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Phytochemical and GC-MS analysis of *Rhododendron arboreum* flowers

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ABSTRACT

Quantitative phytochemical profiling and qualitative analysis of bioactive compounds using GC-MS was done for *Rhododendron arboreum* flowers. The ethanolic extract of flowers was used for phytochemical and GC-MS analysis. Phytochemical screening revealed the presence of saponins, alkaloids, flavonoids and phenolic compounds. High amount of total phenol (65.50 ± 1.12 mg GAE/g) and flavonoid (33.25 ± 0.89 mg RE/g) and low amount of alkaloids (0.03 ± 0.002 mg/g) and saponins (0.11 ± 0.009 mg/g) were found. Large number of different volatile compounds were detected by GC-MS analysis. High amount of phenolic compounds reveals that flowers may have very good antioxidant and antimicrobial activity. Thus flowers of *R arboreum* are a good source of nutraceuticals, functional foods as well as therapeutic agents.

Keywords: *Rhododendron arboreum*; phytochemicals; antioxidant; anti-microbial; nutraceuticals

INTRODUCTION

Secondary metabolites such as phenolic compounds synthesized by plants are helpful in preventing oxidative damage to plants as well as protect humans when these plants are consumed as food (Niciforovic et al 2010). Natural antioxidants are always the first choice because they are better than synthetic antioxidants.

Rhododendron arboreum (Ericaceae) is commonly known as Burans (Himachal Pradesh) and Laliguras (Nepal). It is native to China, Nepal, Malaysia and India. It is an evergreen much branched tree which grows at an elevation of 4500 to 10500 ft amsl (Rai and Rai 1994). Flowering season is from March-April bearing deep red or crimson to pale pink flowers. Simple phenols, flavonoids, anthocyanins and antioxidants are present

in flowers and leaves of *R mucronulatum, R simsii* and other species (Takahashi et al 2001, Krishnaraju et al 2005, Cho et al 2008, Chung et al 1996). Epicatechin, syringic acid, quercetin-3-O-galactoside and quercitrin have been determined from the leaves of *R arboreum, R campanulatum* and *R anthopogon* (Sharma et al 2010).

In recent years secondary metabolites (phyotchemicals) have been investigated as good source of therapeutic agents and with adequate antibacterial efficacy. Thus it can be used for the treatment of bacterial infections (Balandrin et al 1985, Verma et al 2010). Consumption of dietary supplements which are rich in natural phytochemicals helps in reduction of risk of cancer, cardiovascular diseases, diabetes etc (Nagalingam et al 2012, Veerapur et al 2009).

The main aim of this study was to evaluate phenolic compounds, secondary metabolites and GC-MS analysis of the flowers of stately *R arboreum*.

MATERIAL and METHODS

Plant material and extraction

R arboreum flowers were collected from Mandi district, Himachal Pradesh. About 50 g sample of shade dried flowers was extracted with ethanol (350 ml) using Soxhlet apparatus. Extract was concentrated to semi-solid mass by evaporating the solvents and dried in dessicator. The yield was found to be 30 per cent w/w. Sample was kept at -20°C till further use.

Test for Phenol

Total phenolic content was determined according to Folin–Ciocalteu's reagent method (McDonald et al 2001). For this 100 µl of plant extract in DMSO (dimethyl sulfoxide) (1 mg/ml) (Merck India Ltd, Mumbai, India) was mixed with 250 µl of Folin-Ciocalteu reagent. After 5 minutes 1.5 ml 20 per cent Na_2CO_3 solution was added and volume was made to 5 ml. It was allowed to stand for 2 hours and absorbance of the reaction mixture was measured at 760 nm (Hewlett Packard UV/ Vis spectrophotometer). Gallic acid was taken as reference standard. Amount of total phenol was measured in milligram gallic acid equivalent (mg GAE/g) of dried flower extract.

Test for flavonoid

Total flavonoid was determined by aluminium chloride colorimetric method (Chang et al 2002). 150 µl of sample extract in methanol was mixed with 0.45 ml of methanol, 0.03 ml aluminium chloride (10%), 0.03 ml 0.1 M potassium acetate, 0.84 ml distilled water and it was allowed to stand at room temperature for 30 minutes. The absorbance was measured at 415 nm. The absorption of standard rutin solution in methanol was measured under the same conditions. The results were

expressed as mg/g rutin equivalent (mg RE/g) of dried flowers.

Test for alkaloid

For this 2 g of the raw sample was mixed with 100 ml of 10 per cent acetic acid in ethanol and allowed to stand for 2 h at room temperature. The solution was filtered using Whatman filter paper (Fisher Scientific, Nepean, ON, Canada) and concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop-wise to the extract until the precipitation was complete. The precipitate was collected and washed with dilute ammonium hydroxide and again filtered. The dried residue was alkaloid and weighed (Harborne 1973).

Test for saponins

Sample of 2 g was mixed with 44 ml of 20 per cent aqueous ethanol. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55°C. The filtered mixture was mixed with another 44 ml 20 per cent ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separating funnel and 8 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. Six ml of n-butanol was added to it. The combined n-butanol extracts were washed twice with 2 ml of 5 per cent aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight and the saponin content was calculated (Obadoni and Ochuko 2001).

Characterization of secondary metabolites using GC-MS

Mass spectrometric detector (MSD- an Agilent 5975B) was used in scan mode (m/z 35-1050). The automatic RTL screener software in combination with the Agilent NIST'05 library was used to screen volatile and semi-volatiles (Mehra et al 2015).

RESULTS and DISCUSSION

R arboreum was found to have substantial amount of phytochemical content (Table 1). Preliminary phytochemical screening showed the presence of phenolic compounds, alkaloids, flavonoids and saponins. The flowers showed high amount of phenolic content (65.50 ± 1.12 mg GAE/g) and flavonoids (33.25 ± 0.89 mg RE/g). Low amount of alkaloids (0.03 ± 0.002 mg/g) and saponins (0.11 ± 0.009 mg/g) was found.

The presence of these phytochemicals is known to exhibit medicinal as well as physiological activities (Ismaila et al 2011). Both phenolics and flavonoids may possess high scavenging activity due to presence of hydroxyl group (Rajan and Muthukrishnana 2013, Kar

Table 1. Quantitative analysis of phytochemicals in *Rhododendron arboreum* flowers

Total phenolic content (mg GAE/g)	Total flavonoids (mg RE/g)	Alkaloids (mg/g)	Saponins (mg/g)
65.50 ± 1.12	33.25 ± 0.89	0.03 ± 0.002	0.11 ± 0.009

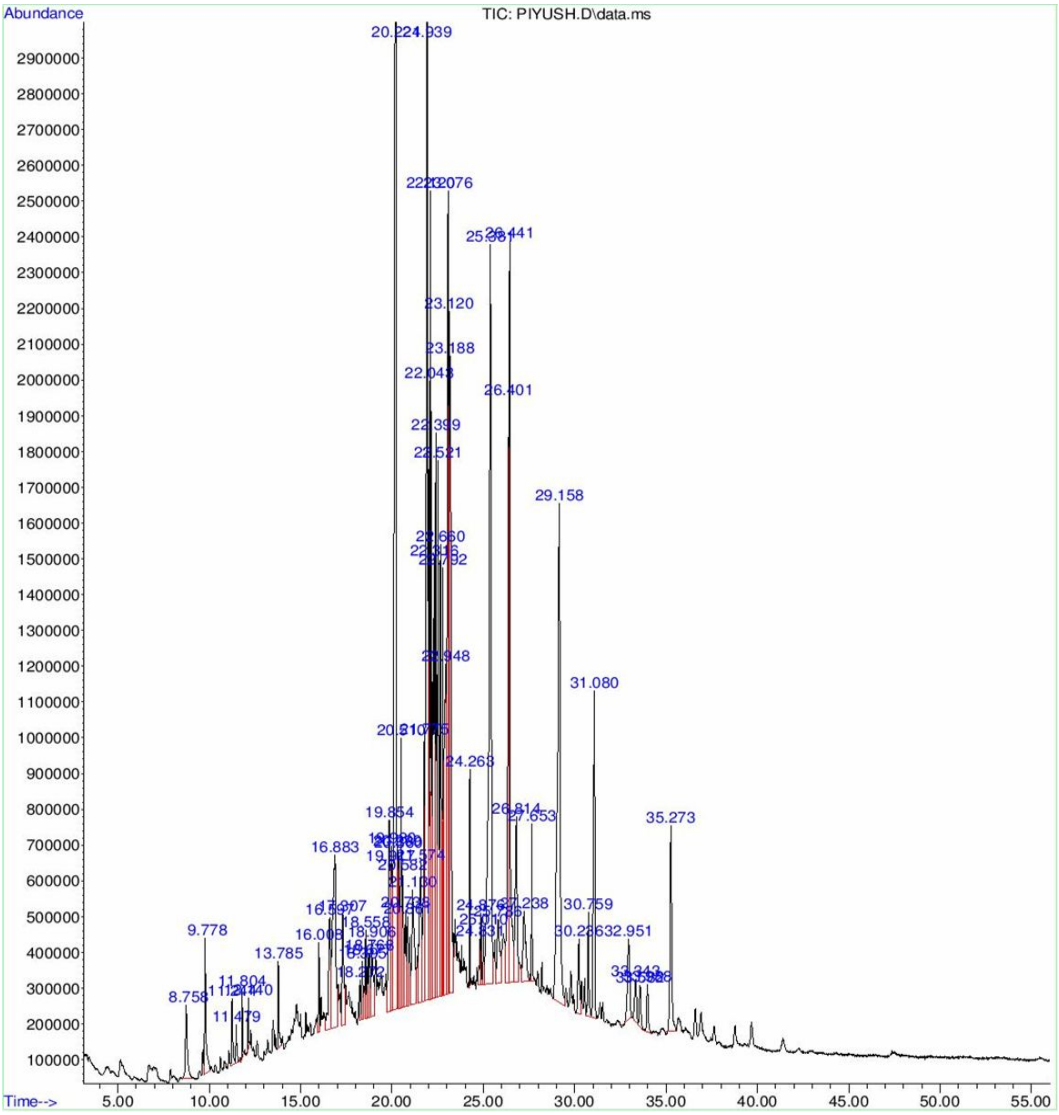

Fig 1. Chromatogram of ethanolic extract of *Rhododendron* flowers

Table 2. List of compounds detected in ethanolic extract of *Rhododendron arboreum*

Compound detected	Area (%)	Retention time
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	0.77	9.783
2-Furancarboxaldehyde, 5-(hydroxymethyl)	0.32	11.241
Butanedioic acid, hydroxy, diethyl ester	0.16	11.801
Butanedioic acid, hydroxy-, (S)	0.16	12.138
Cyclooctasiloxane, hexadecamethyl	0.32	16.007
Cyclononasiloxane, octadecamethyl	0.28	18.553
cis,cis,cis-7,10,13-Hexadecatriena	1.33	19.854
9,12,15-Octadecatrienoic acid, methyl ester	1.33	19.854
9,12,15-Octadecatrienoic acid, ethyl ester	1.33	19.854
Cyclooctene, 3-ethenyl	0.92	19.977
n-Hexadecanoic acid	11.81	20.224
Cyclodecasiloxane, eicosamethyl	0.45	20.302
Hexadecanoic acid, ethyl ester	1.09	20.504
9,12,15-Octadecatrienoic acid	8.61	21.940
9,12,15-Octadecatrien-1-ol	8.61	21.940
Octadecanoic acid	3.18	22.119
7-Heptadecyne, 1-chloro	2.13	22.658
Cyclohexanecarboxaldehyde, 4-(hydroxymethyl)	2.13	22.658
Cyclononasiloxane, octadecamethyl	0.44	24.261
Octadecane, 1-(ethenyloxy)	0.21	24.833
Cyclododecanone, 2-(6-chloro-1-oxohexyl)	0.21	24.833
Eicosane	0.19	24.878
Heptacosane, 1-chloro	0.19	24.878
γ-Sitosterol	7.33	25.383
Stigmasterol, 22,23-dihydro	7.33	25.383
β-Sitosterol	7.33	25.383
Vitamin E	5.49	29.163
Campesterol	0.36	33.346

2007). These phenolic compounds minimize the molecular damage by counteracting the reactive oxygen species. Saponins are known for inhibition of microbial proliferation (Xu et al 1996). Saponins and alkaloids are known to have antibacterial affects (Ani et al 2011).

The mass spectra interpretation of flower extract was conducted by GC-MS. The chromatogram of ethanolic extract of *R arboreum* flowers showing the GC-MS profile of compounds identified is given in Fig 1. The peaks of unknown components present in samples were compared with spectrum of known samples in the library. All the compounds detected in GC-MS analysis are shown along with their area percentage in Table 2. It was found that in ethanolic extract of flowers n-hexadonic acid is present in highest amount with area (11.81%). Along with it α-linolenic acid (1.33%), linolenyl alcohol (8.61%), stearic acid (3.18%), β and γ-sitosterol (7.33%)

and stigmasterol (7.33%) are present in good amount.

Large amount of essential fatty acids was determined in GC-MS analysis of flowers extract. Along with it other fatty acids, vitamin E and other compounds were detected. N-hexadonic acid which is found in largest amount also known as palmitic acid is one of the most common fatty acids. Palmitic acid is widely used as lubricant and additive in food industry. It is also used as antioxidant. Alpha linolenic acid is omega-3 essential fatty acid which is generally found in seeds. It possesses anti-inflammatory, antiarthritic and hepatoprotective properties.

CONCLUSION

In the present study ethanolic extract of flowers of *R arboreum* showed the presence of phenolics, alkaloids, flavonoids, saponins and various secondary metabolites. The obtained compounds have potential antioxidant and antimicrobial activity as well as therapeutic potential. Based on the results it is concluded that flowers of *R arboreum* are a good source for development of nutraceuticals, functional foods as well as therapeutic agents.

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