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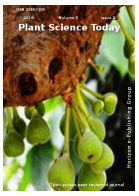

Research Article

GC-MS analysis of root methanolic extract of *Gynochthodes ridsdalei* Razafim. and B. Bremer, an endemic, endangered species of southern Western Ghats of India

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Abstract

The present investigation was carried out to determine the presence of biologically active components in the root methanolic extract of *Gynochthodes ridsdalei* using Gas Chromatography–Mass Spectrometry (GC-MS) analysis. Fresh root of *G. ridsdalei* collected from the forest areas of Ponmudi hills of Thiruvananthapuram district of Kerala State, India was used for the study. The active principles with their retention time, peak area, molecular weight and molecular formula of the compounds were detected. The analysis revealed the presence of 26 components. The components were identified by comparing their retention time and peak area with that of literature available and by the interpretation of mass spectra.

Keywords

Gynochthodes ridsdalei; endangered; Southern Western Ghats; root extract; gas chromatography- mass spectrometry

Citation

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Introduction

Plants are important sources of bioactive components which play a dominant role in the maintenance of human health (1). Knowledge about the chemical constituents of plants is necessary not only for the discovery of new therapeutics but also for transferring the information of new sources of economic phyto-components for the synthesis of complex chemical molecules as well as for recognising the importance of folkloric remedies (2).

Gynochthodes ridsdalei (Syn: *Morinda reticulata*) is a large woody climbing shrub with coriaceous reticulate leaves, that belongs to the family Rubiaceae. This is an endemic and endangered species of southern Western Ghats (3). This plant forms an important constituent in a variety of herbal formulations in traditional medicine (4). The genus *Gynochthodes* (Syn: *Morinda*) is known to contain substantial amounts of anthraquinones particularly in the roots (5) that have been used for dyeing purpose. The present

study has focused on the GC-MS analysis of *G. ridsdalei* roots.

Gas Chromatography–Mass Spectrometry (GC-MS) analysis can be used for the direct analysis of bioactive components in traditional medicine and for separation and analysis of multicomponent mixtures such as essential oils, solvents and hydrocarbons (1).

Materials and Methods

Collection of plant material

Fresh roots of *Gynochthodes ridsdalei* were collected from the forest areas of Ponmudi Hills of Thiruvananthapuram District of Kerala Sate, India. The taxonomical identification of the plant was done using authentic literature (6, 7). A voucher specimen was deposited in the Herbarium of Department of Botany, University of Kerala, Kariavattom (KUBH No. 8095).

Preparation of plant extract

The collected roots were washed thoroughly and chopped into small pieces before shade drying under room temperature for two weeks. The dried material was then milled into coarse powder by mechanical grinder and stored in air tight bottles until further use. About 10 gm of the powdered root sample was subjected to Soxhlet extraction for 6-7 hrs 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 was employed in GC–MS analysis, for the identification of compounds.

GC–MS analysis

The analysis of the extract was performed using GC–MS (Model: GC MS-QP 2010, Shimadzu, Japan) equipped with a VF-5ms fused silica capillary column of 30 m length, 0.25 mm diameter and 0.25 µm film thickness. For GC–MS analysis, electron ionization system with ionization energy of 70eV was used. The carrier gas used was helium (99.9%), at a constant flow rate of 1.2 ml/min. Injector and mass transfer line temperature were set to 200°C and 255°C respectively. The oven temperature was set from 70 to 220°C at 10°C/min for 9 min, and finally raised to 300°C. Two microliters 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 value (8).

Identification of the components

Interpretation of mass spectrum obtained from GC-MS was done using the database of National Institute Standard and Technology (NIST) and Wiley Spectra Libraries. The spectrum of the unknown component was compared with that of the spectrum of known components, which was stored in the NIST08 library source (9). The name, molecular weight and molecular mass of the identified compounds were further confirmed by comparing their retention indices with that of literature data. For quantitative analysis, the concentration of compounds (%) were calculated by combining their corresponding chromatographic peak area.

Results and Discussion

The bioactive components present in the root methanolic extract of *G. ridsdalei* were identified by GC–MS analysis. The gas chromatogram shows the relative concentrations of various compounds eluted as a function of their retention time (Fig. 1). Identification of the compounds was done by comparing their mass spectra and retention indices with those given in the literature and with the authentic samples. The active principles with their retention time (RT), peak area (%) and biological activities are listed by the order of their retention times in Table 1.

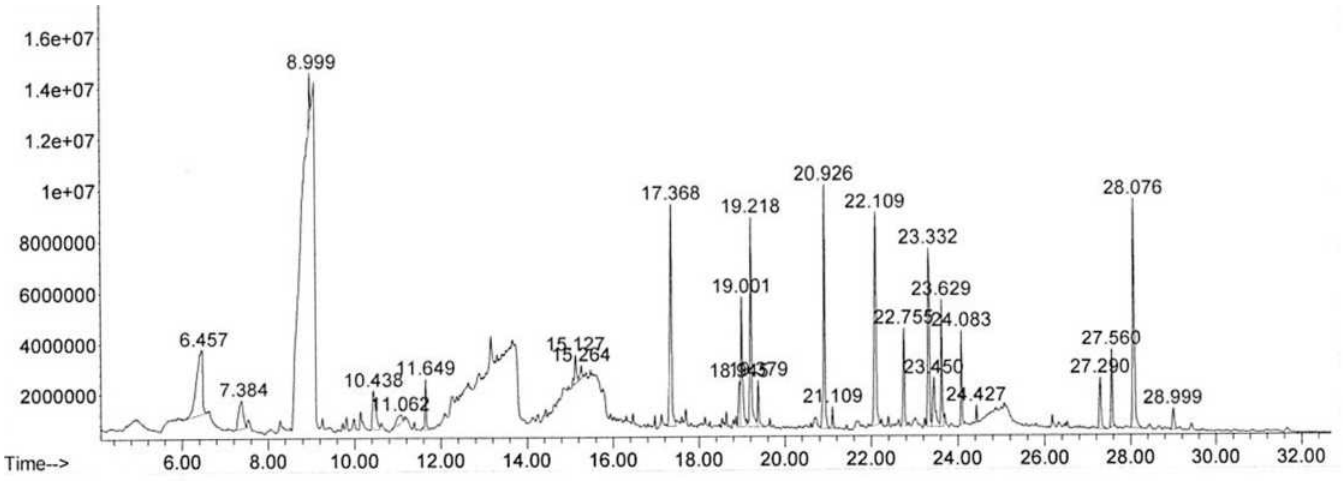

Fig 1. GC-MS Chromatogram of methanolic root extract of *Gynochthodes ridsdalei*

Table 1. Phytocomponents identified in the methanolic root extract of *Gynochthodes ridsdalei* by GC-MS analysis

Sl. No.	Retention time	Peak area%	Name of the compound	Molecular formula	Molecular weight	Nature of compound	Uses
1	6.47	9.35	Maltol	$C_6H_6O_3$	126.1133	Ketone	Flavour enhancer
2	7.385	2.94	4H-Pyran-4-one, 2,3-dihydro-3,5-di hydroxy-6-methyl-	$C_6H_8O_4$	144.126	Organic compound	Unknown
3	8.997	1.11	4-Mercaptophenol	C_6H_6OS	126.176	Phenol	Unknown
4	10.438	2.84	Phenol 2,6-dimethoxy	$C_{11}H_{14}O_3$	194.230	Phenol Eugenol	Perfumery Dentistry
5	11.062	1.54	2-Hydroxyhexadecyl butanoate	$C_{20}H_{40}O_3$	328.529	Ester	Unknown
6	11.649	1.54	4-Aminoresorcinol	$C_6H_8ClNO_2$	161.59	Amine	Unknown
7	15.126	1.24	4-Methylphenylthioacetone	$C_{10}H_{12}OS$	180.27	Acetone	Unknown
8	15.267	0.51	3-Deoxy-d-mannoic lactone	$C_6H_{10}O_5$	162.14	Ester	Unknown
9	17.369	10.16	Hexadecanoic acid	$C_{16}H_{32}O_2$	256.4241	Palmitic acid	Antioxidant Hypocholestero-mic, Nematicide
10	18.994	1.47	9,12-Octadecadienoic acid	$C_{18}H_{36}O_2$	284.4772	Stearic acid	Dietary supplements
11	19.004	5.32	Oleic Acid	$C_{18}H_{36}O_2$	284.4772	Fatty acid	Reducing blood pressure
12	19.219	8.13	9-Octadecanoic acid	$C_{18}H_{36}O_2$	284.4772	Stearic acid	Dietary supplements
13	19.383	1.43	9-Octadecanamide	$C_{18}H_{35}NO$	281.4766	Amide	Depression, Sleep disorders
14	20.928	8.11	9-Octadecenamide	$C_{18}H_{35}NO$	281.4766	Amide	Depression, Sleep disorder
15	21.106	0.51	9-Octadecanamide	$C_{18}H_{35}NO$	281.4766	Amide	Depression, Sleep disorders
16	22.109	9.68	9,10-Anthracenedione, l-hydroxy-2-(hydroxymethyl)-	$C_{15}H_{10}O_4$	254.238	Anthraquinone	Dyes, Medicinal importance
17	22.755	3.09	9,10-Anthracenedione, 1,8-dihydrox y-3-methyl-	$C_{18}H_{18}N_2O_4$	326.346	Anthraquinone	Dyes, Medicinal importance
18	22.335	7.22	Pyrrolo (3,2, F)-9-one	C_9H_7N	129.16	Heterocyclic aromatic organic compound	Manufacture of dyes
19	23.453	2.62	Oleic acid	$C_{18}H_{34}O_2$	282.4614	Fatty acid	Reducing blood pressure
20	23.632	3.64	Octadecanoic acid	$C_{18}H_{36}O_2$	284.4772	Stearic acid	Dietary supplements
21	24.085	2.44	13-Docosenamide	$C_{22}H_{43}NO$	337.5829	Erucic acid (Fatty acid)	Lubricant, Biodiesel fuel precursor
22	24.427	0.44	Squalene	$C_{30}H_{50}$	410	Triterpene	Antimicrobial, Antioxidant, Anti tumour
23	27.287	2.17	Campesterol	$C_{28}H_{48}O$	400.69	Phytosterol	Lowering cholesterol
24	27.561	2.68	Stigmasterol	$C_{29}H_{48}O$	412.6908	Unsaturated phytosterol	Food making
25	28.074	9.25	Sitosterol	$C_{29}H_{50}O$	414.71	Sterol	Lowers blood cholesterol
26	29.003	0.95	Stigmasta-4,6-diene	$C_{39}H_{46}O$	410.686	Phytosterol	Precursor of vitamin D_3

The GC- MS analysis of *G. ridsdalei* revealed the presence of 26 compounds (Fig. 1, Table 1). Hexadecanoic acid showed highest peak (10.16%) which is the dominant component followed by anthracenedione (9.68%), maltol (9.35%), sitosterol (9.25%) and oleic acid (5.32%). Among the identified compounds, hexadecanoic acid is reported to have antioxidant (10) and anti- inflammatory properties (11). Squalene which is a triteterpene, used in cosmetics as a natural moisturiser (1). Recent reports suggest that squalene has chemopreventive activity against colon carcinogenesis (12, 13).

Stigmasterol is an unsaturated phytosterol occurring in the plant fats or oils. Stigmasterol is also found in certain vegetables, nuts, legumes, seeds etc. It is used as a precursor in the production of semisynthetic progesterone, an important human hormone that plays a vital role in the regulatory and tissue rebuilding mechanisms related to estrogen effects. It acts as an intermediate in the biosynthesis of estrogens, androgens and corticoids (14). Phytosterols play significant role in cholesterol metabolism in animals and are suitable ingredients of functional foods (15). Anthraquinones or anthracenedione are one of the most important classes of commercial colorants. Natural anthraquinones are preferred as colouring agents for sweets, beverages and other foods (16).

Conclusion

This is the first report of bioactive components in the roots of *G. ridsdalei.* The result reveals the presence of various bioactive compounds such as sterols, anthraquinone and terpenes and supports the previous reports of therapeutic importance of this endemic, endangered medicinal plant. *G. ridsdalei* is recommended as a plant of phytochemical and pharmaceutical importance. Further studies can be carried out to isolate the active principle of crude extract as well as to elucidate the effect of extract for various diseases.

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Conflict of interest

The authors declare no conflict of interest.

Authors' contribution

RRN wrote the manuscript and AG helped in manuscript corrections. Field visit, collection and identification was done by AG and RRN.

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