ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 9, Issue 1, 2016

ISSN - 0974-2441

Research Article

GAS CHROMATOGRAPHY AND MASS SPECTROMETRY ANALYSIS OF METHANOLIC EXTRACT OF LEAVES OF *RHODODENDRON ARBOREUM*

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Received: 17 August 2015, Revised and Accepted: 07 November 2015

ABSTRACT

Objective: The objective of the present study is to determine the possible phytoconstituents identified by gas chromatography and mass spectrometry (GC-MS) analysis of a methanolic leaf extract of *Rhododendron arboreum* (RAM).

Method: The extraction of RAM was done by accelerated solvent extraction system at room temperature and high pressure using methanol as a solvent. GC-MS analysis of lyophilized methanolic leaf extract of plant samples was carried out by GCMS-QP2010 plus (Shimadzu, Kyoto, Japan).

Results: In RAM, 34 phytochemicals were identified among which 22-stigmasten-3-one showed the highest area (14.59%) and benzyl acetate showed the lowest area (0.15%). The major compounds identified were 22-stigmasten-3-one, 1,1,6-trimethyl-3-methylene-2-(3,6,10,13,14-pentamethyl-3-ethenyl-pentadec-4-enye)cyclohexane, alpha-amyrin, beta-amyrin, linoleyl alcohol, linoleic, beta-citronellol, tetradecane, 9,12-octadecadienoic acid, methyl ester, dibutyl phthalate, L-ascorbic acid 2, 6-dihexadecanoate, dodecane, and heptadecane.

Conclusion: GC-MS analysis revealed the presence of hydrocarbon alkane, ester, terpenes, flavonoids, organic compounds, steroids, and fatty acids in RAM. These active phytoconstituents contribute to the medicinal efficacy of the plant and the plant can be used for the sourcing of these compounds.

Keywords: *Rhododendron arboreum*, Gas chromatography-mass spectrometry, Phytoconstituents.

INTRODUCTION

Currently, medicinal plants are increasingly gaining importance in pharmaceutical and scientific societies, as they are the richest biological resources of traditional medicines, food supplements, and nutraceuticals [1]. The official value of medicinal plants depends on their bioactive phytoconstituents. These phytoconstituents identified in medicinal plants can be used to cure many diseases and disorders today and can be studied to show definite physiological activity in the human body. The screening of chemical constituents present in herbal medicines has been of great interest to the scientists for discovering therapeutic agents/drugs, effective in remedy for several diseases [2].

The *Rhododendron* genus consists of about 1025 species all over the world, which are distributed mostly at higher altitudes in the Sino-Himalayan region [3,4]. *Rhododendron* species were traditionally used in the treatment of different disorders and diseases related to liver, lung, and gastrointestinal disorders, to cure cough, cold, fever, inflammation, mental retardation, headache asthma, arthritis, skin disease, and body detoxification, etc. [5]. In India, the genus comprises about 80 species with 10 subspecies and 14 varieties [6]. *Rhododendron arboreum* (*R. arboreum*) is the state tree of Uttarakhand and state flower of Himachal Pradesh, India. The species in India is restricted to the Himalayan region from Kashmir to Bhutan and to the hills of Manipur and Sikkim at an altitude of 1200–4000 m [7]. In general, *R. arboreum* is known to possess many biomedicinal properties such as anti-inflammatory, anti-diabetic, antibacterial, analgesic, hepatoprotective, and cardio-protective [8]. Flower juice of *R. arboreum* has been used as a health drink for its adaptogenic properties in Uttarakhand since long back. The leaf extract of *R. arboreum* is reported having high antioxidant content with hepatoprotective, immunomodulatory, and antimicrobial activities [9-11]. *R. arboreum* leaves were accounted to have quercetin, hyperoside, ericolin, ursolic acid, alpha-amyrin, glucoside, beta-sitosterol, lupeol, and epifriedelinol [5,12,13]. Although a few phytoconstituents have been explored using a different method, but there is no report of gas chromatography and mass spectrometry (GC-MS) analysis of *R. arboreum*. Therefore, we tried to analyze the phytoconstituents more extensively using GC-MS.

This study will be helpful in the identification of phytoconstituents that can be an alternative for the sourcing of bioactive compounds.

METHODS

Collection and identification of plant materials

R. arboreum leaves were collected from the Chaurangi region of District Uttarkashi. The plant sample was identified and herbarium sample is submitted to the Departmental herbarium of Department of Botany at H.N.B Garhwal University, Srinagar, vide voucher number GUH20742.

Extraction and sample preparation

R. arboreum leaves were dried in sterilized condition and then were crushed to fine powder. The methanolic extract of powdered sample was prepared in accelerated solvent extraction (ASE) system equipped with a solvent controller unit (ASE350, Dionex Corporation, Sunnyvale, CA, USA) at room temperature and high pressure (1500 psi). The lyophilized methanolic extract was then stored at 4°C for further analysis.

The sample was prepared by dissolving lyophilized methanolic extract of *R. arboreum* in methanol at 1 mg/ml of concentration. The volume of 1.0 µl of each sample was injected into the GC-MS system for analysis of possible active phytoconstituents.

GC-MS analysis

GC-MS analysis was done at the University Science Instrumentation Centre, AIRF, Jawaharlal Nehru University, Delhi. GC-MS-QP2010 Plus (Shimadzu, Kyoto, Japan) system was employed for GC-MS analysis, which comprises the headspace sampler (AOC-20s) and autoinjector (AOC-20i). The system was equipped with mass selective detector with an ion source having temperature 220°C and interface temperature 260°C. Capillary column used for MS analysis was Rt × 5MS capillary

column with 30 mm × 0.25 mm (length × diameter) and 0.25 µm of film thickness. The temperature of the injector was adjusted to 250°C, possessing a split injection mode. The initial temperature applied was 80°C (3 minutes), which was further programmed to increase to 280°C at a ramp rate of 10°C/minutes. Helium (>99.99%) was used as carrier gas with 40.5 cm/seconds of linear velocity. The total flow programed was 16.3 ml/minutes, with column flow of 1.21 ml/minutes.

Identification of compounds

Components were identified on the basis of retention time (RT) for GC and interpretation of mass spectrum was done by comparing spectral fragmentation obtained, to the database provided by WILEY8LIB and National Institute Standard and Technology (NIST11LIB).

RESULTS

GC-MS is a combined technique which is used to identify different substances within the sample. It works on separation of the individual compound by GC according to their RT and the separated compounds were further analyzed at a molecular level by MS. The GC-MS analysis revealed the presence of 34 phytoconstituents from which some are higher hydrocarbon alkanes, ester, terpenes, flavonoids, organic compounds, steroids, and fatty acids. The chromatogram in Fig. 1 showed distinct peaks on the basis of RT and area %. The phytoconstituents identified along with their chemical structure in the methanolic extract of *R. arboreum* by GC-MS peak report of total ion chromatogram (TIC) are shown in Table 1.

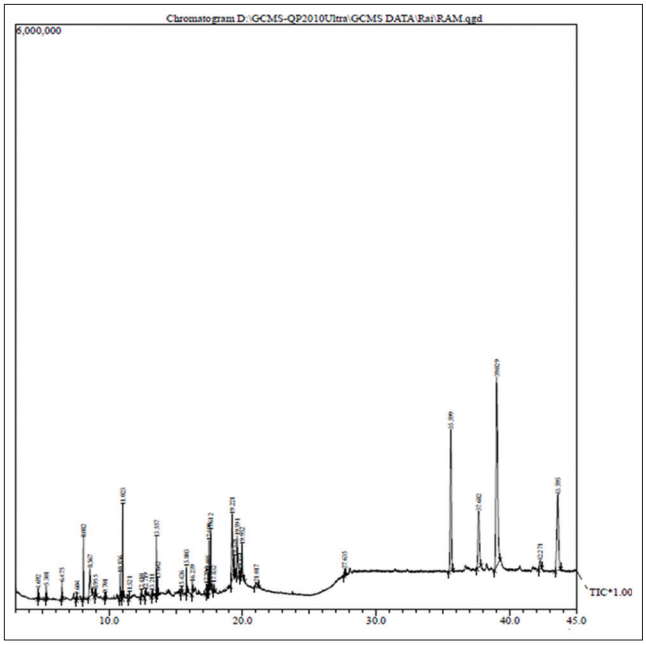
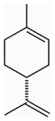
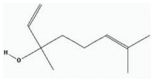
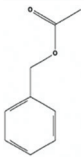
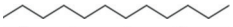
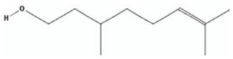
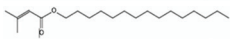
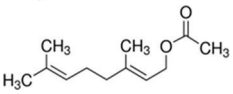
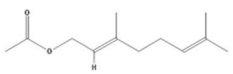
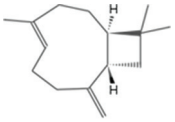
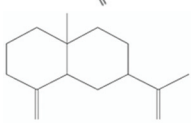
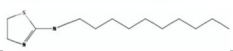


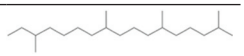
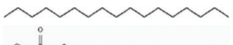
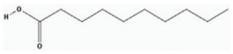
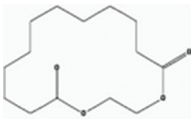
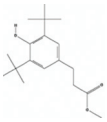
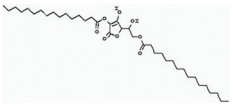
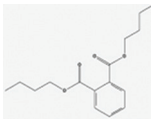
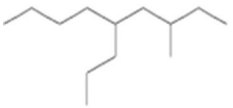
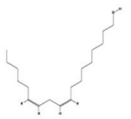
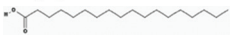
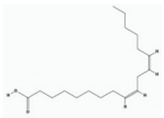
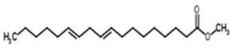
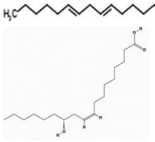
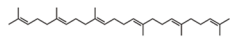
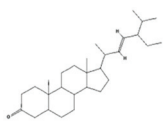
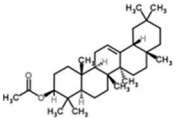
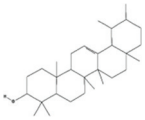
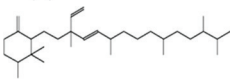
Fig. 1: Gas chromatography and mass spectrometry chromatogram of the phytoconstituents of *Rhododendron arboreum*

Table 1: Phytoconstituents identified in the methanolic extract of RAM by GC-MS peak report of TIC

Peak	IUPAC name	RT	Area %	Formula	Molecular weight	Chemical structure
1	Decane	4.692	0.55	$C_{10}H_{22}$	142	
2	D-limonene	5.301	0.62	$C_{10}H_{16}$	136	
3	Beta-linalool	6.475	0.59	$C_{10}H_{18}O$	154	
4	Benzyl acetate	7.604	0.15	$C_9H_{10}O_2$	150	
5	Dodecane	8.082	1.95	$C_{12}H_{26}$	170	
6	Beta-citronellol	8.567	3.34	$C_{10}H_{20}O$	156	
7	3-methyl-2-butenoic acid, pentadecyl ester	8.955	0.36	$C_{20}H_{38}O_2$	310	
8	3,7-dimethyl-2,6-octadienyl formate	9.701	0.18	$C_{11}H_{18}O_2$	182	
9	Geranyl acetate	10.836	0.83	$C_{12}H_{20}O_2$	196	
10	Tetradecane	11.023	2.82	$C_{14}H_{30}$	198	
11	Caryophyllene	11.521	0.17	$C_{15}H_{24}$	204	
12	7-isopropenyl-4a-methyl-1-methylenedecahydronaphthalene	12.410	0.21	$C_{15}H_{24}$	204	
13	2-[n-decylamino]-2-thiazoline	12.719	0.40	$C_{13}H_{26}N_2S$	242	

(*Contd...*)

Table 1: (*Continued*)

Peak	IUPAC name	RT	Area %	Formula	Molecular weight	Chemical structure
14	2,6,10,15-tetramethylheptadecane	13.211	0.19	$C_{21}H_{44}$	296	
15	Heptadecane	13.557	1.72	$C_{21}H_{44}$	296	
16	Phthalic acid, ethyl pentadecyl ester	13.642	0.58	$C_{25}H_{40}O_4$	404	
17	Decanoic acid	15.426	0.22	$C_{10}H_{20}O_2$	172	
18	Nonadecane	15.803	0.86	$C_{19}H_{40}$	268	
19	Neophytadiene	16.239	0.40	$C_{20}H_{38}$	278	
20	Ethylene dodecanedioate	17.296	0.30	$C_{14}H_{24}O_4$	256	
21	Methyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl) propionate	17.405	0.56	$C_{18}H_{28}O_3$	292	
22	L-(+)-ascorbic acid 2,6-dihexadecanoate	17.490	1.97	$C_{38}H_{68}O_8$	652	
23	Dibutyl phthalate	17.612	2.04	$C_{16}H_{22}O_4$	278	
24	3-methyl-5-propylnonane	17.832	0.23	$C_{13}H_{28}$	184	
25	Linoleyl alcohol	19.221	6.50	$C_{18}H_{34}O$	266	
26	Stearic acid	19.378	0.68	$C_{18}H_{36}O_2$	284	
27	Linoleic	19.591	3.76	$C_{18}H_{32}O_2$	280	
28	9,12-octadecadienoic acid, methyl ester	19.952	2.08	$C_{19}H_{34}O_2$	294	
29	Ricinoleic acid	21.017	0.85	$C_{18}H_{34}O_3$	298	
30	Squalene	27.635	0.33	$C_{30}H_{50}$	410	
31	22-stigmasten-3-one	35.599	14.59	$C_{29}H_{48}O$	412.6	
32	Beta-amyrin	37.682	7.62	$C_{30}H_{50}O$	426	
33	Alpha-amyrin	42.271	1.02	$C_{30}H_{50}O$	426	
34	1,1,6-trimethyl-3-methylene-2-(3,6,10,13,14-pentamethyl-3-ethenyl-pentadec-4-enye) cyclohexane	43.595	12.26	$C_{32}H_{58}$	442	

GC-MS: Gas chromatography and mass spectrometry, TIC: Total ion chromatogram, RAM: *Rhododendron arboreum*, RT: Retention time

In RAM, 22-stigmasten-3-one (14.59%) showed the highest area %, followed by 1,1,6-trimethyl-3-methylene-2-(3,6,10,13,14-pentamethyl-3-ethenyl-pentadec-4-enye)cyclohexane (12.26%), beta-amyrin (7.62%), linoleyl alcohol (6.50%) as major phytoconstituents. These major phytoconstituents were reported with antimicrobial, anticancer, antiarthritic, anti-inflammatory, and antiviral properties.

DISCUSSION

A range of volatile components (especially oils) has been identified by GC-MS in different *Rhododendron* species other than *R. arboreum* [14,15]. The 22-stigmasten-3-one is a steroid compound which possesses several medicinal properties such as, antimicrobial, antiarthritic, anti-asthma, and diuretic properties [16]. Alpha- and beta-amyrin were tested for their pharmaceutical importance and observed with antioxidant, antimicrobial, anti-inflammatory, and anticancer properties [17]. Linoleyl alcohol is a fatty acid derivative and is the product of linoleic acid reduction, whereas linoleic acid is an omega-6-fatty acid and is enormously used in cosmetic industries. The conjugated linoleic acid was reported with anticarcinogenic, fat-reducing, antiatherogenic, and immune-enhancing activity [18]. Citronellol, a monoterpene alcohol**,** is a major constituent of essential oil, hence utilized in cosmetic industries and is also reported with anticonvulsant property [19]. 9,12-Octadecadienoic acid, methyl ester possess hepatoprotective, antihistaminic, hypocholesterolemic, and antieczemic properties [20]. L-(+)-ascorbic acid 2,6-dihexadecanoate, an ester of ascorbic acid, is used as an antioxidant food addictive [21]. Dibutyl phthalate is used for production of flexible plastics and also has low acute and chronic toxicity [22]. Dodecane is used in industries as a solvent and in distillation chaser and scintillator [23]. Heptadecane, a volatile compound**,** consists of antioxidant property and reduces age-related oxidative stress [24]. 1,1,6-trimethyl-3-methylene-2-(3,6,10,13,14-pentamethyl-3-ethenyl-pentadec-4-enye)cyclohexane is found as a second major constituent, which might have several industrial applications like other cyclohexanes. This is the first report on the GC-MS analysis of methanolic leaves extract of *R. arboreum*, worldwide. Methanolic extract of leaves of *R. arboreum* is found to be rich in several bioactive and industrially important compounds.

CONCLUSION

The GC-MS analysis of methanolic extract of leaves of *R. arboreum* showed a highly composite profile of phytoconstituents. Extraction through ASE, at room temperature and high pressure, resulted in the enrichment of medicinally important phenolics. Methanolic leaf extract of *R. arboreum* has shown the presence of diverse classes of compounds having pharmacological and industrial importance. The extraction process can be implied for the sourcing of the compounds of commercial and medicinal importance.

ACKNOWLEDGMENT

We are grateful to the Graphic Era University, Dehradun, for supporting this research and providing us a platform for the execution of our research idea. We are also thankful to Dr. Kshipra Mishra, Sc 'F' and Dr. Raj Kumar Tulswani, Sc 'D', Department of Biochemical Sciences Defence Institute of Physiology and Allied Sciences, DRDO, Delhi-54, for their assistance in the extraction through ASE.

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