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Research Article

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GC-MS Determination of Bioactive Components in *Rhododendron arboreum* Sm. ssp. *nilagiricum* (Zenker) Tagg an Endemic Plant

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ABSTRACT

Rhododendron arboreum Sm.ssp. nilagiricum (Zenker) Tagg is an endemic plant of Southern Western Ghats of India. In this study, the bioactive components were determined from acetone leaves extract of R. arboreum Sm.ssp. nilagiricum by GC-MS analysis. A total of 25 compounds were identified from 7 peaks according to their chromoatographic retention indices and mass spectra. The presence of these bioactive compounds has potent medicinal effects for the treatment of various ailments.

Keywords: *Rhododendron arboreum* Sm.ssp. *nilagiricum;* Bioactive components; GC-MS analysis

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INTRODUCTION

Plants used in traditional medicine contain a wide range of bioactive compounds that can be used to treat various infectious diseases [1]. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins, phenolic compounds and some other secondary metabolites, which have rich valuable biological activities of antioxidant, anti-inflammatory, antitumor, etc. [2]. The genus *Rhododendron*, having about 72 species, 20 sub-species and 19 varieties in India, is mainly distributed in the Eastern Himalayas, while *R. arboreum* ssp. *nilagiricum* is the only endemic species found in western Ghats. *Rhododendron arboreum* leaves were reported to contain flavonoids [3] and phenolic compounds [4] were found to have potent antioxidant [5] anti-inflammatory [6] and hepatoprotective activity [7].

The leaves of *R. arboreum* ssp.*nilagiricum* revealed that the presence of hyperoside (3-D – galactoside of quercetin), ursolic acid and Epifriedelinol, a triterpenoid compound [8]. However, there is no report is available on the any other bioactive components of *R. arboreum* ssp. *nilagiricum*. Gas chromatography-Mass Spectroscopy, a hyphenated system which is a very compatible technique for the identification and quantification of bioactive compounds. The unknown organic compounds in a complex mixture can be determined by interpretation and by matching the spectra [9].

Literature analysis revealed that very few works has been done on the phytochemical characterization of this species. In view of this fact the present study was conducted to find out the bioactive constituents of the leaves of *R. arboreum* ssp. *nilagiricum* using GC-MS technique.

EXPERIMENTAL SECTION

Collection of plant material

Mature leaves of *Rhododendron arboreum* ssp. *nilagiricum* were collected from the Palni Hills of southern Western Ghats, India. The leaves were washed with sterile water, shade dried, chopped into small pieces and ground coarsely to powder using a mixer grinder. 25 g of the dried plant material was used for extraction.

Solvent extraction

Twenty five grams of the powdered material continuously extracted with 100 mL of acetone for 18 hrs at 60°C using Soxhlet apparatus. The extract was filtered through Whatman No.1 filter paper. The filtered extract was evaporated by a rotatory vaccum evaporator. The evaporated residue with constant weight was stored prior to analyses in dark at 4°C. The residue was dissolved in 2 mL of acetone solvent for GC-MS analysis.

GC-MS analysis

GC-MS analyses were carried out in a Shimadzu GC-MS - QP 5050A gas chromatograph fitted with a DB5 (methyl phenyl sylonane, 60 m × 0.25 mm i.d.) capillary column. Carrier gas, helium with a flow rate of 0.6 mL/min; column temperature, 5 min at 60°C, 60-290°C at 3°C/min and finally 2 min at 180°C; injector temperature, 280°C detector temperature, 310°C, Volume injected, 1 µL; Split ratio, 1:12. The MS operating parameters were as follows: ionization potential, 70 eV; ion source temperature; 290°C; quadrupole 100°C, Solvent delay 4.0 min , scan speed 2000 amu/s and scan range 30-600 amu, EV voltage 3000 volts.

Identification of compounds

Identification of compounds of the hydrolyzed acetone extract was based on direct comparison of the retention time and mass spectral data with those for standard compounds and computer matching with the Wiley 229, Nist 107, Nist 21 Library, as well as by comparison of the fragmentation pattern of the mass spectra with those reported in the literature [10,11].

RESULTS AND DISCUSSION

The studies on the active principles content in the acetone extract of R. *arboretum* ssp. *nilagiricum* by GC-MS clearly show the presence of wide spectrum of active components. In the acetone leaf extract, a total of 7 peaks were obtained, out of which 25 compounds were characterized and identified (Table 1; Figure 1).

Table 1: Phytochemical compounds identified in the acetone extract of *R. arboretum* ssp. *Nilagiricum*

Peak No.	RT	Possible compounds	MF	MW	Area %
1	9.72	Ethenetetracarbonitrile	C_6N_4	128	3.46
		3-(2)Methylphenoxy)-3-methoxypropyne	$C_{11}H_{12}O_2$	176	3.46
		4,4-Didauterio-6,6-dimethyl cyclohexanone	$C_8H_{12}D_2O$	126	3.46
		11-(t-Butyldimethylsilyl)-11-azabicyclo(8.2.0)dodeca-3,-7-diyn-112-one	C18H11NOSi	285	3.46
2	14.76	Tetracosane (CAS)	$C_{24}H_{50}$	338	0.86
		Heptacosane(CAS)	$C_{27}H_{56}$	380	0.86
		Nonadecane(CAS)	$C_{19}H_{40}$	268	0.86
		Pentadecane(CAS)	$C_{15}H_{32}$	212	0.86
3	18.2	di-cylohexylacetaldehydeacetal	$C_{14}H_{26}O_2$	226	16.96
		neophytadiene	$C_{20}H_{38}$	278	16.96
		(z)-3-Hexenyl(t-Butyl)carbnonate	$C_{11}H_{20}O_3$	200	16.96
4	24.21	2-Hexadecen-1-ol,3,7,11,15-tetramethyl-,(R-R*,R*(E))-(CAS0	$C_{20}H_{40}O$	296	6.59
		3-Chloromethylfuran	$C_5H_5O_{10}$	116	6.59
5	27.27	(s)-N-(1-Benzyl-2-Chloroethyl)benzamide	$C_{16}H_{16}C_1NO$	273	1.66
		(s)-Hydroxymethyl-2-phenyloxazoline	$C_{10}H_{11}NO_2$	177	1.66
		1-(3-Methoxypropyl)-2-nitro benzene	$C_{10}H_{13}NO_3$	195	1.66
		(R) -4-carbomethoxy-2-phenyl oxazoilen	$C_{11}H_{11}NO_3$	205	1.66
6	30.67	Methyl lithocholate	$C_{25}H_{42}O_3$	390	2.15
		Cyclopentylmethylboronic acid	$C_6H_{13}BO_2$	128	2.15
		(E,E)-1,12-Dibromo-2,10-dodecadiene	$C_{12}H_{20}BR_2$	322	2.15
		(R,Z)-12-Hydroxy-9-octadecenoic acid	$C_{18}H_{34}O_3$	298	2.15
7	34.26	N-cyclohexylmethoxymethylketenimine	$C_{10}H_{17}NO$	167	1.32
		(2R,3S)-4-(3-Hydroxy-5,7-dimethoxychroman-2-yl)benzene 1,2-diol	$C_{17}H_{18}O_6$	318	1.32
		1-(Ferrocenyl)-3-phenylpropanone	$C_{17}H_{18}FeO$	318	1.32
		Bis(u(5)-cyclopentadienyl) bis(cyanodithioformiato)-titanium	$C_{14}H_{10}N_2S_4Ti$	382	1.32

RT- Retention time; MF-Molecular Formula, MW-Molecular weight

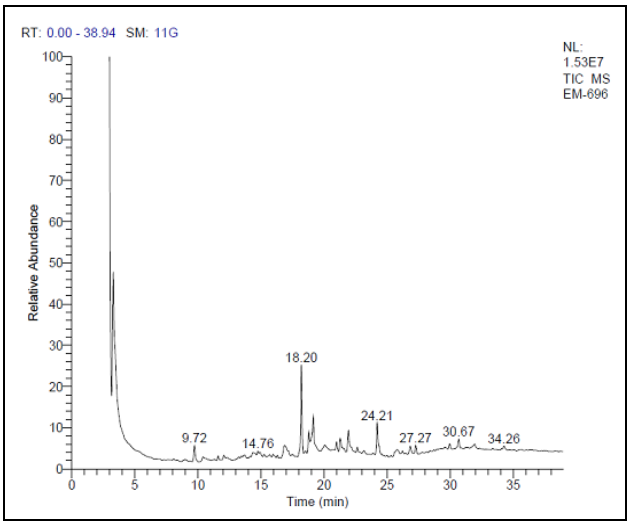

Figure 1: Chromatogram of acetone extract of *R. arboretum* ssp. *nilagiricum*

The major compounds of di-cylohexylacetaldehydeacetal, neophytadiene and (z)-3-Hexenyl (t-Butyl) carbnonate were found (16.96%) followed by other compounds like Ethenetetracarbonitrile, 3-(2) Methylphenoxy)-3-methoxypropyne, 4,4-Didauterio-6,6-dimethyl cyclohexanone and 11-(t-Butyldimethylsilyl)-11-azabicyclo(8.2.0)dodeca-3,-7-diyn-112-one (3.46%) had next highest concentration (Figure 2). The comparison of the mass spectrum of GC-MS with the data base gave more than 90 % match and confirmatory chemical compound structure match.

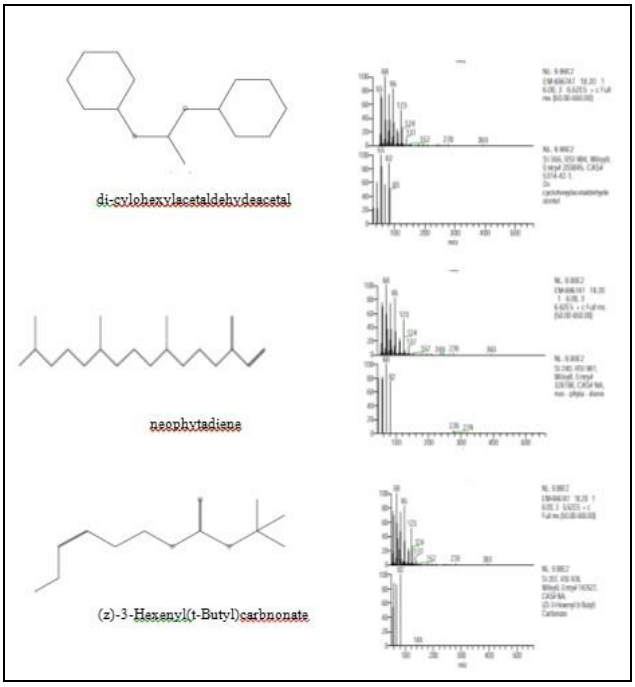

Figure 2: Structure of phytochemical constituents having high peak area % (16.96%)

The various identified phytochemicals which contribute to the medicinal activity of the plant. Out of 25 compounds identified, some compounds have reported bioactive properties. The bioactive compound Hexadecane reported from the present study possesses antibacterial and antifungal properties [12-14]. An antifungal terpenoid, Neophytadiene have antioxidant, antipyretic, analgesic and vermifugic, including a topical application for sores and inflammation

[15-18]. According to the recent concept, the medicinally important properties of plants have a positive correlation with phytochemical constituents present it. Isolation and purification of identified compounds may lead to the identification of new drugs for various ailments.

CONCLUSION

The present study concludes that the plant *Rhododendron arboretum* Sm.ssp. *nilagiricum* has potential source of biologically active compounds with phytopharmaceutical value and reported 25 compounds isolated by GC-MS. Thus, the present study may give valuable information about active principles in the medicinal plants. Further, the identification of the compound can be used for finding novel drugs to treat various ailments.

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