}O_2$	340.5836	Behenic acid) carboxylic acid	Detergents, floor polishes
30	22.636	2.20	9,10-Anthracenedione, l-hydroxy-2-(hydroxymethyl)-	$C_{15}H_{10}O_4$	254.238	Anthraquinone	Dyes, Medicinal importance
31	22.777	1.31	9,10-Anthracenedione,1,8-dihydrox y-3-methyl-	$C_{18}H_{18}N_2O_4$	326.346	Anthraquinone	Dyes, Medicinal importance
32	23.238	1.44	4-Ethenyl-2-methoxyphenol	$C_9H_{10}O_2$	150.18	Aromatic compound	Flavouring agent
33	23.342	2.09	Benzoic acid, heptadecyl ester	$C_{23}H_{28}$ N O_3	380.48	Aromatic carboxylic acid	Food preservative
34	23.439	0.59	Fumaric acid, cis-hex-3-enyl tetra decyl Ester	$C_4H_4O_4$	116.07	Unsaturated fatty acid	Food industry
35	23.632	1.69	Octadecanoic acid, 2,3-dihydroxypr opyl ester	$C_{21}H_{42}O_4$	358.5558	Glycerol ester of stearic acid	Food additive
36	24.107	7.31	13-Docosenamide, (Z)-	$C_{22}H_{43}NO$	337.5829	Erucid acid (Fatty acid)	Lubricant, biodiesel fuel precursor
37	24.865	0.62	22-Tricosenoic acid Triacontyl acetate	$CH_3(CH_2)_{21}$ COOH	354.61	Fatty acid	Oil paints, lubricants
38	25.073	0.67	4,5-Dibenzopyrene	$C_{20}H_{12}$	302.35	Aromatic hydrocarbon	Naturally emitted coal tar
39	25.964	0.69	gamma.-Tocophero beta.-Tocopherol	$C_28H_{48}O_2$	416.680	Vitamin E	Antioxidant
41	27.301	2.49	Campesterol 5-Cholestene-3-ol,24-methyl-	$C_{28}H_{48}O$	400.69	Phytosterol	Lowering cholesterol
42	27.569	3.46	Stigmasterol	$C_{29}H_{48}O$	412.6908	Unsaturatd phytosterol	Food making
43	28.096	7.59	gamma.-Sitosterol beta.-Sitosterol Stigmast-7-en-3-ol,	C29H50O	414.71	Sterol	Lowers blood cholesterol
44	28.497	2.22	Cholest-4-en-3-one Pregn-4-ene-3, 20-dione, (8. alpha, 10. alpha.)-	$C_{27}H_{46}O$	386.65	Sterol	Food making
45	28.661	1.74	Tetradecanoic acid	$C_{14}H_{28}O_2$	228.37	Fatty acid	Antioxidant, Cancer preventive
46	28.817	2.23	4,22-Stigmastadiene-3-one Spinasterone	$C_{39}H_{46}O$	410.686	Phytosterol	Precursor of vitamin D_3
47	29.010	0.65	Cyclohex-2-enone, 2	C_6H_8O	96.13	Ketone	Synthesis of pharmaceuticals and

							fragrances
48	29.463	6.71	Stigmast-5-en-3-one	$C_{29}H_{50}O$	414.72	Beta-Phytosterol	Food preparations
49	29.567	0.91	Imidazolidine	$C_3H_8N_2$	72.109	Diamine	Muscle relaxant
50	31.447	1.04	Cholestan-3-one	$C_{27}H_{48}$	372.68	Triterpene	Food industry
51	31.494	2.83	Stigmasterol	$C_{29}H_{48}O$	412	Sterol	Precursor of vitamin D_3
52	32.561	1.00	Gibbane-1,10-diarboxyli acid	$C_{19}H_{22}O_6$	182.22	Heterocylic acid	Fungicides, making dyes

The analysis revealed the presence of 52 photo components. Major compounds detected were sterols, anthraquinones, terpenes, vitamins etc. Sitosterol (7.59%) showed highest peak (dominant component), followed by docos enamide. Among the identified compounds, the diterpene alcohol, phytol is vital in the dispensation of glucose and can trigger enzymes within the body that have strong positive effects on insulin level. This means that phytol in the human diet could perhaps help reinstate the metabolic activities of those with type-2 diabetes [11, 12]. It is also a constituent of chlorophyll in plants and precursor for the manufacture of synthetic forms of vitamin E [13].

Stigmasterol is an unsaturated phytosterol occurring in the plant fats or oils. Stigmasterol is also found in various vegetables, legumes, nuts, seeds etc. Stigmasterol is used as a precursor in the manufacture of semisynthetic progesterone, a valuable human hormone that plays an important physiological role in the regulatory and tissue rebuilding mechanisms related to estrogen effects, as well as acting as an intermediate in the biosynthesis of androgens, estrogens, and corticoids [14]. It is also used as the precursor of vitamin D_3. Recently squalene possesses chemo preventive activity against colon carcinogenesis [15, 16].

CONCLUSION

This is the first report on the analysis of bioactive components present in *G. ridsdalei.* The result reveals the existence of various bioactive compounds and validates the earlier reports of therapeutic importance of the plant. *G. ridsdalei* is recommended as a plant of phytochemical and pharmaceutical importance. Further studies can be done to isolate the active principle of the methanolic extract as well as to elucidate the effect of extract for various diseases.

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CONFLICT OF INTERESTS

Declare none

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