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Research Article

GC-MS (Gas chromatography and mass spectroscopy) analysis of methanol leaf extract of *Rhododendron arboreum* Sm. of District Sirmaur, Himachal Pradesh

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Abstract

Plants are a rich source of secondary metabolites or chemical constituents with interesting biological activities. Therefore, a thorough validation of the herbal agents has emerged as a new branch of science emphasizing and prioritizing the standardization of the natural drugs and products because several of these phytochemicals have complementary and overlapping mechanism of action. Mass spectrometry coupled with chromatographic separations such as Gas chromatography (GC-MS) is generally used for direct analysis of components existing in traditional medicines and medicinal plants. Gas chromatography separates the components of the mixture and mass spectroscopy analyses each of these components separately. In recent years, GC-MS studies have been increasingly applied for the analysis of medicinal plants as this technique has proved to be a valuable method for the analysis of non-polar components, volatile essential oils, fatty acids, lipids, alkaloids etc. The present study was aimed to find out the bioactive compounds present in methanol extract of *Rhododendron arboreum* Sm. by using Gas chromatography and Mass spectroscopy. GC-MS chromatogram showed 10 peaks indicating the presence of ten phytochemical constituents in this plant. The results revealed Limonene (18.48%), Caryophyllene (13.01%), 3-Heptanoicacid methyl ester (11.00%) and Phenol, 2-(2-methyl-2-propenyl) (5.52%) as the major components in the methanol extract. The results support the use of *R. arboreum* in traditional medicines for controlling bacterial, degenerative and other diseases. Thus, the present study approves the medicinal value of this plant and scientifically validates it for use as a component of medicinal preparations.

Keywords: *Rhododendron arboreum*, methanol, leaf extract, phytochemicals, Gas chromatography and Mass spectroscopy (GC-MS)

INTRODUCTION

Phytochemicals (from the Greek word *phyto* meaning 'plant') are naturally occurring chemical compounds synthesized during various metabolic processes. These chemicals are often called secondary metabolites and serve as plant defence mechanisms against pathogenic microbes such as bacteria, viruses and fungi[1]. They protect or defend plants from disease and damage and contribute to the plant's colour, aroma and flavour. In short, the plant chemicals that protect plant cells from biological as well as environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are known as phytochemicals[2,3]. Now, it is clearly known that these phyto-constituents have roles in the protection of human health when their dietary intake is significant. Till date, more than 4,000 phytochemicals have been catalogued and classified based on their protective functions, physical and chemical characteristics[4].

These phytochemicals are classified as phenols, flavonoids, alkaloids, quinones, tannins, terpenes, glycosides and polysaccharides and accumulate in different parts of the plants such as roots, stems, leaves, flowers, fruits or seeds[5,6]. The quantity and quality of phytochemicals present in different plants may differ from one part to another depending upon the variety, processing and environmental conditions[7].

The knowledge of phytochemicals is desirable not only for the discovery of therapeutic agents but also because such information may be of great value in revealing new sources of economic phyto-compounds for the synthesis of complex chemical substances and thereby discovering the actual significance of folkloric remedies[8]. Gaining this knowledge requires overcoming many analytical challenges posed by these complex mixtures because they normally exhibit huge variations in component amounts, chemical structures and their functionalities[9]. The most popular and prevalent method of studying phytochemical composition of a plant extract is Gas Chromatography and Mass Spectroscopy (GC-MS) as this is very compatible and the most commonly used technique for the identification as well as quantification purpose[10]. The Mass spectrometry (MS) coupled with chromatographic separations such as Gas chromatography (GC) is generally used for direct analysis of components existing in traditional medicines and medicinal plants[11]. Gas chromatography separates the components of the mixture and eventually mass spectroscopy analyses each of the component/s separately[12]. In recent years, GC-MS studies have been increasingly applied for the analysis of medicinal plants as this technique has proved to be a valuable method for the analysing non-polar components, volatile essential oils, fatty acids, lipids and alkaloids[13,14].

Rhododendron arboreum Sm. (Family: Ericaceae) is an evergreen much branched tree up to 14 m in height & 2.4 m in girth. The leaves generally are oblong lanceolate, 10-20 cm

long and 3.6 cm wide. The flowers of this plant range in colour from a deep scarlet to red with white markings, pink to white, capsule-curved central column composed of fine lobes, ribbed, up to 3.8 cm long and 1.25 cm wide. The seeds are minute, dark-brown, compressed, thin linear with an obvolute membrane[15]. Originally reported in North Central India, the plant is found in the Himalayas from Kashmir to Bhutan & in the hills of Assam & Manipur at an altitude of 1200-4000 meters. It grows at an elevation of about 4,500 to 10,500 ft & grows up to 40 to 50 ft high even sometimes attaining over 100 ft[16].

The dried flowers of *R. arboreum* are highly efficacious in checking diarrhoea and blood dysentery[17]. The young leaves are usually known to be poisonous (associated with intoxication in higher quantities) as well as medicinal and applied on the forehead to alleviate headache[18]. Therefore, the present study was undertaken to find out the bioactive compounds present in methanol extract of *Rhododendron arboreum* Sm. leaves by using Gas chromatography and Mass spectroscopy.

MATERIALS AND METHODS

Collection and processing of plant material

The fresh leaves of *Rhododendron arboreum* Sm. were plucked, collected and were brought to the laboratory for further examinations. The leaves were washed thoroughly under tap water and then treated with 2% Mercuric chloride. Thereafter, the plant parts were cut into smaller pieces for quick drying. The plant material thus obtained after drying was crushed into fine powder with the help of pestle mortar and stored in air-tight containers at room temperature.

GC-MS analysis

GC-MS analysis was carried out using thermo GC model TRACE 1300 and thermo MS model TSQ 8000 (triple quadrupole) equipment employing the following conditions i.e Thermo TG 5MS Column having dimensions of "30 m × 0.25 mm × 0.25 µm", operated in electron impact mode at 70 eV. Helium (He 99.99%) was used as carrier gas at a constant flow rate of 1 mL per minute with an injection volume of 1 µL. An injector temperature of 250°C and an ion-source temperature of 280°C were regularly employed in this process. The Oven program consisted of maintaining the temperature at 60°C for about 2 minutes followed by an increase to 250°C at the rate of 15°C/minute and maintaining this temperature of 250°C for a further 15 minutes duration. Samples or extracts dissolved in methanol solvent were run fully at a range of 50-1000 m/z. The mass spectra were detected in approximately 26 minutes and the percentage of each chemical constituent/component was calculated by comparing the average peak area to the total area.

Identification of component/s

Interpretation of mass spectrum of GC-MS was done by using the database of National Institute Standard and Technology (NIST) comprising more than 62,000 patterns. Thereafter, the spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST Library. The name, molecular weight and structure of the components of the test materials were ascertained eventually.

RESULTS AND DISCUSSIONS

GC-MS chromatogram of methanol extract of *Rhododendron arboreum* leaves showed 10 peaks indicating the presence of ten phytochemical constituents. The results revealed that Limonene (18.48%), Caryophyllene (13.01%), 3-Heptanoicacid methyl ester (11.00%) and Phenol, 2-(2-methyl-2-propenyl) (5.52%) were found to be the major components in methanol extract. Essential oils of some *Rhododendron* species have been investigated and examined. Mostly terpenes such as *α*- humulene, caryophyllene, limonene, *α*-pinene formed the main constituents of the essential oils[19]. Limonene, a cyclic terpene, has been demonstrated to possess bacteriostatic activity against several microorganisms[20,21]. Limonene also demonstrated strong chemo-preventive effects in rodent lymphomas and mammary, gastric, skin, liver and lung cancers also[22]. Venkata et al.[23] reported that caryophyllene possesses antimicrobial, antioxidant, anti-tumour, analgesic, antibacterial, anti-inflammatory, sedative and fungicide properties. Terpenes (caryophyllene) are known to be active against a wide variety of microorganisms, including Gram-positive and Gram-negative bacteria and some fungal species[24]. 3-Heptanoicacid methyl ester and Phenol, 2-(2-methyl-2-propenyl) have been investigated to have cancer preventive, insectifuge, antioxidant, hypo-chloesterolemic, nematicide, pesticide, lubricant, antiandrogenic, haemolytic, 5-alpha reductase inhibitor activities[25].

Many studies on green leaves of *Rhododendron arboreum* revealed the presence of glucoside, ericolin (arbutin), ursolic acid, α-amyrin, epifriedelinol, triterpenoid campanulin, quercetin & hyperoside, lupeol and epifriedelinol. Quercetin-3-rhamnoside which is a crystalline chemical compound has been reported from the flowers of this plant[26]. The bark of this plant is found to be the richest source of single triterpenoid substance taraxerol and ursolic acid acetate[27].

Table 1: GC-MS spectral analysis of methanol extract of *Rhododendron arboreum*

Peak No.	Retention time	Name of compound	Molecular formula	Peak area %
1	8.18	Geraniol formate	$C_{11}H_{18}O_2$	1.76
2	10.68	1-Hexadecanol	$C_{16}H_{34}O$	1.08
3	12.89	Limonene	$C_{10}H_{16}$	18.48
4	13.20	Phenol, 2-(2-methyl-2-propenyl)	$C_{10}H_{12}O$	5.52
5	13.98	Caryophyllene	$C_{15}H_{24}$	13.01
6	14.57	1-Cyclohexene1butanal,a,2,6,6-tetramethyl	$C_{14}H_{24}O$	0.99
7	16.91	2-Undecanone,6,10-dimethyl	$C_{13}H_{26}O$	1.38
8	19.53	Phytol	$C_{20}H_{40}O$	1.81
9	25.42	Methyl octanoate	$C_9H_{18}O_2$	1.04
10	32.21	3-Heptanoicacid methyl ester	$C_8H_{14}O_2$	11.00

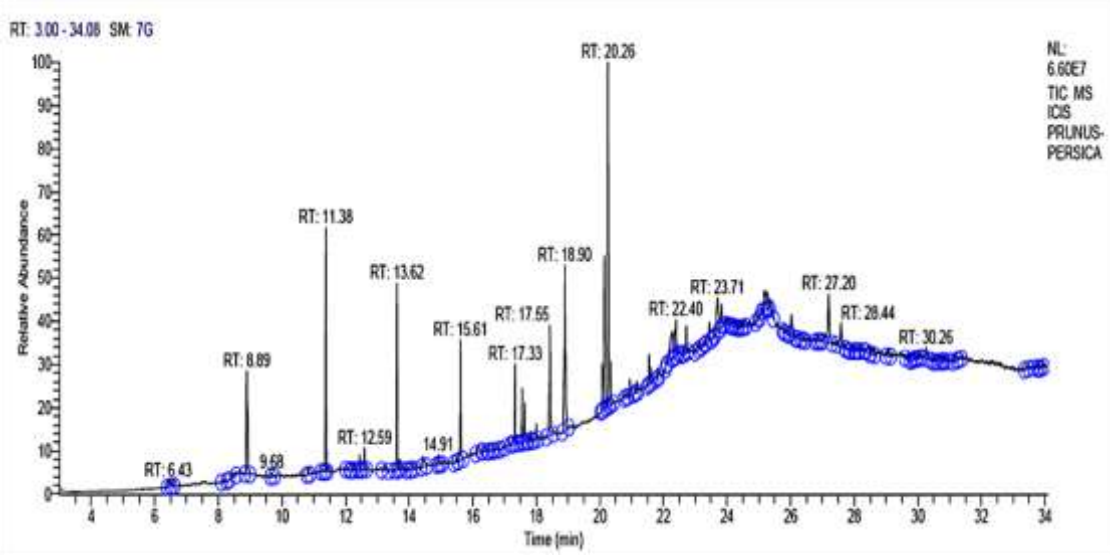

Figure 1: GC-MS chromatogram of methanol leaf extract of *R. arboreum*

CONCLUSION

The results support the use of *Rhododendron arboreum* in traditional medicines for controlling bacterial, degenerative and other diseases. Thus, the present study approves the medicinal value of this plant and scientifically validates it for use as a component of medicinal preparations. The study was conducted to find out the bio-active constituents present in methanol extract of *Rhododendron arboreum* leaves by using Gas chromatography and Mass spectroscopy. GC-MS chromatogram of leaf extracts revealed the presence of numerous phytochemicals by showing different peaks. GC-MS analysis revealed the presence of 10 major peaks in methanol leaf extract. The major chemical compounds were reported to be Limonene (18.48%), Caryophyllene (13.01%), 3-Heptanoicacid methyl ester (11.00%) and Phenol, 2-(2-methyl-2-propenyl) (5.52%). The presence of these phyto-constituents in this plant may be directly or indirectly associated with its valuable medicinal properties.

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Competing interests

Authors hereby declares that no competing interests exist.

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