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Short Communication

GC-MS ANALYSIS OF METHANOLIC EXTRACT OF LEAVES OF *RHODODENDRON CAMPANULATUM*

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ABSTRACT

Objective: *Rhododendron campanulatum* is a native of high altitude and is known for its medicinal properties. The present study is aimed to identify the phytochemical constituents in the leaf extract of *R. campanulatum* using Gas Chromatography and Mass Spectroscopy (GC-MS).

Methods: The methanolic leaf extract was prepared using Accelerated Solvent Extraction system at room temperature and high pressure. Phytochemical screening of methanolic extract of *R. campanulatum* was performed using GCMS-QP2010 Plus (Shimadzu, Kyoto, Japan) and the spectrum was interpreted on the basis of the databases of National Institute Standard and Technology (NIST11LIB) and WILEY8LIB.

Results: The GC-MS analysis revealed the presence of 49 phytochemical compounds in the methanolic leaf extract. Baccharis oxide (9.99%), betuligenol (8%), alpha and beta-amyrin (7.38 and 2.64%), geranyl acetate (5.91%), (R)-(-)-14-methyl-8-hexadecyn-1-ol (5.19%) and phthalic acid (5.16%) were identified as major constituents.

Conclusion: The methanolic leaf extract of *R. campanulatum* contains various phyto-compounds of pharmaceutical and industrial importance.

Keywords: *Rhododendron campanulatum*, Gas Chromatography-Mass Spectrometry, Phytochemical compounds.

Rhododendron genus is comprised of about 1025 species, which are mostly found at higher altitudes [1, 2]. In India, there are around 80 species and 14 subspecies of *Rhododendron*, distributed in the Himalayan region at the altitude ranging 1500-5500 meters [3]. Different species of *Rhododendron* are known for their ethnopharmacological values [4]. *R. campanulatum* is a very important member of the genus *Rhododendron*, which is known for its traditional medicinal importance for the different ailments like body ache, sore throat, digestion, skin diseases, rheumatism, syphilis, cold and fever, etc. [4-9]. *R. campanulatum* is found at altitudes between 2500-4300 m [2]. There are very few scientific studies on phytochemical constituents and therapeutic potential of the *R. campanulatum*. Analysis through High Performance Thin Layer Chromatography (HPTLC), High-Performance Liquid Chromatography (HPLC) and Nuclear Magnetic Resonance (NMR) have shown the presence of epicatechin, syringic acid, quercetin, chlorogenic acid, gallic acid, proto catechic acid and oleanane triterpenoid in the different extracts of leaves of *R. campanulatum* [6, 9, 10]. Antibacterial properties have also been shown in the leaf extract of *R. campanulatum* [8]. In the present study, we attempted to identify the phytochemical constituents in the methanolic leaf extract of *R. campanulatum* using GC-MS method of analysis.

R. campanulatum leaves were collected from the Govind ghat region of Chamoli District, Uttarakhand, India. Plant sample was authenticated by Department of Botany at H. N. B Garhwal University (Srinagar), Uttarakhand. The Herbarium specimen was deposited in the Department of Botany; H. N. B Garhwal University vide voucher number GUH-0743.

The leaves of *R. campanulatum* were shade dried at sterilized condition and pulverized with the grinder. The powder was successively extracted with methanol (100%) in the Accelerated Solvent Extraction System, which was equipped with a solvent controller unit (ASE350, Dionex Corporation, Sunnyvale, CA, USA) and further the extract was subjected to lyophilization [11]. The lyophilized leaf extract was then stored at 4 °C for further investigation.

1 µl of prepared sample (1 mg/ml lyophilized extract in methanol) was applied for GC-MS analysis.

GC-MS analysis was performed at University Science Instrumentation Centre, AIRF, Jawaharlal Nehru University, Delhi. For GC-MS analyses of plant extract, GC-MS QP2010 Plus (Shimadzu, Kyoto, Japan) system was utilized. The system was equipped with an auto injector (AOC-20i), head space sampler (AOC-20s), a mass selective detector with an ion source (220 °C) and an interface (260 °C). Rtx-5 MS capillary column having 30 mm X 0.25 mm of length X diameter and 0.25 µm of film thickness was used for MS analyses. The mass range of 40-650 m/z with 1,000 ev of the threshold was purposed. The injector was set in the split injection mode having 250 °C of temperature. The ratio applied for split mode was 10.0. The starting temperature was adjusted to 80 °C (3 min), which afterwards increased to 280 °C with a ramp rate of 10 °C/min. Helium (>99.99 %) with 40.5 cm/s of linear velocity was employed as a carrier gas. The system was programmed with 16.3 ml/min of total flow rate and 1.21 ml/min of column flow.

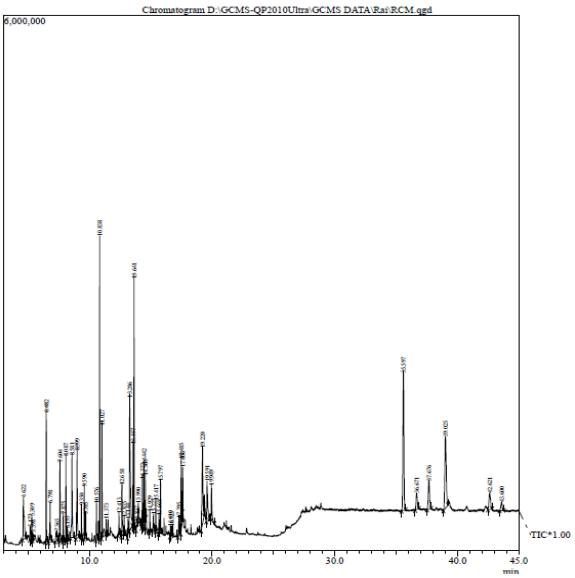
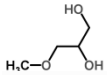
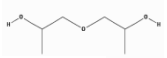
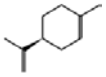
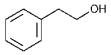
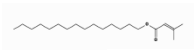
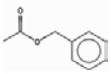
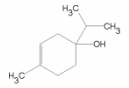
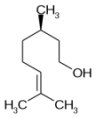
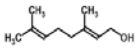
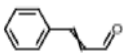
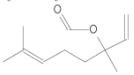
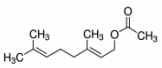
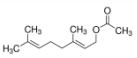
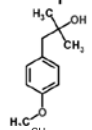
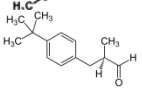
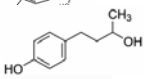
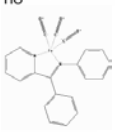
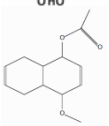
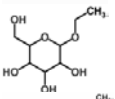
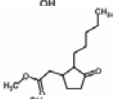
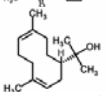
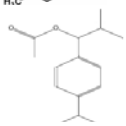
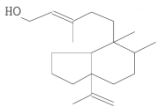
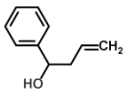
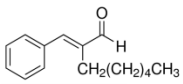
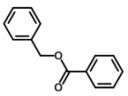
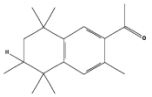
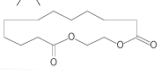
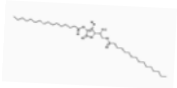
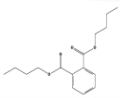
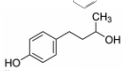
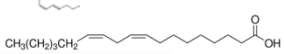
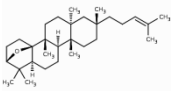


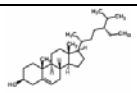
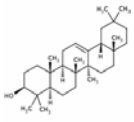
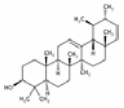
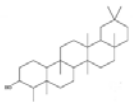
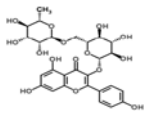
Fig. 1: GC-MS chromatogram of the constituents of methanolic leaf extract of *R. campanulatum*

Components were recognized by their retention time (RT) and elucidation of mass spectra. The spectral fragmentation of unknown components was compared with the known and standard components provided by the databases of WILEY8LIB and NIST11LIB. The I. U. P. A. C name, molecular weight (MW) and chemical structure of the unknown components are mentioned in table 1. The GC-MS chromatogram of the extract of *R. campanulatum*, revealed the presence of 49 phytochemicals, which manifest the presence of several classes of compounds like alkane, fatty acid, terpenes, organic compounds, ester, steroids and flavonoids (fig. 1).

Table 1: The phytochemical compounds identified in *R. campanulatum* by GC-MS

Peak	IUPAC name	RT	Area%	Formula	MW	Chemical structure
1.	3-Methoxypropane-1,2-Diol	4.622	2.84	$C_4H_{10}O_3$	106	
2.	Bis(2-Hydroxypropyl) Ether	5.177	0.63	$C_6H_{14}O_3$	134	
3.	D-Limonene	5.309	0.73	$C_{10}H_{16}$	136	
4.	Eucalyptol	5.39	0.29	$C_{10}H_{18}O$	154	
5.	Beta-Linalool	6.482	3.35	$C_{10}H_{18}O$	154	
6.	Phenethyl Alcohol	6.798	1.66	$C_8H_{10}O$	122.17	
7.	Pentadecyl 3-Methyl-2-Butenoate	7.365	0.76	$C_{20}H_{38}O_2$	310	
8.	Acetic Acid, Phenylmethyl Ester	7.604	2.14	$C_9H_{10}O_2$	150	
9.	1-Isopropyl-4-Methyl-3-Cyclohexen-1-Ol	7.875	0.68	$C_{10}H_{18}O$	154	
10.	Dodecane	8.087	1.85	$C_{12}H_{26}$	170	
11.	Beta-Citronellol	8.581	3.44	$C_{10}H_{20}O$	156	
12.	Geraniol	8.999	3.39	$C_{10}H_{18}O$	154	
13.	Phenylacrylaldehyde	9.350	0.96	C_9H_8O	132	
14.	Thymol	9.590	1.13	$C_{10}H_{14}O$	150	
15.	1,6-Octadien-3-Ol, 3,7-Dimethyl-, Formate	9.705	0.55	$C_{11}H_{18}O_2$	182	
16.	3,7-Dimethyl-2,6-Octadienyl Acetate	10.576	1.01	$C_{12}H_{20}O_2$	196	
17.	Geranyl Acetate	10.838	5.91	$C_{12}H_{20}O_2$	196	
18.	Tetradecane	11.027	2.14	$C_{14}H_{30}$	198	
19.	Alpha-Gurjunene	11.373	0.29	$C_{15}H_{24}$	204	
20.	7-Isopropenyl-4a-Methyl-1-Methylenedecahydronaphthalene	12.413	0.55	$C_{15}H_{24}$	204	
21.	1-(4-Methoxyphenyl)-3-Methylpropanol	12.658	1.81	$C_{11}H_{16}O_2$	180	
22.	Lily Aldehyde	12.835	0.56	$C_{14}H_{20}O$	204	

23.	Hedycaryol	13.144	0.41	$C_{15}H_{26}O$	222	
24.	Betuligenol	13.286	8.00	$C_{10}H_{14}O_2$	166	
25.	Iron, Tricarbonyl[N-(Phenyl-2-Pyridinylmethylene)Benzenamine-N,N']	13.557	1.63	$C_{21}H_{14}FeN_2O_3$	398	
26.	Phthalic Acid	13.641	5.16	$C_{12}H_{14}O_4$	222	
27.	4-Methoxy-1,4,4a,5,8,8a-Hexahydro-1-Naphthalenyl Acetate	13.837	0.38	$C_{13}H_{18}O_3$	222	
28.	Ethyl hexopyranoside	13.990	1.52	$C_8H_{16}O_6$	208	
29.	Methyl (3-Oxo-2-Pentylcyclopentyl)Acetate	14.322	1.53	$C_{13}H_{22}O_3$	226	
30.	2-(4,8-Dimethyl-3,7-Cyclodecadien-1-Yl)-2-Propanol	14.442	2.00	$C_{15}H_{26}O$	222	
31.	1-(4-Isopropylphenyl)-2-Methylpropyl Acetate	14.564	0.92	$C_{15}H_{22}O_2$	234	
32.	5-(7a-Isopropenyl-4,5-Dimethyl-Octahydroinden-4-Yl)-3-Methyl-Pent-2-En-1-Ol	14.929	0.58	$C_{20}H_{34}O$	290	
33.	1-Phenyl-3-buten-1-ol	15.225	0.73	$C_{10}H_{12}O$	148	
34.	Alpha-Hexylcinnamyl Aldehyde	15.417	0.73	$C_{15}H_{20}O$	216	
35.	Benzoic Acid, Phenylmethyl Ester	15.668	0.41	$C_{14}H_{12}O_2$	212	
36.	Chlorooctadecane	15.797	1.21	$C_{18}H_{37}Cl$	288	
37.	Tonalid	16.723	0.24	$C_{18}H_{26}O$	258	
38.	1,4-Dioxacyclohexadecane-5,16-Dione	17.295	0.45	$C_{14}H_{24}O_4$	256	
39.	L-(+)-Ascorbic Acid 2,6-Dihexadecanoate	17.485	2.15	$C_{38}H_{68}O_8$	652	
40.	Dibutyl Phthalate	17.606	0.93	$C_{16}H_{22}O_4$	278	
41.	(R)-(-)-14-Methyl-8-Hexadecyn-1-ol	19.220	5.19	$C_{17}H_{32}O$	252	
42.	10,12-Hexadecadien-1-Ol	19.591	2.59	$C_{16}H_{30}O$	238	
43.	Cis-9,Cis-12-Octadecadienoic Acid	19.949	1.58	$C_{18}H_{32}O_2$	280	
44.	Baccharis Oxide	35.597	9.99	$C_{30}H_{50}O$	426	

45.	Stigmast-5-En-3-Ol	36.671	1.43	$C_{29}H_{50}O$	414	
46.	Beta-Amyrin	37.676	2.64	$C_{30}H_{50}O$	426	
47.	Alpha-Amyrin	39.025	7.38	:$C_{30}H_{50}O$	426	
48.	4,4a,6b,8a,11,11,12b,14a-Octamethyl-Docosahydro-Picen-3-Ol	42.621	1.82	$C_{30}H_{52}O$	428	
49.	Flavone 4'-OH,5-OH,7-Di-O-Glucoside	43.600	0.86	$C_{27}H_{30}O_{15}$	594	

Among these 49 phytoconstituents, baccharis oxide showed the highest area (9.99 %) followed by betuligenol (8.00 %), alpha-amyrin (7.38 %) geranyl acetate (5.91 %), phthalic acid (5.16), linalool (3.35%), citronellol (3.44%) and geraniol (3.39%).

Most of the major phytochemical compounds are either pharmacologically active compounds or the compounds useful for various industries. Baccharis oxide is a type of triterpene, known as a precursor of steroids in both plants and animals [12]. Betuligenol also known as Rhododendrol is an inhibitor of melanin synthesis hence is used in cosmetic industries [13, 14]. A few pharmacological investigations on alpha and beta-amyrin have proven its antioxidant, antimicrobial, anti-inflammatory and anticancer properties [15]. Grenayl acetate, an organic monoterpene, is known to possess antioxidant properties and specific fragrance due to which it is used as cleanser in industries [16, 17]. Citronellol is a monoterpene alcohol found in essential oils and is reported to have anti-covulsant property. It is also used in cosmetic industries [18]. Linalool, a terpene alcohol is a natural compound being used in toothpaste and gargling solution due to its anti-inflammatory and antibacterial activities [19, 20]. Geraniol showed anticancer activity against human colon cancer cell lines (Caco-2) at 400µM of concentration [21]. Phthalic acid is used in industries for the preparation of other important chemicals [22]. From above discussion, it is obvious that the compounds of methanolic extract of leaves of *R. campanulatum* have diverse medicinal properties and different industrial applications. Therefore, the extract can be used for the sourcing of these compounds for various applications.

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CONFLICT OF INTERESTS

The author hereby declares no conflict of interest regarding the manuscript and experimentation done

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